Ecological Monitoring 2016

***rare* Charitable Research Reserve**

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Cover Photography by Allie Abram. Clockwise from top right; Monarch butterfly, Yellow Birch, Clouded Sulphur butterfly and Juvenile Eastern Red-backed Salamander.

Ecological Monitoring Report Executive Summary

The ***rare*** **Charitable Research Reserve** is a not-for-profit urban land trust and environmental institute that preserves 900+ acres of land along the Grand River in Waterloo Region, Ontario. In 2006, ***rare*** joined Environment Canada's Ecological Monitoring and Assessment Network (EMAN) to establish long-term ecological monitoring programs for the property with the objective of determining the status of ***rare's*** ecosystems and tracking how they change over time. Since 2006, several ongoing monitoring programs have been established at ***rare*** and have been carried out in each subsequent year. In 2016 ecological monitoring programs occurred for butterflies, plethodontid salamanders, forest health, and soil humus decay rates.

Butterfly Monitoring

Butterfly monitoring occurs at ***rare*** across four separate transects for fourteen weeks during the late spring and summer. Butterfly monitoring in 2016 was the second most abundant in butterfly observations after 2012 with 5,820 individual butterfly observations. Both years were very hot and dry, and proceeded by years with relatively high early rainfall, further suggesting that butterfly abundances are closely tied to weather conditions. However, low abundances observed in Transects Three and Four in 2016 relative to all years indicate that there may be other factors at play.

Warm and dry weather conditions appeared to have had negative effects on species that require specific host plants, resulting in a low diversity of species relative to the previous two years. Despite low diversity observed during monitoring, two new species were observed on ***rare*** property outside of monitoring, bringing the total number of species observed at ***rare*** to 75 species.

The most abundant butterfly species were Cabbage White (*Pieris rapae*), followed by Clouded Sulphur (*Colias philodice*) and the European Skipper (*Thymelicus lineola*). Cabbage White and European Skipper are both non-native butterflies, which have seen high abundances in past years. Although Cabbage whites were seen in low abundances relative to previous years, European Skippers appear to generally be increasing in abundance, possibly increasing competition for native butterfly species that use grasses as host plants. Observations of Monarch butterflies continue to be low during monitoring at ***rare***, and it is too soon to know the impacts of milkweed planting on milkweed abundance and Monarch butterflies populations.

Despite annual fluctuations, no significant directional trend in butterfly abundances has occurred over the last seven monitoring years. This may be indicative of stable abundances over time, or of a need for longer term data collection.

Plethodontid Salamander Monitoring

Monitoring of lungless (Plethodontid) salamanders occurs at ***rare*** by turning over pre-placed wooden cover boards in Indian Woods and the Hogsback once a week for nine-weeks each fall. Eastern Red-backed Salamanders (*Plethodon cinereus*) were the most abundant species found in both Indian Woods and the Hogsback in 2016 and in every other monitoring year. Observed salamander abundances in both plots have fallen outside threshold levels, and species diversity was low relative to previous years. Abundance decreases were particularly apparent in the Hogsback, which was previously considered to be relatively stable. The proportion of adult salamanders to other size classes was also the lowest of all years in both plots.

These changes are likely tied to low moisture levels in plots during monitoring, in combination with a variety of other environmental conditions. As the salamander monitoring program acts as a warning sign for environmental change, falling numbers coupled with ongoing human pressures from agriculture, development projects, and the potential for accumulative effects from aggregate extraction highlight the need for continued salamander monitoring at ***rare***.

Forest Canopy and Tree Biodiversity Monitoring

The forest canopy and tree biodiversity monitoring program at ***rare*** occurs in in all three major forest areas; the Hogsback, Indian Woods and the Cliffs and Alvars. Three permanent plots are set-up within each area to track changes in the health of the trees within these forests. 2016 monitoring occurred in all plots with a modification to tree height monitoring, which has been difficult to accurately measure in previous years. It is suggested that this method continue in future years to improve accuracy of collected data, and that height monitoring occur every five years. Forest diversity, heath, and size class distribution have been fairly consistent across monitoring years with the exception of the loss of most ash trees from forest plots. As prevalence of Emerald Ash Borer and Beech Blight Disease continues to be observed within forest stands, it is important that targeted monitoring protocols be developed and implemented in addition to general forest health monitoring. As forest stand composition does not change quickly and results of analysis are not likely to be apparent year to year, it is suggested that monitoring and reports be completed every 5 years.

Emerald Ash Borer Monitoring

As an extension of a pilot project completed in Cliffs and Alvars in 2015 to assess the condition of ash trees, emerald ash borer presence, and the impact of invasive species on native species rejuvenation, a pilot project was completed in Indian Woods with a modified protocol. Ash trees made up less than five percent of trees in survey plots, with confirmed EAB presence in more than 50 percent of trees, and the remaining trees being classified as high risk for EAB. A modified protocol has been proposed to allow for a more thorough assessment of ash tree presence and health, while limiting the time spent on forest and rejuvenation inventories. Monitoring of ash trees and rejuvenation in forest plots should be expanded to the Hogback and plots should be monitored every two to three years to importantly inform management decisions at ***rare.***

Soil Humus Decay Rate Monitoring

Decay rate monitoring occurs in late October and early November around one of the permanent forest canopy plots in each forest area. Decay rates are measured by burying wooden tongue depressors below the soil surface and comparing their mass lost over a period of a year to those left on the soil surface. Analysis of results was not included in this report; however 2016 results will be incorporated in future year’s reports.

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1.0 Introduction

1.1 Ecological Monitoring

Ecological monitoring involves measuring a set of environmental variables at regular intervals over a long period of time (Vaughan et al. 2001). The consistent monitoring of these abiotic or biotic environmental variables can provide information about the environmental changes that are occurring within an ecosystem (Lovett et al. 2007). The fundamental reasons for conducting long term ecological monitoring are to establish baseline data, which represents the current status of an ecosystem, and to facilitate the detection of environmental changes over time. Observations of environmental variables that exceed the natural variation in baseline data can be indicative of an environmental change (Vaughan et al. 2001).

The importance of continued long term ecological monitoring has been stressed in the scientific literature as it can provide important information for evaluating ecosystem health (Wolfe et al. 1987; Jeffers 1989; Vos et al. 2001; Lovett et al. 2007). The results of monitoring programs should be considered during policy development in order to create suitable strategies for mitigating and responding to environmental changes (Wolfe et al. 1987; Noss 1990; Beever 2006; Lovett et al. 2007).

Due to the broad scope of biological diversity throughout an entire ecosystem, the limited time, personnel, and money available for monitoring programs often means that only the highest priority indicators can be monitored (Beever 2006). Therefore, measuring the occurrence of a few indicator species is much more feasible than conducting comprehensive species inventories throughout the entire ecosystem (Fleishman et al. 2005). Indicator species are particularly sensitive to changes in their environment and are relatively cost effective and easy to monitor, making them ideal representatives for identifying changes in ecosystem health (Noss 1990).

1.2 Ecological Monitoring and Assessment Network

In 1994, Environment Canada initiated the Ecological Monitoring and Assessment Network (EMAN) which connected the various groups and individuals conducting ecological monitoring across Canada (Craig & Vaughan 2001). These members worked towards the collective goal of determining “what is changing and why in Canadian ecosystems” by achieving the following objectives: 1) determine how Canada’s ecosystems are being influenced by environmental stresses, 2) demonstrate scientific rationale for resource management policies, 3) evaluate the effectiveness of resource management policies, and 4) promptly detect new environmental issues (Vaughan et al. 2001).

The EMAN coordinating office was responsible for developing standardized protocols for the ecological monitoring of marine, freshwater and terrestrial ecosystems across Canada (Environment Canada 2012). The use of standardized protocols improves the ability to detect, describe, and report ecosystem changes by encouraging the collection of comparable data sets. In addition, collected data was uploaded to a shared database to facilitate the analysis of large scale ecosystem changes (Vaughan et al. 2001).

The EMAN coordinating office was closed in September 2010 and the future of EMAN is currently unknown. Protocols can still be accessed from the Environment Canada website but data can no longer be uploaded or accessed.

1.3 Ecological Monitoring at *rare* Charitable Research Reserve

The ***rare* Charitable Research Reserve** provides a unique opportunity for monitoring. Located at the confluence of the Speed and Grand River within Waterloo Region, it is 900+ acres of preserved land surrounded by expanding urban development. A high diversity of habitats supports a wide biodiversity of flora and fauna, providing a good representation of local species (Appendix A, Figure A.3).

An ecological monitoring program was established at ***rare*** in 2006 following EMAN protocols, with the goal of developing baseline data and the hope of creating a long-term protocol to observe changes over time. Due to limitations, such as funding and manpower, monitoring is restricted to indicator species, which are closely tied to environmental changes. Butterfly monitoring began in 2006 on two transects, Cliffs and Alvars and South Field, and was expanded in 2009 to include the newly acquired Thompson's Tract, and again in 2010 to Blair Flats. Plethodontid salamander monitoring began in 2006 in Indian Woods and was expanded in 2008 to include the Hogsback forest. Benthic invertebrate monitoring occurred at Bauman and Cruickston creeks in 2006, and, continuing on a three year cycle, occurred again in 2009 and 2012. In 2009, the monitoring program was expanded to include forest canopy tree biodiversity plots in the Indian Woods and Cliffs and Alvars forests, with soil humus decay rate monitoring also occurring within the Cliffs and Alvars plot. In 2010, an additional forest health plot was added to the Hogsback forest, and soil humus decay rate monitoring was included in all forest plots. Here, the results of the 2016 monitoring year are reported and discussed.

Lists of Acronyms

Acronym	Description
EMAN	Ecological Monitoring and Assessment Network
ACO	Artificial Cover Object
IN	Indian Woods
HO	Hogsback
CA	Cliffs and Alvars
SVL	Snout-Vent Length
VTL	Vent-Tail Length
ANOVA	Analysis of Variance
CPUE	Catch Per Unit Effort
AIC	Akaike's Information Criterion
SD	Standard Deviation
DBH	Diameter at Breast Height

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2.0 Butterfly Monitoring

2.1 Introduction

2.1.1 Lepidoptera Taxonomy

The order Lepidoptera, meaning “scaled wings”, is comprised of butterflies and moths. There are six families of butterflies, including five true families (*Papilionidae*, *Nymphalidae*, *Pieridae*, *Lycaenidae*, and *Riodinidae*) and the skipper family (*Hesperiidae*). Approximately 13,750 of an estimated 17,500 species of butterflies are true butterflies (Robbins and Opler 1997). There are general rules that can be used to distinguish moths and butterflies from one another. Butterflies are predominately diurnal, have clubbed antennae, and fold their wings vertically over their body while at rest, whereas moths are predominately nocturnal, have feathered or tapering antennae, and hold their wings out flat when resting (Pyle 1981).

2.1.2 Why Monitor Butterflies?

Long term monitoring of butterfly populations can provide valuable insight into the overall health of ecosystems and environmental change. Butterflies have short life spans, and thus respond quickly to various ecological pressures, both locally and on a broader scale (Fleischmann and Murphy 2009). Butterflies are sensitive to regional weather conditions, as unseasonably cold or wet periods can delay their development and reproduction (Pollard 1988). Further, global climate change can result in an extension or shift of butterfly populations outside of their typical ranges (Roy et al. 2001). Climate warming is expected to allow butterflies to expand their ranges to higher elevations and latitudes, particularly along the geographic margins of their current ranges (Oliver et al. 2012). However, this ability to increase geographic range is largely species specific, with species with high dispersal ability or those willing to use a range of host plants appearing to best exploit warmer temperatures (Kallioniemi 2013). Therefore, the presence or absence of butterfly species within geographic regions could provide useful information to better understand environmental change, as well as adaptability of different species of butterflies.

Throughout their life cycle, butterflies have specific requirements, namely the host plants they require for egg laying and feeding as both caterpillars and adults. Changes in the availability of host plants through both natural and human-caused disturbances (i.e. habitat loss, exotic species introduction) can have negative effects on butterfly populations. Invasive plant species can out-compete the native plants butterfly require. However, some butterfly species are now taking advantage of these alien plants and effectively using them as hosts (Tallamy and Shropshire 2009). For example, the Wild Indigo Duskywing (*Erynnis baptisiae*) was historically uncommon and restricted to habitats in southwestern Ontario where its larval food plant, Wild Indigo (*Baptisia tinctora*), was found (Hall 2009). However, its range has been expanding as a result of it using a non-native foodplant, Crown Vetch (*Coronilla varia*). Since 2010, it has been more commonly observed in the Waterloo Region (Linton 2012) and at ***rare***.

In addition to their rapid responses to ecological change, butterflies are good indicator species because of the ease in which they can be monitored (Fleischmann and Murphy 2009).

Their size and the colourful distinctions between species make observation and identification relatively simple for most butterfly families.

Finally, butterflies are a good choice for monitoring because they are a charismatic species that invoke a positive response from the public, allowing for recruitment of volunteers and the promotion of citizen science programs. Charismatic species can act as important symbols for conservation, and also inspire the public's intrinsic desire to protect them and their habitat.

2.1.3 Importance of Butterflies

Butterflies are important components of ecosystems and human practices. For example, butterflies are important members of the foodchain, supplying nutrients to many animals such as bats, frogs, birds and mice. A decrease in butterfly availability as a food item may result in effects at higher trophic levels. Butterflies are also important pollinators of natural and anthropogenic vegetation (Faegri and Pijl, 1997; Ghazoul 2006). In agriculture, approximately 70 percent of crops used for human consumption depend on pollinators (Klein et al. 2007; Potts 2010) with insect pollination is valued at over $215 billion per year for the world (Gallai et al. 2007). Although honeybees account for approximately 90 percent of insect pollination (Klein et al. 2007), there has been a decline in honeybee populations around the world over the last few decades (Sluijs et al. 2013), increasing the need for alternative pollinators such as butterflies.

Over the past few decades, populations of butterflies have declined at an alarming rate in many parts of the world (Merckx et al. 2013). Habitat loss, pesticide use, habitat degradation, and fragmentation are just some of the proposed causes of these declines (Merckx et al. 2013). This decrease in butterfly populations may be indicative of broader scale ecosystem changes. Continued pressure from these sources and the response from butterfly populations highlight the urgent need for monitoring efforts to help make conservation management decisions to protect these charismatic and important species.

*2.1.4 Butterfly Monitoring at **rare Charitable Research Reserve***

The standardized Ecological and Monitoring Assessment Network (EMAN) protocol for long term butterfly monitoring was developed and piloted at ***rare*** in 2006. The purpose of this pilot program was to determine if the Transect Walk Method (Pollard 1977) was a feasible technique to examine butterfly abundance and diversity in Canada (Grealey 2006), and it marked the start of the long term monitoring program at ***rare***.

In 2006, two transects were established: one located in the Cliffs and Alvars and one in South Field/Sparrow Field. Baseline data were collected over a five week period during the initial pilot study. Butterfly monitoring at ***rare*** continued in 2009 and during which time two more transects were established: one in 2009 in the newly acquired Thompson Tract, and one in 2010 in Blair Flats. Monitoring took place over thirteen weeks in 2009, and fourteen weeks in 2010 through to 2016. It is important to note that due to a change in property boundaries, the South Field/Sparrow Field transect had to be slightly altered in 2014; the changes are described below (Section 2.2.2).

2.2 Methods

2.2.1 Monitoring Protocol

One of the most commonly used monitoring methods around the world is the Transect Walk Method, originating in Britain in 1976 (Pollard 1977; Pollard and Yates 1993). This method involves walking established routes (i.e. transects) at a uniform pace, and making observations within a given radius (Pollard 1977). Butterfly monitoring at the ***rare*** **Charitable Research Reserve** is conducted using the Transect Walk Method, as it does not require extensive effort or time, and limits disturbances to the butterflies' behaviour.

Ideally, butterfly monitoring programs should take place over a 26 week period, from April to September (Layberry et al. 1998). At ***rare***, this time period has been reduced due to time and monetary constraints. Monitoring typically begins on the third Monday of May; however, this may be either advanced or delayed, depending on weather conditions (i.e. particularly cold or warm local temperatures). Butterflies are most active during the warmest part of the day, and thus monitoring is completed between the hours of 10am and 3pm (Grealey 2006). Monitoring is completed on sunny days, when the temperature is above 13°C. If it is overcast, the temperature must be at least 17°C (UK Butterfly Monitoring Scheme; Butterfly Monitoring Scheme Germany). Wind should also be less than five on the Beaufort Wind Scale (refer to table C.1. in Appendix C).

Each transect is broken into sections based on habitat, each section with a stopping point, as described in Appendix A. Each individual section was created based on changes in habitat type. Prior to beginning monitoring, the observer walked all transects and flagged the section breaks and stopping points, as required. Observations were recorded during optimal weather conditions. Provided there was no rain, observations were recorded in suboptimal conditions when necessary, as this is more valuable than not collecting data at all. In order to minimize observer bias, all observations were made by one individual with occasional assistance from volunteers.

In 2016, monitoring began on May 16, and each of the four transects were walked once weekly for fourteen weeks. A recommended list of field equipment can be found in Appendix B, Figure B.1. At the start and end of each transect, the time was recorded, and a hand-held Kestrel 3000© (Nielson-Kellerman, Boothwyn, PA, USA) was used to determine air temperature. Transects were walked at a uniform pace, and butterflies observed within a ten metre radius were recorded. Approximately halfway through each section, ten minute stops were made at predetermined locations, recording any butterflies observed within a ten metre radius. At each stopping point, the percent of blue sky was estimated (0-100; 0 being full cloud cover) and the Kestrel 3000© was used to determine average wind speed. Butterflies were visually identified in the field, and caught with a net when necessary to aid in identification. Unknown species were photographed and sent to local experts for identification. If identification was not possible, the individual was recorded as the most common possibility. While walking each transect, occasional stops were permitted to properly identify butterflies, and recording continued from where the stop was made. All observations were recorded in a standard field form, found in Appendix C and on the ***rare*** server.

2.2.2 Transect Descriptions

Butterfly monitoring occurred across the following transects at the ***rare*** **Charitable Research Reserve**. Refer to Appendix A for a map of the property which outlines the transect routes.

The **Cliffs and Alvars** transect is 3.5 km and follows primarily the River and Grand Trunk trails. A large part of the transect consists of mature hardwood forest stands, dominated by American Beech (*Fagus grandifolia*) and Sugar Maple (*Acer saccharum*). This transect also passes through deciduous swamps, limestone cliffs, open alvar habitats, and an extensive floodplain.

The **South Field/Sparrow Field** transect is 2.9 km, running along the edge of agricultural fields, hedgerows, and through a recently restored tall grass prairie. Several fields in the area are currently in agricultural production, including hay in South Field West and corn in South Field East in 2015. Sparrow Field has gradually been removed from agriculture production and is being restored to native vegetation, with an approximately 20 hectare portion involved in tallgrass prairie restoration research. Prior to the 2014 monitoring year, this transect traveled along the south-eastern perimeter of Indian Woods. However, due to a change in the ***rare*** property boundary in early 2014, this part of the transect (formerly section 6 and 7) was eliminated and an alternative route was used. To minimize the effects of this change, the new section is referred to as 6/7 (allowing the other sections to remain as they were).

The **Thompson Tract** transect is 2.2 km and follows established trails through meadows, plantations, and lowland and upland forest dominated by American Beech (*Fagus grandifolia*) and Sugar Maple (*Acer saccharum*). Thompson Tract is located at the western boundary of the ***rare*** property.

The **Blair Flats** transect is a 1.3 km loop that walks the perimeter of a restored tallgrass prairie. Prior to 2010, Blair Flats was in agriculture production. As part of a long term study, the area was restored to a tall grass prairie, and is currently dominated by Goldenrod (*Solidago*), Queen Anne's Lace (*Daucus carota*), Black Eyed Susan (*Rudbeckia hirta*), and Tansy (*Tanacetum vulgare*) as well several grass species. In 2015, Blair Flats was burned as part of a five year prescribed burn program, which intends to encourage and promote native prairie plants and overall tallgrass prairie ecosystem health. Beginning at the large Bur Oak (*Quercus macrocarpa*) just off of Blair Road, the transect heads north towards the river, turns west and runs parallel to the river, then turns south and follows the property boundary, and finally traveling eastward, parallel to the road and ending at the Bur Oak.

2.2.3 Data Analysis

Data analysis was conducted using R 3.2.4 and Microsoft Excel 2010. Due to variations in transect lengths and habitats, some analyses consider each transect individually over time, rather than pooling transects in global analyses. However, if appropriate, transects were pooled in global analyses to represent the property as a whole. Data previous to the 2010 monitoring season was excluded in analyses because 2010 was the first year that all four transects were monitored.

The Shannon Diversity Index and Species Evenness were calculated to measure global butterfly diversity for each monitoring year at ***rare***, as well as diversity within each transect.

Species Evenness refers to the relative abundance of individuals of different species, and the Shannon Diversity Index takes into account the Evenness and the total number of species to produce a score from 0-4. Zero (0) indicates very low diversity, while 4 is very high diversity; real world values typically fall between 1.5 and 3.5 (Magurran 2004).

$$H = -\sum_{i=1}^{S} p_i \ln p_i$$

Shannon Diversity Index: Where p_i is the proportion of individuals belonging to the *i*th species and S is the number of species.

$$E_H = H/\ln(S)$$

Species Evenness: Where H is the Shannon Diversity Index and S is the number of

To test whether there has been a significant change in abundance across monitoring years and between transects (i.e. whether butterfly populations varied over time and with transect), a generalized linear model with a quasi-poisson error distribution was run. The number of observed individuals was the response variable, and year and transect were the independent variables. Potential interaction effects were also tested in the model. The significance of interactions and main effects was tested with an iterative F-test (Crawley 2007). An alpha value of 0.05 was used to determine significance. This continuous analysis was included due to the importance of identifying directional global abundance trends occurring over all monitoring years and to lay the foundation for analysis of long term trends in future years.

To determine if abundance at a given transect was significantly different between years, transects were analyzed independently and year was converted to categorical data. First, a generalized linear model was fit to data from a single transect. For each transect, an ANOVA was performed using the generalized linear model to determine if significant differences exist between one or more monitoring years. Pairwise comparisons (Tukey HSD tests) were performed where ANOVA results were significant to determine where differences occurred. An alpha value of 0.05 was used to determine significance. This analysis considered transects individually rather than globally as in the above analyses, and allows for identification of differences between years for each transect.

2.3 Results

2.3.1 Overall Abundance and Diversity

Across all transects at ***rare*** **Charitable Research Reserve**, 5,820 individuals were recorded belonging to 51 butterfly species during the 2016 monitoring season. This year was the second most abundant year for total butterfly observations, after the 7,866 individuals observed in 2012. Species richness (S=51) was at the median of monitoring years. The Shannon Diversity Index (H=2.56) and Species Evenness (E=0.65) were both the third highest of all monitoring years. Total number of observed individuals, Species Richness, the Shannon Diversity Index, and Species Evenness values for all transects pooled are presented with those from previous monitoring years in Table 2.1.

The most abundant species observed during monitoring were: Cabbage White (N=1,410), Clouded Sulphur (N=1,183), European Skipper (N=565), Inornate Ringlet (N=539), Northern Crescent (N=344), Common Wood Nymph (N=311), and Little Wood Satyr (N=278). For the second year in a row, the three most abundant species were Cabbage White, Clouded Sulphur, and European Skipper. Abundances of each butterfly species as well as global abundances are presented in Table 2.2.

Table 2.1: Summary of observed number of individuals, the Shannon Diversity Index, and Species Evenness for all transects pooled from 2010 to 2016.

	Number of Individuals (n)	Species Richness (S)	Species Evenness (E)	Shannon-Diversity Index (H)
2010	4,049	41	0.54	2.00
2011	3,808	46	0.54	2.06
2012	7,866	52	0.58	2.28
2013	5,262	45	0.65	2.47
2014	4,105	53	0.69	2.73
2015	4,931	55	0.72	2.90
2016	5,820	51	0.65	2.56

Table 2.2: Summary of observed butterflies during 2016 monitoring season at the ***rare* Charitable Research Reserve**. The Waterloo Regional Status for each of the observed species is also included from Linton (2012).

	Transect					
Species	1	2	3	4	Total	Regional Status
American Lady	3	1	0	0	4	Common
Appalachian Brown	3	0	1	0	4	Uncommon
Arctic Skipper	0	0	1	0	1	Rare
Banded Hairstreak	2	0	0	1	3	Uncommon
Black Dash	1	0	0	0	1	Uncommon
Black Swallowtail	13	37	5	8	63	Very Common
Bronze Copper	5	0	0	0	5	Very Common
Cabbage White	590	499	201	120	1,410	Very Common
Clouded Sulphur	46	1002	106	29	1,183	Very Common
Common Sooty Wing	1	39	0	0	40	Rare
Common Wood Nymph	50	50	117	94	311	Very Common
Crossline Skipper	1	0	0	0	1	Rare
Delaware Skipper	1	3	2	5	11	Common
Dun Skipper	6	0	10	6	22	Very Common
Eastern Comma	5	0	4	1	10	Very Common
Eastern Tailed Blue	1	15	3	24	43	Uncommon
Eastern Tiger Swallowtail	18	9	16	1	44	Very Common
European Skipper	288	23	236	18	565	Very Common
Eyed Brown	18	0	0	0	18	Very Common
Giant Swallowtail	14	3	2	1	20	Uncommon
Great Spangled Fritillary	13	2	31	0	46	Very Common
Grey Comma	0	0	1	0	1	Uncommon
Hobomok Skipper	18	3	10	0	31	Common
Inornate Ringlet	71	151	277	40	539	Common
Juvenal's Duskywing	18	6	18	0	42	Rare
Least Skipper	6	0	0	0	6	Uncommon
Little Glassywing	1	0	0	0	1	Uncommon
Little Wood Satyr	141	4	132	1	278	Very Common
Milbert's Tortoiseshell	1	1	0	0	2	Uncommon
Monarch	19	17	18	2	56	Very Common
Mourning Cloak	10	5	4	1	20	Very Common
Northern Broken Dash	7	4	1	7	19	Common
Northern Crescent	90	67	159	28	344	Uncommon
Northern Pearly Eye	27	16	91	0	134	Common
Orange Sulphur	2	127	10	2	141	Very Common
Painted Lady	0	5	1	1	7	Common

Pearl Crescent	13	62	13	13	101	Common
Peck's Skipper	1	0	0	0	1	Very Common
Question Mark	1	1	0	1	3	Very Common
Red Admiral	30	10	12	7	59	Very Common
Red Spotted Purple	18	6	9	1	34	Common
Silver bordered Fritillary	0	1	18	0	19	Rare
Silver-Spotted Skipper	1	0	3	2	6	Uncommon
Silvery Blue	0	1	6	0	7	Unknown
Spring Azure	48	6	21	1	76	Common
Striped Hairstreak	2	0	0	0	2	Uncommon
Summer Azure	20	6	4	4	34	Very Common
Tawny Emperor	6	0	1	9	16	Uncommon
Tawny-edged Skipper	9	1	2	0	12	Common
Viceroy	6	4	3	0	13	Very Common
Wild Indigo Duskywing	7	0	2	2	11	Unknown
Total	**1,652**	**2,187**	**1,551**	**430**	**5,820**	

2.3.2 Global Abundance Trends

Global butterfly abundances at ***rare*** have not changed significantly over time ($p>0.05$). Abundances vary significantly by transect ($p<0.05$), however the interaction effect between year and transect is not significant ($p>0.05$), indicating that the effect of year on abundance does not differ between transects, and the effect of transect on abundance does not vary by year. Because there is no interaction effect, it cannot be stated that transects vary significantly in abundance over time. However, pairwise comparison between years in a given transect can indicate significant differences in abundance between years. This information can be useful to compare to other variables such as weather and restoration efforts to infer possible explanations of population trends.

Although there is no significant trend in global abundances over monitoring years, a visual representation of abundances reveals a slight upward trend in global abundance over monitoring years (Figure 2.1). Transects Three and Four also exhibit an upward trend, while Transects One and Two appear relatively stable.

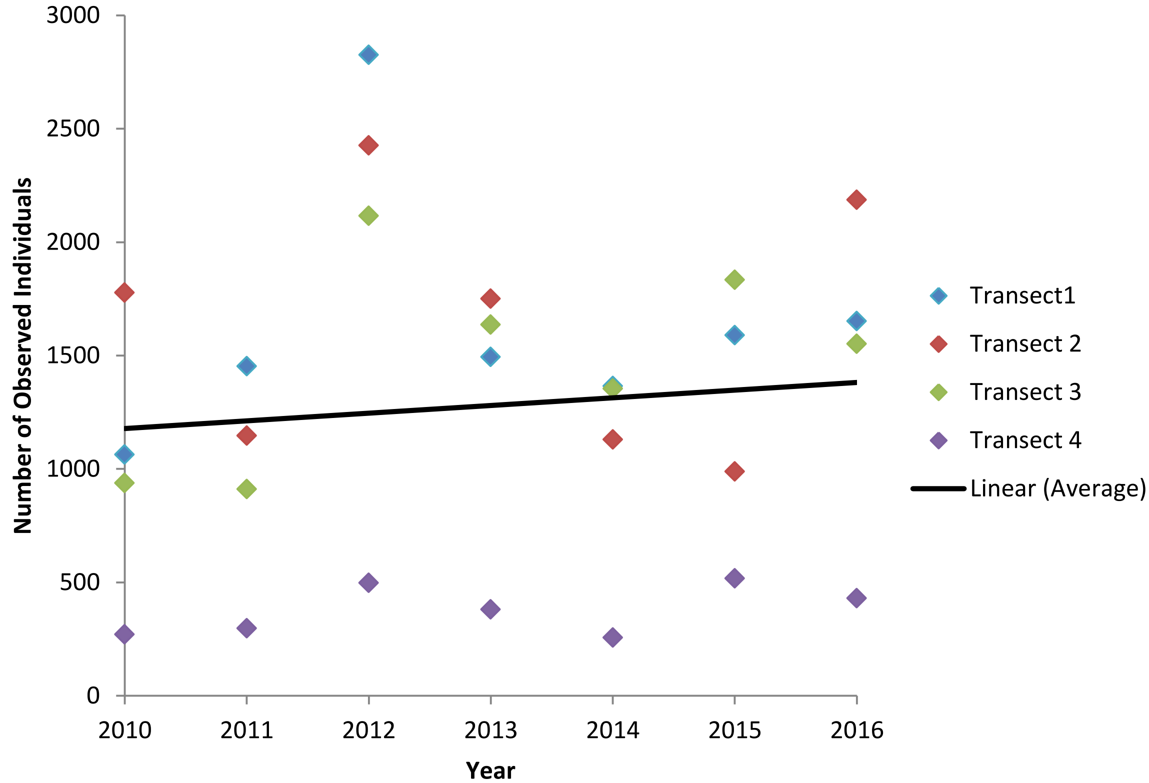

Figure 2.1: Abundances of butterflies across years for each transect.

2.3.3 Within Transect Comparisons

Transect One: Cliffs and Alvars

A total of 1,652 butterflies were observed from 46 species in Transect One during the 2016 monitoring period. The total number of individuals observed in Transect One this year was the second highest after the 2,826 individuals observed in 2012, which has consistently been the highest on record. Species Evenness (E=0.64) and the Shannon Diversity Index (H=2.44) were both mid-range in comparison to previous years, and lower than the last three years (Table 2.3).

There was a significant difference in the number of individuals observed in Transect One between years (X^2 =18.733, df=6, $p<0.01$). The pairwise comparison did not reveal any significant differences between observations in 2016 and other years. Significant differences exist between 2012 and 2014 ($p<0.05$), and 2012 and 2010 ($p<0.01$) monitoring years (Figure 2.2).

The species with the highest observed individuals in Transect One in 2016 were Cabbage White (590), European Skipper (288), and Little Wood Satyr (141), accounting for 62 percent of all observations. These species were also the three most abundant species observed in Transect One in 2015, with the European Skipper and Cabbage White being among the three most abundant species observed across monitoring years. Three species had the highest observations of all years in Transect One in 2016 (Spring Azure, Tawny Emperor, and Wild

Indigo Duskywing). Species observations for each year in Transect One are shown in Appendix H (Figures H.1 and H.2).

Table 2.3: Summary of observed number of individuals, the Shannon Diversity Index, and Species Evenness in Transect One from 2010 to 2016.

	Number of Individuals (n)	Species Richness (S)	Species Evenness (E)	Shannon-Diversity Index (H)
2010	1,063	33	0.59	2.07
2011	1,453	35	0.50	1.77
2012	2,826	46	0.57	2.19
2013	1,494	43	0.65	2.45
2014	1,365	47	0.71	2.72
2015	1,590	43	0.76	2.85
2016	1,652	46	0.64	2.44

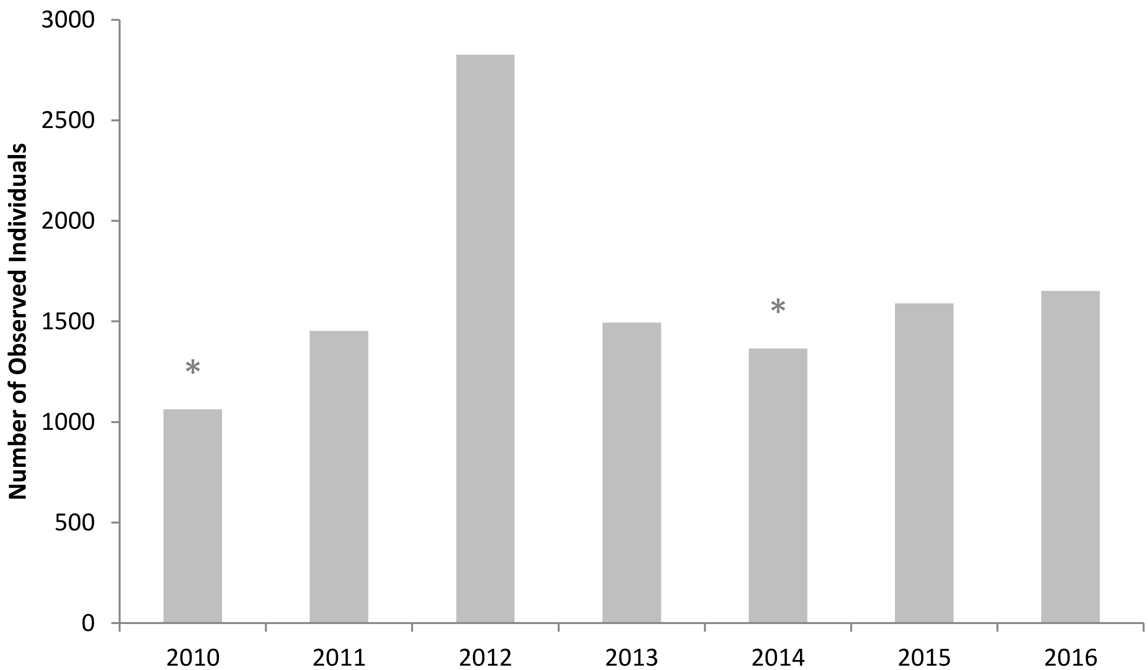

Figure 2.2: Total number of butterflies observed during each monitoring year at Transect One. Asterisk denotes a significant difference between observations in the monitoring year and those in 2012 (p<0.05).

Transect Two: South Field/ Sparrow Field

A total of 2,187 butterflies were observed from 34 different species in Transect Two during the 2016 monitoring period. The total number of individuals observed in Transect Two in 2016 was the second highest after the 2,427 individuals observed in 2012, which is has consistently been the highest on record. Species Evenness (E=0.53) and the Shannon Diversity Index (H=1.86) were both mid-range in comparison to previous years, and lower than the last three years (see Table 2.4).

Although there was a significant difference in the number of individuals observed in Transect Two between monitoring years (X^2 =15.303, df=6, $p<0.05$), the pairwise comparison revealed that no two years differed significantly at the $p>0.05$ significance level. Figure 2.3 demonstrates observations across monitoring years.

Species with the highest observations were Clouded Sulphur (1,002), Cabbage White (499), and Inornate Ringlet (151), accounting for 76 percent of all observations. This is consistent with the three most observed species in 2015, with Cabbage White and Clouded Sulphur butterflies being the two most abundant species in Transect Two across all monitoring years. Four species had the highest observations in Transect two of all years (Clouded Sulphur, Inornate Ringlet, Northern Crescent, Orange Sulphur). Species observations in Transect Two for each year are shown in Appendix H (Figures H.3 and H.4).

Table 2.4: Summary of observed number of individuals, the Shannon Diversity Index, and Species Evenness in Transect Two from 2010 to 2016.

	Number of Individuals (n)	Species Richness (S)	Species Evenness (E)	Shannon-Diversity Index (H)
2010	1,778	26	0.44	1.42
2011	1,146	30	0.47	1.60
2012	2,427	37	0.49	1.76
2013	1,751	35	0.57	2.02
2014	1,130	31	0.62	2.12
2015	989	38	0.67	2.43
2016	2,187	34	0.53	1.86

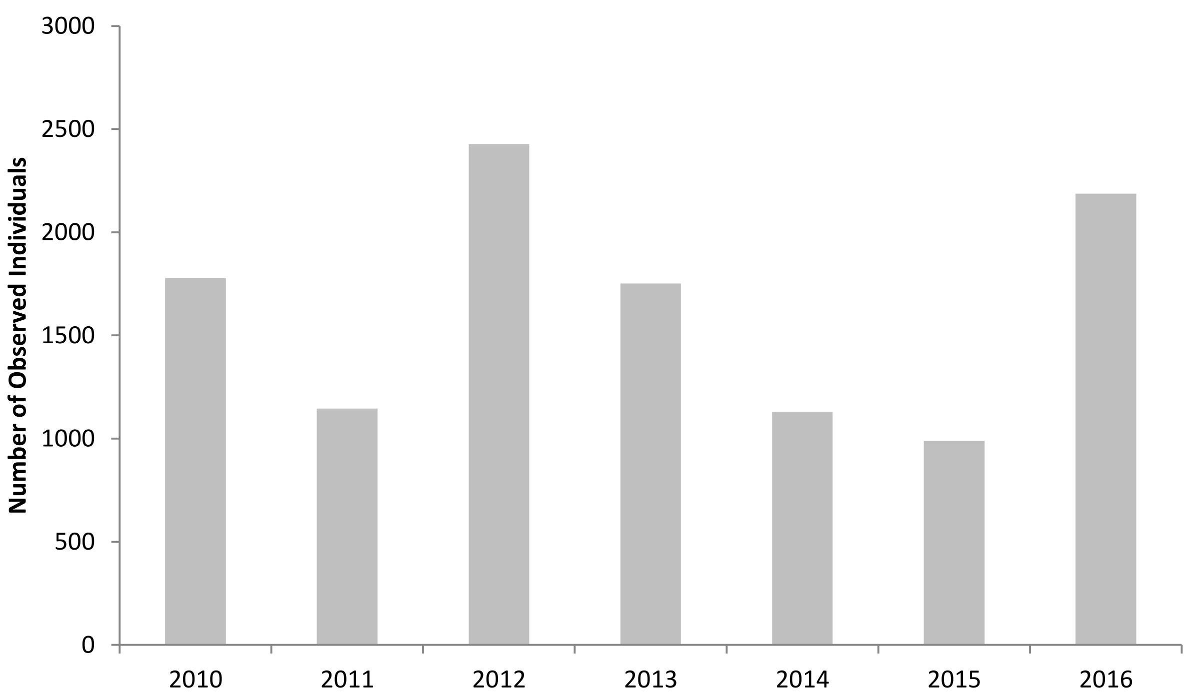

Figure 2.3: Total number of butterflies observed during each monitoring year at Transect Two.

Transect Three: Thompson Tract

A total of 1,551 butterflies were observed from 38 different species in Transect Three during the 2016 monitoring period. Although the number of individuals was mid-range compared to other years, species richness matched the second highest on record, after the 44 observed in 2015. Species Evenness (E=0.71) and the Shannon Diversity Index (H=2.58) were both lower than those of the previous two monitoring years, and very similar to 2012 (E=0.71, H=2.56) (Table 2.5).

There was a significant difference in observations between monitoring years in Transect Three (X^2 =18.068, df=6, $p<0.01$). No significant differences occurred between 2016 and other monitoring years ($p>0.05$). Significant differences occurred between 2012 and 2010 ($p<0.05$) and 2012 and 2011 ($p<0.05$) monitoring years (See Figure 2.4).

Species with the highest observations were Inornate Ringlet (277), European Skipper (236), and Cabbage White (201), accounting for 46 percent of all observations. This is the first year the Inornate Ringlet has had the highest number of observations. Cabbage White butterflies have been in the top three observed in all monitoring years except 2015, while European Skippers have been in the top three over only the last three monitoring years (2014-2016). Three species have had the highest observations in Transect Three to date during 2016 monitoring (Black Dash, Inornate Ringlet, Silvery Blue). Species observations in Transect Three for each year are shown in Appendix H (Figure H.5 and H.6).

Table 2.5: Summary of observed number of individuals, the Shannon Diversity Index, and Species Evenness in Transect Three from 2010 to 2016.

	Number of Individuals (n)	Species Richness (S)	Species Evenness (E)	Shannon-Diversity Index (H)
2010	938	30	0.70	2.37
2011	911	35	0.72	2.56
2012	2,116	38	0.71	2.56
2013	1,636	36	0.71	2.55
2014	1,354	38	0.72	2.62
2015	1,834	44	0.73	2.75
2016	1,551	38	0.71	2.58

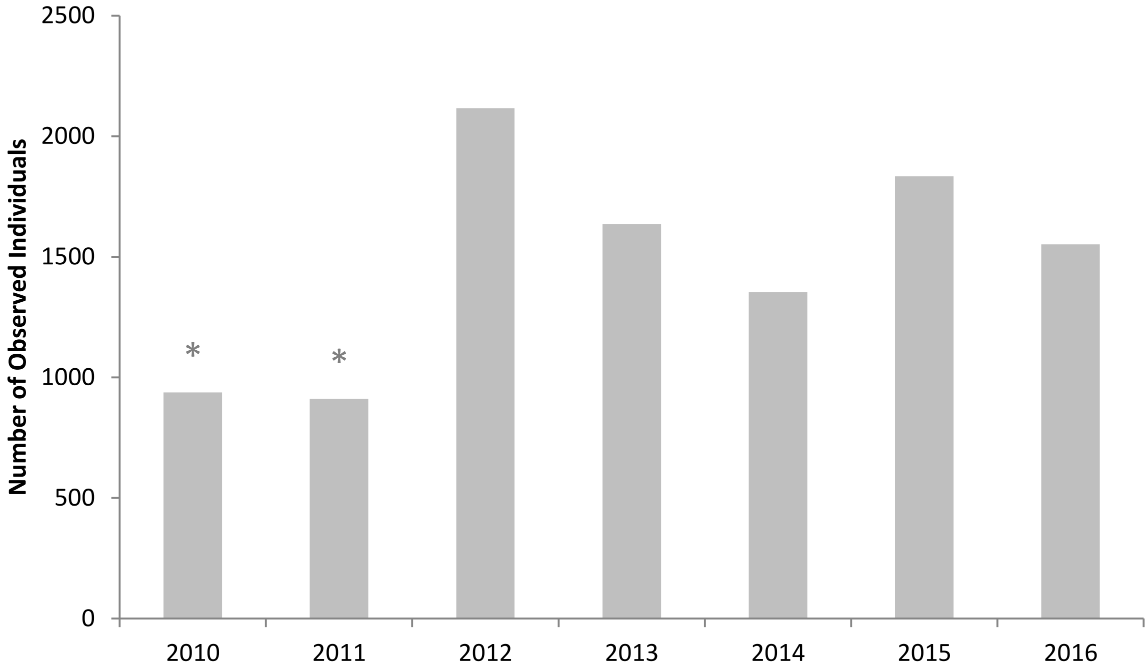

Figure 2.4: Total number of butterflies observed during each monitoring year at Transect Three. Asterisk denotes a significant difference between observations in the monitoring year and those in 2012 ($p<0.05$).

Transect Four: Blair Flats

A total of 430 butterflies were observed from 29 different species in Transect Four during the 2016 monitoring period. Total observations are third highest after 2015 (N=518) and 2012 (N=497). Species richness is the same as 2015, and second highest after 2012 (S=29). Species Evenness and Shannon Diversity Index (E=0.70, H=2.35) were second highest after 2014 (E=0.74, H=2.42) (Table 2.6).

There was no significant difference between observations in monitoring years ($X^2 = 7.6901$, df=6, $p>0.05$) for Transect Four (See Figure 2.5).

Cabbage White (N=120), Common Wood-Nymph (N=94), and Inornate Ringlet (N=40) were the most common species observed in 2016, accounting for 59 percent of all observations in total. Cabbage White and Inornate Ringlet butterflies have been the most abundant across all monitoring years, but this is the first year Clouded Sulphur has not been one of the top three observed butterfly species in Transect Four. Four Species had the highest observations in 2016 (Eastern Tailed Blue, Inornate Ringlet, Northern Crescent, Common Wood-Nymph). Species observations in Transect Four for each year are shown in Appendix H (Figure H.7 and H.8).

Table 2.6: Summary of observed number of individuals, the Shannon Diversity Index, and Species Evenness in Transect Four from 2010 to 2016.

	Number of Individuals (n)	Species Richness (S)	Species Evenness (E)	Shannon-Diversity Index (H)
2010	270	14	0.49	1.30
2011	298	20	0.42	1.26
2012	497	35	0.60	2.12
2013	381	21	0.63	1.93
2014	256	26	0.74	2.42
2015	518	29	0.67	2.26
2016	430	29	0.70	2.35

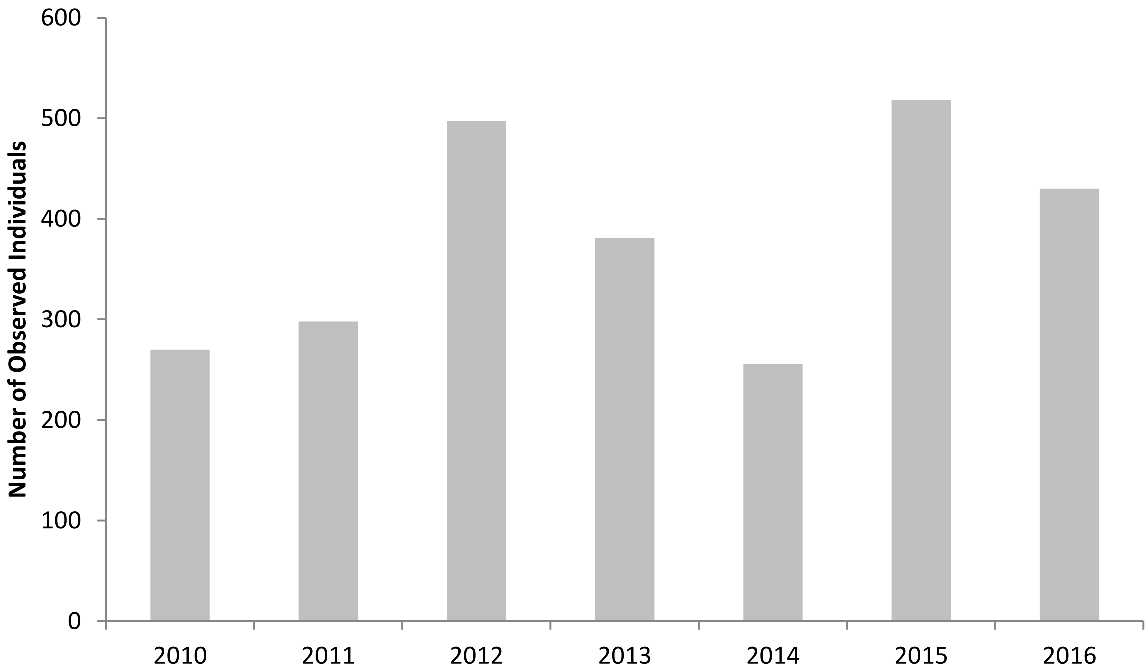

Figure 2.5: Total number of butterflies observed during each monitoring year at Transect Four.

3.4 Noteworthy Species and Species of Special Concern

The number of recorded Monarch Butterflies (*Danaus plexippus*) dropped steeply in 2013, and numbers have remained low but steady in the subsequent monitoring years. The percentage of total observed individuals that are Monarchs dropped from 6.74 percent in 2010 to less than 2 percent since 2013. This year 56 Monarchs were observed during monitoring, accounting for only 0.96 percent of all observed individuals. A graphical representation of observed abundances is shown in Figure 2.6.

At 1,410 individuals observed, Cabbage Whites accounted for 24.22 percent of the total number of individuals observed in 2016 during monitoring. Both numbers have increased from the previous monitoring season and are third lowest among all seasons. Although less than half of the observations in 2012, 2016 observations are almost two-fold 2015 observations (Figure 2.7).

European Skippers have generally shown an upward trend in abundance; however observations are down from 687 individuals observed in 2015 to 565 observed in 2016 during monitoring. Similarly, European Skippers made up 9.71 percent of total observed individuals in 2016 in comparison to 13.93 percent in 2015, their most abundant year during to date (Figure 2.8).

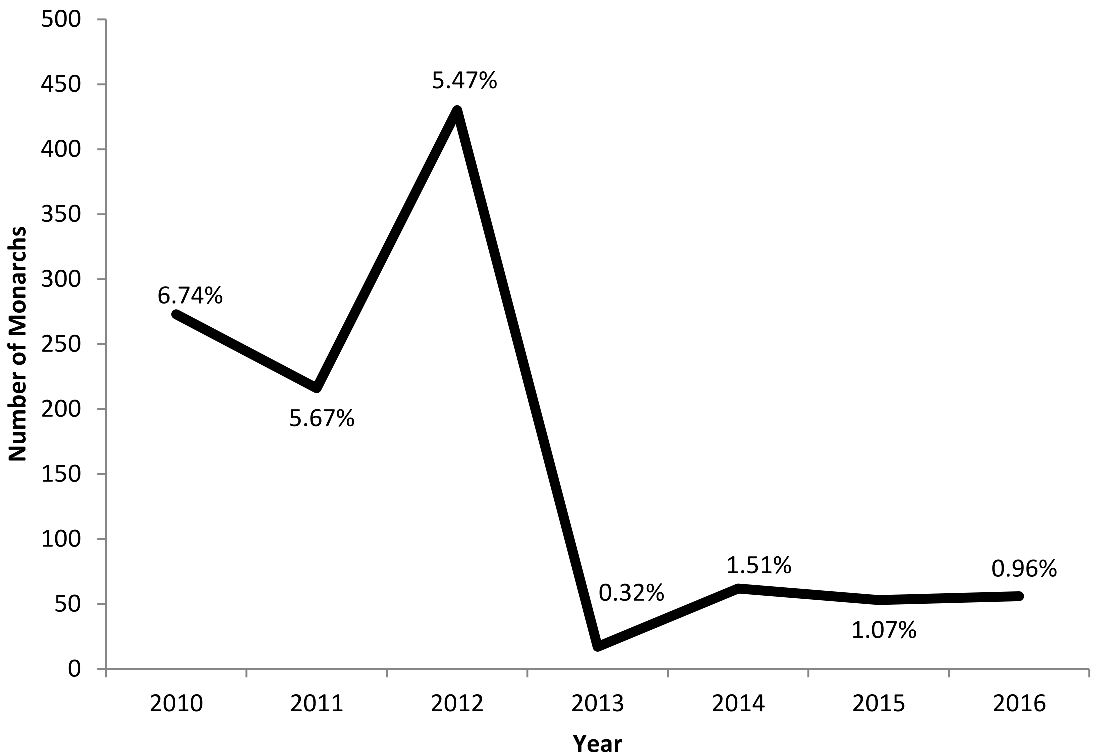

Figure 2.6: Number of observed Monarch individuals recorded for each monitoring year between 2010 and 2016.

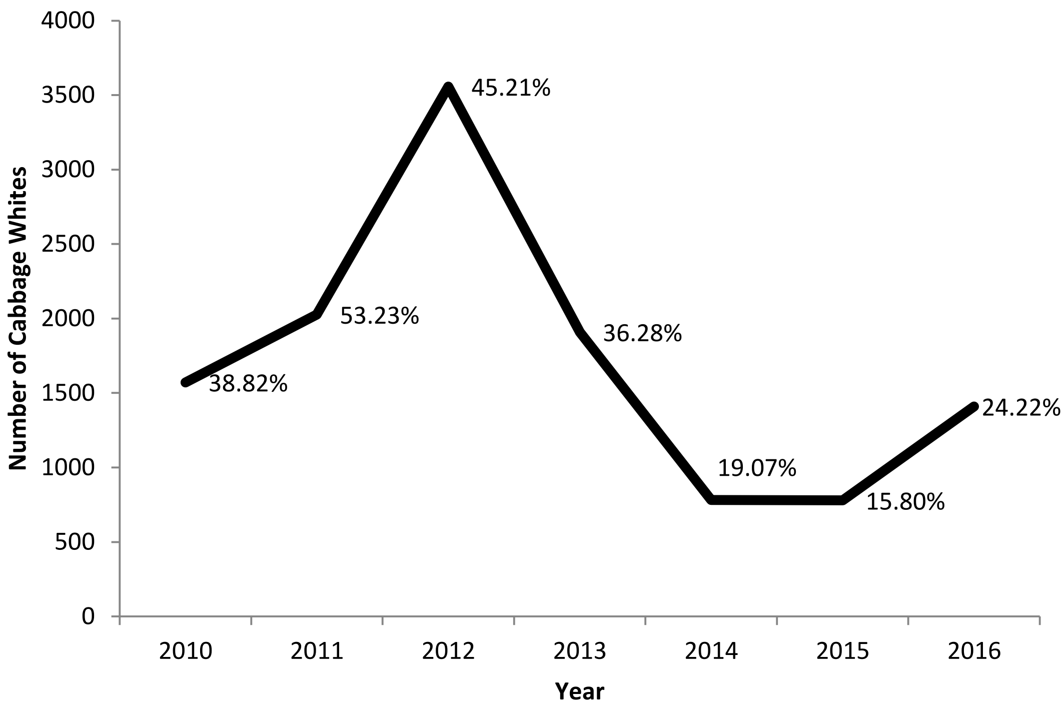

Figure 2.7: Number of observed Cabbage White individuals recorded for each monitoring year between 2010 and 2016.

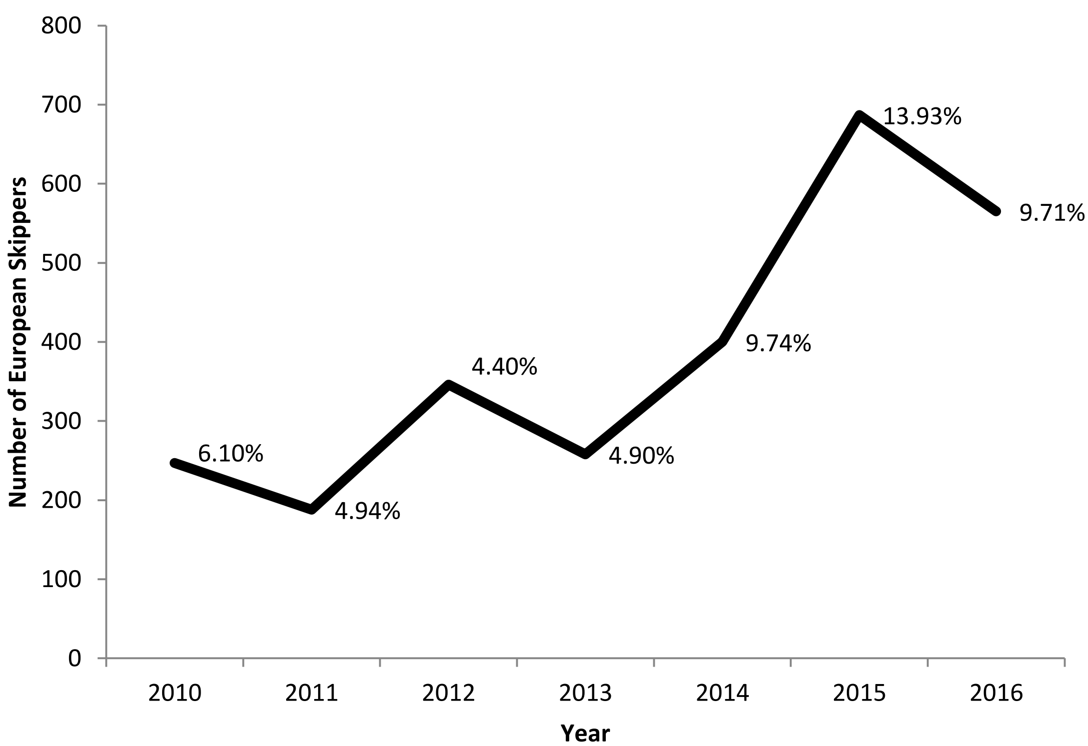

Figure 2.8: Number of observed European Skipper individuals recorded for each monitoring year between 2010 and 2016.

2.3.4 Comparison with Baseline Data

EMAN protocol suggests the first five years of monitoring data be used to create a baseline for monitoring programs in order to accurately identify trends and averages for populations. 2013 was the fifth year that butterfly monitoring at ***rare*** took place for either 13 or 14 weeks, and thus data from these years were used to identify averages and standard deviations for both abundance and species richness across the four transects (Table 2.7). Using these data, we can compare the 2016 results to the averages for each transect to determine if this monitoring season fell within or outside of these averages. Values that are outside of the given ranges may indicate environmental change that has potentially had either positive or negative impacts on the populations. A wide range of values are considered acceptable, due to the large variation in observations between years.

The number of Individuals in Transects One, Two, Three, and Four were above baseline averages but fall within the average range of the baseline data. Transects Two, Three, and Four were above baseline averages for species richness but also fell within the average range of the baseline data. Transect One was above average species richness and did not fall within average ranges, with 46 species observed (Table 2.7). In 2016, no transects fell below baseline levels for species richness or number of individuals.

Table 2.7: Average butterfly abundance and species richness, with standard deviations, for monitoring seasons 2009-2013. Numbers falling outside of baseline range are bolded.

	Number of Individuals			**Species Richness**		
Transect	Average	Standard Deviation	*2016*	Average	Standard Deviation	*2016*
Transect One	1,491	+/- 825	*1,652*	36	+/- 8	***46***
Transect Two	1,563	+/- 655	*2,187*	30	+/- 6	*34*
Transect Three	1,203	+/- 670	*1,551*	35	+/- 3	*38*
Transect Four	361	+/- 101	*430*	23	+/- 9	*29*

2.3.5 Weather Conditions

Although mean temperature (18.2°C) was the third highest of all years, the season started cool in comparison to other monitoring years. The mean temperature in May (13°C) was the second lowest of all years. 2016 temperatures increased relative to previous years, with the mean temperature in August being the highest of all years (21.4°C) (Figure 2.9).

The precipitation mean for 2016 (71.55 mm) was second lowest of all years, despite August having the highest precipitation of all years (100.9 mm). June precipitation was the lowest of all years at 39.3 mm (Figure 2.10). Butterfly monitoring was very minimally disrupted due to weather conditions, with rain occurring during monitoring only once.

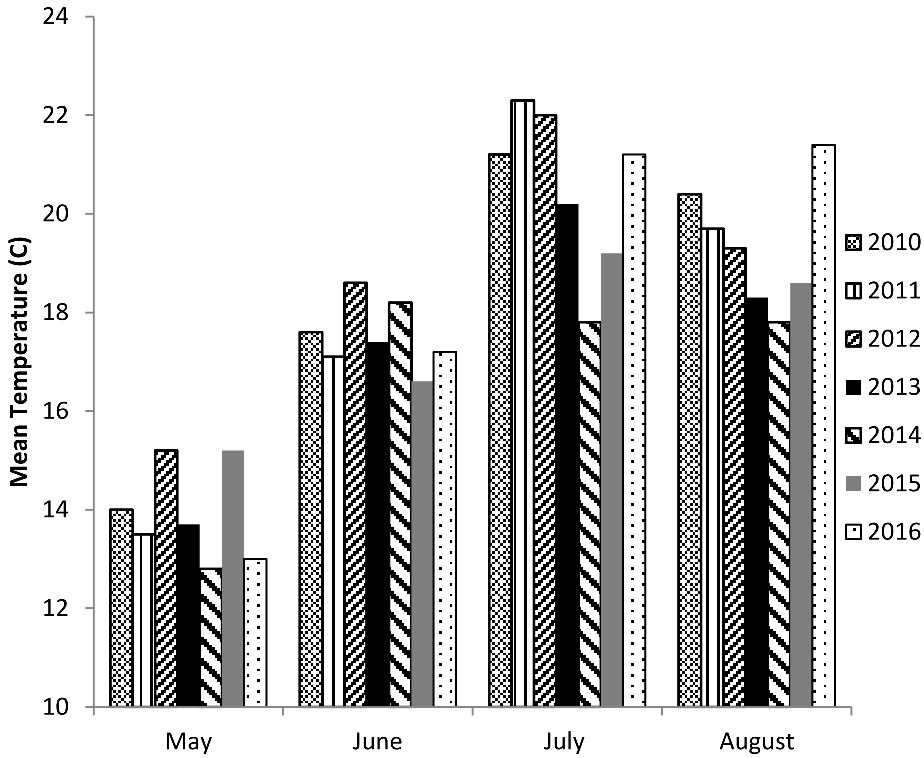

Figure 2.9: Mean monthly temperatures for Kitchener/Waterloo for all months of all butterfly monitoring seasons at the ***rare* Charitable Research Reserve**. Data for 2010-2016 are from the Kitchener Waterloo Weather Station (Accessed from Environment Canada 2016).

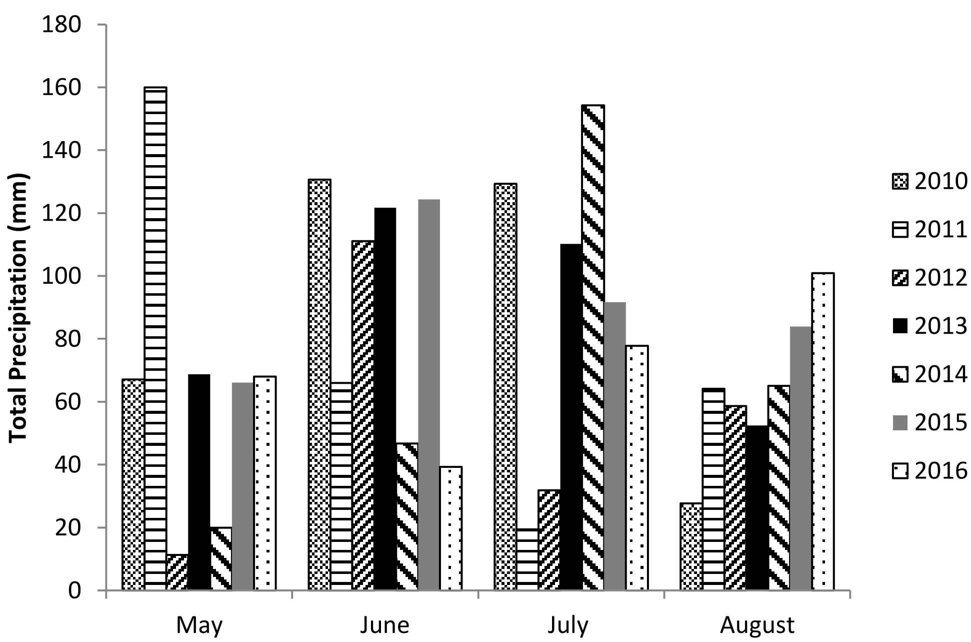

Figure 2.10: Total monthly precipitation for Kitchener/Waterloo for all months of all butterfly monitoring seasons at the ***rare* Charitable Research Reserve**. Data for 2010-2016 are from the Kitchener Waterloo Weather Station, with the exception of July 2015 due to missing values. July 2015 data was taken from Roseville Weather Station 43°21'13.026" N 80°28'25.056" W (Accessed from Environment Canada 2016).

2.3.6 2016 Butterfly Count

The 11th Annual Butterfly Count was held at ***rare*** **Charitable Research Reserve** on July 16, 2016. In total, 418 individuals were observed from 39 butterfly species. Results have been submitted to the North American Butterfly Association and can also be seen below. Results from previous years are included in Appendix E.

Observations: E. Tiger Swallowtail 2, Cabbage White 63, Clouded Sulphur 22, Orange Su. 3, E. Tailed-Blue 3, 'Summer' Spring Azure 2, Gr. Spangled Fritillary 14, Aphrodite Fr. 2, Silver-bordered Fr. 5, Meadow Fr. 4, Pearl Crescent 26, N. Cr. 19, Baltimore Checkerspot 1, Question Mark 1, Red Admiral 6, Red-spotted Admiral 1, Viceroy 1, Tawny Emperor 1, N. Pearly-eye 9, Eyed Brown 2, Little Wood-Satyr 27, Com. Wood-Nymph 87, Monarch 2, Silver-spotted Skipper 8, Wild Indigo Duskywing 12, Com. Sootywing 2, Least Sk. 3, European Sk. 9, Peck's Sk. 13, Tawny-edged Sk. 6, N. Broken-Dash 18, Little Glassywing 5, Delaware Sk. 5, Mulberry Wing 1, Broad-winged Sk. 2, Dion Sk. 1, Black Da. 10, Dun Sk. 18. **Unidentified:** Hairstreak sp 1, Greater Fritillary sp 1. **Total:** 39 species, 418 individuals.

2.4.0 Discussion

2.4.1 Overall Abundance and Diversity

Observations in 2016 were the second highest of all monitoring years with 5,820 individuals observed, after 7,866 individuals observed in 2012. Weekly monitoring in 2016 was very seldom interrupted by unfavourable weather conditions, allowing for more monitoring days with optimal monitoring conditions.

Butterflies are very sensitive to local weather conditions and patterns (Wikström et al. 2009). Generally, butterflies respond positively to warm and dry weather by increasing development rates during egg and larval stages, and through reproductive success (Roy et al. 2001). Additionally, there are strong associations between high rainfall early in the previous year and higher abundances in the current year (Pollard 1988; Roy et al. 2001). The 2012 and 2016 monitoring years were also the hottest and driest summers and were preceded by summers with wet beginnings (2011 and 2015, respectively), which may explain the high abundances seen in those years.

Despite annual changes in abundances, there has been no upward or downward trend in global abundances of butterflies (all transects pooled) from 2010 to 2016. Many butterflies undergo population cycling, having abundances that fluctuate annually between high and low. Factors such as population cycling and annual weather variations influence annual fluctuations in abundance (Harrison et al. 2015) and likely contribute to the annual variations in abundance that have been observed. The absence of a trend over monitoring years may indicate that there has been no significant response (either positive or negative) to changes in appropriate habitat and changing climatic conditions. However, is also possible that abundance trends cannot be captured within such a short time frame, especially due to annual fluctuations and dependence of butterfly abundances on seasonal weather conditions.

Considerable efforts have been made by ***rare*** to restore areas previously used for intensive agriculture to areas that host more native species, including areas of Transect Two and Transect Four. Although significant increases in abundances have not occurred in either plot to date, there has been a general increase in species richness overtime at Transect Four. Richness has not shown an increase in Transect Two, however unavoidable changes made to the length and route of the Transect may have reduced the habitat diversity of the transect and as a result lowered the number of species observations. These changes may also have affected the observed abundances in Transect Two over the three most recent monitoring periods, however more time will be needed to confirm the effects of the modification. The prescribed burn that occurred in 2015 in Transect Four may have also affected observed abundances due to temporal loss or suppression of butterfly host plants during part of the 2015 season. Due to limited years of monitoring and recent changes to both transects that have had restoration effort, changes in butterfly abundances may not yet be apparent. Annual fluctuations in weather, in addition to population cycling may also be contributing to observed abundances at Transects Two and Four, and continued monitoring is necessary to determine abundance trends and efficacy of restoration efforts.

Although 2016 yielded high counts of butterflies, species richness was mid-range of all monitoring years at 51 species. It is possible that this drop in observed species richness is a result of droughts during the monitoring season. Sensitive and volatile species have shown responses to weather conditions that affect their foodplants (Harrison et al. 2015). Due to their reliance on particular foodplants, reproduction of habitat specialists has been found to decrease in response to drought (WallisDeVries et al. 2011). Population cycling is also a possible explanation for the low diversity observed. However, many of the species that were not observed in 2016 and have been observed in previous seasons are considered rare in Waterloo region (Linton 2012), and few individuals (often only one for each species) have been observed in previous monitoring years (e.g. White Admiral, Columbine Duskywing, Coral Hairstreak, Crossline Skipper, Eastern Pine Elfin, Harvester). As such, it is more likely that the rarity of species has affected observations during monitoring. Species presence at ***rare*** can be cross checked with observations posted to eButterfly, and observations at the Annual Butterfly Count to provide more insight on presence or absence. A few butterfly species that were not observed during monitoring in 2016 were observed incidentally or during the Annual Butterfly Count. However, most of these species are also classified as rare in Waterloo region, and it cannot be confirmed if sighting occurred along transect routes. Due to the rarity of species and different observers each year, it is also likely that observation bias plays a role in the number of species observed each year. Although protocol and training aims to limit bias to the extent possible, monitoring protocols based on count data are subjected to a certain degree of observational bias (Dennis et al. 2006).

Species Evenness for all transects pooled was lower this year than the previous two monitoring years. The drop in Evenness compared to the previous two years is likely influenced by the almost doubling of Cabbage White butterflies in 2016 compared to 2015 and 2014 monitoring seasons (Figure 2.7). The Shannon Diversity Index also dropped in comparison to the previous two years of monitoring, which is not surprising given the drop in Evenness and increased number of total individuals.

2.4.2 Transect Comparisons

Consistent with global butterfly abundance fluctuations at ***rare***, the number of butterflies observed in Transects One and Two in 2016 were the second highest of all monitoring years after 2012. Favourable weather conditions in years prior to 2012 and 2016, as well as favourable conditions during these years may explain the high abundances observed. However, observed abundances in Transects Three and Four were mid-range in 2016 compared to previous years (note there was no significant difference between 2016 and other years in either transect). This somewhat negates suggestions above that abundances are heavily correlated with what are considered ideal weather conditions. If this were the case we would expect that observations in Transect Three and Four in 2016 would be higher than the majority of other years. Despite fluctuations, no transects have shown a significant directional trend over monitoring years, suggesting that abundances over time are relatively stable, or possibly longer term data must be collected to see a significant change.

Transects One and Two have relatively large lengths coupled with an abundance of open habitat, which likely explains why they have generally seen the highest number of observed butterflies in comparison to other transects. Transects One and Three have consistently had the highest species richness, which is not surprising considering these to transects have the greatest diversity of habitat meeting the needs of a variety of butterfly species. Transect Two, and, in particular Transect Four, have less diverse habitats and are able to support fewer species of butterflies and host plants.

Mid-range Species Evenness and Diversity in Transect One in 2016 may be due to higher counts of a few of common species. For example, the three most abundant species in Transect One accounted for 61.7 percent of observations in 2016, compared to 42.1 percent, 48.1 percent, and 58.0 percent in 2015, 2014, and 2013 respectively. It is likely that a similar explanation be used for the mid-range Species Evenness and Diversity Index in Transect Two in 2016, with Clouded Sulphur and Cabbage White butterflies accounted for 45.8 and 22.8 percent of all observations respectively. Together, the two species accounted for 68.6 percent of total observations. At Transect Three, the Species Evenness and Shannon Diversity Indices have remained relatively constant across monitoring years. This indicates some stability in butterfly populations over the last seven years. The stability of butterfly populations may be influenced by the stability of the surrounding environment, as no major landscape changes have occurred on Transect Three throughout the monitoring program. At Transect Four, Evenness and the Shannon Diversity Index have been the highest over the past three monitoring seasons, likely in part due to the a decrease in Cabbage White butterflies observed in Transect Four during the last three monitoring seasons (see Appendix H, Figure H.8). Cabbage Whites account for a substantial proportion of total annual abundances in Transect Four (more than 50 percent per year in 2010-2013 to less than 30 percent per year in 2014-2016).

It is important to note that mowing occurred in Transect Two in South field East and West field during the tenth week of monitoring and observations of species common to areas that were mowed decreased substantially. Total butterfly counts in week ten were less than half of the previous week. Clouded Sulphurs generally accounted for at least 40 percent of all observations, with their highest abundances observed in South field East. Clouded Sulphurs have multiple, overlapping generations, with abundances highest between mid-June and mid-

September (Hall et al. 2014). It is unlikely that a low between generations is responsible for drops in observed abundances, and is probable that without mowing, total abundances observed over the fourteen weeks of monitoring would have been higher.

2.4.3 Species of Special Interest

Cabbage White butterflies (*Pieris rapae*) are invasive, habitat generalists that have spread rapidly throughout Canada (Layberry et al. 1998), and have consistently been the most abundant butterfly species observed at ***rare*** across all monitoring years. This butterfly's foodplants belong to the Mustard Family, including another non-native species, Garlic Mustard (*Alliaria petiolata*). This plant is widespread throughout the ***rare*** property (***rare*** Environmental Management Plan 2014; Robson et al. 2012), and both laboratory and field studies have demonstrated successful reproduction of Cabbage White butterflies on this host (Davis and Cipollini 2016; Heinen et al 2016).

Although Cabbage Whites were the most abundant species observed this year, they accounted for only about 24 percent of the total number of butterflies observed in 2016 (Figure 2.7). In the last four years, Cabbage White observations have been much lower than the initial years of monitoring. Low abundances in 2016 may be attributable to low abundances in the previous three seasons. It is also possible that changes in habitat composition, such as in the tall grass prairie, has increased competition for potential host plants. However, due to the generalist nature of Cabbage White butterflies and increasing abundance of garlic mustard as a potential host, it is unlikely host availability is main driver of decline. Regardless, the apparent decline in the invasive butterfly is positive as they are considered garden pests, and a decline in number would also decrease host competition for native species that share potential host plants, such as the Virginia White (*Pieris virginiensis*) and Mustard White (Hall et al. 2014).

European Skipper butterflies (*Thymelicus lineola*) were in the top three species observed at ***rare*** for the second year in a row. The European Skipper is another invasive species that has been present in Canada for quite some time. Original introduction of the species occurred near London Ontario around 1910 in seed for livestock feed (Layberry et al. 1998). Since that time, the European Skipper has attained incredibly abundant populations throughout Ontario, aided by their fondness for using many common invasive plants as hosts, such as Timothy Grass (*Phleum pratense*), Orchard Grass (*Dactylis glomerata*) and Quackgrass (*Elymus repens*) (Layberry et al. 1998).

Despite a decrease in abundance and percent of all butterflies since last year, European Skippers were the third most abundant species observed in 2016, and were still observed in higher numbers than all other previous monitoring years. Graphical representation of European Skipper abundances across monitoring years shows annual population fluctuations between 2010 and 2014, and it is possible the decrease in 2016 abundances is a result of natural population cycling (refer to Figure 2.8). Regardless, there has been a clear upward trend in abundances of European Skippers at ***rare*** and close monitoring should continue.

Although Monarch Butterflies are considered Very Common in the Waterloo Region, observed abundances have declined at ***rare***, mirroring trends of other migrant Monarch populations. Monarch observations have been consistently low since a peak in 2012, with less than 100 individuals overserved per year between 2013 and 2016. Despite hopes that larger overwintering populations in Mexico in the winter of 2015-2016 would yield higher Monarch populations in the summer of 2016, monitoring in 2016 did not increase substantially from 2015 numbers.

The Monarch is listed as a species of Special Concern, and the Eastern population which is observed at ***rare*** has significantly declined over the last 15 to 20 years (Environment and Climate Change Canada 2016). The Management Plan for the Monarch (*Danaus plexippus*) in Canada - 2016 indicates that loss of overwintering habitat due to forest loss and degradation, and loss of breeding and nectaring habitat due to the widespread use of pesticides are the threats of highest concern. Recent research by Flockhart et al. (2014) has also suggested that a critical cause of their decline is caused by the loss of Milkweed. Until 2014, Ontario had Common Milkweed (Asclepias syriaca) on its list of noxious weeds (OMAFRA, 2015). Positively, the management plan recommends promoting removal of native species of milkweed from provincial Weed Control Acts as a control measurement. However, large numbers of the plants have already been removed not only in Ontario, but throughout the United States, where some states still consider this plant a noxious weed (OMAFRA, 2015). As the Monarch requires adequate numbers of foodplants as it makes its way back north in each successive generation throughout the summer (Pleasants and Oberhauser 2012), it remains vulnerable if milkweed is not available along the way.

Efforts have been made at ***rare*** to increase viable habitat for Monarchs, mainly through the seeding of Milkweed. A Milkweed monitoring protocol was developed in 2015 and data have been collected on the abundance of Milkweed in four areas of the property in 2015 and 2016. 2016 Milkweed abundances were lower in all four plots. Although three of the four milkweed monitoring plots were seeded in 2015 with milkweed and other nectaring plants, numbers in 2016 were lower than 2015. It is possible that newly germinated plants were killed by frost, or that plants have not matured to form a large enough colony and thus the effects of seeding have yet to be realized. It is also important to note that the ECO centre area was disturbed (much of the vegetation was flattened by machinery) by the construction of a new trail, and the plot also had to be slightly modified to avoid the trail. As such, it is unknown whether seeding efforts have had any impact on Monarch populations at ***rare***. Continued monitoring of Milkweed and Monarch butterfly abundances at rare may shed light on the efficacy of seeding efforts. Results and maps of 2015 and 2016 Milkweed Monitoring are included in Appendix F.

Recent literature identified a third species of Azure Blues that may be present in the Carolinian forest zone (Schmidt and Layberry 2016). Monitoring years to date have considered only two species of Azure: *Celastrina ladon* and *Celastrina neglecta.* However, Schmidt and Layberry (2016) have identified *Celastrina lucia* and *C. neglecta* as the species that occur in the Kitchener area, with the possibility of a third species, *C. ladon*, also occurring in the area. Contrary to previous literature, there is also evidence that both *C. lucia* and *C. neglecta* have a second, summer flying generation. Monitoring at ***rare*** has previously considered Spring and Summer flying Azures to be different, univoltine species. This recent literature suggests closer attention will need to be invested in identifying *Celastrina* butterflies in future monitoring years.

As knowledge of butterfly taxonomy progresses, it is important to ensure that the program is updated based on the best scientific knowledge to reduce possible identification error. It will likely be necessary to take pictures of *Celastrina* butterflies observed in subsequent monitoring years to ensure proper identification by experts, particularly because these species look so similar.

2.4.4 Noteworthy Observations

Of the 51 species observed in 2016 during monitoring, 30 are considered very common or common, 14 uncommon, five rare, and two unknown, according to the Waterloo Regional Status assignment (Linton, 2012).

Common Sooty Wing (*Pholisora catullus*) butterflies are considered rare, but sightings have increased over the past two monitoring seasons, with 27 observed in 2015 and 40 observed in 2016. Previous to 2015, the highest number observed during monitoring was seven in 2013. Although considered rare in the Region of Waterloo, this butterfly has been observed somewhat consistently during butterfly monitoring at ***rare*** (with the exception of 2010). The majority of observations have occurred along Transect Two, likely because they prefer open, disturbed areas (Hall et al. 2014), and because hedgerows along Transect Two contain their primary foodplant, lamb's-quarters *(Chenopodium album)***.**

One Arctic Skipper (*Carterocephalus palaemon*) was observed along Transect Three, and has previously been observed during monitoring in 2010, 2013, and 2014, and during the Annual Butterfly Count in 2010. Although this species is considered common throughout its Canadian range, it has been observed in limited spots around the Waterloo Region, and is thus considered rare (Grealey et al. 2010).

A Grey Comma (*Polygonia progne*) was observed for the second year in a row during monitoring. Previous to 2015, Grey Commas have not been observed during monitoring, but have been observed incidentally on ***rare*** property and submitted to eButterfly. Grey Commas are considered regionally uncommon.

A Crossline Skipper (*Polites origins)* was observed in Transect One. This is the second year this skipper has been observed during monitoring. Previous to 2015, the species had only been observed during the Annual Butterfly Count in 2011. Crossline Skippers are considered regionally rare.

Juvenal's Duskywing (*Erynnis juvenalis*) are considered regionally rare but continue to be observed in high numbers considering their status. However, observations decreased from 70 in 2015 to 42 in 2016. This year at ***rare***, a research project with the University of Guelph conducted a study involving capturing, marking, and releasing Juvenal's Duskywing butterflies. Although it is unlikely this study contributed to observed populations during monitoring, it is important to note that these butterflies were handled more than during a regular monitoring year.

19 Silver-Bordered Fritillary (*Boloria selene*) individuals were observed in 2016. This is the second year in row that this species was seen frequently during monitoring, predominantly in Transect Three. This species is considered regionally rare, however it is possible that relatively high observations in the past two years are indicative of a growing population at ***rare***.

Silvery Blue *(Glaucopsyche lygdamus)* butterflies are considered regionally rare, but were observed for the second year in a row during monitoring in 2016. The presence of Silvery Blue butterflies is probably due to the increasing range of non-native plants the butterfly will use as a host, such as Cow Vetch *(Vicia cracca*) (Layberry et al. 1998).

Several commonly abundant species had the highest observed abundances in 2016. Clouded Sulphur (*Colias philodice*), a regionally very common species, had its highest abundance of all monitoring years in 2016. With 1,183 individuals observed, it was the second most abundant species in 2016. This number nearly doubles all years with the exception of 2012, with 797 individual Clouded Sulphurs observed. Orange Sulphur (*Colias eurytheme*) butterflies also had their most abundant year at 141 individuals. Inornate Ringlet butterflies (*Coenonympha tullia*) had their highest year at 539 observed individuals. One possible explanation is the association between increased numbers of butterflies in the current year, and wet conditions in the previous year (Pollard 1988; Roy et al. 2001) as well as warm conditions in both the current and previous years (Roy et al. 2001). Another is that possible that ideal weather conditions in 2015 and 2016 contributed to the high number of common butterflies observed in 2016. As none of these species are habitat specialists, it is unlikely they were negatively affected by the drought in 2016. It is also possible that negative effects of the drought on other butterfly species reduced competition for the aforementioned species, but this cannot be stated conclusively.

Two butterfly species new to ***rare*** were observed in 2016. The first is Aphrodite Fritillary (*Speyeria aphrodite)*, which was observed during the annual butterfly count on July 16th, 2016. The second was Leonard's Skipper (*Hesperia leonardus)*, observed during the BioBlitz on August 14, 2016. Both were later observed on the ***rare*** property outside of monitoring and reported to eButterfly. It should be noted that observations of Leonard's skippers occurred outside of monitoring areas, possibly explaining why they were not observed during monitoring. Another explanation could be that they typically have later flight seasons, with peak abundance outside of monitoring weeks (mid-August and mid-September) (Hall et al. 2014). The Aphrodite Fritillary is considered a rare species in the Region of Waterloo and the status of Leonard's Skipper is unknown.

Several butterfly species have been recorded only incidentally or during annual counts that have not been observed during monitoring. These species include: Mulberry Wing (*Poanes Massasoit*), Little Yellow (*Eurema lisa*) and Acadian Hairstreak (*Satyrium acadicum*). The Mulberry Wing is regionally rare has been observed during three annual butterfly counts (2013, 2015, 2016), the Acadian Hairstreak is regionally uncommon and has been observed during one annual count (2013), and the Little Yellow during is regionally rare and has been observed during one annual count (2006).

The Meadow Fritillary (*Boloria bellona*) is considered to be very common in the area. However, official sightings have been very low and temporally sporadic. According to eButterfly, there have been six incidental sightings in 2016, and two individuals were observed during the Annual Butterfly Count. Due to similarity in Fritillary butterflies and given that butterflies are often identified during flight, it is possible that these butterflies have been misidentified during monitoring. This may also be the case with the Aphrodite Fritillary, which is very similar to Great Spangled Fritillary (*Speyeria cybele*) butterflies, which often fly very high and quickly and are identified during flight. Special attention should be given to Fritillary butterflies in future monitoring years.

2.5.0 Conclusions and Recommendations

Observed butterfly abundances in 2016 were second highest after the 2012 monitoring season. Both years were very hot and dry, and proceeded by years with relatively high early rainfall, further suggesting that butterfly abundances are closely tied to weather conditions.

However, low abundances observed in Transects Three and Four in 2016 relative to all years indicate that there are likely other factors at play.

Despite annual fluctuations, no significant directional trend in butterfly abundances has occurred over the last seven monitoring years. This may be indicative of stable abundances over time, or of a need for longer term data collection. Analysis of an abundance trend across years should be continued in addition to analysis between years to determine whether abundances are experiencing a directional trend over time.

Abundances and indices of diversity have been heavily influenced by abundant species across monitoring years. To eliminate skewed abundances in analyses due to more prevalent species (e.g. Cabbage Whites), future analysis should consider independent analysis of each species, or grouping species into biologically and/or phenologically relevant groups such as habitat generalists and habitat specialists, or endemic, migrant, and non-native species.

It cannot be concluded whether restoration efforts in Transects Two and Four have had positive effects on butterfly populations to date. Annual fluctuations in abundance and species richness make it difficult to determine a trend in the short term, and further years of data will be necessary to shed light on the efficacy of management efforts.

It is recommended that the monitoring program at ***rare*** continue in its full capacity in the years to come. Extending monitoring by several weeks in the fall is also recommended, as it would allow for more appropriate capture of species with late summer/ fall flights. With a constant urban growth surrounding the ***rare*** property, including new subdivisions, increased vehicle traffic, and continued aggregate mining, the butterfly monitoring program will play a key role in detecting changes in ecosystem health. Identifying potential issues early on will also allow for further creation and implementation of management plans for the property. The data collected during butterfly monitoring at the ***rare* Charitable Research Reserve** will also continue to be useful on a broader scale, adding to the knowledge of environmental health in the Region of Waterloo as a whole.

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3.0 Plethodontid Salamander Monitoring

3.1.0 Introduction

3.1.1 Salamander Taxonomy

Ontario is home to salamanders representing four different families (Proteidae, Salamandridae, Ambystomatidae, and Plethodontidae), of which two families are known to be present at ***rare***. The mole salamanders (Ambystomatidae) are large burrowing salamanders with an aquatic juvenile phase and a terrestrial adult phase (Conant and Collins 1998). Members of this family such as Yellow-spotted salamanders (*Ambystoma maculatum*) and Blue-spotted salamanders (*A. laterale*) are occasionally observed at ***rare***. Members of the *jeffersonian-laterale* complex are also present on the property. An additional report on the occurrence of these species can be found on the ***rare*** server.

The lungless salamanders (Plethodontidae) are the most frequently observed salamander family at ***rare.*** Primarily observed are Eastern Red-backed salamanders (*Plethodon cinereus*), with occasional sightings of Four-toed salamanders (*Hemidactylium scutatum*). Plethodontids are the largest family of salamanders worldwide representing 27 genera and over 370 recognized species (Larson et al. 2006). These salamanders are generally long and slender and are lungless, breathing through their thin, moist skin (Behler and King 1979). This reliance on cutaneous respiration across moist body surfaces makes plethodontid salamanders particularly sensitive to environmental changes in their micro-habitat (Zorn et al. 2004). Gas exchange requires skin to be moist (Welsh and Droege 2001) resulting in high absorption rates potentially exposing the salamander to contaminants in the soil.

The Eastern Red-backed salamander is the most abundant plethodontid in Eastern Canada (Zorn et al. 2004) and at ***rare***. They are completely terrestrial and therefore do not require ponds or vernal pools for development. They can generally be found in moist soil under downed woody debris in mature forests (Conant and Collins 1998). There are two main colour phases of the Eastern Red-backed salamander: a red-backed morph that has dark grey sides and a rough edged red stripe down the back, and a lead-backed morph that lacks the red stripe and is entirely grey.

3.1.2 Global Amphibian Decline

It is estimated that one-third of all amphibian species worldwide are endangered or threatened with extinction (Stuart et al. 2004), with more than 40 percent of all known species in decline (International Union for Conservation of Nature, 2016). Most amphibians experience both aquatic and terrestrial stressors, and therefore are uniquely valuable as indicators of environmental stress. As such, there is significant concern over the noted amphibian declines world-wide; however, the causes of such declines are still largely undecided, and are seemingly both variable and context dependent (Blaustein and Kiesecker 2002, Caruso and Lips 2012). Alford and Richards (1999) suggest the decline of amphibian populations is a global problem with complex local causes. Habitat destruction and alteration, global climate change, diseases, contaminants, and introduced species are all examples of such causes that have likely contributed to this global decline (Blaustein and Kiesecker 2002, Hof et al. 2011, Bruhl et al. 2013). Given the difficulty in neutralizing or reversing these threats, the future for amphibians around the world is seemingly bleak (Beebee and Griffiths 2005).

3.1.3 Plethodontid Salamanders as Indicator Species

Woodland plethodontids, which complete their entire life cycle on the forest floor, are useful indicator species for forested ecosystems (Welsh and Hodgson 2013). This is due to their life history traits, sensitivities to anthropogenic stresses, and population sampling properties (Zorn et al. 2004).

Under normal conditions, plethodontid salamanders typically have stable population sizes due to long life spans (10+ years), high annual survivorship, and low birth rates. They have small home ranges (13m^2 for males and juveniles and 24m^2 for females (Kleeberger and Werner 1982)) and display site fidelity, with some species exhibiting occasional territorial behaviours (Peterson et al. 2000; Maerz and Madison 2000). Due to these traits, observed changes in population from long-term monitoring are more likely to be indicative of ecosystem stresses than typical home range shifts or population fluctuations. The role of plethodontid salamanders in the forest ecosystem is an important one. They are efficient predators and quickly metabolize insect and other invertebrate prey, which can result in plethodontid densities equalling or surpassing other vertebrate groups (Butron and Likens 1975). These high densities provide an ample food source for predators such as snakes, rodents, and birds. Therefore, their role in transferring energy up trophic levels is invaluable (Zorn et al. 2004). Walton's 2013 study supports a hypothesized top-down regulatory role of plethodontid salamanders in the terrestrial detrital food web. As predators of invertebrate species that have substantial impact on decomposition and nutrient cycling on the forest floor, plethodontid salamanders help in managing these important ecosystem functions.

Being lungless, plethodontid respiration is strongly affected by body moisture and the contact between their skin and contaminants (Droege et al. 1997). This sensitivity makes woodland plethodontids useful indicators of ecological stresses, as they are influenced by their micro-climate and water and air quality. Potential stresses include human activities, (development, pollution, etc.) natural disturbances (storms, fires, etc.) or any event that may alter soil moisture, quality, or sun exposure (Zorn et al. 2004).

Finally, monitoring and identifying plethodontid salamanders can be done with relative ease. With a limited number of salamander species inhabiting the area, accurate identification can occur with minimal training, and reliable data can be collected from year to year with varying observers and/or volunteers. Additionally, since woodland plethodontids are attracted to artificial cover boards (ACOs) they can be easily sampled, avoiding destruction of habitat and unnecessary stress or harm to individuals.

*3.1.4 EMAN Plethodontid Salamander Monitoring at **rare***

In 2004, the Ecological Monitoring and Assessment Network (EMAN) and Parks Canada published a joint National Monitoring Protocol for plethodontid salamanders. The goals of this protocol were to work alongside a suite of other standardized protocols to act as an early detection of ecological change and to environmental issues. First and foremost, this protocol aims to provide a standardized methodology for plethodontid monitoring across Canada (Zorn et al. 2004). The protocol involves the establishment of permanent forest monitoring plots which contain a series of wooden ACOs (artificial cover objects) spaced evenly across the forest floor. Zorn et al. (2004) suggest that monitoring should ideally occur in both spring and fall of each year to achieve the best results relating to salamander abundance and community structure as an indicator of ecosystem health.

The salamander monitoring program at ***rare*** is conducted exclusively in the fall due to monetary and time constraints. The program was established in 2006 with the installation of 29 ACOs in Indian Woods. Following a pause in 2007, the monitoring resumed in 2008 and was expanded to include a second monitoring plot in the Hogsback consisting of twenty ACOs, running for only five weeks. In 2009, the program was once again expanded with the addition of three ACOs to the already established monitoring plot in Indian Woods, bringing the total number of ACOs in that plot to 32 and increasing the length of monitoring in the Hogsback to the full nine weeks. Monitoring has been ongoing with consistent a nine-week sampling effort each fall since 2009 at both sites.

Salamanders successfully began using the ACOs within weeks of establishment and continue to use them despite resultant disturbances from the monitoring process. The initial years of this monitoring have resulted in the collection of valuable baseline data regarding salamander populations at ***rare*** with which data from future years can be compared in order to determine how ***rare*****’s** salamander populations are changing over time. Additionally, McCarter (2009) identified specific research questions regarding the goals and mandates of this monitoring initiative at ***rare***:

1. What is the current state (species diversity, abundance, age structure) of the salamander populations in ***rare*** forests, and how do they compare to one another?
2. What are the long-term trends in Eastern Red-backed salamander abundance and population structure taking place within Indian Woods and the Hogsback?
3. Is the ecosystem integrity of Indian Woods and the Hogsback being maintained or improved under ***rare*** management?
    - Ecosystem integrity is defined as an ecosystem that has its native abiotic and biotic components intact and likely to persist (Parks Canada 2009)
4. Is either the ecological health or integrity of Indian Woods and the Hogsback being affected by on-site and nearby changes in land use (i.e. restoration, agriculture, residential development and aggregate extraction)?
    - Ecosystem health is defined as an ecosystem that has the capacity to resist and recover from a range of disturbances, while maintaining its functions and processes (Styers et al. 2010; Twery and Gottschalk 1996)

3.2.0 Methods

3.2.1 Monitoring Locations

Indian Woods (IW) is an old-growth Sugar Maple-American Beech (*Acer saccharum-Fagus grandifolia*) dominated forest located on the western side of the ***rare*** property, south of Blair Road and north of Whistle Bare Road. The forest covers approximately 20 acres and contains trees as old as 240 years. The Indian Woods salamander monitoring plot is located on the east side of an ephemeral pond near the south edge of the forest (Appendix A, Figure A.3). The plot is accessed by parking at the South Gate on Whistle Bare Road, and walking north along the Grand Allée trail until a second path merges from the west (left) side. This second trail is marked by a blue square sign with a white arrow. From the point of the trail junction, walk east (right) into the forest towards a large ephemeral pond (approximately 100m). The 32 ACOs are distributed in a large square made up of four lines of eight ACOs each. Boards five, six, and seven were missing prior to 2009.

The Hogsback (HO) is a 57-acre forest located approximately 700m southeast of Indian Woods, south of Blair Road, and just west of the Newman Drive subdivision. It is comprised of mixed swamp interspersed with ridges of upland forest characterized by Red Maple (*Acer rubra*) and White Pine (*Pinus stroba*). The Hogsback salamander plot can be accessed from the Springbank Community Gardens by travelling across the farm field adjacent to the gardens to the edge of the forest. At the forest's edge, on foot, keep left and walk north and then east along the edge of the forest, finally heading south into the stand at an area of downed fence marked by pink flagging tape on a fallen log. Continue south into the stand for approximately 50m to the monitoring plots. Twenty ACOs are distributed in a large rectangle with eight ACOs on the north and south sides and two ACOs on the east and west sides (Appendix A, Figure A.3). Each board is identified with a writeable aluminum tag marked as follows: SITE-YEAR-NUMBER (ex.HB-08-01) and is flagged with pink or orange flagging tape on an adjacent shrub or tree.

3.2.2 Monitoring Protocol

Approximately three weeks prior to the start of monitoring, all ACOs in both Indian Woods and the Hogsback were visited to ensure proper positioning and clear labelling. If necessary, boards were repositioned so that they were flush against the soil and reoriented into their original location. As the boards have been in place for multiple years, the proper positioning is generally noticeable as an area of bare soil. Labels and flagging tape were replaced as needed, and any holes in the boards were packed with soil to prevent salamanders from hiding during monitoring. Boards that were missing or too damaged or decomposed to be viable were replaced by newly cut boards, and relabelled with the current year.

Each plot was monitored once a week for nine successive weeks from the end of August to the end of October. At the beginning of each monitoring session, water was collected into a squeeze bottle from the education pond behind Lamb's Inn. This water was used to calibrate the soil moisture meter (Lincoln Irrigation Corporation, Lincoln, Nebraska, USA) by adjusting the meter with a screw driver so that it read a moisture rating of "10: saturated" when the probe was completely immersed in the water. The start time for the entire monitoring plot and Beaufort's wind and sky codes were recorded on the data sheet at the start of monitoring (see Appendix C, Tables C.1 and C.2 and Figures C.2 and C.3). Presence or absence of precipitation in the 24 hours previous to monitoring was recorded as 0 or 1 rather than recording precipitation values. This change was made to account for the fact that the Kitchener Waterloo data was not consistent with personal observations of precipitation on ***rare*** property (i.e. rain would be recorded for Kitchener Waterloo which did not occur at ***rare***). In Indian Woods, the depth of the ephemeral pond was recorded using the measuring stick permanently in place.

Boards were always visited in sequential order starting with one. Soil temperature (°C) and moisture measurements were collected at each ACO by inserting the probes of the soil thermometer (Ashcroft® Thermometers, USA) and soil moisture meter to a depth of 10cm, as marked with tape on the probes, in the soil beside the board. Canopy cover was also recorded at each ACO, and starting in 2016 a light reading was also taken using a luster leaf rapitest light and moisture reader.

The ACO was then gently turned over and any salamanders underneath were collected by the observers wearing nitrile gloves and placed into a plastic container with a sponge dampened with pond water previously collected in squeeze bottle. Each salamander was identified to species (colour phase was indicated for Eastern Red-backed salamanders) and any noticeable physical defects were recorded. A list of common and scientific names for all salamanders observed at ***rare*** and their abbreviated codes is available in Appendix D, Table D.2. Salamanders were weighed on a digital scale (Equal Digital Scale, model #23-D-50, capacity 50g) in grams to two decimal places. Snout-vent length (SVL) and vent-tail length (VTL) were recorded for each individual using a set of digital calipers (TuffGrade IDI, Commercial Solutions, Alberta, Canada). To ensure measurements were recorded accurately from the vent, individuals were measured through a clear lid while pressed up against moist sponges in the base of the container to secure the salamander and view the ventral side. Following measurements, salamanders were released next to the board. Disturbances under or near the ACOs (e.g. snakes, ant nests, turkey scratches, fungus/mold, ACO movement) were also recorded. Data sheets can be found in Appendix C and on the ***rare*** server.

In each monitoring plot, specific ACOs were assigned the status of weather station and each weather station represents a specific subset of ACOs. Table 3.1 and 3.2 show which ACOs are associated with each weather station in Indian Woods and the Hogsback. When each weather station is reached during the monitoring of boards in sequential order, weather variables including average wind speed (taken as the average after ten seconds), air temperature (°C) and percent relative humidity were collected using the Kestrel 3000 (Nielson-Kellerman, Boothwyn, PA, USA). Additionally, soil samples for pH testing were collected from both Indian Woods and the Hogsback at each weather station on the last day of monitoring. A complete list of required equipment is available in Appendix B, List B.2.

Table 3.1: Weather stations and the artificial cover objects (ACOs) associated with them in Indian Woods salamander monitoring plot.

Weather Station ACO Number	Associated ACOs
3	1,2,3,4
7	5,6,7,8
11	9,10,11,12
15	13,14,15,16
18	17,18,19,20
23	21,22,23,24
27	25,26,27,28
31	29,30,31,32

Table 3.2: Weather stations and the artificial cover objects (ACOs) associated with them in the Hogsback salamander monitoring plot.

Weather Station ACO Number	Associated ACOs
2	1,2,3,4,5
7	6,7,8,9,10
12	11,12,13,14,15
17	16,17,18,19,20

3.2.3 Data Analysis

Data analysis was conducted using R 3.2.4 and Microsoft Excel 2010. Prior to analysis, assumptions of parametric testing were examined. When transformation was required, the most appropriate transformations were preformed and assumptions were retested with each model. Each salamander monitoring plot (Indian Woods and the Hogsback) was interpreted as representing a unique population, and each ACO within that plot was interpreted as representing a sample of that population.

Each monitoring plot had a differing number of ACOs and since in 2006 and 2008 the Indian Woods monitoring plot had three less ACOs than in later years, data had to be standardized to allow for comparisons of count data. Abundance was therefore transformed into catch per unit effort (CPUE) for each monitoring session, as is commonly used in fisheries science (Krebs 2001). To calculate CPUE, the total salamander count for each monitoring session was divided by the number of ACOs in that plot to get the mean weekly catch per ACO. The CPUE calculation included only Eastern Red-backed Salamanders due to very low numbers of other species. A two-way ANOVA with plot and year as independent variables and CPUE as the dependent variable was performed to determine differences in CPUE between years and plots, and the interaction between year and plot variables. Where interactions occurred, data were split or combined appropriately for subsequent testing. This was followed by Bonferroni post hoc testing to determine where the differences between the levels occurred.

A univariate ANOVA split by plot was used to investigate weekly differences in Eastern Red-backed salamander abundance, with week as an independent variable. As abundance was used as the dependent variable, plots were considered separately to account for uneven sample numbers (ACOs) at each plot. This was followed by Bonferroni post hoc testing to determine where the differences between the levels occurred.

Two-way ANOVAs were used to examine differences in species composition in each plot across all years. The first test included abundance as the dependent variable and species and plot as independent variables. The second test included abundance as the dependent variable and species and year as independent variables. Interactions between independent variables were tested in both models. Where interactions occurred, data were split appropriately for subsequent testing. This was followed by Bonferroni post hoc testing to determine where the differences between the levels occurred. Lead-backed and Red-backed phases of Eastern Red-backed salamanders were considered together in this analysis.

To determine differences in the ratios between colour phases of Eastern Red-backed salamanders over monitoring years and between plots, the ratio of Lead-backed to Red-backed individuals was calculated and fitted to a general linearized model, with ratio as the dependent variable and year and plot as independent variables. The significance of effects was tested with an iterative F-test (Crawley 2007).

Only Eastern Red-backed salamanders (both colour phases) were considered in a size class comparison. Individuals were classified as adult, intermediate, or juvenile based on their snout-vent length as outlined in Zorn et al. (2004). Age classes were defined as follows: juveniles <25mm; intermediates 25mm-35mm; adults >35mm. Eastern Red-backed salamanders are capable of tail autonomy (Wise and Jaeger 1998), and so while vent-tail length was also measured it is not a reliable indicator of size class. An ANOVA with three fixed factors (plot, year, and size class) was used to look for differences in salamander size class. Interactions between factors would represent that a size class varies among plots or years. Bonferroni post hoc testing followed to determine where differences occurred.

Each plot was analysed separately for their relationship with environmental parameters, as sampling effort varied with plot. Data from 2006 and 2008 were eliminated from this analysis since its sampling effort varied from other years. Variables were considered for models based on their inherent relationship with salamanders (i.e. since salamanders live in the soil, soil factors were likely important). A correlation matrix was created to identify multicollinearity between parameters. In this analysis, multicollinearity is considered to be present when correlation is greater than 0.7 and less than -0.7. R squared values of each correlated pair were compared to which variables to include in model. Multiple linear regressions were used for each plot to determine which environmental factors (soil temperature, soil moisture, soil pH, pond depth, precipitation, sky and wind codes, wind speed, relative humidity, air temperature and disturbance) affected total salamander abundance. Hierarchal multiple regressions followed with total abundance as the dependent variable and related parameters as the independent variables. How well each model predicted the dependent variable- the goodness of fit of each model- was tested using the Akaike's Information Criterion (AIC) model selection technique.

3.3.0 Results

3.2.1 Total Abundance

A total of 125 salamanders were observed between August 30 and October 26 at the ***rare* Charitable Research Reserve** in 2016. In Indian Woods, 78 salamanders were observed, and 47 were observed in the Hogsback. This is the second lowest abundance in Indian Woods after 2011, the lowest in the Hogsback of monitoring years with equal sampling effort, and the lowest total observed abundance (both plots combined) of any year. Two species of salamanders were observed in the Hogsback (Eastern Red-backed and Four-toed) and one species was observed in Indian Woods (Eastern Red-backed).

3.2.2 Eastern Red-backed Salamander Abundance

Plot differences varied with years (interaction $F_{8,144}$=8.692, p<0.001), so both factors were considered simultaneously in an nineteen-level combination variable of plots and years (Leech et al. 2008), and significant differences occurred between these levels (ANOVA $F_{18,144}$=10.08, p<0.001). In 2016, CPUE in the Hogsback was lowest of all years. Significant differences occurred between 2016 CPUE and CPUE in 2009, 2013, 2014 and 2015 (p<0.01). In Indian Woods, 2016 CPUE was the second lowest, after CPUE in 2011. Significant differences occurred between 2016 CPUE and CPUE in 2008 and 2006 ($p<0.001$). In most years, CPUE was higher in the Hogsback than Indian Woods, with the exception of 2008 and 2016 (Figure 3.1). Significant differences occurred between plots in 2008 (CPUE IW >CPUE HO, $p<0.001$), 2013 and 2014 (CPUE HO> CPUE IW, $p<0.001$), and 2015 (CPUE HO> CPUE IW, $p<0.05$). There was no significant difference between CPUE in the Hogsback and Indian Woods in 2016.

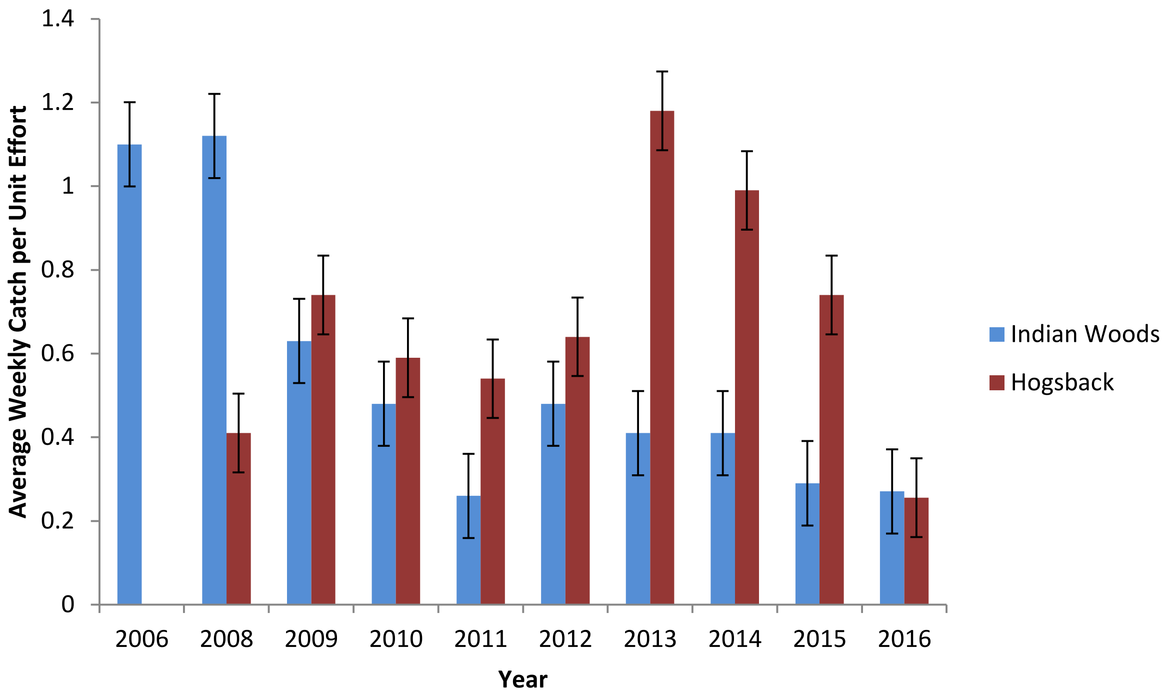


Figure 3.1: Average weekly salamander observation per artificial cover object (ACO) (Catch per Unit Effort) for both Indian Woods and Hogsback for all monitoring years. Error bars represent +/- one standard error.

Differences in salamander abundance were examined across weeks (Figure 3.2). This analysis used total weekly salamander abundance as the dependent variable as opposed to CPUE, and excluded years 2006 and 2008 as sampling efforts differed. Since the number of ACOs in each plot differed, Indian Woods and Hogsback were examined independent of one another. No significant differences occurred between weeks at the Hogsback ($F_{8, 63}=1.723$, $p=0.1$). Significant differences occurred between weeks at Indian Woods ($F_{8, 63}=2.454$, $p<0.05$), with week 7 having significantly higher abundances than week 2($p<0.05$). Figure 3.2a. demonstrates this trend, with abundances in Indian Woods peaking week 7 and tend to be lowest in week 2. This is consistent with what was observed overall in 2016.

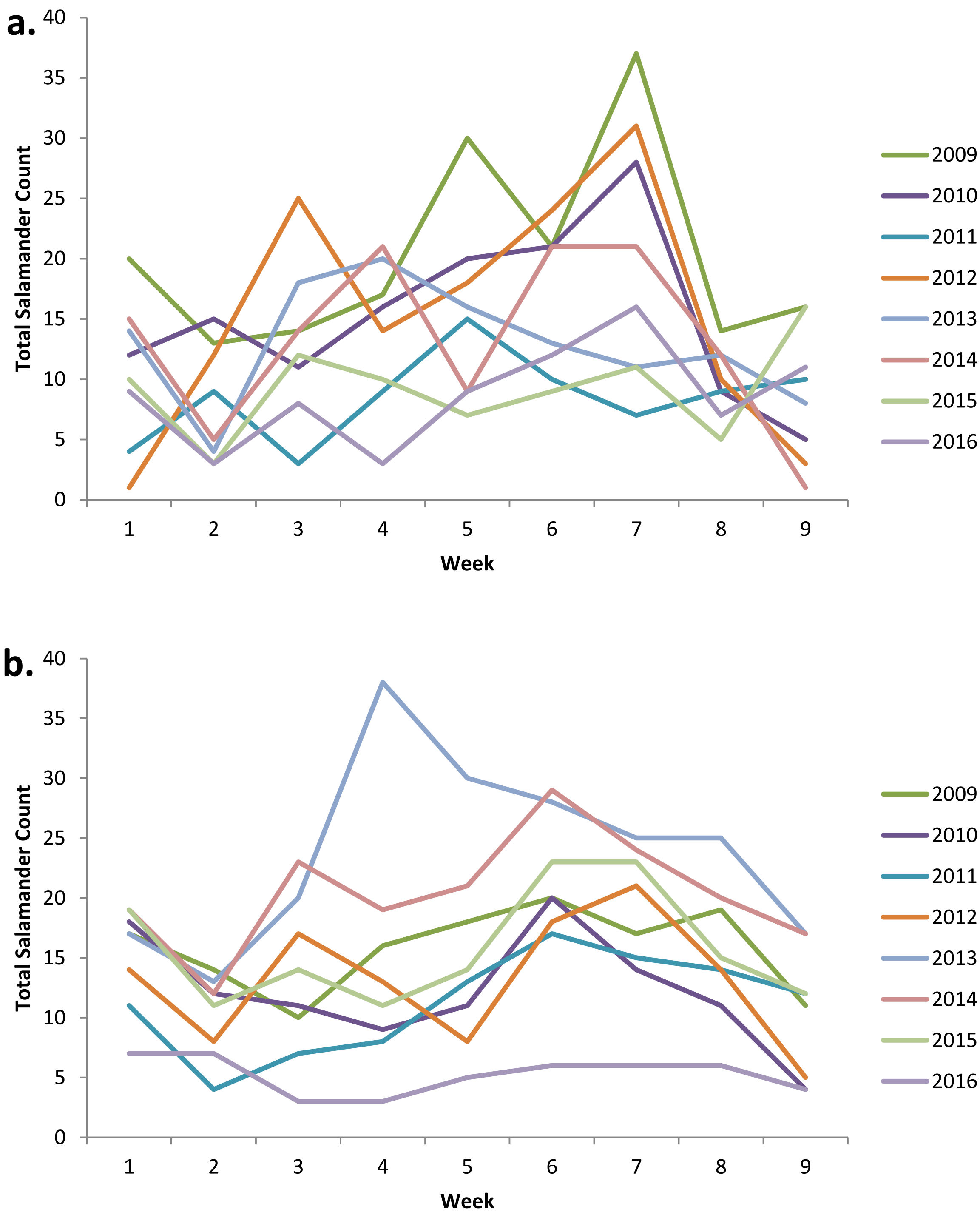


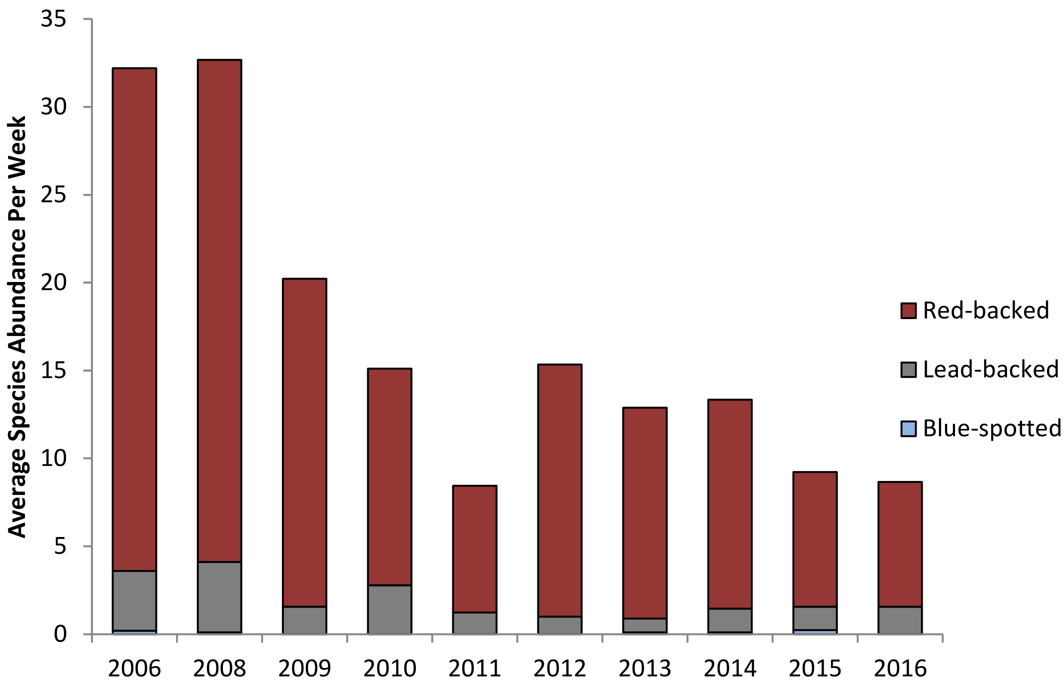
Figure 3.2: Total weekly salamander counts in Indian Woods (a.) and the Hogsback (b.) from 2009-2016. Data from 2006 and 2008 is excluded due to unequal sampling effort.

3.2.3 Salamander Species Composition

An interaction occurred between plot and species (interaction $F_{3,68}$=5.652, *p*=0.007) so data were split by plot (Zar 1999) and Indian Woods and Hogsback were each considered independently of one another. In both plots, significant differences occurred between species (p<0.001), with significantly more Eastern Red-backed salamanders occurring than other species, regardless of year. Four species have been observed in the Hogsback since 2008, and only two species have been observed in Indian Woods since 2006 (Figure 3.3). In 2016, Eastern Red-backed salamanders were observed in Indian Woods in both lead-backed and red-backed phases. In the Hogsback, both phases of Eastern Red-backed salamanders were observed, as well as a Four-toed salamander.

No significant differences exist between the ratio of Eastern Red-backed colour phases between years (p=0.8177). Significant differences exist between plots (p<0.001), with less lead-back salamanders occurring in the Hogsback (Figure 3.4).

a.



b.

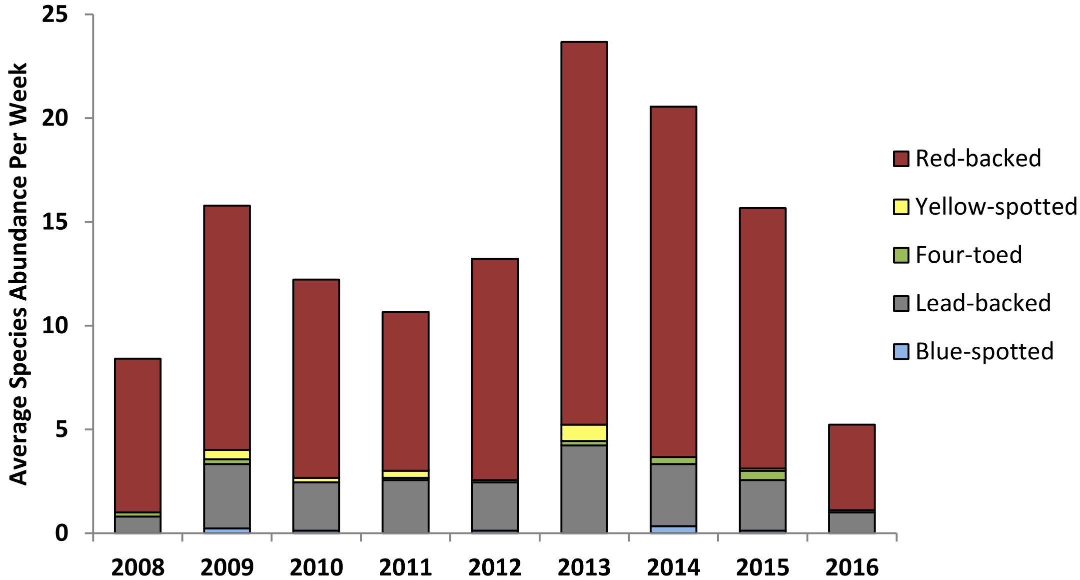


Figure 3.3: Mean salamander abundance by species for each monitoring year in Indian Woods (a.) and the Hogsback (b.). Red-backed and Lead-backed are two colour morphs of the same species, the Eastern Red-backed Salamander.

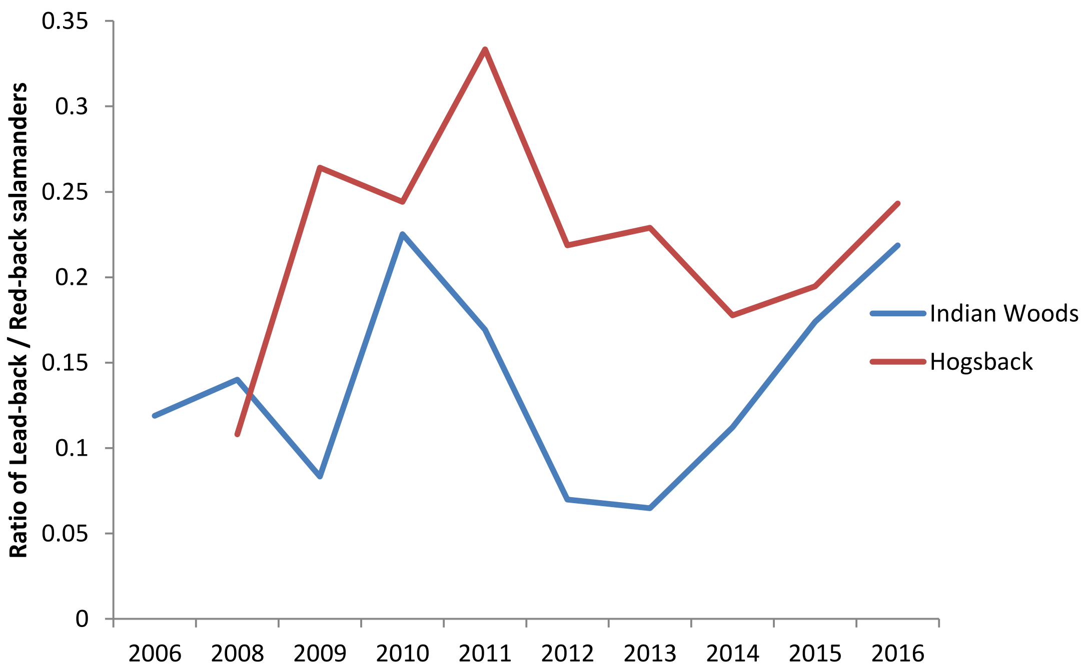
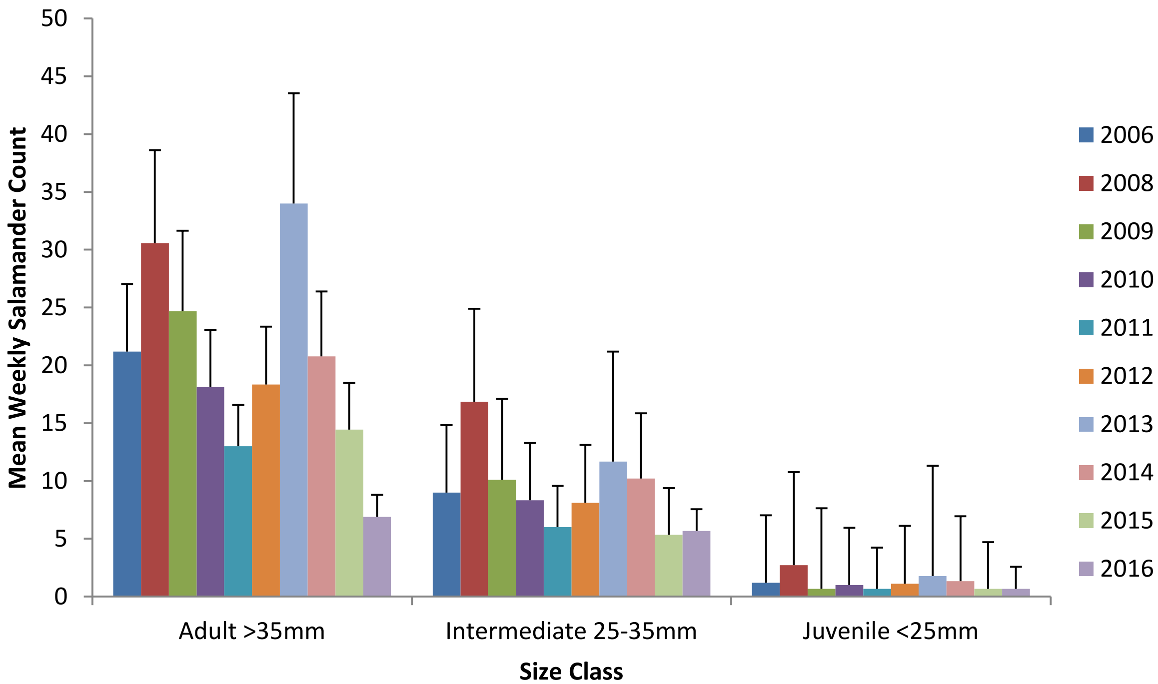


Figure 3.4: Ratio of Lead-backed to Red-backed colour phases of Eastern Red-backed salamanders in Indian Woods and Hogsback plots.

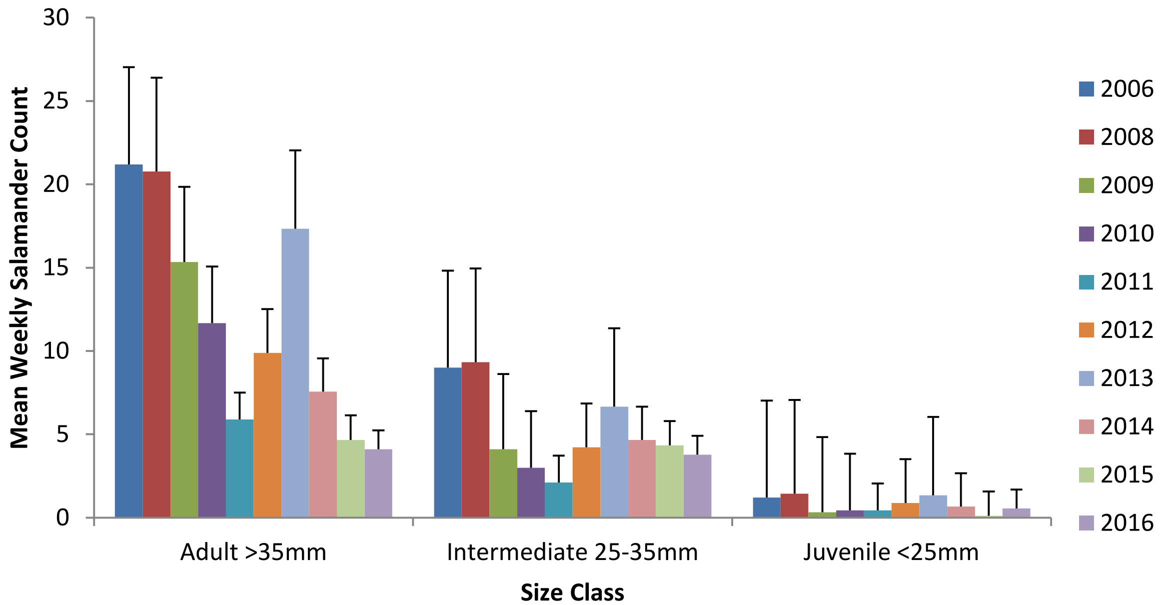
3.2.4 Eastern Red-backed Salamander Size Class Distribution

Size class did not interact with plot ($F_{2,50}$=0.589, p=0.5586) or year ($F_{8,27}$=0.519, p=0.9241), so both plots were considered simultaneously in a univariate ANOVA with abundance as the dependent variable and size class as the independent variable. Significant differences between size class exist ($F_{2,54}$=0.8062, p<0.001), with significantly more adults than intermediates and juveniles, and significantly more intermediates than juveniles (p<0.001). These differences are represented by Figure 3.5. Individual figures representing size class distribution in Indian Woods and the Hogsback are also included for reference.

a.



b.



c.

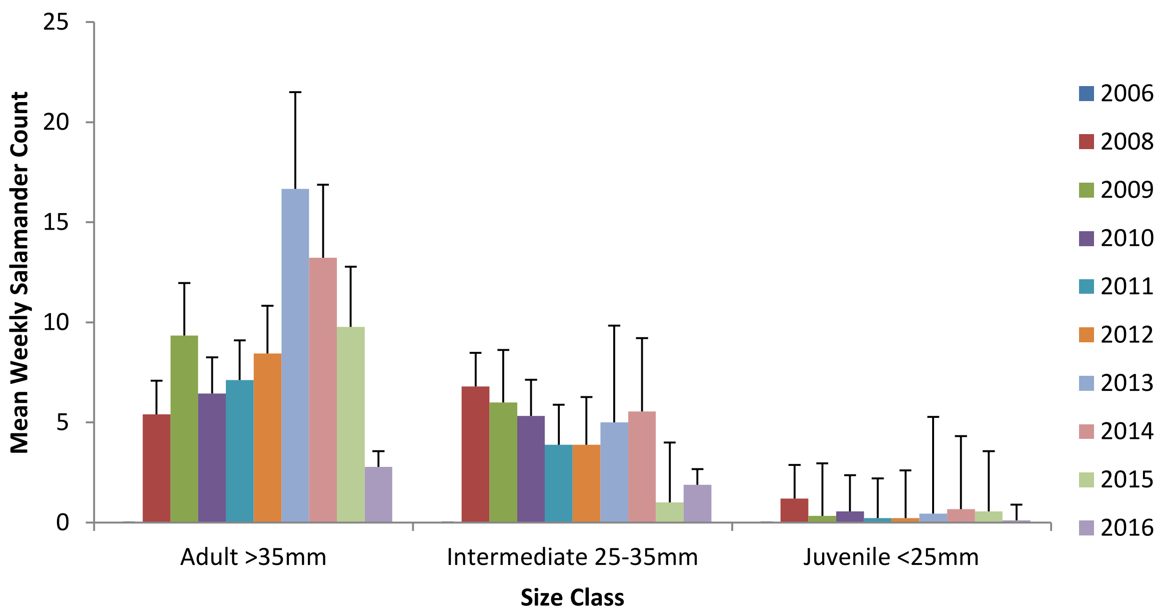


Figure 3.5: Mean size distribution of salamanders observed weekly during monitoring in Indian Woods and the Hogsback combined (a.), Indian Woods (b.), and the Hogsback (c.). from 2006-2016. Error Bars represent + one standard error. 2006 represents weekly averages in Indian Woods. 2008 represents both Indian Woods and the Hogsback average weekly abundances adjusted for uneven sampling duration.

3.2.5 Environmental Parameters

Correlation analysis between environmental parameters in Indian Woods identified a positive correlation between soil temperature and air temperature (r^2=0.869). Soil temperature was excluded from the model because it had a lower R^2 value that air temperature (r^2=0.113 and 0.179 respectively). The best model predicting salamander abundance in the Indian Woods included soil moisture, air temperature, wind code, sky code, and year ($F_{5,66}$=8.125, p<0.001, r^2=0.381). Soil moisture had a significant positive effect on salamander abundance (p<0.05), while air temperature, and Beaufort sky code had significant, negative relationships with salamander abundance in Indian Woods ($p<0.005$).

Correlation analysis between environmental parameters in the Hogsback identified a positive correlation between soil temperature and air temperature ($r^2=0.869$). Soil temperature was excluded from the model because it had a lower R^2 value than air temperature ($r^2=0.0647$ and 0.06792 respectively).The best model for predicting salamander abundance in the Hogsback included soil moisture, air temperature, wind code, sky code, and disturbance ($F_{5,66}=9.675$ $p<0.001$, $r^2=0.423$). Soil moisture had a positive, significant relationship with salamander abundance ($p<0.001$), while Beaufort wind code, Beaufort sky code, and disturbance had significant negative relationships with abundance ($p<0.05$).

Graphical representation was included for parameters that had significant relationships with observed salamander abundance in both plots across years (Figures 3.6, 3.7 and 3.8).

Temperature was second highest in September and highest in October compared to other monitoring seasons (Figure 3.9). Precipitation was the second lowest in September and the lowest in October compared with other monitoring seasons (Figure 3.10)

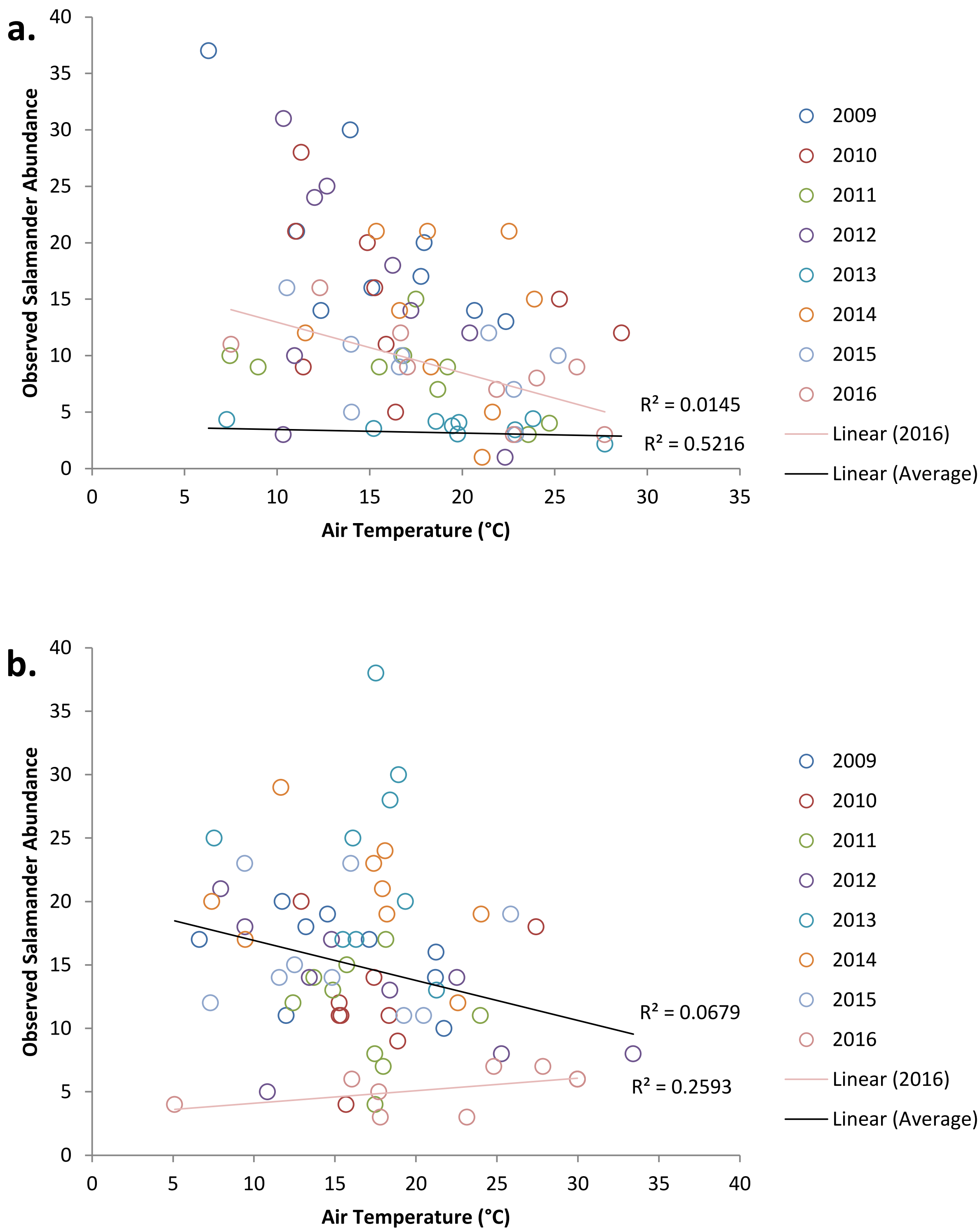


Figure 3.6: Relationship between total salamander abundance in Indian Woods (a.) and the Hogsback (b.) and measured air temperature for 2009-2016. Trend lines are included for the linear average for all years, as well as 2016 with corresponding R^2 values.

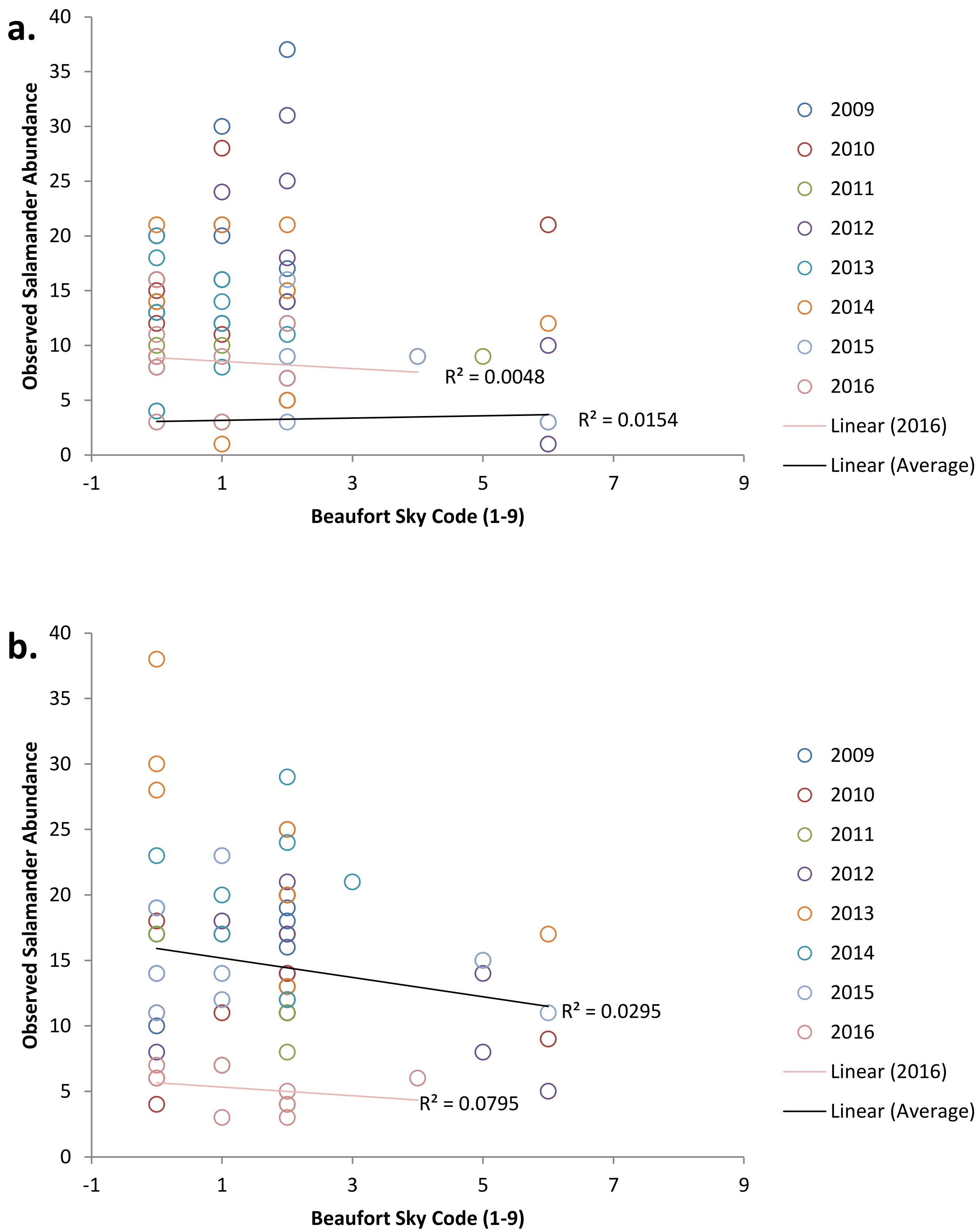


Figure 3.7: Relationship between total salamander abundance in Indian Woods (a.) and the Hogsback (b.) and Beaufort Sky codes for 2009-2016. Trend lines are included for the linear average for all years, as well as 2016 with corresponding R^2 values.

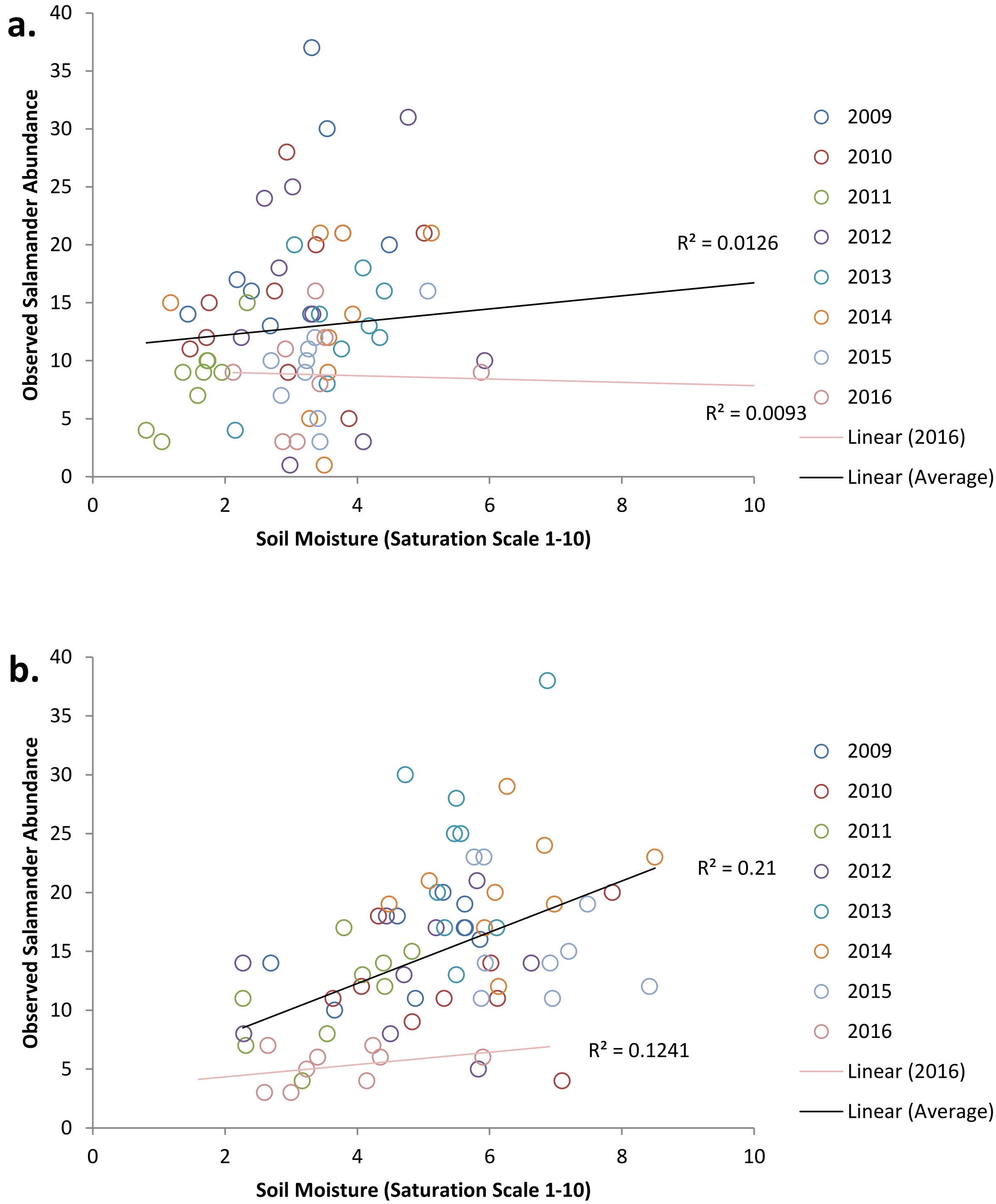


Figure 3.8: Relationship between total salamander abundance in Indian Woods (a.) and the Hogsback (b.) and measured soil moisture for 2009-2016. Trend lines are included for the linear average for all years, as well as 2016 with corresponding R^2 values.

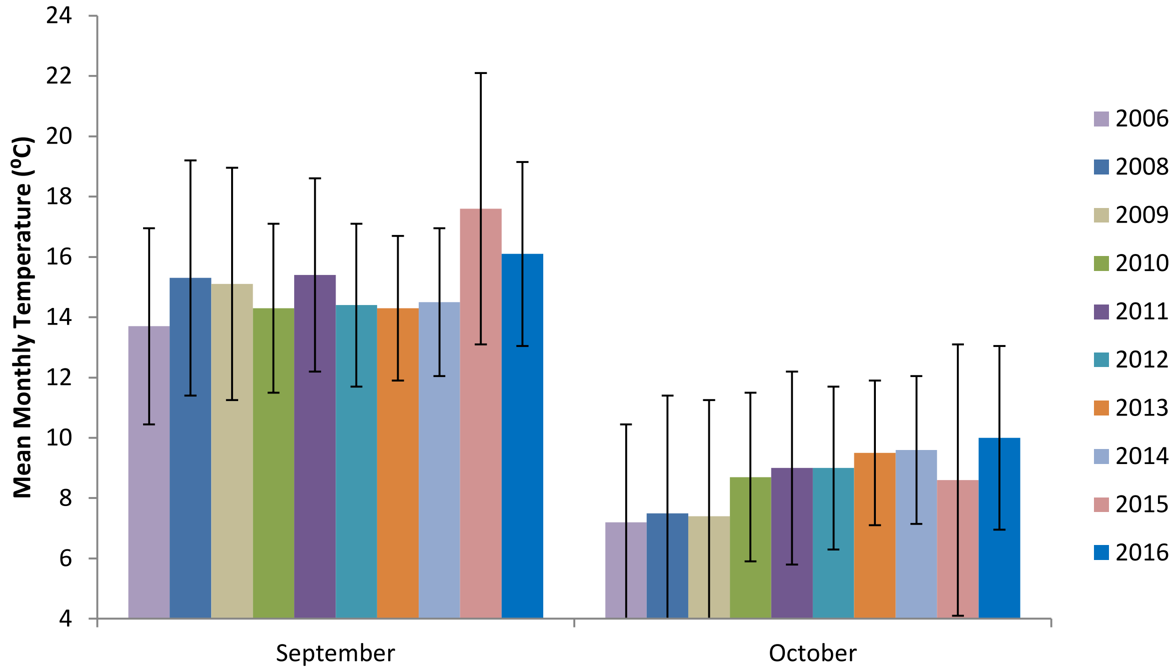


Figure 3.9: Mean monthly temperatures for Waterloo Region during the salamander monitoring season in 2006, 2008-2016 (Environment Canada- 2006, 2008-2009 data from Waterloo International Airport Weather Station, and 2010-2016 data from Kitchener-Waterloo Weather Station). Error bars represent +/- one standard error.

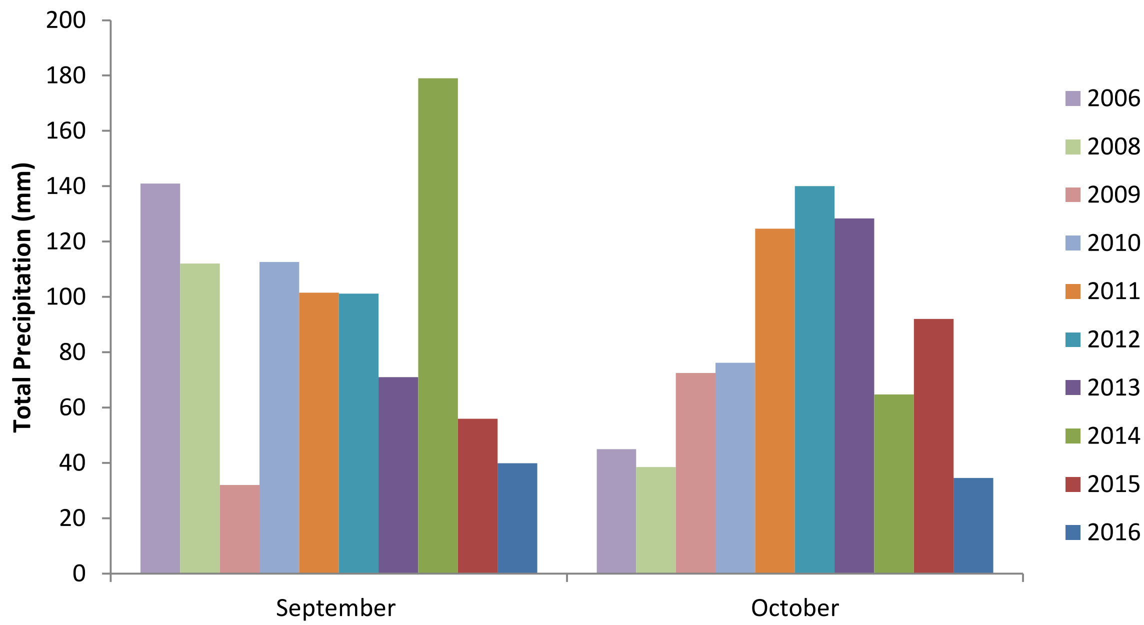


Figure 3.10: Total monthly rainfall for Waterloo Region during the salamander monitoring season in 2006, 2008-2016 (Environment Canada- 2006/2008-2009 data from Waterloo International Airport Weather Station, and 2010-2016 data from Kitchener-Waterloo Weather Station).

3.4.0 Discussion

3.4.1 Eastern Red-Backed Salamander Abundance

Given their importance in food web dynamics and their sensitivity to changes in forest floor conditions, significant changes in plethodontid salamander populations over time may be an early warning of ecosystem stress. Recognizing a population change that may be acting as an early warning sign as opposed to natural population fluctuations requires a monitoring target or threshold to be set (Zorn et al. 2004). Zorn et al. (2004) recommends a monitoring threshold set at “a statistically significant change in plethodontid counts at a plot level over 5 or more years”. With variable sampling effort in the first years of data collection, five consecutive and consistent years of data collection were achieved in 2013. Information gathered on salamander populations in the inaugural years does not contribute to the EMAN protocol for testing monitoring thresholds. Thresholds for the first five consistent and consecutive years of salamander monitoring (2009-2013) are: **Indian Woods: 130 +/- 31** and **Hogsback: 136 +/- 38**.

Several results distinguish the 2016 monitoring season from previous years. The 2016 monitoring season is the lowest year to date for total salamander observations in both plots, and abundances were not within the threshold ranges for either plot. Similarly, CPUE was the lowest of all monitoring years in the Hogsback, and the second lowest in Indian Woods. 2016 is also the first year that more salamanders were observed in Indian woods than the Hogsback of all years that plots were given equal sampling efforts. Examination of Figure 3.1 yields concern about salamander trends at ***rare***, particularly in the Hogsback. There has been a decline in salamander abundance in the Hogsback over the last four monitoring periods after a spike in 2013. Indian Woods abundances have a similar but less drastic decline in abundances as well. There are several possible explanations for these low occurrences.

Environmental parameters may help explain low abundances in 2016. Considerably low abundances observed in both plots may be explained by relatively low precipitation and moisture levels in both plots during monitoring. Combined precipitation in September and October was also the lowest of all years, and moisture level at ACOs in both plots were also the lowest (Figure 3.10, Table 3.3). Additionally, although there was above average precipitation in August, June and July were both below average for precipitation, further contributing to dry conditions. Terrestrial salamanders will only spend time on the surface if moisture conditions are adequately high; if it is too dry salamanders will retreat underground to stay moist (O’Donnell and Semlitsch, 2015). Although Jaeger (1972, 1980) reports that cover objects become more important during dry periods by acting as a moisture refuge for salamanders, it is possible that moisture levels were so low that salamanders retreated underground to reach the moisture levels they need to survive.

The temperatures during the 2016 monitoring period were the highest of all monitoring years (at least 4 degrees Celsius higher than any other year), and may also have contributed to low abundance observations. High temperatures cause salamander skin to dry out more quickly and, as a consequence, limit their surface activity (Spotila 1972, Feder and Pough 1975). Air temperature was found to have a significant, negative relationship with salamander abundance in both plots, was correlated with soil temperature, and likely contributed to low observations in 2016.

Beaufort sky codes are a measurement of the amount of sunlight, cloud cover, and rain, and are therrefore representative of the temperature and precipitation on a given monitoring day (see Appendix C, Table C.2. Higher Beaufort sky codes indicate more precipitation and less sun. Although a significant negative relationship exists between Beaufort Sky codes and salamander abundance in both plots, sky codes in 2016 were below average and likely were not a contributing factor. Also, given that a lower sky code would generally indicate a higher temperature, it is contradictory to results that temperature has a significant negative relationship with salamander abundance. Considering that air temperature had higher R^2 values in models for Indian Woods and the Hogsback, and therefore accounted for more of the variance, it is likely air temperature is a more influential predictor in the models. Beaufort wind code also had a significant negative relationship with abundance in the Hogback, however 2016 wind codes were below average and likely did not contribute to the observed decline.

Low survivorship during winter months previous to the monitoring seasons may be another explanation for a low number of observations in 2016. The major winter strategy of Eastern Red-backed salamanders is avoidance of sub-zero temperatures by retreating into the soil column (Storey and Storey 1986), and these salamanders have been observed as deep as one meter in the soil (Grizzell 1949; Hoff 1977). Harsh winters may have been particularly difficult to survive in if there was little or no snow covering the soil during these years. Snow pack acts as an insulator against ambient air temperatures protecting animals beneath the snow (Aitchinson 2001). The 2016 winter was on average warmer and had more precipitation than the previous three winters; indicating winter survivorship likely is not a contributing factor to low 2016 salamander counts. However, if there was no snow accumulation on particularly cold days it is possible that salamander winter survivorship was indeed a contributing factor to salamander decline in 2016. Given that weather measurements such as precipitation fall and precipitation accumulation are not taken in monitoring plots, and information is gathered from weather stations that may or may not represent weather at ***rare***, it is difficult to draw conclusions. In-situ measurement of snow accumulation during the winter months would be useful for determining the effects of weather on salamander populations, and should be considered in the future as part of the monitoring program at ***rare***.

Several boards were replaced in both plots (one in the Hogsback and three in Indian Woods) less than a month prior to monitoring. It is possible that the late replacement of these boards did not allow enough time for weathering, which would make boards more attractive to salamanders. However, there is uncertainty in the literature about the attractiveness of new cover boards, including placement in areas that had previously had a board (Hesed, 2012). The replaced board in the Hogsback was not used throughout the 2016 monitoring period, however was used at least 6 weeks during the previous three monitoring periods. Although use of replaced boards in Indian Woods were on average lower than in previous years, they do not exhibit the more drastic trend observed in the Hogsback.

The pond in Indian Woods has been dry for the last two fall monitoring seasons, likely due to low levels of rainfall during 2015 and 2016, which had less total annual rainfall than all other monitoring years. It is also possible that anthropogenic disturbances such as aggregate mining operations have had a part in lowering the water table. Regardless, if the pond fails to fill with water in the future, this area may cease to be a productive breeding site for salamanders with aquatic juvenile phases. Although Red-backed salamanders live a completely terrestrial life not requiring this pond for breeding, a reduced water table may mean they must burrow deeper into the soil to find moisture in periods of drought, potentially reducing their visibility during salamander monitoring, and limiting the amount of time they can spend on the surface foraging before finding moisture refuge. Regardless of the cause, loss of moisture in the plots likely play a heavy role in observed salamander abundance considering the positive relationship soil moisture has with Eastern Red-backed salamander abundance in both plots (See Figure 3.8).

In all likelihood there is no one cause of the abundances seen in any year and factors including temperature, moisture, available cover, and anthropogenic disturbances influencing the environment can be having an impact (Heatwole 1962; Spotila 1972; Feder and Pough 1975; Jaeger 1972, 1979, 1980; Feder 1983; Feder and Londos 1984; DeMaynadier and Hunter 1998; Herbeck and Larsen 1999).

Weekly patterns in abundance in 2016 generally mirror those of previous years, with Indian Woods abundances lowest in the second week and highest in the seventh week. Although Figure 3.2 (a) illustrates this trend, this is the first year significant differences have existed between weeks in either plot, indicating the importance of long term data collection in the understanding of salamander population dynamics. Weekly patterns in the Hogsback are less clear, although there also appears to be a spike in abundance during the sixth or seventh week. Figure 3.2 (b) clearly shows that 2016 abundances in the Hogsback were consistently lower than other years across weeks.

Drastic decreases observed in both plots in 2016 may be a concern for salamander populations at ***rare***, and it recommended that monitoring continue to help attribute cause and severity of changes to Eastern Red-backed salamander populations.

Table 3.3: Average soil moisture levels during the salamander monitoring season in 2009-2016 at Indian Woods and the Hogsback.

Plot	Year	Mean Soil Moisture Level
Indian Woods	2009	3.02+/-0.93
	2010	2.87+/-1.14
	2011	1.58+/-0.460
	2012	3.53+/-1.18
	2013	3.68+/-1.91
	2014	4.65+/-2.55
	2015	3.4+/-2.12
	2016	4.25+/-3.13
Hogsback	2009	4.87+/11.07
	2010	5.47+/-1.42
	2011	3.65+/-0.912
	2012	4.63+/-1.37
	2013	5.63+/-2.34
	2014	4.94+/-2.45
	2015	6.7+/-2.77
	2016	3.73+/-2.1

3.4.2 Salamander Species Composition

While the monitoring program at ***rare*** is primarily designed for plethodontid salamanders (Zorn et al. 2004), other species have also been observed on the property.

During five of ten monitoring years in Indian Woods, Blue-spotted salamanders have been observed in low numbers (1-2 individuals), however no Blue-spotted salamanders were observed in 2016. More information on this species can be found in the report on the jeffersonanium-laterale complex investigation completed in spring 2015. Mole salamanders are more easily found in the spring during their breeding season (Whitford and Vinegar 1966) and therefore low numbers in the fall likely are not unusual. The presence of Blue-spotted salamanders in Indian Woods is likely connected to the vernal pond near ACOs, as the species may use the pond for breeding. The lack of water in the vernal pool in 2015 and 2016 consecutively may have contributed to the lack of observations in 2016. If interest in Blue-spotted salamanders grows at ***rare***, it would be useful to include spring as part of the monitoring season to get more accurate information on their populations.

Species diversity is higher in the Hogsback than Indian Woods (Figure 3.3). Four-toed salamanders, another member of the plethodontid family, were observed in low numbers (1-4 individuals) in three of nine monitoring years in the Hogsback. These salamanders are typically found in sphagnum moss or boggy woodlands (Conant and Collins 1998), the latter of which is found in the Hogsback forest stand. Multiple mole salamanders have been observed; Blue-spotted salamanders in five of nine monitoring years (1-3 individuals). Yellow-spotted salamanders have been observed in six of nine monitoring seasons with a peak of seven observations in 2013. From 2009 to 2012, it has been suggested that the same individual Yellow-spotted salamander was repeatedly observed as it was roughly the same size and consistently observed under the same ACO near what appeared to be a burrow or underground tunnel. This suggests salamanders may exhibit fidelity to ACOs. High site fidelity for salamanders has also been seen in other studies (Marvin 2001, Peterson et al. 2000). Expanding monitoring efforts at ***rare*** to include individual identification and possibly gender identification may be of benefit, and would help eliminate oversampling of individuals. However, methods such as toe-clipping are invasive and would require additional permitting. Programs have been explored in other studies to identify individual Yellow-spotted salamanders based on the location of their spots (Grant and Nanjappa 2006). This is perhaps something less invasive that ***rare*** could apply, but would only work for species with easily identified unique markings like Yellow-spotted salamanders.

In 2016, one Four-toed salamander was observed in addition to Eastern Red-backed salamanders in the Hogsback. Dry conditions in 2016 may have contributed to a relatively low diversity observed in the Hogsback, as breeding grounds were dry compared to previous years. Similar to Indian Woods, it would be beneficial to collect data in the spring to gain more accurate population data, particularly for mole salamanders which breed in the spring.

Eastern Red-backed salamanders have been dominant in both plots across monitoring years (Figure 3.3), with the proportion of red-backed phase individuals being consistently higher than the proportion of lead-backed phase individuals (Figure 3.4). This is unsurprising, as the lead-backed phase salamanders are known to experience preferential predation pressures (Moreno 1989; Venesky and Anthony 2007) and the red-backed phase is known to be proportionately higher in more areas and at higher latitudes (Lamond 1994; Harding 1997). Studies of spatial variation indicate that the lead-backed phase is more closely associated with warmer, drier climates, experiencing higher mortality in colder sites, and retreating from the surface earlier than red-backed individuals in the fall (Lotter and Scott 1977; Moreno 1989). Since there is a temperature preference between colour morphs, Gibbs and Karraker (2006) suggest increasing global temperatures may be resulting in a shift from red-backed dominance to lead-backed dominance in temperate areas. This change has been observed at ***rare*** over the last two seasons in the Hogsback and the last three seasons in Indian Woods. Although no significant difference in proportion exist between years, analysis of changes should continue as they may be indicative of important global temperature changes affecting the entire forest ecosystem.

3.4.3 Eastern Red-backed Salamander Size Class Distribution

Consistent with previous monitoring years, the greatest proportion of Eastern Red-backed salamanders in 2016 fell within the snout-vent length range of 35mm-45mm. Based on size class categories outlined in Zorn et al. (2004), significantly more adults were found in both plots than intermediates and juveniles, and further there are significantly more intermediates observed than juveniles (Figure 3.5). A significant positive correlation between unsexed salamander size and age in their first four years has been documented (LeClair et al. 2006). Based on their results, the majority of salamanders found under ACOs at ***rare*** are between the ages of two and six. If other size class distinctions had been used to categorize salamanders at ***rare***, such as those outlined in Sayler (1966) and subsequently used in additional studies (Brooks 1999; Ballantyne 2004), data would have shifted toward more intermediate sized salamanders. In either case, few juveniles (or first year young) have been found under the ACOs at ***rare*** in either forest stand over monitoring years.

In 2016, there was a decrease in adult salamander populations at ***rare***, while intermediate and juvenile populations remained relatively stable (Figure 3.5 (a)). Although there was no significant interaction between size class and plots, there are differences in trends at each plot that should be noted. In general, size class distribution in Indian Woods did not vary greatly from other years. The most notable change is the decrease in adult salamander observations in the Hogsback, which were almost a third of 2015 adult observations, and almost doubling of intermediate observations from 2015. The drop in adults contributes to the overall drop in all salamander abundance in the Hogsback as well as in both plots combined. Despite the two-fold increase in intermediates in the Hogsback, observation levels in 2016 were still far below average (Figure 3.5 (c)).

The decrease in adult populations in the Hogsback is likely influenced by a drop in adult and intermediate salamander populations in 2015, which would decrease both the number of adults that carried over, and the intermediates that would mature into adults from last year. Disturbance under ACOs may have played a role, as disturbance levels were highest in 2016 and 2015 respectively. There was a significant negative relationship between salamander abundance and disturbance in the Hogsback, where the greatest decrease in adult salamanders was observed; indicating that increased disturbance levels at ACOs may be contributing to lower observations of adult populations. It is possible that adult salamanders may be avoiding disturbances as they are more likely to come into contact with them under ACOs due to their large size, although no supporting literature has been found to support this speculation. Disturbances are varied and include white mould, rodent activity, ants and use of boards by snakes.

Plethodontid salamander growth can be influenced by weather conditions including temperature and rainfall. Caruso et al. (2014) identified a reduction in plethodontid size in response to warmer and dryer environmental conditions. Similarly, Grant et al. (2015) identified reduction in the size of observed plethodontid adults over drier seasons, and an increase in size of observed adults during seasons with more rainfall. However, Grant et al. (2015) importantly notes that individuals of the same population are not detected as easily in some weather conditions as others, and observations will be biased by the sampling method itself. For example, larger salamanders were observed to be more active during dry periods and therefore less likely to be detected during monitoring. Therefore, it is possible that decreased growth due to warm, dry temperatures in 2016, and changes in adult activity during dry periods contributed to the decrease in size class seen during monitoring

As mentioned, it is likely a combination of factors, including yearly weather, soil moisture, and temperature working in tandem with high-levels of disturbance to cause low abundances. Should abundances in Indian Woods continue to decrease, the possibility of disturbance playing a role should not be overlooked.

It should also be noted that juvenile populations may be underrepresented by ACO sampling. Adults may be exhibiting territorial behaviours that outcompete juveniles for space (Marsh and Goicochea 2003), or, in the fall, this behaviour could be in connection to mating (Van Wieren 2003). Low observations of juveniles in tandem with intermediates or adults at ***rare*** indicates that territorial behaviour may be occurring. Although Red-backed salamanders have also been shown to exhibit kin selection, allowing related juveniles into their territories in stressful conditions (Horne and Jaeger 1988; Jaeger et al. 1995; Simons et al. 1997) this seems to be occurring minimally, if at all, during the fall months at ***rare***.

Another likely hypothesis for low juvenile representation is that larger salamanders prefer the wider cover provided by ACOs. Mathis (1990) and Moore et al. (2001) found significant positive correlations between salamander size and cover object size. Therefore, ACOs used in this study may be more attractive to larger adults. Gabor (1995) found this relationship with cover object size and salamander size existed only where direct sunlight reached the board. In cases where direct sunlight does not heat the boards, cover objects were chosen in relation to food quality and quantity in surrounding areas. Although there have been a reduction in the number of adults found under boards during monitoring over the last two years, the proportions of size classes represented across years support this hypothesis.

3.4.4 Environmental Parameters

A more comprehensive comparison of changes in environmental parameters and their relationship with salamander abundance can be found in previous sections.

The use of a light reader this year in addition to the canopy cover was tested. A quick correlation analysis revealed that there is no correlation between readings of canopy cover used in previous years, and light readings (r^2= -0.049). Salamander abundance is less correlated with readings from the light reader (r^2= -0.052) than with canopy cover estimations (r^2= -0.076), however neither are very correlated as r^2 values are close to zero. Experimentation with the reader during monitoring revealed that readings from the light reader did not assess the amount of light from directly above the board like canopy cover estimations. The light reader was sensitive to all light (not just light from directly above), so if there was strong sun coming from the east in the morning it would assess that amount of light, rather than the light directly above the board. The amount of light coming from directly above the board would have the greatest effect on the temperature under the board, as the sun at noon is the hottest sun. Light readings only measure the amount of light at the time of day monitoring occurs, whereas canopy cover measures how much light could potentially reach boards at the hottest point of the day. Three methods of measurement have been used over the past four years to estimate the amount of light reaching the boards, which has limited the collection of consistent data for analysis. It is recommended that one method be selected and continued to facilitate continuity of data and power of analysis.

3.5.0 Conclusions and Recommendations

After eight years of consistent and consecutive monitoring, this program has established baseline data of expected salamander populations in both Indian Woods and Hogsback and will continue to compare future years to these baselines. Observed salamander abundances in both plots have fallen outside threshold levels, and species composition was low relative to previous years. Abundance decreases were particularly apparent in the Hogsback, which was previously considered to be relatively stable. The proportion of adult salamanders to other size classes was also the lowest of all years in both plots. Low precipitation and high temperatures of the 2016 season may have contributed to low observations, but at this point the cause of declines in ***rare*** forests is unknown. As this program acts as a warning sign for environmental change, falling numbers coupled with ongoing human pressures from agriculture, development projects and the potential for cumulative effects from aggregate extraction highlight the need for continued monitoring at ***rare***. Only by continuing long-term monitoring, can ***rare*** best assess the impact of land management decisions both on and adjacent to the property. Including a spring monitoring season in addition to fall monitoring would be helpful to capture abundances of mole salamanders. Therefore, it is recommended that a full nine week fall monitoring program continue at both forest sites, in addition to spring monitoring if resources allow.

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4.0 Forest Canopy and Tree Biodiversity Monitoring

4.1Introduction

4.1.1 Forest Health Monitoring

Forests are critical to environmental health and stability (Environment Canada and Canadian Forest Service 2004). They house a significant amount of the world's biodiversity and provide numerous ecosystem services such as; soil conservation, water cycling, and air quality mediation (Butt 2011). Establishing global policies and protocols related to the safeguarding of forests are of high priority.

In southern Ontario, forests have experienced a great deal of change in the past 200 years. Prior to European settlement, southern Ontario was largely covered by a patchwork of deciduous and mixed hardwood forests (Ontario Ministry Natural Resources 1999). Due to rapid development and land use changes, forest species have been removed and land cover has been significantly altered. What remains are highly fragmented forests which are much smaller in size than they were historically (Waldron 2003). Forests are also under pressure from many other biotic and abiotic factors. Widespread invasive species have caused drastic changes to forest stand composition and forest nutrient cycles, threatening to alter the ecology of forest systems profoundly (Moser et al. 2009). Impacts to forests from climate change are thought to be equally far-reaching (Allen et al. 2010). Natural disturbances to forests from insects and disease will become more severe with warmer climates (Weed et al. 2013). Forests will also have to adapt to more instances of extreme weather such as storms and drought (Allen et al. 2010). These factors demonstrate the number of pressures impacting our forests and highlight the need to monitor the health of our remaining forest stands.

Establishing long-term ecological monitoring across a network of forest sites can help develop a more thorough understanding of baseline levels of both variability and health in natural systems (Gardner 2011). Monitoring crown conditions and stem defects is essential to detect early warning signs and recognizing changes in tree health of Canadian forests and Canada's urban areas (Environment Canada and Canadian Forest Service 2004). Records of tree damage and mortality can help with identifying and understanding the causes and effects of tree and forest decline. Information on populations and species decline can be used as a platform to launch conservation initiatives (Gardner 2011), and may influence management objectives when considering human-impact on forests.

Although the age, diversity, and overall health of a forest stand can be derived from canopy tree monitoring, it says little about the likely successional trajectory of the stand. Beneath the canopy, the rate of sapling recruitment and survivorship in the shrub and small tree stratum can be informative of the health and progression of a forest stand (Roberts-Pichette & Gillespie 1999). Shrub and small tree monitoring can provide valuable insight into the successional direction of a forest stand by observing saplings that may eventually be a part of the forest canopy. Historical records can aid in understanding a forest's past dynamics and structure, while ongoing, long-term monitoring of both canopy tree and shrub/small tree forest strata can shed light on the present influential factors affecting its development. Together, these can contribute to effective long-term best management practices that have been developed to meet the challenges of dynamic forest ecosystems.

*4.1.2 EMAN Forest Monitoring at **rare***

With the rapid development of southern Ontario, there are very few undisturbed remnant old-growth forests remaining (Ontario Ministry Natural Resources 1999). At the ***rare*** **Charitable Research Reserve**, one such remnant old growth exists: a Sugar Maple-American Beech (*Acer saccharum – Fagus grandifolia*) dominated forest named Indian Woods, which has trees more than 240 years old. Additional forest stands at ***rare*** include the Cliffs and Alvars, a mixed deciduous forest that was partially grazed by cattle within the last century, and the Hogsback, a relatively undisturbed mixed swamp forest. All of these forest ecosystems contribute invaluable services to the region by sequestering carbon dioxide and improving air and water quality (Führer 2000), as well as providing increasingly uncommon habitat to countless plants and animals that require mature forest interior (Ontario Ministry of Natural Resources 1999).

Forests face diverse challenges in the landscape of Waterloo Region; ***rare*** is bordered by conventional farm fields, aggregate mining operations, subdivisions, and busy roads. Many of these neighbouring lands are scheduled for drastic changes and development within the next few years. By acquiring baseline records of the conditions of the ***rare*** forests and continuing long-term monitoring, changes in the forest stands may be detected early, allowing for the development and implementation of an effective management plan to protect ***rare*** forest ecosystems.

The research questions being addressed with long-term forest canopy tree biodiversity monitoring were identified at the establishment of the program (McCarter 2009) and subsequent questions were asked based on new objectives established in 2013:

1. What is the current state (biodiversity, composition, health) of ***rare's*** forests, and how do they compare to one another?
2. What are the long-term trends in tree mortality, recruitment, and replacement taking place within the forests at ***rare***?
3. Is the ecosystem integrity of the forests being maintained or improved under ***rare*** management?
4. Is either the ecological health or integrity of ***rare*** forests being affected by on-site and nearby changes in land use (i.e. restoration, agriculture, residential development, and aggregate extraction)?
5. How does the canopy tree stratum influence the species composition of the shrub and small tree stratum?
6. What is the most likely successional trajectory as suggested by the recruitment and mortality rates of saplings in the forests at ***rare***?

The forest canopy tree biodiversity monitoring program at the ***rare*** **Charitable Research Reserve** began in 2009 with the establishment of three plots in the Cliffs and Alvars forests and three plots in the Indian Woods. Preliminary monitoring data, such as trees specie, location within the plot, and diameter at breast height (DBH), were collected in this first year. In the 2010 monitoring year, three plots were established in the Hogsback forest so that all three major

forested areas on the ***rare*** property would be represented in the monitoring program. An Ecological Monitoring and Assessment Network (EMAN) Tree Health Protocol was added to the monitoring program in 2010, and all nine forest plots have been monitored in full each subsequent year. In 2013, a shrub and small tree monitoring program was added as a pilot study to the existing protocol at ***rare***. Based on the results of the pilot study, a more tailored shrub and small tree monitoring program specific to this forest stratum was developed and implemented in 2014. Shrub and small tree monitoring is to occur every five years with 2014 as the initial year. Methodology, results and discussion for shrub and small tree monitoring can be found in the Forest Canopy and Tree Biodiversity report from 2014 and on the ***rare*** server.
Tree heights have been recorded during forest monitoring at rare since 2009 as part of the Forest Canopy and Tree Biodiversity Monitoring program. Tree height data has not been used in reports to date due to the high variation of measurements. Many large changes in tree height have been recorded for individual trees year to year (up to 31 meters), including negative changes in growth. While it is possible that negative changes are the result of tree injury or death, the number of these negative changes, in combination with unrealistic large positive changes for many individuals indicates the need to refine tree height measurement protocol in order to yield more accurate results.

2016 Protocol Changes

It has been indicated in previous monitoring reports that tree height measurements be taken after leaves have fallen in autumn to reduce the error in measurements. In previous year's data, there have been changes up to 23 meters from one year to the next, and many years have registered large negative changes in height. As a result, it is difficult to make accurate comparisons in tree heights between years. To address this problem and develop a protocol to improve tree height measurement accuracy, a review of literature and consultation with the MNRF regarding tree height measurements was conducted. The complexity of measuring tree heights accurately is not unique to ***rare*** (Sharma and Parton 2007; personal communications, 2016); practiced technicians at the MNRF often differ between 20 to 30 centimeters when measuring the same tree at the same time, and this margin is considered acceptable by the MNRF (personal communications, 2016). No literature was found regarding the accuracy of the instrument used at ***rare*** (Haglöf Electronic Clinometer). General guidelines from the MNRF (2016) manual have been incorporated into a revised tree height monitoring protocol, which was conducted in the fall after the leaves had fallen to improve sightlines of the top of tree crowns (See Section 5.2.4)

4.2 Methods

4.2.1 Forest Plot Locations

Forest biodiversity monitoring plots are established in three forest stands on ***rare*** property. Each of these stands contains three monitoring plots, which together are used to describe their respective stands.

Cliffs and Alvars: A mature Sugar Maple-American Beech dominated forest located on the north side of Blair Road, bordered by Cruickston Creek on the west, Newman Creek on the east and the Grand River to the north. The three plots in the Cliffs and Alvars forest are located approximately 50m north of the Grand Trunk Trail, arranged parallel to the trail (Appendix A). To access these plots, walk from the ECO Centre to the Grand Trunk Trail. Follow the Grand Trunk Trail to the east (right) until completely under the canopy (approximately 200m). Shortly after, the forest opens up and a small seasonal trail heads north towards the river. The plots are located to the left and right of this trail, past the large fallen trees. Plot corners are marked with pigtail stakes and orange or pink flagging tape.

Indian Woods: A remnant old-growth forest located south of Blair Road and north of Whistle Bare Road, on the west side of the property. The three forest plots in Indian Woods are oriented in a north-south line in the centre of the forest, approximately 100m east of the Grand Allée. The third plot can be accessed by turning east into the forest off the Grand Allée towards the salamander monitoring plot and continuing to the top of the hill overlooking the pond. The first and second plots can be found by heading north from the third plot (Appendix A). The plots are approximately 30m apart and the flagging tape on the corners of each plot should be visible from the adjacent plot.

Hogsback: Located at the south-west corner of the property, the Hogsback is bisected by Cruickston Creek and bordered by the Newman Drive subdivision to the west. The Hogsback is a mixed swamp forest with upland ridges dominated by White Pine, Red Maple, American Beech, and Sugar Maple. The three forest biodiversity plots were established on these elevated ridges as the lower areas will likely be too swampy to access in wetter years. The second forest plot overlaps with the Hogsback salamander monitoring plot. The first plot is found approximately 30m north of the second plot on the same elevated ridge, and the third plot is located 30m southwest of the second plot, separated by a small boggy area (Appendix A). This area can be accessed by driving east down South Gate Road to edge of the forest stand, and following the hedgerow around the forest (north, east, north, east). Alternatively, the site can be accessed by parking at Springbank Gardens, turning south at the pavilion, travelling south along the small hedgerow, then east along the forest perimeter. The forest can be entered at part of fence lowered with a fallen log, at the southern edge of Hogsback Field (303).

4.2.2 Plot Establishment

Following the EMAN Forest Canopy Tree Biodiversity Monitoring Protocol (Environment Canada and Canadian Forest Service 2004), the plots established in 2009 and 2010 at ***rare*** are permanent 20m x 20m plots located in the forest interior. According to EMAN, plots should not be closer than three times the average tree height to any forest edge (estimated at 90m-100m for our forests); however this was not always possible due to the small size of Indian Woods and swampy topography of the Hogsback; in these cases, plots were established as far from any edge as possible. The plots were oriented along the cardinal directions and the corners were marked with galvanized steel pigtail stakes with labelled flagging tape (Figure 4.1). All trees within the plot with a diameter equal to or greater than 10cm at breast height (DBH) were included in the monitoring. Trees in Indian Woods and Hogsback were labelled with pigtail stakes inserted in the ground at the base of the tree with pre-printed aluminum tags attached. The trees in the Cliffs and Alvars forest plots were originally marked with forestry tags, each with unique identification codes (ex. CA-02-08, Cliffs and Alvars – Plot 2 – Tree 8) which were fixed to the tree with a downward angled nail. In 2013, these forestry tags were removed from the trees in Cliffs and Alvars and were replaced with steel pigtails with numbered aluminum tags in a manner consistent with Indian Woods and Hogsback.

The trees were tagged in a clockwise spiral inward from the northwest corner of the plot. The species of each tree was recorded at the time of plot establishment, and its distance to two plot corners was recorded for plot map generation. In this plotting technique, one observer stands with their back to the tree, facing the nearest line (i.e. edge) of the plot. The line number was recorded, and the "A" distance and "B" distance were measured; "A" distance was measured from the tree to the corner to the right-hand side of the observer facing the line, while the "B" distance was measured from the tree to the corner to the left-hand side of the observer (Figure 4.1). Trees that split into multiple stems under breast height had each stem measured independently.

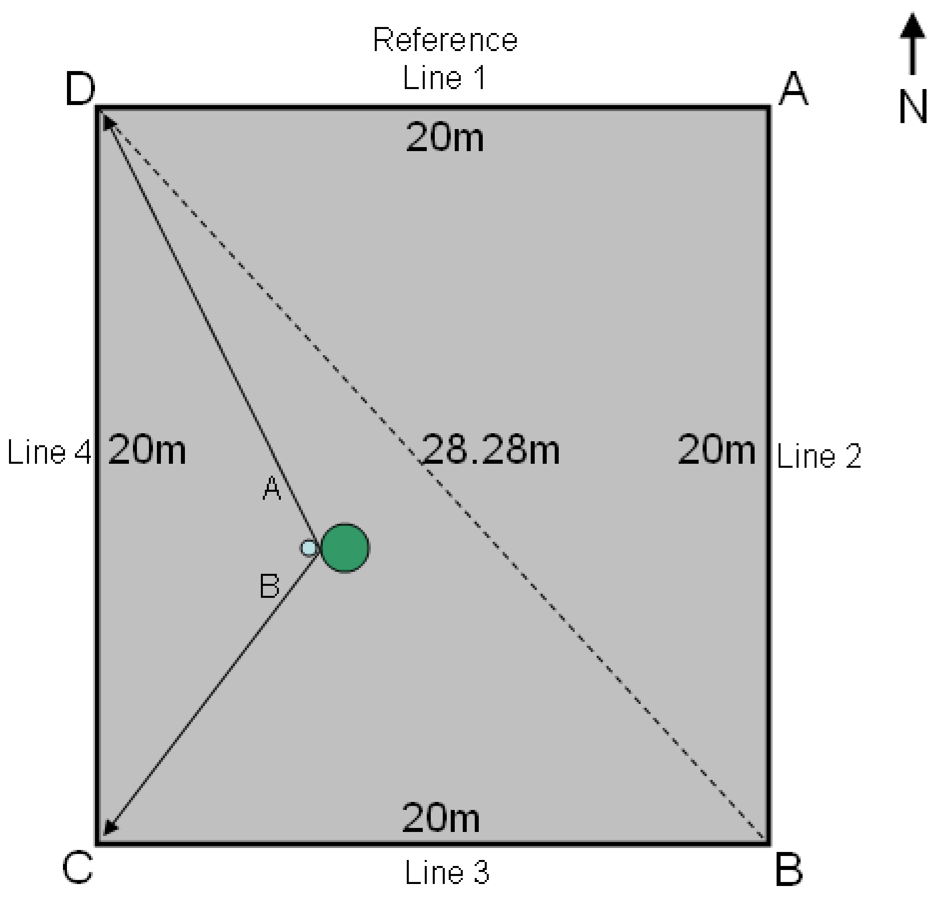

Figure 4.1: Diagram of an EMAN forest canopy tree biodiversity plots from McCarter 2009. The A and B distances are used to map the position of the tree within the plot. The A distance is measured from the tree to the corner to the right of the observer standing facing the reference line. The B distance is measured to the corner on the left side of the observer.

4.2.3 Summer Monitoring Procedure: Canopy-Tree Monitoring

Each plot is visited once in the summer while the leaves are still present for ease of identification and canopy assessments. At each plot, the following variables were recorded for each tree in the monitoring plots: diameter at breast height (Woven Fibre Glass 5m Diameter Tape, Richter Measuring Tools), and tree condition based on Environment Canada and Canada Forestry Services EMAN codes (Table 4.1). Tree health was monitored by recording stem defects, crown class, crown rating (Table 4.2), and any other health notes, again based on Environment Canada's EMAN protocol. Marginal trees in each plot were checked to see if they had graduated into the 10cm DBH size class (minimum for inclusion). No marginal trees were large enough to be added to the plots in 2016.

4.2.4 Fall Monitoring Procedure: Tree Height Measurements

Each plot is visited once in the fall after all the leaves have fallen to allow for more accurate height measurements. At each plot, tree heights were measured using the Haglöf Electronic Clinometer and the Mastercraft© Fibre glass measuring tape. An updated protocol based on MNRF protocol aimed at improving tree height accuracy is included below. Due to time constraints, measurements were taken multiple times by one observer in 2016, but would ideally be taken by two to achieve the most accurate results.

Surveying Method:

1. Measurements will be taken with the HEC by two observers.
    a. Make sure that the batteries in the instrument are fully charged (many electronic clinometers will continue functioning with poor batteries, but will not provide accurate measurements).
2. Total height is considered the tree base to highest live part of the crown (Figure 4.2)
3. Walk around the tree to determine the highest live part of crown, and evaluate lean.
4. Choose one sighting position that allows a view of the highest live part of the crown.
    a. If the tree is leaning (the high point of the crown is offset from the base of the crown by more than 1 m) an offset sighting position should be used (see figure 4.2).
5. Each observer will follow HEC manual instructions to take height measurements.
6. Record measurements for each observer at the sighting position.
    a. Each observer takes multiple measurements until measurements of the two observers are within 20-30 centimeters, and two measurements are averaged.

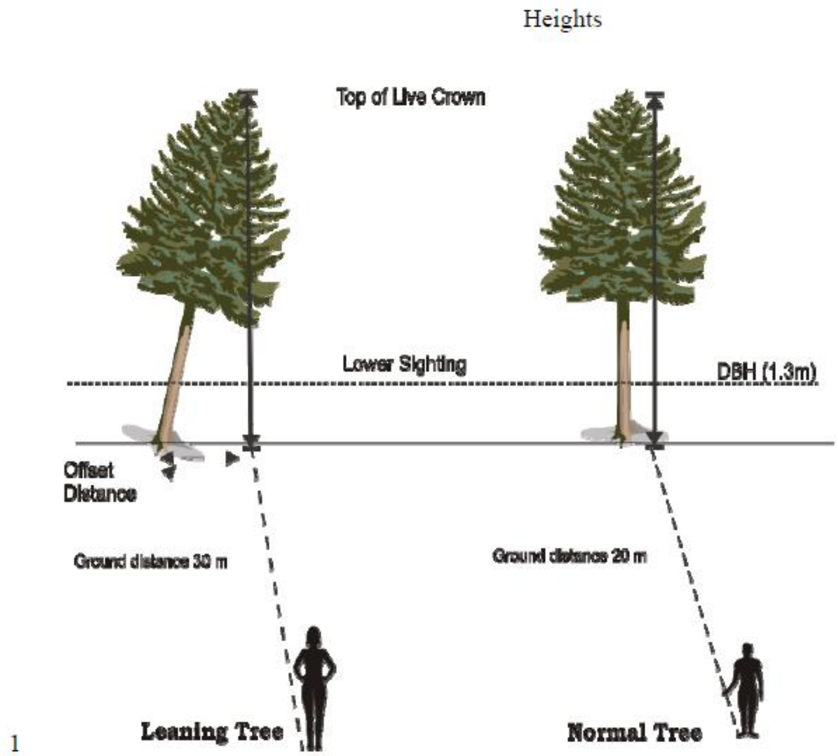

Figure 4.2: Sighting positions for leaning and normal trees (MNRF Procedural Manual, 2016).

Table 4.1: Tree condition codes from EMAN protocol (Environment Canada and Canada Forestry Service 2004).

Code	Condition
AS	Alive Standing
AB	Alive Broken
AL	Alive Leaning
AF	Alive Fallen/Prone
AD	Alive Standing with Dead Top
DS	Dead Standing
DB	Dead Broken
DL	Dead Leaning
DF	Dead Fallen/Prone

Table 4.2: Crown class and rating codes from EMAN protocol (Environment Canada and Canada Forestry Service 2004).

Crown Class	Code	Crown Rating
Dominant: Crown extends above the general canopy level and receives full sunlight from above and partly from the sides; larger than the average trees in the stand	**1**	**Healthy**: Appears in good health, no major branch mortality, <10% branch/twig mortality
Co-dominant: Crown forms the general canopy level and receives full sunlight from directly above and comparatively little from the sides	**2**	**Light-Moderate Decline**: Branch and twig mortality <50% of the crown, <50% branch/twig mortality
Intermediate: Shorter than the two preceding classes, and receiving little direct sunlight from above and from the sides; their crowns extend into the base of the canopy of the dominant and co-dominant trees	**3**	**Severe Decline**: Branch and twig mortality >50% of the crown, >50% branch/twig mortality
Suppressed: Receives no direct sunlight from above or the sides, their crowns are entirely below the general level of the crown cover.	**4**	**Dead, Natural**: Tree is dead; either standing or downed
Open: Exposed to full sunlight from directly above and on all sides; typically growing in a field or along a boulevard.	**5**	**Dead, Human**: Tree cut down, removed, or girdled

4.2.5 Data Analysis

All data were analyzed using SPSS Statistics Version 20 and Microsoft Excel 2010. For each location, summary statistics were calculated by combining the data from the three plots which together represent the forest stand. For each stand, the number of trees present, the mean diameter at breast height, and the total basal area (sum of cross sectional area of all trees within a plot, based on DBH measurements) were recorded. These data were used to calculate the Shannon Diversity Index (H) and Species Evenness Value (E_H) for each forest stand. The relative density, relative frequency, and relative dominance were also calculated, and results were combined to give an Importance Value to each species within each stand (Roberts-Pichette & Gillespie, 1999). Only living trees were included in these calculations; formulas used for all calculations are found in Figures 4.3 to 4.6

In addition to the summary statistics, a univariate analysis of variance (ANOVA) was used to investigate differences between size classes at each forest stand. Trees were assigned to one of eight size classes based on their DBH measurements in meters (0.1-0.19, 0.2-0.29, 0.3-0.39, 0.4-0.49, 0.5-0.59, 0.6-0.69, 0.7-0.79, 0.8+; hereon referred to as size class 1 through 8). For each forest stand, data from 2009 to 2015 were used to conduct the ANOVA, where year and size class were the independent variables and tree abundance was the dependent variable. When results were significant, a Tukey HSD Post-hoc test was used to determine where the differences existed. An alpha level of 0.05 was used to determine significance.

$$\textbf{Relative Density} = \frac{\text{Number of tree species A in plots}}{\text{Total number of trees in the plots}} \text{ x } 100$$

Figure 4.3: Formula for calculating the relative density of tree species in a forest stand, with all three plots per stand combined.

$$\textbf{Relative Frequency} = \frac{\text{Frequency of species A in plots}}{\text{Total frequency of in all trees in the plots}} \text{ x } 100$$

$$\text{Where: Frequency} = \frac{\text{number of plots with species A}}{\text{total number of plots in the stand}}$$

Figure 4.4: Formula for calculating the relative frequency of tree species in a forest stand, with all three plots per stand combined.

$$\textbf{Relative Dominance} = \frac{\text{Basal area of species A } (\text{m}^2)}{\text{Total basal area of all species in the plots } (\text{m}^2)} \text{ x100}$$

Figure 4.5: Formula for calculating the relative dominance of tree species in a forest stand, with all three plots per stand combined.

$$\textbf{Importance Value} = \text{Relative Density} + \text{Relative Frequency} + \text{Relative Dominance}$$

Figure 4.6: Formula for calculating the importance value of each tree species in a forest stand.

4.3 Results

3.1 Canopy Tree Monitoring: Abundance and Dominance

The Cliffs and Alvars forest plots were comprised of five species, representing five different families (Figure 4.7). Similar to previous years, Sugar Maple and American Beech were the two most dominant species in all plots, and thus in the forest stand as a whole. No new trees were added to the canopy monitoring in the Cliffs and Alvars and there were no mortalities. Both the Shannon Diversity Index and the Species Evenness Value were the lowest on record, at 1.3 and 0.67 respectively (Table 4.3).

Indian Woods forest plots had the lowest number of species with only four different species present from two different families (Figure 4.8). Indian Woods experienced no changes in recruitment or mortality between 2015 and 2016. Sugar Maple is the dominant species for Plot Two and Plot Three and was previously the dominant species in Plot One. American Beech is the most dominant species in Plot One. The Shannon Diversity Index and Species Evenness were the same as 2015 and the highest to date at 0.848 and 0.611, respectively (Table 4.3).

The Hogsback Forest has consistently has the highest species abundance across all forest stands, with ten different species representing six different families (Figure 4.9). Sugar Maple was the dominant species for the forest stand as a whole, but American Beech and Hop Hornbeam (*Ostrya virginiana*) were both dominant species in individual plots. Two new mortalities occurred between 2015 and 2016, a Black Ash and an American Beech. Shannon Diversity Index values were the lowest for any monitoring period at 2.01 and evenness was the second lowest at 0.87, however changes are small from previous years (Table 4.3).

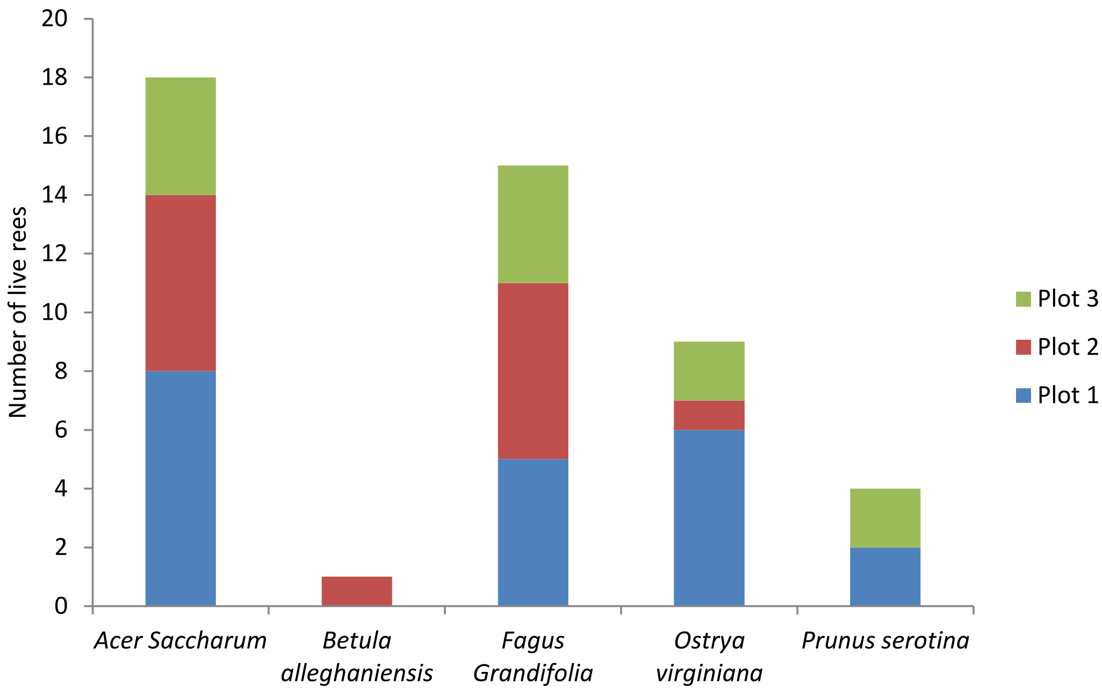

Figure 4.7: Tree species composition and abundance from the three forest plots in Cliffs and Alvars

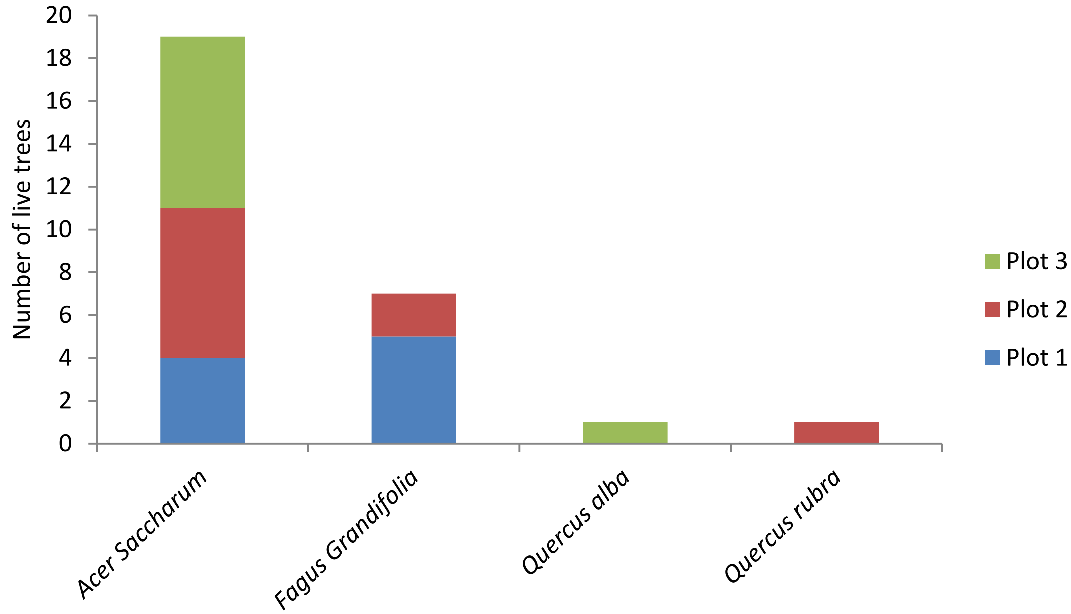

Figure 4.8: Tree species composition and abundance from the three forest plots in Indian Woods.

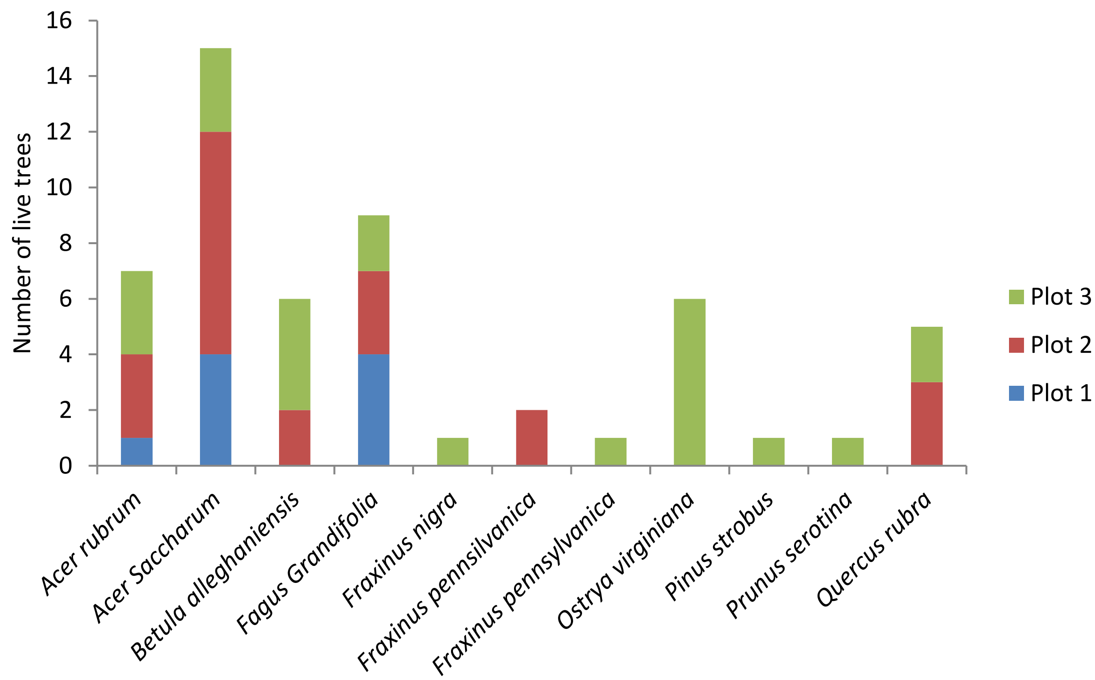

Figure 4.9: Tree species composition and abundance from the three forest plots in the Hogsback.

Table 4.3: Summary of the forest canopy monitoring observations, along the Shannon Diversity Index and Species Evenness values, for each forest stand across all monitoring years.

		Measures					
		Number of Live Trees	Number of dead trees	Number of Species	Mean Stem DBH (cm)	Shannon-Weiner Diversity Index	Species Evenness Value
Cliffs and Alvars	2009	48	7	7	23.07	1.49	0.83
	2010	50	6	7	23.34	1.56	0.80
	2011	49	8	7	23.30	1.48	0.76
	2012	49	9	6	23.40	1.40	0.78
	2013	49	9	6	23.40	1.40	0.78
	2014	48	10	6	23.90	1.41	0.79
	2015	47	11	5	24.30	1.34	0.83
	2016	47	11	5	24.55	1.30	0.67
Indian Woods	2009	34	4	5	32.97	0.84	0.52
	2010	32	7	4	32.11	0.75	0.54
	2011	32	7	4	32.30	0.75	0.54
	2012	29	10	4	33.10	0.79	0.57
	2013	31	10	4	32.90	0.76	0.55
	2014	30	10	4	33.30	0.78	0.56
	2015	28	14	4	30.10	0.85	0.61
	2016	28	14	4	31.10	0.85	0.61
Hogback	2010	54	6	10	24.92	2.08	0.90
	2011	54	6	10	25.10	2.08	0.90
	2012	54	6	10	24.49	2.08	0.90
	2013	56	6	10	25.30	2.05	0.89
	2014	57	6	10	24.70	2.04	0.86
	2015	56	7	10	25.20	2.03	0.88
	2016	54	9	10	25.92	2.01	0.87

4.3.2 Canopy Tree Monitoring: Stand Characteristics and Size Class Abundance

Across all monitoring stands, Sugar Maple had the highest Importance Value. The Importance Value, along with the Abundance, Basal Area, Relative Density, Relative Frequency, and Relative Dominance for all species present in the three forest stands can be found in Table 4.4.

When investigating the differences in the number of trees within each size class by year, it was found that monitoring year did not have a significant effect ($p>0.05$ for all forest stands). However, within each forest stand, there were significant differences across size classes.

In all forest stands, the mean abundance of size class one was significantly greater than all other size classes ($p>0.001$), and in general, small size classes were more abundant than larger size classes. In the Cliffs and Alvars, the mean abundance of trees in size class one was significantly greater than all other size classes ($p>0.001$), the mean abundance of size class two was significantly greater than all size classes except size class one ($p>0.001$), the mean abundance of size class three was significantly greater than all size classes except one, two, and four ($p>0.001$), and size the mean abundance of size class four was greater than those of size classes three and five though eight ($p>0.001$) (Figure 4.10).

In Indian Woods, the mean abundance of trees in size class one was also significantly greater than all other size classes ($p>0.001$), the mean abundance of size class two was significantly greater than all size classes except size class one and four ($p>0.001$), the mean abundance of size class three was significantly greater than size classes four through eight ($p>0.001$) and the mean abundance of size class five was greater than that of size classes six through eight ($p>0.001$), and the mean abundance of size class six was significantly greater than size class seven and eight ($p>0.05$) (Figure 4.11).

In the Hogsback, the mean abundance of trees in size class one was also significantly greater than all other size classes ($p>0.001$), the mean abundance of size class two was significantly greater than all size classes except size class one ($p>0.001$), the mean abundance of size class three was significantly greater than size classes five-eight ($p>0.001$), the mean abundance of size class four was significantly greater than that of size classes five through eight ($p>0.01$), the mean abundance of size class five was greater than that of size classes six through eight ($p>0.001$) and the mean abundance of size class six was significantly greater than size class seven $p>0.005$) (Figure 4.12).

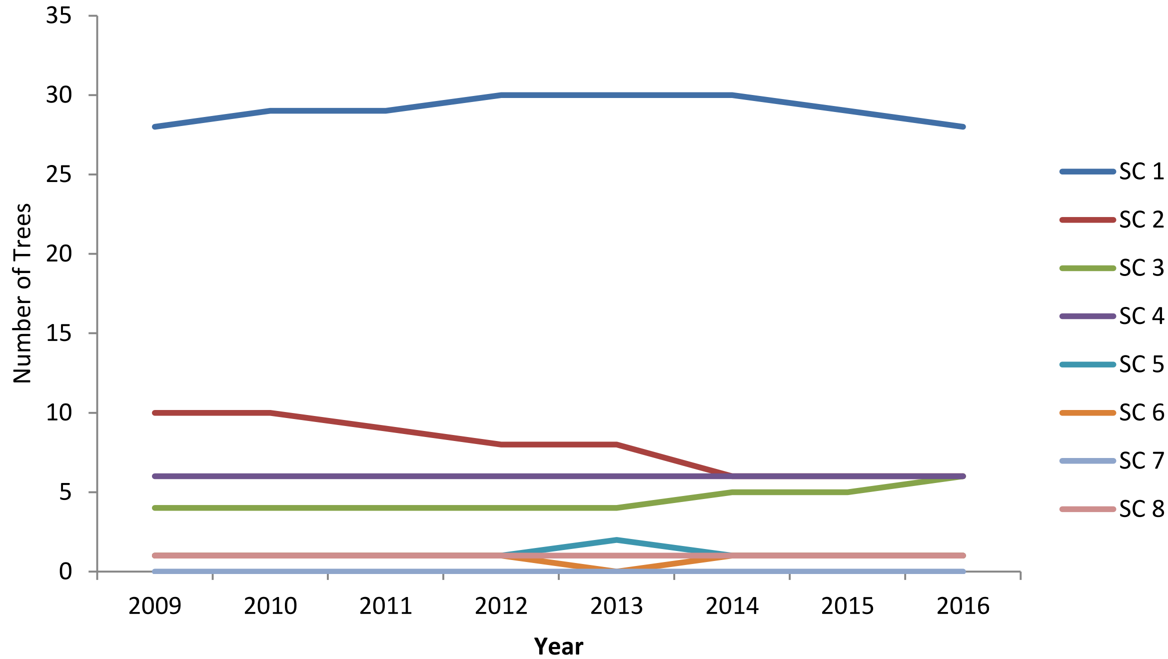

Figure 4.10: Number of trees in each size class across years in Cliffs and Alvars.

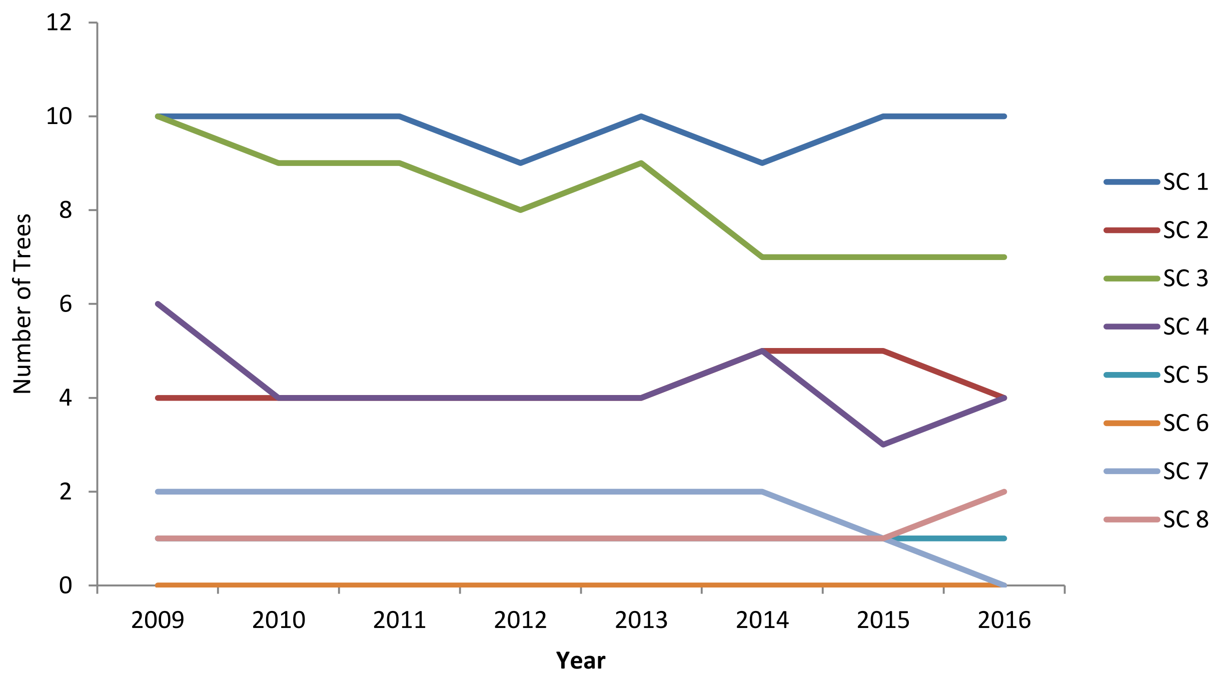

Figure 4.11: Number of trees in each size class across years in Indian Woods.

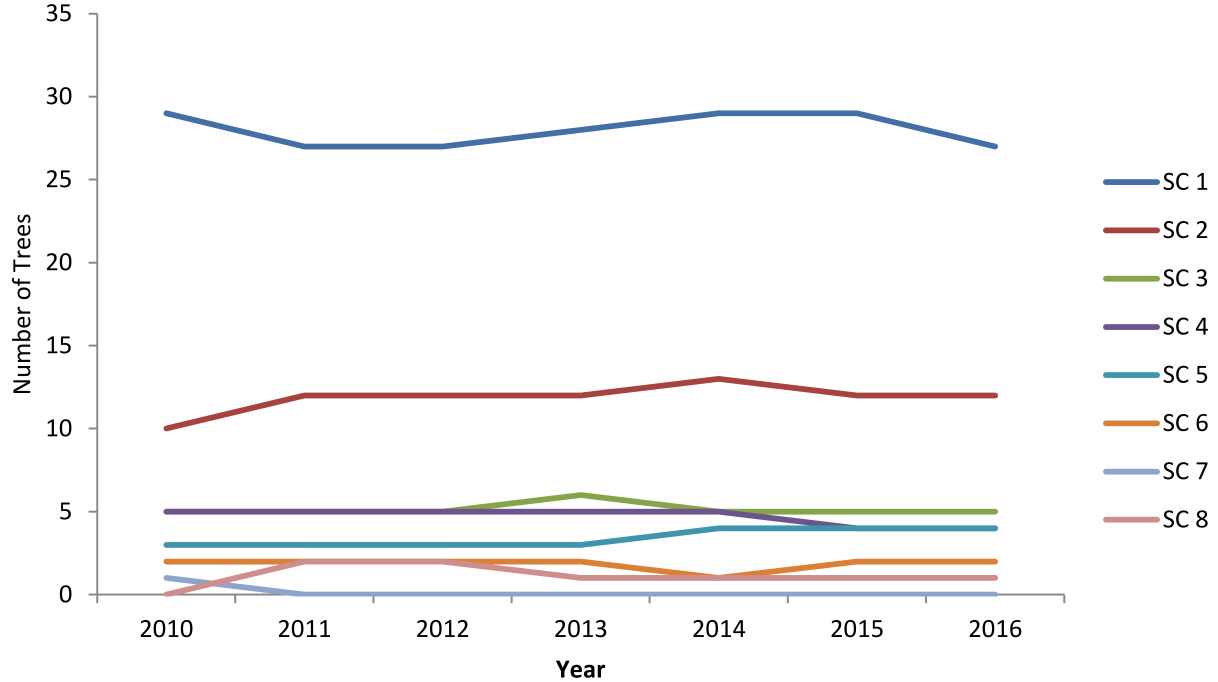

Figure 4.12: Number of trees in each size class across years in the Hogsback.

Table 4.4: 2016 tree species composition and summary statistics for the three forest stands monitored at ***rare***.

Location	Species name	Abundance	Basal Area (m2)	Relative Density	Relative Frequency	Relative Dominance	Importance Value
Cliffs and Alvars	*Acer saccharum*	18	1.66	37.50	25.00	49.76	112.26
	Betula alleghaniensis	1	0.16	2.08	8.33	4.77	15.18
	Fagus grandifolia	17	1.17	35.42	25.00	34.95	95.36
	Ostrya virginiana	9	0.10	18.75	25.00	3.13	46.88
	Prunus serotina	3	0.25	6.25	16.67	7.40	30.31
Indian Woods	*Acer saccharum*	19	2.00	67.86	42.86	68.46	179.17
	Fagus grandifolia	7	0.23	25.00	28.57	7.73	61.30
	Quercus alba	1	0.52	3.57	14.29	17.85	35.71
	Quercus rubra	1	0.17	3.57	14.29	5.96	23.82
Hogsback	*Acer rubrum*	7	1.16	12.73	15.00	17.09	44.81
	Acer saccharum	15	0.95	27.27	15.00	13.91	56.18
	Betula alleghaniensis	6	1.03	10.91	10.00	15.08	35.99
	Fagus grandifolia	9	0.68	16.36	15.00	10.05	41.41
	Fraxinus nigra	1	0.68	1.82	10.00	10.05	21.86
	Fraxinus pennsylvanica	3	0.68	5.45	10.00	10.02	25.48
	Ostrya virginiana	7	0.68	12.73	5.00	10.02	27.75
	Pinus strobus	1	0.55	1.82	5.00	8.09	14.90
	Prunus serotina	1	0.22	1.82	5.00	3.21	10.03
	Quercus rubra	5	0.17	9.09	10.00	2.49	21.58

4.4.0: Discussion

4.4.1 Tree Species Diversity and Health

All the forest areas monitored are characterized by two species; American Beech and Sugar Maple. For further information on species composition, please refer to Appendix D for species lists. Species composition within forest stands has been relatively stable, with the exception of the loss of the majority of ash trees within plots. Unsurprisingly, ash species are faring poorly, likely in large part due to the Emerald Ash Borer epidemic. White Ash trees no longer exist within the forest plots and for Green/Red and Black Ash some trees are dead while the majority are in severe decline. For more information on emerald ash borer presence and monitoring at ***rare***, please refer to Section 3 of this report.

In addition to concerns of Emerald Ash Borer, Beech Bark Disease (BBD) is another concern for forests at ***rare***. There was one Beech mortalities between 2015 and 2016, although BBD cannot be attributed as the cause. BBD is caused by an infestation of one or more species of a fungus called *Neonectria* (Cale et al. 2015). The fungus typically enters a tree that has been stressed due to feeding from a non-native scale insect called *Cryptococcus fagisuga* (Cale et al. 2015). The fungal infestation causes a whole range of health problems from reduced growth, to crown dieback and potentially death. Mortality from individuals infected by the fungus can be up to 50% (Kasson and Livingston 2011). The non-native scale insect has been known to be present in southern Ontario since 2003 (Morin et al. 2007) and has been documented in the Kitchener area in Steckles Woods (Burtt 2005). Potential instances of BBD have been identified by forest health monitoring on ***rare*** property since 2010, and pictures of suspected BBD are on the ***rare*** sever. For further discussion on Diversity and Health of ***rare*** forests, please refer to the 2015 Ecological Monitoring report.

4.4.2 Stand Characteristics and Size Class

The importance value (IV) in forestry is calculated as a means of characterizing the importance of a particular species to the forest community (Roberts-Pichette and Gillespie 1999). The IV examines each species within forest stand, and takes into consideration how abundant that species is, as well as the total amount of forest area that species occupies within each plot (i.e. basal area). From a forest management perspective, the IV is indicative of the overall influence of a particular species in the community structure and contributes to defining a community based upon its species assemblage.

Despite the differences between Indian Woods, Cliffs and Alvars, and the Hogsback forests, Sugar Maple was found to have the highest IV across all stands. Consistent with the trends in species dominance, the IV of American Beech was second highest in all three forest stands. This combination is commonly associated with late-successional northern hardwood forests, and is typical of the Carolinian forest region (Takahasi & Lechowicz 2008).

The size class distribution is useful baseline data for future comparisons examining recruitment and replacement patterns of each stand (Forrester & Runkle 2000; Parker 2003). All three forest stands had greater abundances of trees in size classes one and two than other size classes. This indicates that, although the forests stands are late-successional, many younger individuals, particularly shade tolerant species such as Sugar Maple and American Beech, are successfully becoming part of the larger canopy. For further discussion on stand characteristics and size classes, please refer to the 2015 Ecological Monitoring report.

4.4.3 Tree Heights

Tree height differences between years were calculated to evaluate which years had the greatest differences in tree heights. The greatest differences, in general, have been between trees greater than 20 m, which is unsurprising given the increased difficulty of locating the top of the crown of tall trees, particularly when the canopy is still full with leaves. Average differences in height for each plot fall between 4.39 and 7.79 metres, although these numbers vastly underestimate the degree to which tree differences have been over or under estimated. The differences between years also vary with tree, making it difficult to deduce an estimated accuracy of data collection in a given year.

In addition to the new protocol, it is suggested that tree height be measured every five years, in order to more easily capture changes in tree height, and to decrease sampling effort. Many mature tree experience little to no upward growth, such as mature sugar maples that grow between 30 and 40 centimeters per year (Burns and Honkala 1990). This range only slightly exceeds that of the acceptable measurement error used by the MNRF (20 to 30 cm). Allowing for 5 years of growth between measurements would increase certainty that the observed changes in height are due to tree growth rather than measurement error. Additionally, limiting tree height measurements to once every five years may offset the increased sampling effort of the proposed protocol.

4.5.0 Conclusions and a Summary of Recommendations

Over the past eight years of monitoring the forests at ***rare***, there have been few changes in the forest stands in terms of diversity, size class, dominance, and canopy composition. The most appreciable difference over the monitoring period has been the decline of ash trees as a result of Emerald Ash Borer. American Beech are also showing signs of decline and developing management and/or monitoring programs targeting species of special concern is of the utmost importance.

Long term data collection and analysis is required in order to fully understand if the integrity of the three forest stands is in fact being maintained or improved through management strategies, which have come as a result of forest health monitoring. With constant changes in the surrounding land use, continued monitoring will be important so that any changes in the health of the forests can be detected early on. However, due to very small changes across years, effort could be reduced by increasing the period of time between monitoring. The following are recommendations for this monitoring program;

1. Tree height monitoring should occur every five years, after the leaves have fallen from the trees.
2. Monitoring, data analysis, and report production should occur every 3-5 years.
3. Targeted monitoring for disease such as Emerald Ash Borer, Beech Blight Disease, and Butternut canker should be developed and conducted either in conjunction with or in addition to forest plot monitoring to better capture the changes in health throughout forest stands.

4.6.0 Literature Cited

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5.0 Emerald Ash Borer 2016

5.1.0 Background

Emerald Ash Borer (EAB; *Agrilus planipennis*) is a major pest for Ash trees in North America as it is capable of infesting and killing even the healthiest of Ash trees (OMNR 2010). Native to Asia and Eastern Russia, Emerald Ash Borer was first detected in Canada in 2002 just outside of Windsor, Ontario (OMNR 2010). Management of EAB has been challenging due to EAB's ability to rapidly spread; they are capable of travelling up to 20km per year (Prasad et al. 2010). Additionally, the difficulty of early detection of EAB (Cappaert et al. 2005) may result in delayed management efforts, furthering the probability of spread. EAB has spread over much of southern Ontario and Quebec, making its first appearance in the Waterloo Region in 2010 at Highway 401 and Homer Waterson Boulevard in Kitchener (Region of Waterloo 2010); a location only a few kilometers away from the ***rare*** property.

Evidence of EAB within the forests at ***rare*** has been noted, although no individual adults have actually been observed. Evidence includes small D-shaped exit holes left by adults that emerge from under the bark, as well as reduction and death of ash tree crowns. In 2015, a literature review was conducted in conjunction with a pilot monitoring project in Cliffs and Alvars to answer the following questions related to presence and impacts of EAB in ***rare*** forest stands:

1. What is the current state of ash condition in relation to its status in the overall forest community and which management strategies are feasible to reduce impact of expected canopy decline?
2. How do forest invaders, such as European (*Rhamnus cathartica* L.) and Glossy Buckthorn (*Rhamnus frangula* L), respond to suddenly emerging canopy gaps in comparison to natural tree rejuvenation?

For more a complete literature review and preliminary findings from the 2015 pilot year, a report can be found on the ***rare*** server. In 2016, the pilot study was extended to Indian Woods based on a modified sampling protocol to further evaluate forest habitats at ***rare***.

5.2.0 Sampling Method

5.2.1 Plot Selection

A systematic sampling approach was used to capture species composition and ash occurrence within Indian Woods. A 100 by 100 meter grid was chosen based on the number of plots relative to the size of the forest. As the Indian Woods forest stand is smaller than Cliffs and Alvars forest, a decreased grid size was necessary to offer a comparable number of potential monitoring plots. Eighteen plots were identified within the Indian Woods forest stand, including plots in the Dry-Fresh Sugar Maple-Oak Deciduous Forest and the Yellow Birch Mineral Deciduous Swamp (Figure 5.1). Plot locations were located within the forest using a Garmin etrex 20 GPS unit.

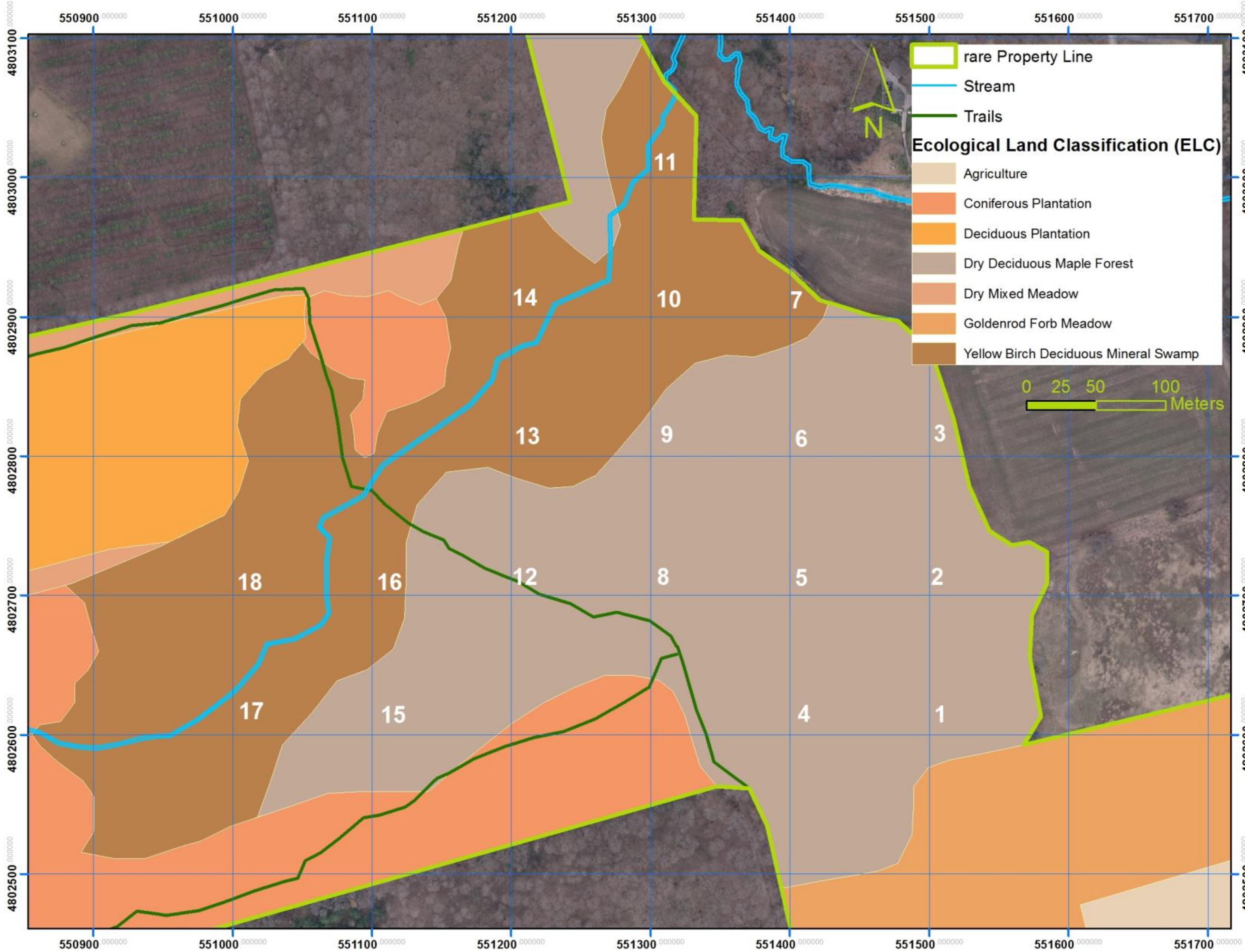

Figure 5.1: Plot location of forest inventories in Indian Woods in 2016. Plots are located on grid nodes and numbered 1-18.

5.2.2 Monitoring protocol

Monitoring was conducted during the first week of October. Due to seasonal time constraints, it was not possible to complete all plots, and the ten plots within the Dry-Fresh Sugar Maple-Oak Deciduous Forest were chosen for monitoring. Monitoring of each plot included a Forest Inventory, Rejuvenation/Invasive Species Distribution Inventory, and Ash Tree Assessment Surveys.

Forest Inventory: Upon location of a plot, a pigtail stake was placed in the centre point determined by GPS coordinates. Within a 10 m radius, all live trees ≥ 7cm DBH (1.30m) were identified by species and the cardinal orientation from and distance to the center point established (Figure 5.2). These measurements were taken to allow for ease of identification of individual trees in each plot in subsequent monitoring years. Generally, a direct line to the center point was maintained to ensure consistency. DBH measurements were taken of each tree using DBH tape. For trees with a leaning less than 45°, DBH was measured at 1.30 meter stem length instead of height and in case of significant diameter anomalies (e.g. canker) the measurement was taken at its lowest point. The same procedure was applied to standing coarse woody debris at all degrees of decay, if clearly identifiable as ash.

Rejuvenation/ Invasive Species Distribution: Beginning at the centre point, the first 20 individuals of a given species were identified within a 5 m radius (if more than three individuals of a species were present), and were graded within a five point height system (1 = 0-20cm; 2 = 21-40cm; 3 = 41-80cm; 4 = 81-130cm; 5 = 131-199cm). Canopy cover was also estimated over plots within a 5 m radius using 5 percent increments.

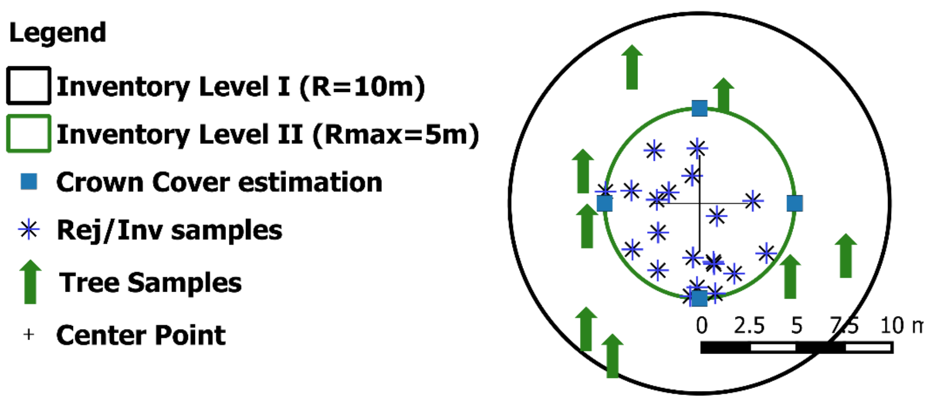

Figure 5.2: Sampling Plot designed from 2015 Protocol by David Winger.

Ash Tree Surveys: Based on a protocol for ash trees in urban areas provided by the University of Toronto (Melamed & Zhou, 2012), a variety of criteria were considered as indicators of Emerald Ash Borer presence. A visual scan for these criteria was conducted on ash trees within forest plots (Appendix G, Table G.1). An identification guide from the University of Toronto Emerald Ash Borer Management Plan was used as reference material (Appendix G, Figure G.1). Ash trees were then scored based on criteria to identify degree of EAB concern (Table 5.1).

Table 5.1: Emerald Ash Borer risk rating system based on seasonal crown parameters, stem defects and growth restrictions for ash (*Fraxinus spp.*) in forest environments (adopted from Melamed & Zhou, 2012*).*

Score	Rating	Criteria
0	Low risk of EAB	• EAB scores total (excluding feeding & exit holes) ≤ 3
1	Medium risk of EAB	One or more of: • Defoliation = 2 • Bald spots on trunk = 3 • Debarking = 1/2 • Epicormic shoots = 2 • EAB scores total (excluding feeding and exit holes) = 4
2	High risk of EAB	One or more of: • Defoliation = 2/3 • Animal feeding = 1 • Debarking = 3 • Epicormic shoots = 3 • EAB scores total (excluding feeding and exit holes) ≥ 5
3	EAB confirmation	Visual ID of D-shaped exit holes (= 1)

4.3.0 Results

Eight species of tree were identified in Indian Woods plots. Few ash trees were found within plots, with only three of ten plots containing ash trees. Ash trees accounted for less than five percent of all trees in plots (7/147), and four out of seven were classified as dead. All three living ash trees were White ash, along with one of the dead trees. The remaining dead trees were identified as ash species, but could not be further identified. Based on the presence of nearby White ash saplings, it is likely that unidentified dead ash trees were White ash as well.

Visual surveys of ash trees confirmed EAB infestation in three ash trees through presence of D-shaped exit holes. The remaining ash trees were classified as having “High Risk of EAB” presence with risk ratings of 2 (see Table 5.1). However, these scores must be interpreted cautiously due to late sampling and possible inaccuracy of criteria scores.

In rejuvenation/ invasive species surveys, eight species were identified for major tree recruitment, including one unknown species (Figure 5.3). Sugar maples (n=168) were the most abundant in forest plots, followed by White ash (n=149) and American Beech (n=103). Low numbers of invasive species existed in plots relative to other species (Glossy Buckthorn (n=24) and European Buckthorn (n=7)). Average height scores of both invasive species (Glossy Buckthorn=2.33, European Buckthorn=2.5) were lower than average height scores of all other recruits with the exception of Sugar maples (1.58) (Figure 5.4).

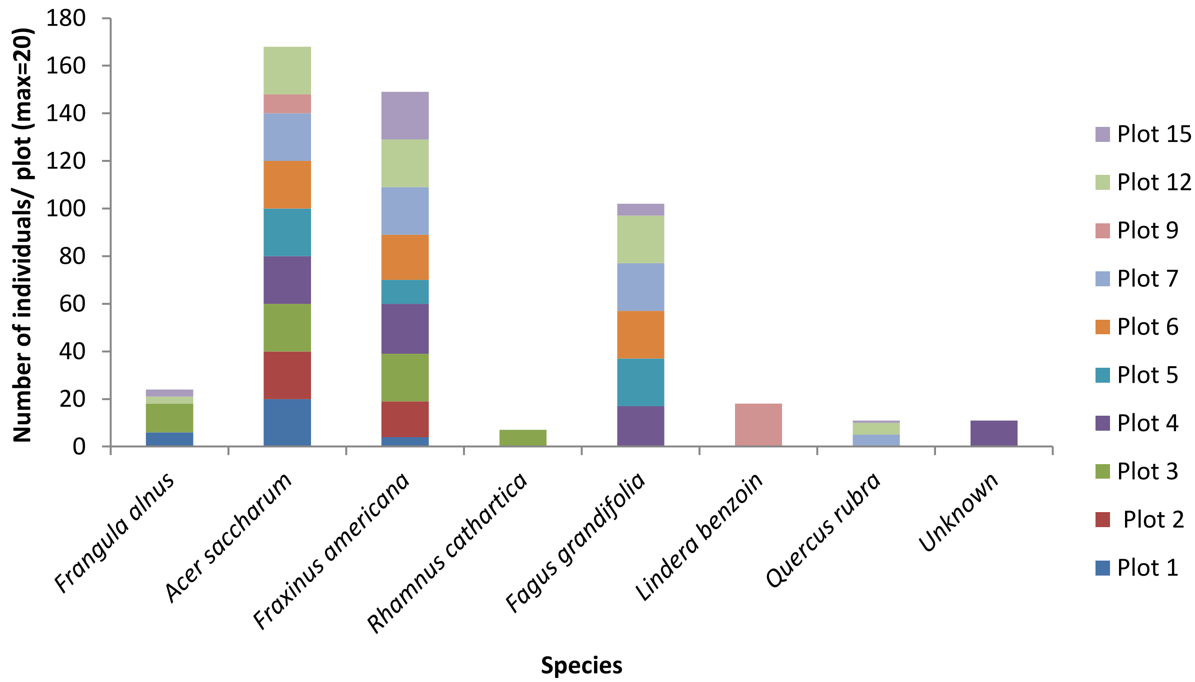

Figure 5.3: Number of individuals observed per species at each plot. Note that individuals of a given species were counted to a maximum of 20.

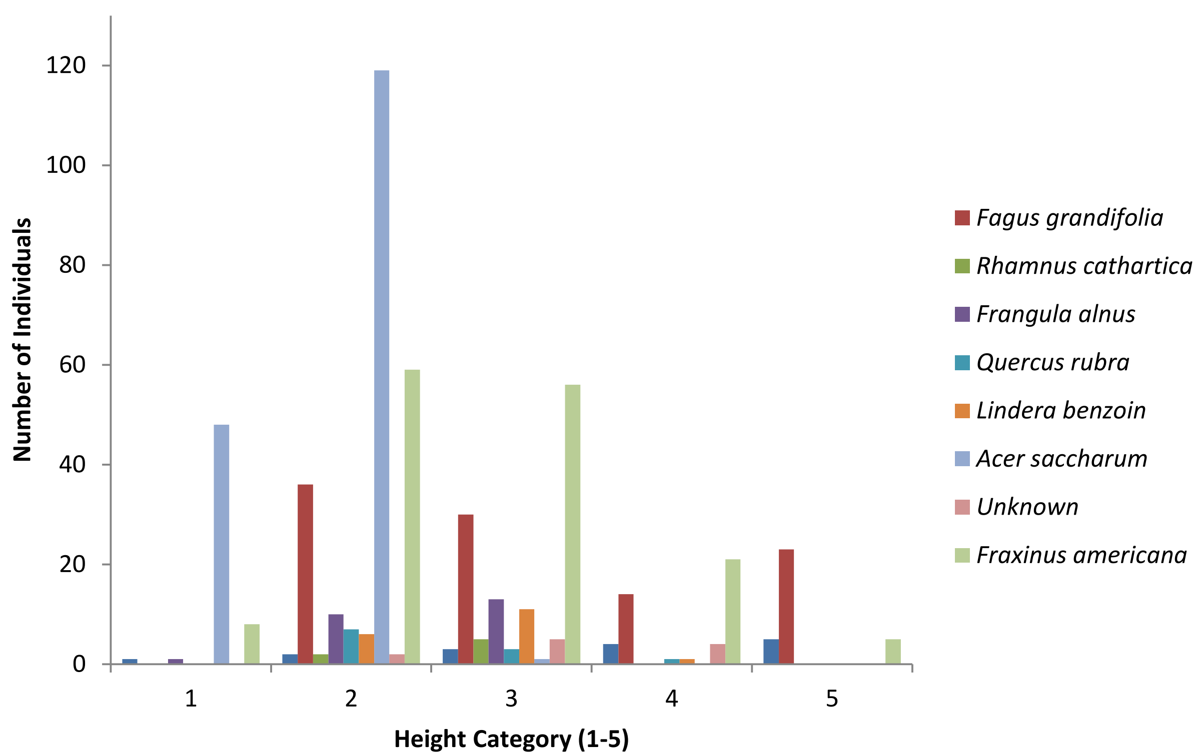

Figure 5.4: The number of individuals of a given species in each height category. Note that individuals of a given species were counted to a maximum of 20. Height categories are: 1 = 0-20cm; 2 = 21-40cm; 3 = 41-80cm; 4 = 81-130cm; 5 = 131-199cm.

5.4.0 Discussion

EAB surveys in 2016 revealed that there is a low proportion of mature ash trees in Indian Woods (less than five percent), and those which existed within plots showed confirmed or high risk of EAB presence. In 2011, NRSI conducted an ecological land classification at ***rare*** and the proportion of ash trees in Indian Woods was approximately 17 percent, which is more than three times the proportions of this year's surveys. This indicates that live ash trees have declined substantially in numbers over the last five years, which mirrors trends that have been observed in ***rare*** forest plots during annual monitoring. However, sampling method and effort varied between surveys, and continued monitoring with a greater sampling effort should occur to yield more reliable results.

Considering that four of eleven assessment criteria involve assessment of the crown, late surveying could have resulted in incorrect scores being assigned to criteria, and the resultant risk score being over estimated. However, observation of EAB exit holes confirms infestation of three trees. All but one of the remaining four trees that did not exhibit visible exit holes were classified as dead, indicating that crown assessment was likely accurate, and that six out of seven ash trees sampled were either dead or infested with EAB. Only seven ash trees were identified within sampling plots, which is a low sample size. Ash trees represented less than five percent of all trees in monitoring plots, which may be indicative of the proportion of ash trees at the Indian Woods site. Increasing the number of plots would increase validity of this extrapolation and increase usability of data for statistical testing. Despite strong evidence of EAB presence, results should be interpreted carefully, as the sample size is low and sampling occurred late in the season when leaves had already begun to change colour and fall.

Species with individuals which have a diameter of less than 7 cm and a height of more than 2 meters are not captured within the rejuvenation/invasive species distribution protocol. For example, two of ten plots have large ash saplings that were not included in the forest inventory or rejuvenation/ invasive species surveys. Similar observations were made for young Maple and American Beech Trees that did not fall within monitoring ranges. Generally, larger saplings have the greatest chance of taking over available resources when, for example, a canopy gap opens up and are therefore perhaps most representative of the species that would fill in such a gap. It may be beneficial to add a sixth category of more than 2 metres (but less than the minimum diameter) to ensure all saplings are accounted for. Plots that contained larger saplings also contained seedlings and small saplings of the same species. Therefore sampling seedlings and small saplings may be sufficient to make predictions about the species that are most likely to benefit from a canopy opening, and the future diversity of the forest. The only exception is ash; plots with large ash saplings did not always contain seedlings. This is not surprising considering that ash trees do not flower until they are at least 8-10 cm DBH (Kennedy 1990; Schlesinger 1990), and plots with large saplings did not contain trees of this DBH. Considering that EAB will attack ash stems with as low as 2.5 cm DBH (McCullough et al. 2008; Rebek et al. 2008), whether or not ash saplings are captured in this protocol is somewhat irrelevant, as it is unlikely ash trees will grow to maturity barring the elimination of EAB from forests. Due to the challenges of EAB management, this is unlikely to happen. Regardless, to determine which species are most likely to benefit from current or upcoming openings, inclusion of larger saplings in monitoring is required.

Contrary to results of the 2015 survey, invasive species were well represented in forest plots in Indian Woods generally falling within the stated size categories. However, as most forest plots were placed away from the path, it is possible that fewer individuals of invasive species were counted than if plots were adjacent to paths. The presence of invasive species was low in comparison to rejuvenates within plots, which may be a good sign for natural rejuvenation in Indian Woods. Although it is unlikely that young ash tree recruits will survive to replace dying ash trees in the canopy the composition of species found in rejuvenation studies indicates that native species will. Sugar maples are the most likely to fill the canopy, with the most saplings present in plots and the ability to grow rapidly in the presence of light availability (NRCS 2016). American Beech trees were also abundant and were also more evenly distributed in height class (2-5), whereas Sugar Maples were almost all in lower height classes (1 and 2). Given that the Sugar Maple and American Beech trees account for 86 percent of the canopy trees in monitoring plots (65 and 21 percent respectively), it is likely that the forest diversity will continue to decline and composition will continue to shift towards dominance of these species, as is typical of a later-successional old growth forest. Continued management of invasive plant species in Indian Woods will help reduce the presence of these species, and promote growth of native species.

Several important modifications to the monitoring protocol were identified. The modifications will simultaneously decrease sampling effort devoted to forest plot inventories and rejuvenation/ invasive species inventories, and increase the sample size of ash trees for Emerald Ash Borer surveys. Modification suggestions and justifications follow:

1. The grid size used to determine the number of plots and their locations at each site (Indian Woods, Hogsback, or Cliffs and Alvars) should be determined based on the size of the forest stand as well as the number of plots a grid size produces. Grid sizes in Indian Woods and the Hogsback should be decreased to at the largest 75 metres by 75 meters to produce more possible forest plots (between 25 and 30 per site), and also be comparable to the number of plots in Cliffs and Alvars (27). This will allow for a more thorough survey and representation of forest composition, as well as increase possible encounters of ash trees.
2. Complete forest and rejuvenation/invasive species inventories should be limited to plots which contain ash trees with diameters greater than 7 cm within the 10 m radius. In plots which do not contain ash trees (DBH>7 cm), trees (DBH>7) within the 10 m inventory radius should be counted and identified to species. This modified survey will provide important information regarding species composition while limiting sampling effort. Plots without ash trees will not need to be visited in future monitoring years, and thus locations of trees within forest plots without ash trees is unnecessary. Monitoring rejuvenation/ invasive species monitoring in plots where canopy opening may occur in response to EAB infestation and ash tree loss will focus efforts of monitoring and reduce overall sampling efforts.
3. It was suggested in the report from the original pilot study in 2015 that ash condition should be monitored every two to three years. As relocation of plots and ability to identify individual trees within plots will be necessary for reassessment, and GPS accuracy is not high in forested areas, it is suggested the centre points of plots are marked with pigtail stakes and flagging tape.
4. Changes to rejuvenation surveys should be made for ease of data collection and analysis. Instead of counting and measuring heights of only the first 20 individuals within

the 5 meter inventory radius, I suggest that monitoring occur within a 3 meter radius and all individuals are counted.

5. Finally, saplings over two metres tall and less than 7 cm DBH should be included in rejuvenation/invasive species studies to determine which species are most likely to benefit from upcoming changes in the canopy.

*A complete updated protocol and data sheets is included in Appendix G and C.

4.5.0 Conclusion and Recommendations

Forest inventory and EAB monitoring conducted in 2016 provided important preliminary information on the abundance of ash trees in Indian Woods as well as a preview of the condition of ash trees at the site. Overall, low numbers of ash trees and high occurrence of Emerald Ash Borer indicators mirror the declining trends in ash trees across North American forests. Continued monitoring of ash condition, loss of canopy, and species composition of rejuvenates will allow ***rare*** to maintain the native canopy in forest plots through invasive species management. Monitoring should be expanded to the Hogsback, and plots should be revisited every two to three years in late summer. It is suggested that to avoid difficulties in crown assessment, monitoring should occur in late summer in future monitoring years to ensure accuracy of surveys.

4.6.0 References

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Appendix A: Maps and Coordinates

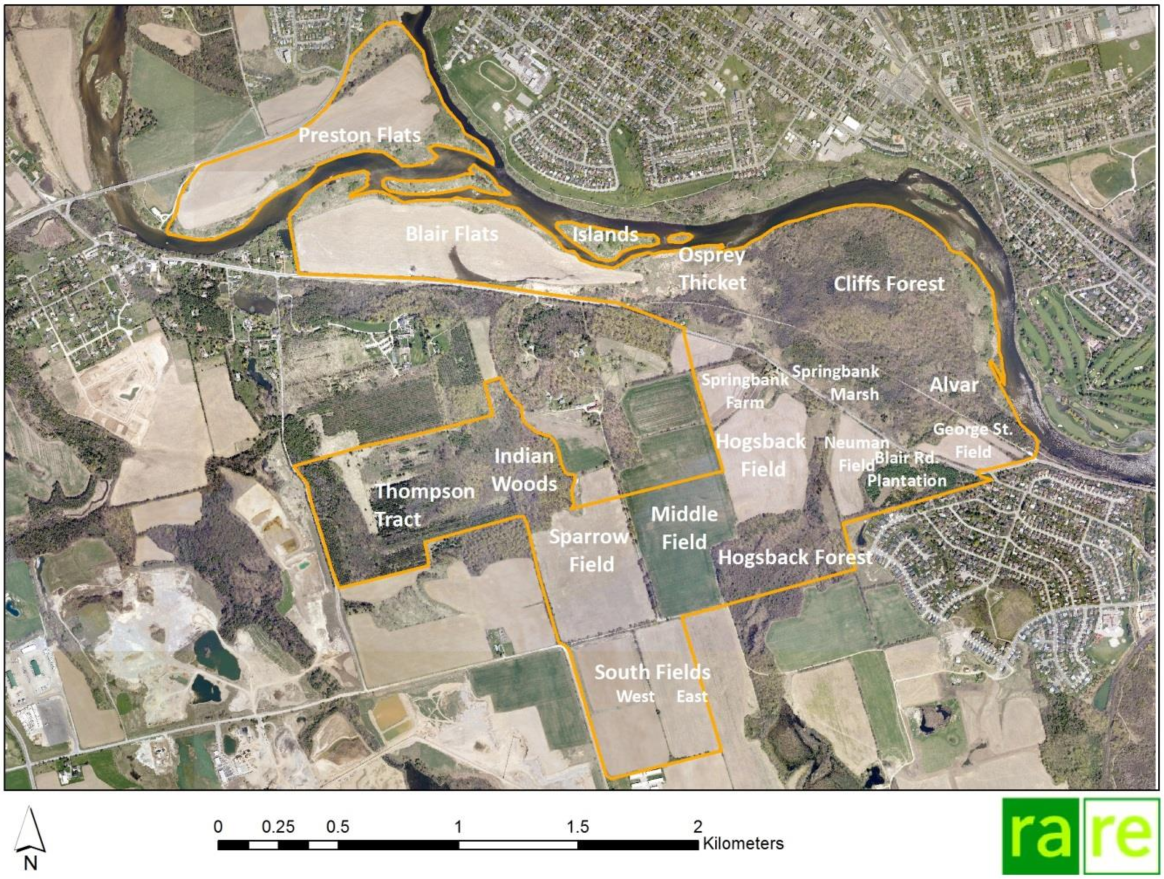


Figure A.1: Property map of the ***rare* Charitable Research Reserve** with colloquial names of properties.

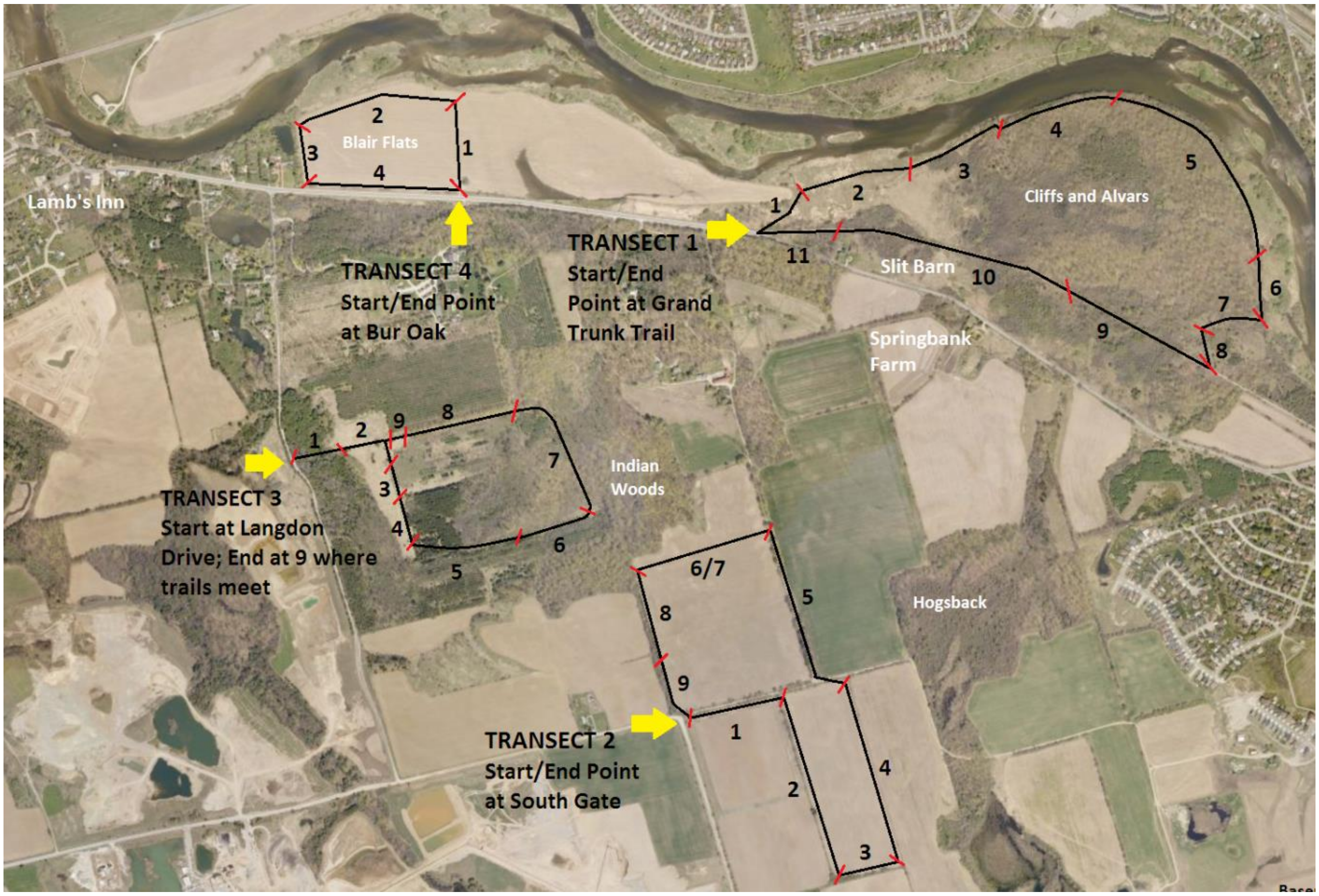


Figure A.2: Location of the four butterfly monitoring transects at the ***rare*** **Charitable Research Reserve** with start/end points and section breaks.

List A.1: Description of **Transect One** sections with stopping point coordinates (GPS coordinate accuracy less than 10m).

*** Enter transect across from big dead tree, cut in by rocks, avoid trail reinforcement**

Section one (N 43° 22.980’ W 80° 21.541’)
- Riparian grassland (milkweed, goldenrod, grasses)
- Stop past the sedge wetland, toward the river at the solitary shrub

Section two (N 43° 23.025’ W 80° 21.426’)
- Riparian meadow with trees and shrubs on south side (starts when pass single tree)
- Generally walk towards wooden post
- Stop at old fallen tree in middle of field, within direct view of the osprey tower, 100m

Section three (N 43° 23.058’ W 80° 21.222’)
- Riparian area with trees on south side (grasses, sedges, small shrubs, goldenrod) (starts when cross stream on trail)
- Stop in open grass area with small hill on right hand side just after trail turns away from river, before continuing into forest

Section four (N 43° 23.120’ W 80°21.017’)
- Mainly coniferous forest trail with open canopy areas, on cliffs
- Stop when path forks to small lookout over the river to the left, break in cedar dominance

Section five (N 43° 22.986’ W 80°20.625’)
- Deciduous forest trail (after stake in ground)
- Stop at large fallen tree over trail, trail has moved around log; cliffs on south side and open meadow (milkweed, raspberry, goldenrod, one Oak) on north side

Section six (N 43° 22.761’ W 80°20.617’)
- Open shrub land (at the turn after the hill)
- Stop at alvar on the left hand side of trail right after the old car on the right hand side, large red pine on trail edge and large white pine further back near alvar

Section seven (N 43° 22.767’ W 80° 20.697’)
- Deciduous forest trail (turn to go into alvar after woods stop and open up again)
- Stop at large alvar, ~10m after tall Oak tree

Section eight (N 43° 22.749’ W 80° 20.734’)
- Open shrub land (after big left turn)
- Stop on second boardwalk

Section nine (N 43° 22.793’ W 80° 20.901’)
- Grand Trunk Trail, deciduous forest
- Stop at culvert in wetland

Section ten (N 43° 22.901’ W 80° 21.250’)
- Grand Trunk Trail, dense shrub growth on both sides of trail
- Stop at entrance to Osprey Tower path to the north, and path to Slit Barn to the south

Section eleven (N 43° 22.927' W 80° 21.546')

- Grand Trunk Trail, wetland on either side of trail, after barn (sedges, cattail, milkweed, goldenrod, purple loosestrife)
- Stop at culvert near Blair Road entrance to Grand Trunk Trail, several Trembling Aspen trees, just after post.

List A.2: Description **of Transect Two** sections with stopping point coordinates (GPS coordinate accuracy less than 10m).

***Park at L bend/ south gate**

Section one (N 43° 22.177' W 080° 21.691')

- Agricultural field (mix of alfalfa, red fescue, perennial wild rye, buckwheat, winter wheat and oats) to south of transect, deciduous trees and shrubs to the north
- Stop at north side of South Field West in naturalized buffer, directly across from silo at farm to the south

Section two (N 43° 22.048' W 080° 21.560')

- Hedgerow along soybean field edge, mostly open with some shrubs
- Stop halfway along west side of South Field East, near solitary Buckthorn shrub & old collapsed wooden structure

Section three (N 43° 21.909' W 080° 21.438')

- Hedgerow of deciduous trees along edge of soybean field
- Stop halfway along south side of South Field East, at the end of the tree line to the north, before the row of three single trees

Section four (N 43° 22.050' W 080° 21.404')

- Hedgerow on east side of soybean field, mostly open with few shrubs along fence
- Stop halfway along field edge, blue post on east side of fence

Section five (N 43° 22.386' W 080° 21.608') **change in coordinates this year*

- Deciduous hedgerow of mostly Oak trees; bordering soybean field on east side and naturalized agricultural field on west side
- Stop after open canopy, once there is partial coverage again, by large Maple

Section six/seven (N 43° 22.423' W 080° 21.771')

- Naturalized agricultural field, now with grasses, wildflowers, and some saplings (maple)
- Stop halfway across field, just before the bird boxes to the South

Section eight (N 43° 22.299' W 080° 21.892')

- Hedgerow of deciduous trees (mostly Maple) bordering naturalized agricultural field
- Stop at top of hill at fallen tree, can see apartment building to the east

Section nine (N 43° 22.212' W 080° 21.857')

- Hedgerow (east of Grand Allee Trail) of mainly shrubs, vines and grasses bordering naturalized agricultural field
- Stop on incline past large group of young maple trees, 20 meters before path to Grand Allee

List A.3: Description of **Transect Three** sections with stopping point coordinates (GPS coordinate accuracy less than 10m).

Section one (N 43° 22.584' W 080° 22.569')
- Coniferous forest (Ash trees, Cedar trees, shrubs)
- Stop at swampy meadow just past culvert (goldenrod, cattails, milkweed)

Section two (N 43° 22.601' W 080° 22.469')
- Meadow (milkweed, goldenrod, grasses, sedges)
- Stop at junction of trails

Section three (N 43° 22.541' W 080° 22.454')
- Black Locust plantation and meadow
- Stop halfway through plantation area, where tree has grown around top wire of fence on east side

Section four (N 43° 22.482' W 080° 22.430')
- Meadow (milkweed, goldenrod, grasses, sedges) on west side of transect, Spruce tree forest on east side
- Stop at third large Spruce tree on east side, about halfway down the straight portion of the trail

Section five (N 43° 22.424' W 080° 22.301')
- Spruce and deciduous forest
- Stop where wet area ends (will change from year to year), small clearing to the north, several small trees leaning across path

Section six (N 43° 22.476' W 080° 22.064)
- Meadow (grasses, sedges) and Walnut tree plantation
- Stop halfway down straight section of walnut trees, dead and fallen White Pine on north side with young maples around it

Section seven (N 43° 22.568' W 080° 22.158')
- Grand Allee Trail in Indian Woods (deciduous forest of Sugar Maple, Beech and Oak trees with woodland plants and flowers such as may apple, solomon's seal, trillium and ferns)
- Stop on cement bridge over Bauman Creek

Section eight (N 43° 22.635' W 080° 22.273')
- Maple Lane Trail (deciduous forest of Sugar Maple and shrubs)
- Stop near small pile of logs on south side of trail

Section nine (N 43° 22.606' W 080° 22.437')
- Meadow (vetch, goldenrod, grasses, sedges, scattered trees and shrubs)
- Stop halfway before the junction of trails, between two stumps on north side of trail

List A.4: Description of **Transect Four** sections with stopping point coordinates (GPS coordinate accuracy less than 10m).

***Park before first driveway**

Section one (N 43° 23.090' W 080° 22.307')

- Weedy meadow planted for tall grass prairie, recovering from agricultural use (horseweed, black-eyed susan, goldenrod)
- Walk from Bur Oak toward tower in distance, stop halfway before field edge in between two University of Guelph plant enclosures

Section two (N 43° 23.131' W080° 22.523')

- Regeneration area to the north side of transect and planted tall grass prairie to the south (black-eyed susan, burdock, goldenrod, horseweed, tansy, thistles)
- Stop halfway along field edge, just after the bird boxes

Section three (N 43° 23.056' W 080° 22.641')

- Hedgerow of shrubs and trees to the west of transect and planted tall grass prairie to east of transect (black-eyed susan, burdock, goldenrod, horseweed, tansy, thistles)
- Stop halfway along field edge, just before stack of rocks on west side.

Section four (N 43° 22.998' W 080° 22.473')

- Hedgerow along Blair Road to the south of transect and planted tall grass prairie to north of transect (black-eyed susan, horseweed, Manitoba Maple, poison ivy, tansy, thistles, shrubs)
- Stop halfway along field edge, where shrubs are tallest

Table A.1: GPS coordinates of artificial cover objects (ACO) used for plethodontid salamander monitoring in Indian Woods and the Hogsback (from McCarter 2009).

Monitoring Plot	ACO	Latitude and Longitude	UTM (zone 17T)
Indian Woods	1	N43°22'32.05" W80°21'55.49"	551408E 4802718N
	9	N43°22'31.97" W80°21'53.71"	551448E 4802716N
	17	N43°22'30.97" W80°21'53.63"	551450E 4802685N
	25	N43°22'30.85" W80°21'55.37"	551411E 4802681N
Hogsback	1	N43°22'23.93" W80°21'12.74"	552372E 4802475N
	8	N43°22'22.99" W80°21'13.32"	552359E 4802446N
	11	N43°22'22.44" W80°21'12.84"	552370E 4802429N
	18	N43°22'23.57" W80°21'12.30"	552382E 4802464N

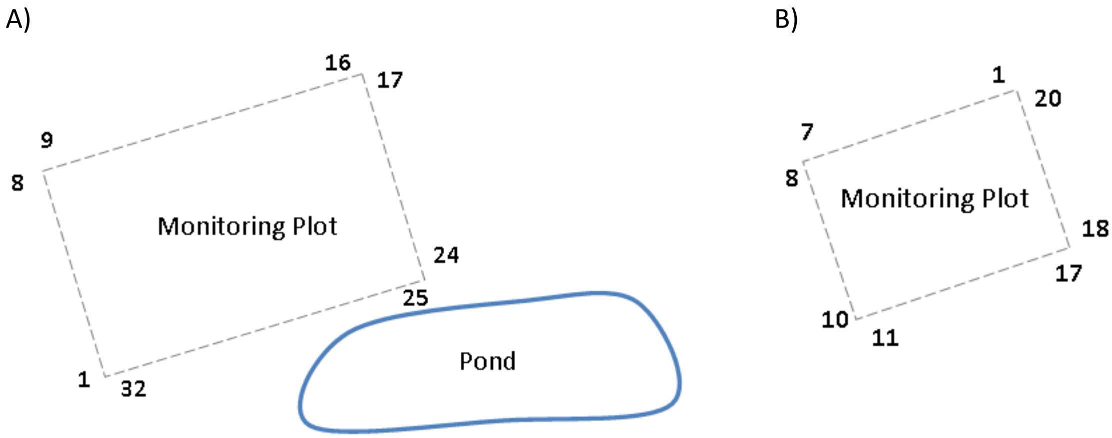
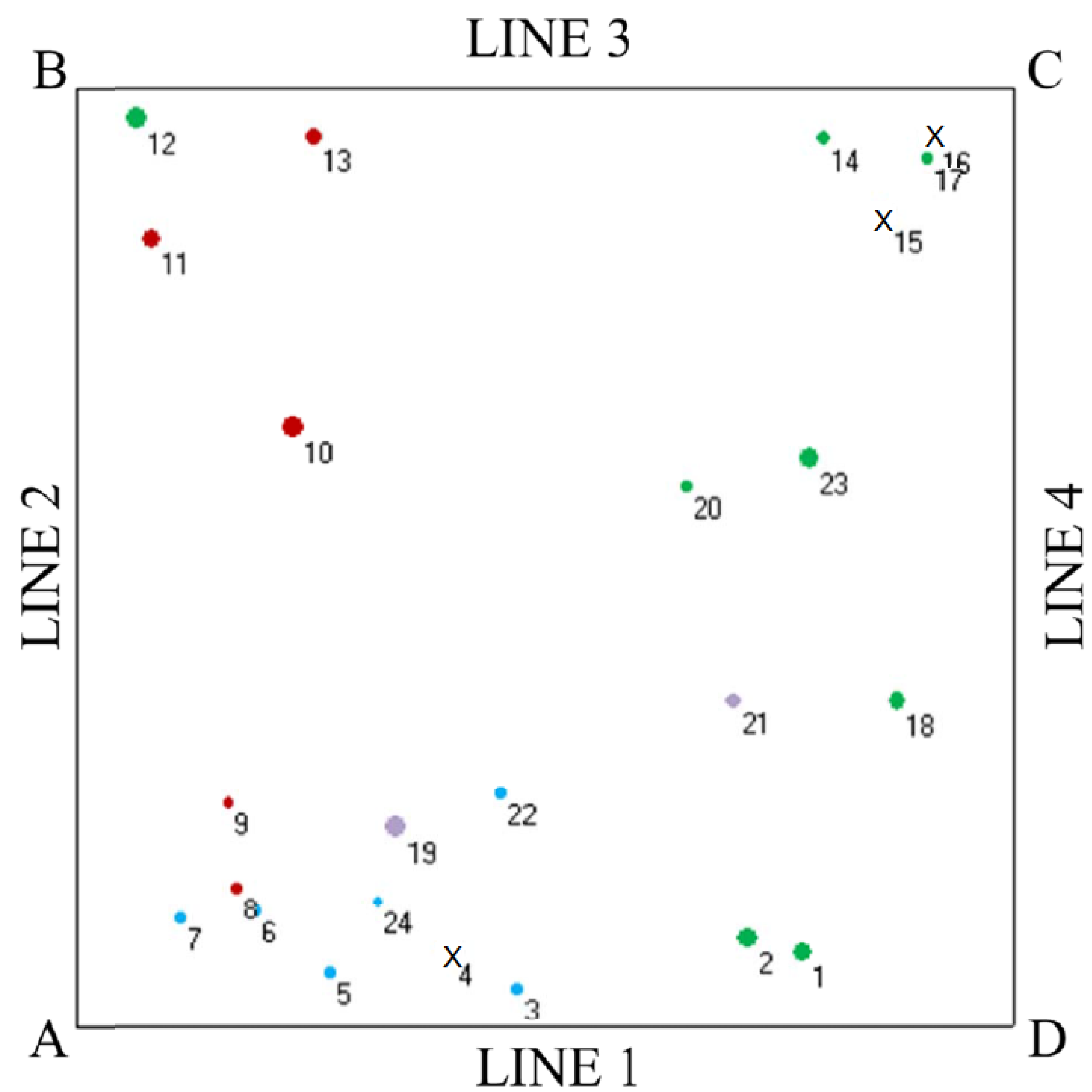


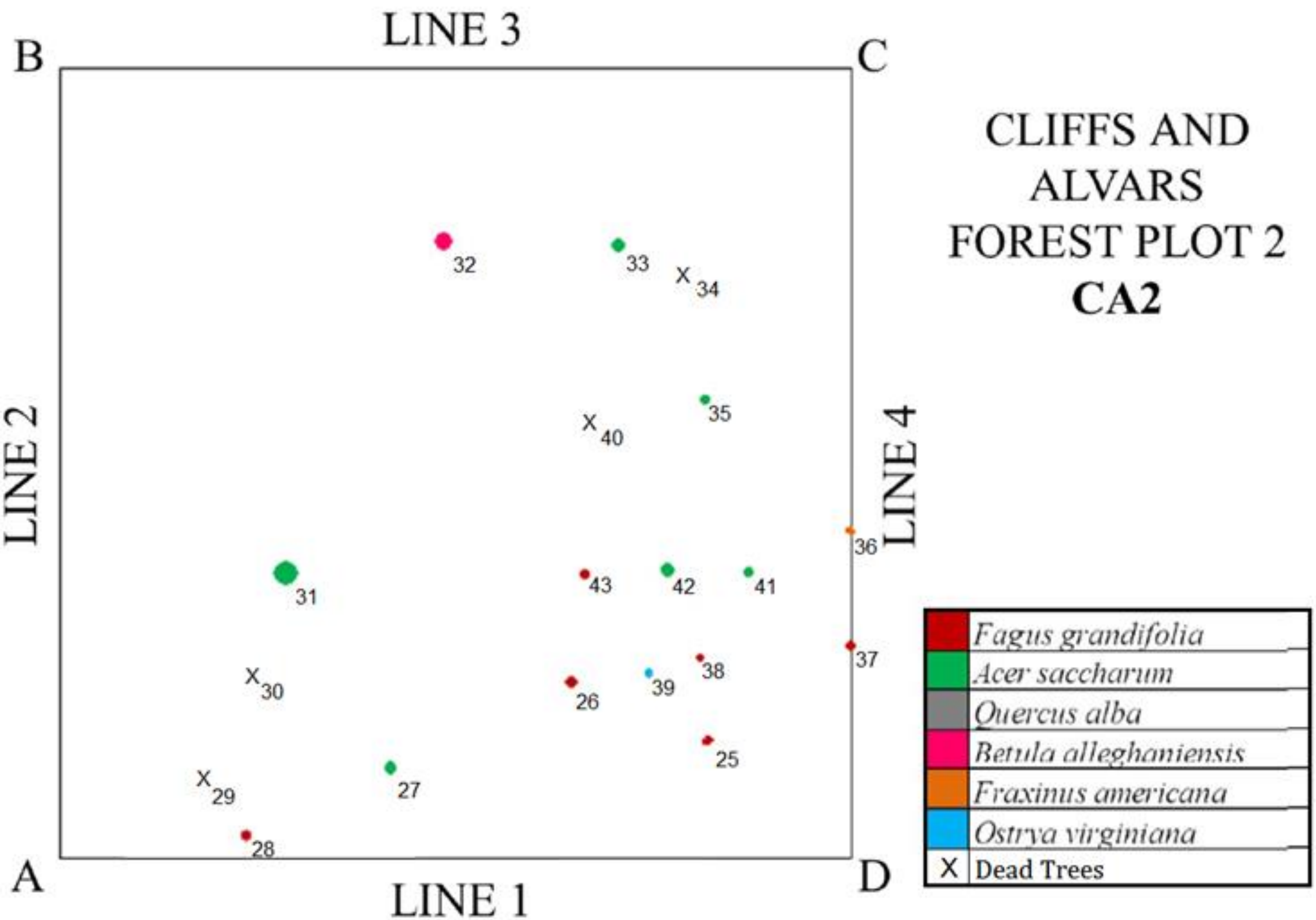
Figure A.3: Layout of artificial cover objects (ACOs) on salamander monitoring plots in A) Indian Woods and B) Hogsback

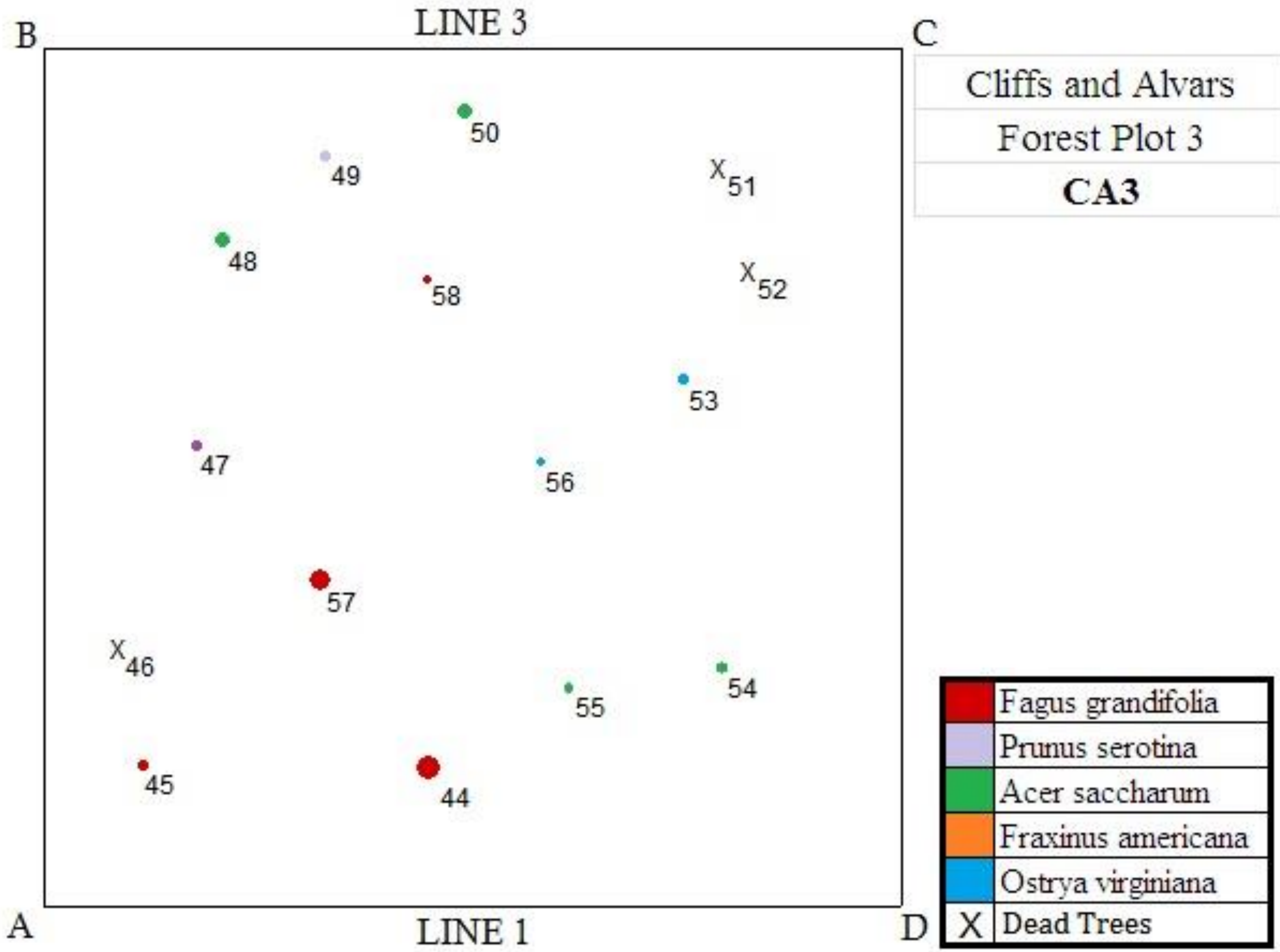


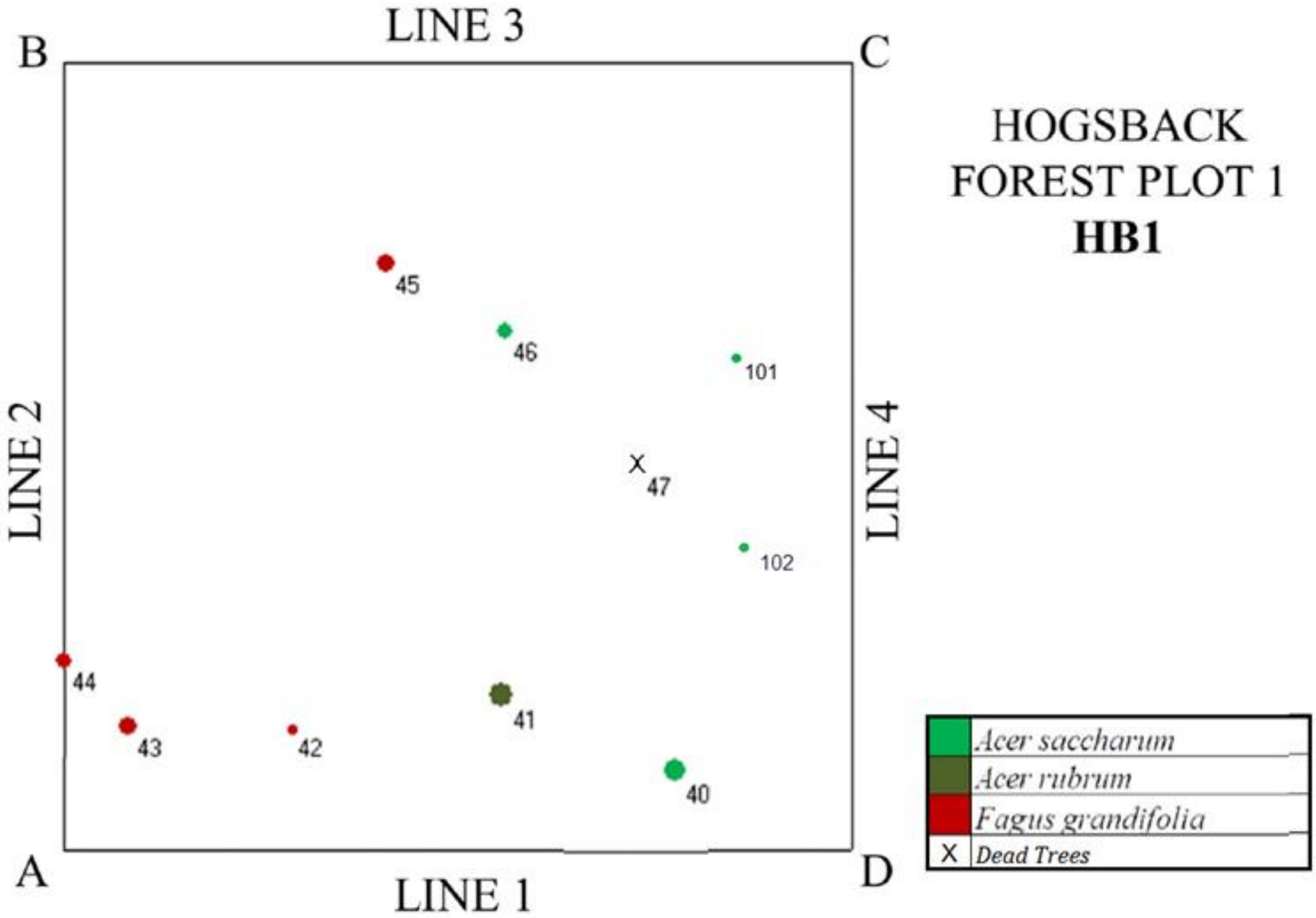
CLIFFS AND ALVARS FOREST PLOT 1

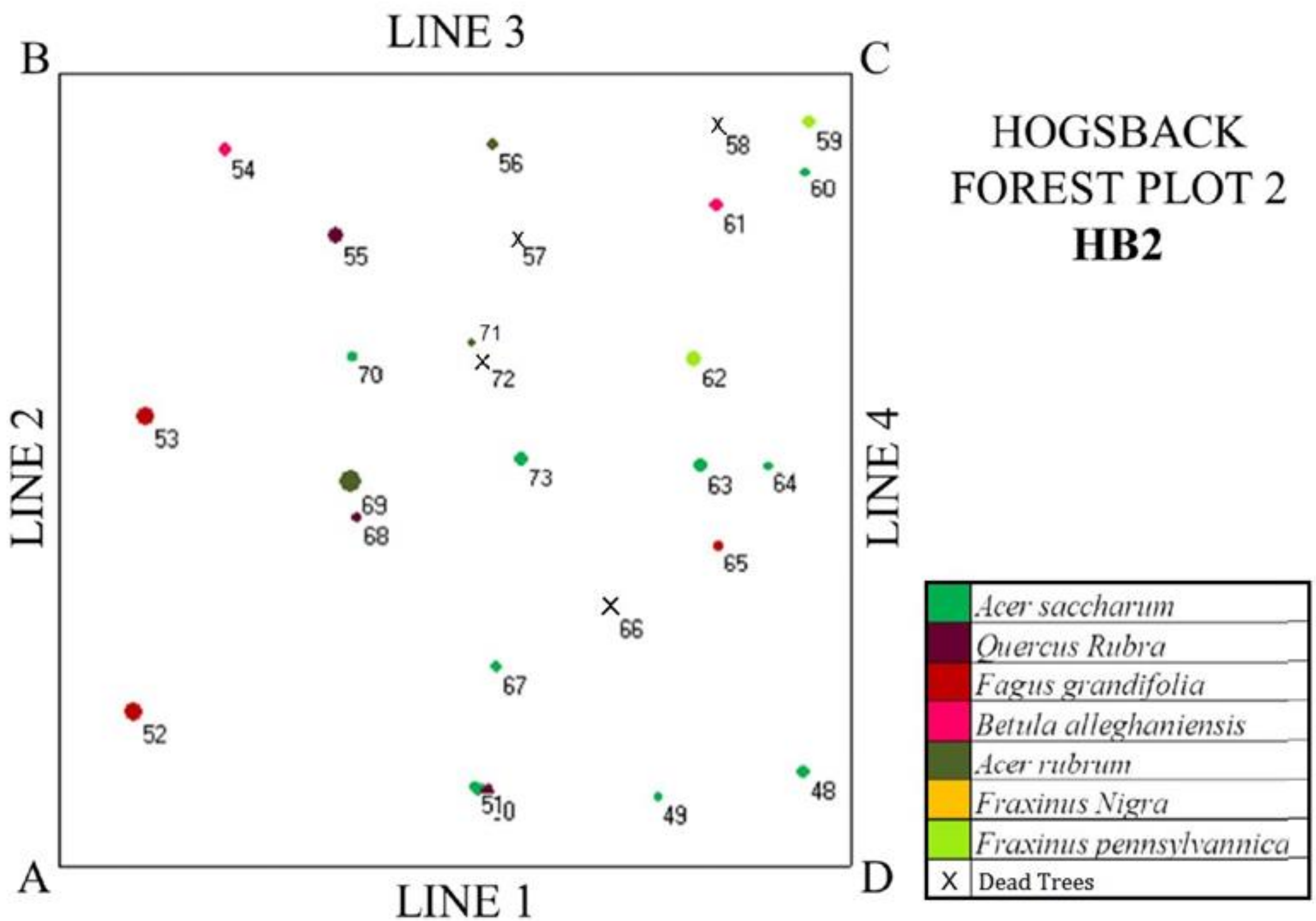
CA1

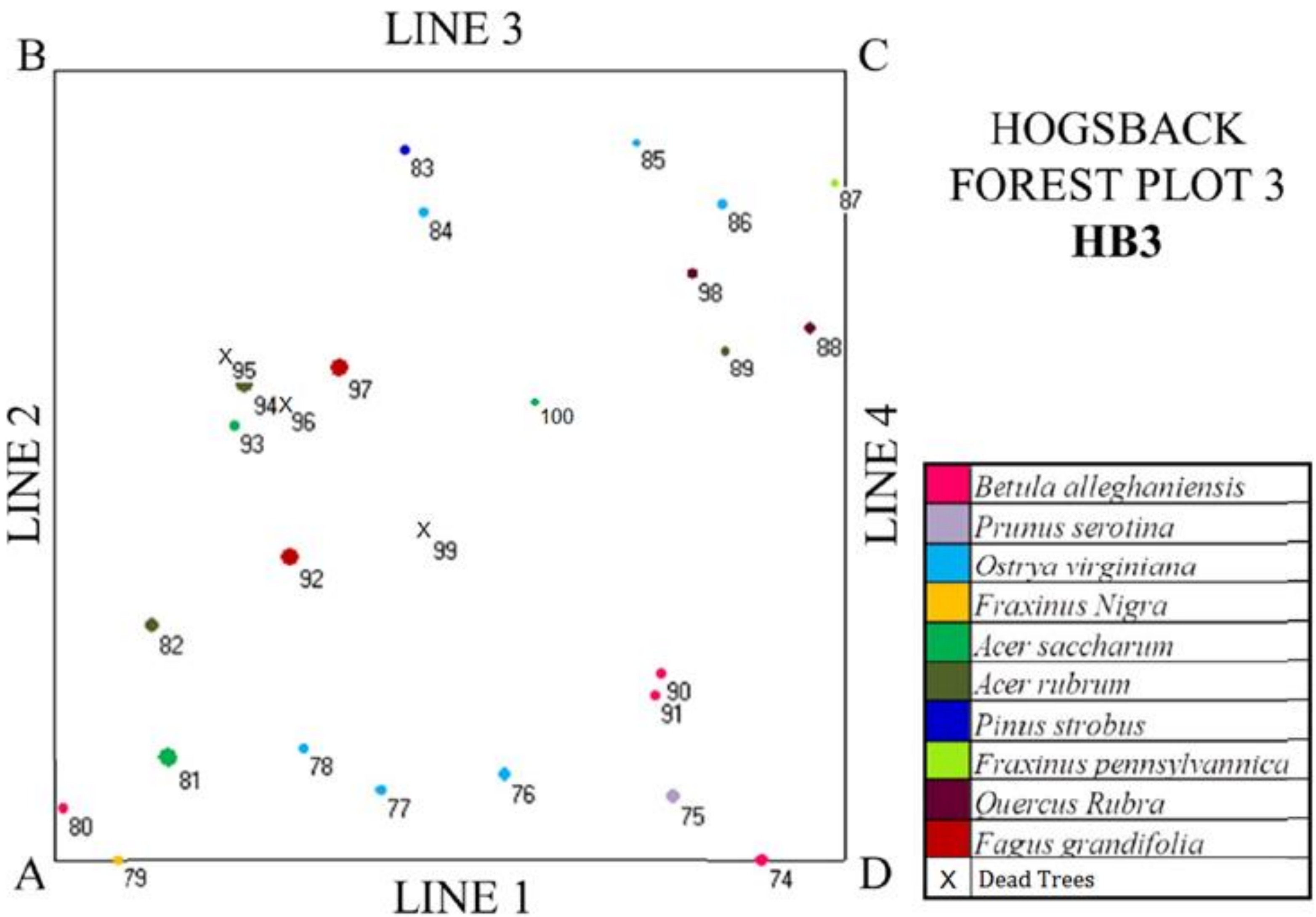
	Acer saccharum
	Ostrya virginiana
	Fraxinus americana
	Fagus grandifolia
	Juglans cinerea
	Prunus serotina
X	Dead Trees

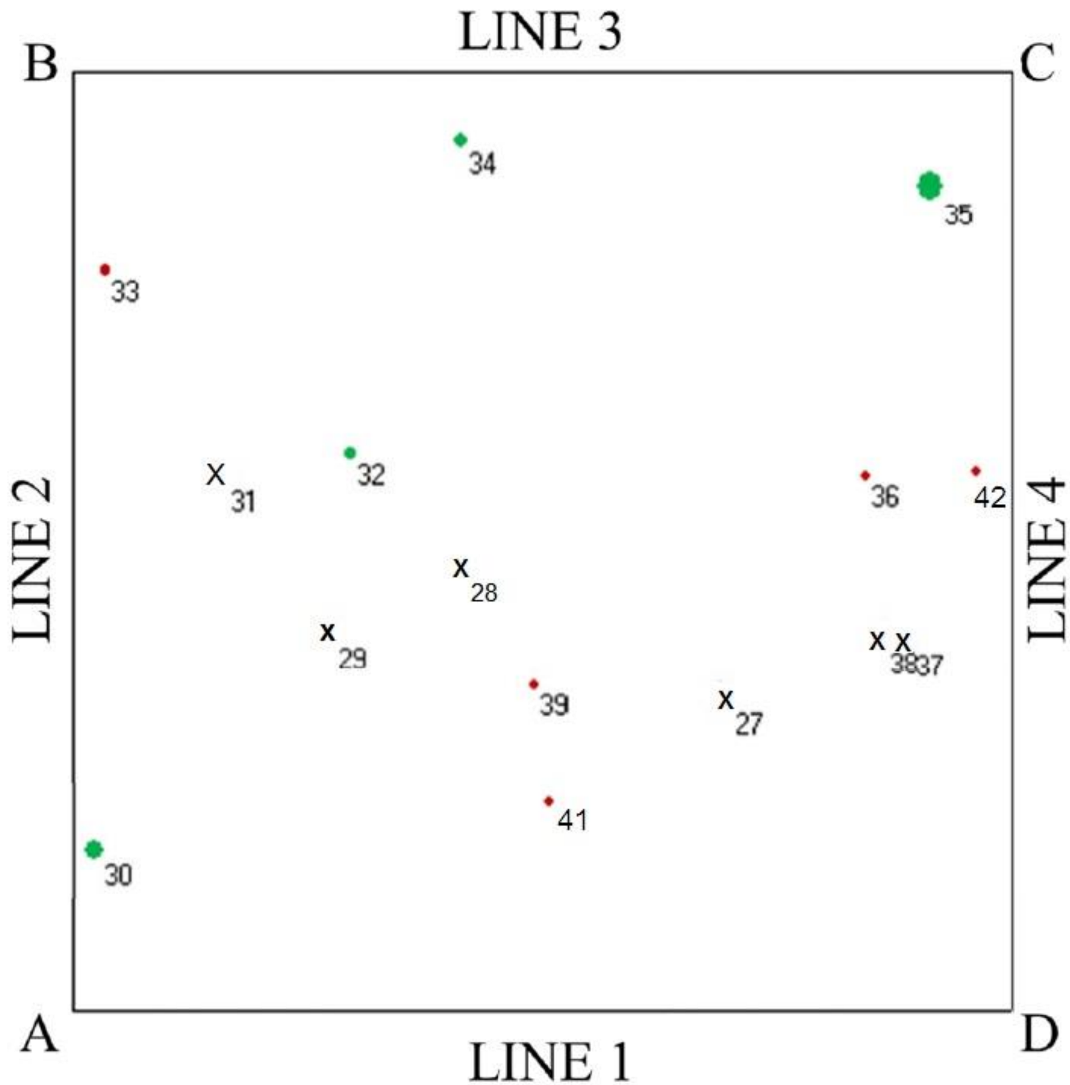

LINE 3
B
C
CLIFFS AND ALVARS FOREST PLOT 2
CA2
LINE 2
LINE 4
32
33
34
35
40
36
31
43
42
41
37
38
39
26
30
25
29
27
28
A
LINE 1
D
Fagus grandifolia
Acer saccharum
Quercus alba
Betula alleghaniensis
Fraxinus americana
Ostrya virginiana
X Dead Trees


LINE 3
B
C
Cliffs and Alvars
Forest Plot 3
CA3
50
49
X51
48
58
X52
53
47
56
57
X46
55
54
45
44
Fagus grandifolia
Prunus serotina
Acer saccharum
Fraxinus americana
Ostrya virginiana
X Dead Trees
A
LINE 1
D


HOGSBACK
FOREST PLOT 1
HB1
LINE 1
LINE 2
LINE 3
LINE 4
A
B
C
D
40
41
42
43
44
45
46
47
101
102
Acer saccharum
Acer rubrum
Fagus grandifolia
X Dead Trees

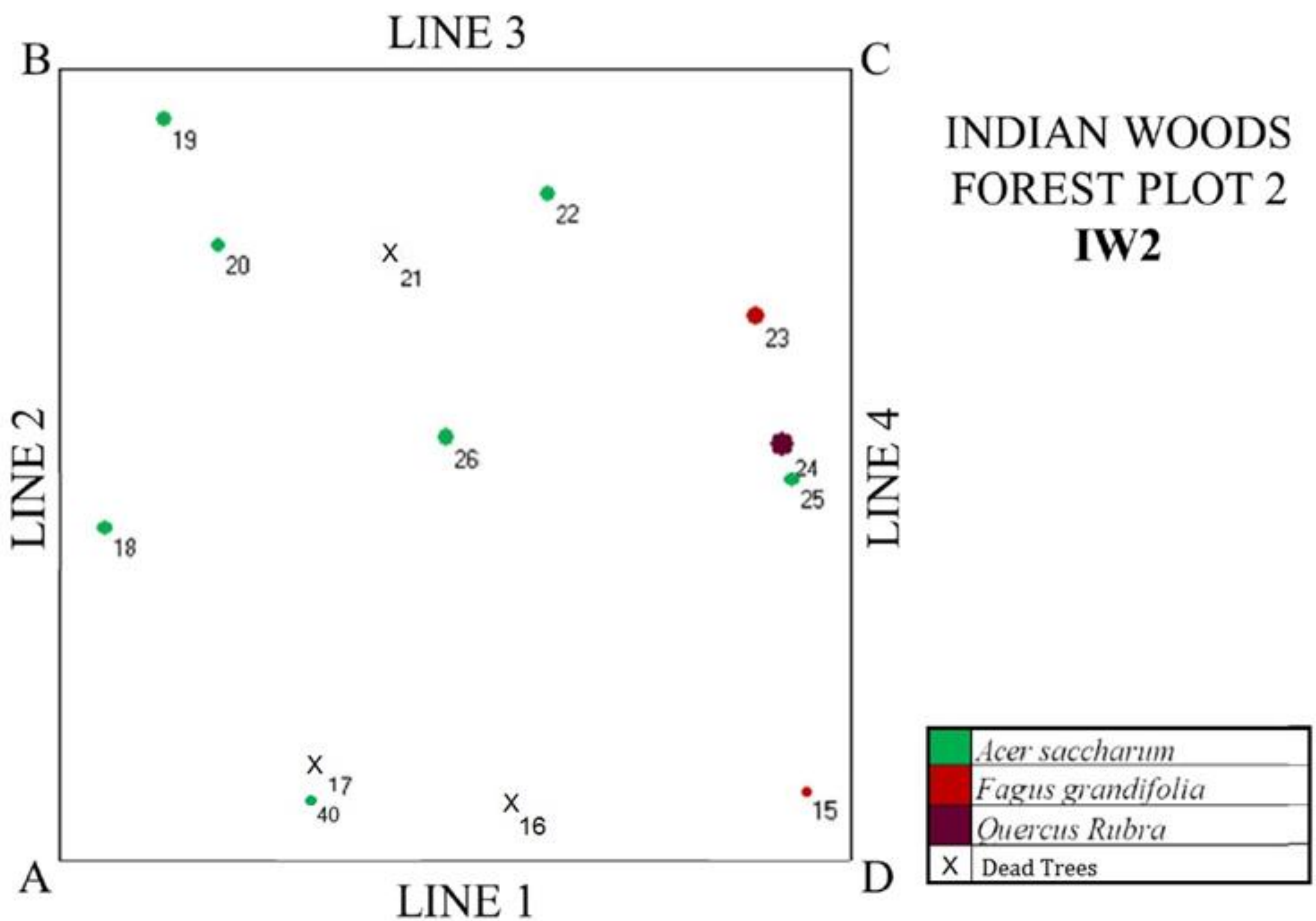

LINE 3
B
C
LINE 2
LINE 4
A
D
LINE 1
54
56
58
59
60
61
55
57
71
70
72
62
53
73
63
64
69
68
65
66
67
52
48
51
10
49
HOGSBACK
FOREST PLOT 2
HB2
Acer saccharum
Quercus Rubra
Fagus grandifolia
Betula alleghaniensis
Acer rubrum
Fraxinus Nigra
Fraxinus pennsylvannica
X Dead Trees


LINE 3
B
C
LINE 2
LINE 4
A
D
LINE 1
83
85
87
84
86
98
88
95
97
89
94
96
100
93
99
92
82
90
91
81
78
76
77
75
80
79
74
HOGSBACK
FOREST PLOT 3
HB3
Betula alleghaniensis
Prunus serotina
Ostrya virginiana
Fraxinus Nigra
Acer saccharum
Acer rubrum
Pinus strobus
Fraxinus pennsylvannica
Quercus Rubra
Fagus grandifolia
X Dead Trees



INDIAN WOODS
FOREST PLOT 1
IW1

	Acer saccharum
	Fagus grandifolia
X	Dead Trees


LINE 3
B
C
LINE 2
LINE 4
A
D
LINE 1
19
20
21
22
23
26
24
25
18
17
40
16
15
INDIAN WOODS
FOREST PLOT 2
IW2
Acer saccharum
Fagus grandifolia
Quercus Rubra
X Dead Trees

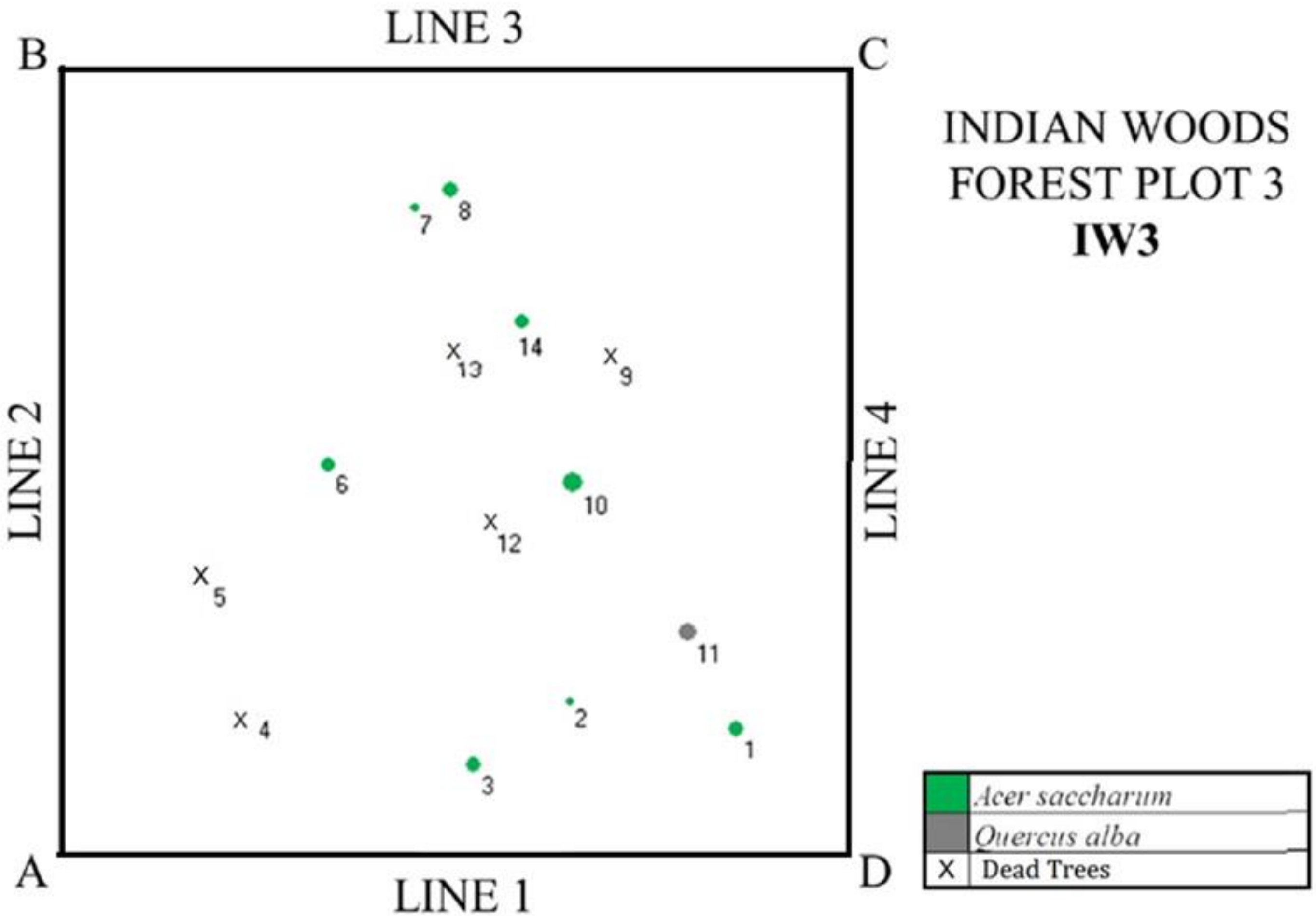


Figure A.3-A.11: Maps of Cliffs and Alvars, Indian Woods, and the Hogsback forest biodiversity monitoring plots showing location of all standing, live trees with a diameter at breast height (dbh) greater than 10.0cm. Sizes of circles are proportional to real tree diameters, colours indicate different species

APPENDIX B: Field Equipment

List B.1: Suggested butterfly monitoring field equipment

- Field data sheet
- Clipboard
- Pencils
- Stopwatch
- Kestrel 3000©
- Butterfly net
- Field guide (Recommended: Carmichael, I. and Vance, A. 2003. Photo Field Guide to the Butterflies of Southern Ontario. St. Thomas Field Naturalist Club Inc., St. Thomas, ON.)
- Clear jar with mesh lid
- Binoculars
- Digital Camera

List B.2: Salamander monitoring equipment list

- Field data sheets A and B on waterproof paper
- Clipboard
- Pencils
- Nitrile gloves
- Kestral 3000 pocket weather station
- Soil moisture meter (calibrated with screw driver)
- Soil thermometer
- Digital calipers
- Ruler
- Digital pocket scale (with spare batteries)
- Sandwich sized plastic container filled with moist sponges
- Larger plastic container with some moist sponges
- Wash bottle filled with pond water from education pond
- Flagging tape
- Aluminum tags
- Digital camera
- Luster Leaf Rapitest Digital Soil pH Meter

List B.3: Forest canopy tree biodiversity monitoring equipment list

- Blank canopy-sample and tree condition field data sheets on waterproof paper
- Past year data sheets & EMAN reference package
- Clipboard
- Pencils
- Flagging tape
- Diameter tape

- Two nylon tape measures (30m)
- Field guide
- Binoculars
- Clinometer

List B.4: Emerald Ash Borer Surveys

- GPS Garmin etrex SUMMIT HC
- Suunto Baseline Compass
- Diameter tape
- Binoculars
- Measuring tape 50 m
- Field Guide
- Pigtail stakes
- Flagging tape
- Data Sheets 1-3

APPENDIX C: Field Codes and Data Sheets

Table C.1: Beaufort wind codes (Zorn et al. 2004)

Wind Code	Wind Speed (mph)	Wind Speed (km/h)	Description
0	1	1.6	Calm. Smoke rises vertically.
1	2	3.2	Light. Smoke drifts.
2	5	8	Light breeze. Leaves rustle.
3	10	16	Gentle breeze. Lighter branches sway.
4	15	24	Moderate breeze. Dust rises. Branches move.
5	21	33.6	Fresh breeze. Small trees sway.
6	28	44.8	Strong breeze. Larger branches move.
7	35	56	Moderate gale. Trees move.
8	42	67.2	Fresh gale. Twigs break.
9	50	80	Strong gale. Branches break.
10	59	94.4	Whole gale. Trees fall.
11	69	110.4	Storm. Violent blasts.
12	75	120	Hurricane. Structures shake.

Table C.2: Beaufort sky codes (Zorn et al. 2004)

Sky Code	Description
0	Clear. No clouds.
1	Partly cloudy. Scattered or broken clouds.
2	Cloudy (broken) or overcast.
3	Sandstorm. dust storm, or blowing snow.
4	Fog, thick dust or haze.
5	Drizzle.
6	Rain.
7	Snow, or snow rain mixed.
8	Shower(s).
9	Thunderstorm(s).

BUTTERFLY MONITORING FIELD NOTES									
DATE:				START:			TEMP_START:		
TRANSECT:				FINISH:			TEMP_END:		
1			S:	SUN:	2			S:	SUN:
W1:				WIND:	W1:				WIND:
W2:					W2:				
3			S:	SUN:	4			S:	SUN:
W1:				WIND:	W1:				WIND:
W2:					W2:				
5			S:	SUN:	6			S:	SUN:
W1:				WIND:	W1:				WIND:
W2:					W2:				
NOTES:									

Figure C.1: Sample butterfly monitoring field data sheet. Available on the ***rare*** server.

Field Data Sheet A								
Plot Name: Indian Woods				Group Name: ***rare*** Charitable Research Reserve				
Observer Name(s):								
Pond depth (mm; Indian Woods):				Date:			Time:	
Precip.(last 24hrs):			Beaufort Sky Code:			Beaufort Wind Code:		
ACO Number	Species	Count	ACO: Type	Age	Soil: Temp	Moisture	Canopy Cover/Light Reader	ACO Disturbance
Additional Comments:								
Canopy Cover	C: Complete (90-100%)			I: Incomplete (10-90%)			N: No Cover (0-10%)	

	North Perimeter		**East Perimeter**		**South Perimeter**		**West Perimeter**	
ACO #:	IN-02-03	IN-02-07	IN-02-11	IN-02-15	IN-02-19	IN-02-23	IN-02-27	IN-02-31
WS (mph)								
RH (%)								
AT (C)								

WS= Wind Speed RH= Relative Humidity AT= Air Temperautre

Figure C.2: Sample of salamander monitoring field sheet A (available on ***rare*** server).

Field Data Sheet B							
Plot Name:				Group Name: ***rare*** Charitable Research Reserve			
Observer Name(s):							
Pond depth (mm; Indian Woods):				Date:		Time:	
Precip.(last 24hrs):			Beaufort Sky Code:			Beaufort Wind Code:	
ACO Number	Cumulative Number of Salamanders	Species	Length (mm) S-V	V-T	Total	Weight (g)	Comments
Additional Comments:							

Figure C.3: Sample of salamander monitoring field sheet B (available on ***rare*** server)

CANOPY-TREE SAMPLE: FIELD DATA SHEET (1-ha. plot or 20 m x 20 m stand-alone quadrats). Stand name.. Date...
Stand location (lat. & long.) ....Hectare plot and quadrat nº...........................**OR** Stand-alone quadrat nº............ Av. stand height...
Identification manual................................ Observer(s)...

Tag #	Species name	Number of stems	dbh (cm)	Line (1,2,3,4)	A distance (m)	B distance (m)	Height (m)	Condition	Notes

Figure C.4: Sample of forest canopy tree biodiversity monitoring sheet (available on ***rare*** server).

CANOPY-TREE SAMPLE: SUMMARY SHEET. Stand name.. Date..........................

Stand location (lat. & long.)........................ Number of 1-ha plots .... **OR** Number of 20 m x 20 m stand-alone quadrats ....

Sample area (m^2)............. Average canopy height .... Data processor(s)..

Species name	Abundance	Density	Basal Area	Dominance	Frequency	Relative Density	Relative Frequency	Relative Dominance	Importance Value	Notes

Figure C.5: Sample of forest canopy tree health monitoring field sheet, tree condition (available on ***rare*** server

Date:		Observers:			
Plot:		Coordinates:			
FOREST INVENTORY					
Species	Tree #	Condition	Cardinal Orientation	Distance to Centre point	DBH (> 10 cm)

REJUVENATION/INVASIVE SPECIES INVENTORY

	Height Category (tally)					
Species	1	2	3	4	5	6

*Height Categories: 1 = 0-20cm; 2 = 21-40cm; 3 = 41-80cm; 4 = 81-130cm; 5 = 131-199cm, 6= >200cm

Figure C.6: Complete Forest and Rejuvenation/ Invasive Species Inventory field data sheet (available on ***rare*** server).

Date:			
Observers:			
RDUCED FOREST INVENTORY			
Plot/ coordinates	Species	Number of Individuals	

Figure C.7: Reduced Forest Inventory field data sheet (available on ***rare*** server)

Date:

Observers:

VISUAL EAB SURVEYS

Plot	Tree #/ Condition	Canopy Cover (%)	Crown Reduction	Unbalanced Crown	Weak or Yellowing Foliage	Crown Defoliation	Cracks	D-Shaped exit holes	Bird/ Animal Feeding	Peeling Bark in Crown	Bald Spots on Trunk	Epicormic Shotos	Dead Crown

Figure C.8: Visual EAB Surveys field data sheet (available on ***rare*** server).

APPENDIX D: Species Lists

List D.1: Common and scientific names of all butterflies observed at the ***rare*** **Charitable Research Reserve** during all previous butterfly monitoring seasons and annual butterfly counts since 2006. A total of 75 butterfly species have been observed.

Common Name	Scientific Name
Acadian Hairstreak†	*Satyrium acadicum*
Aphrodite Fritillary†	*Speyeria aphrodite*
American Lady	*Vanessa virginiensis*
American Snout†	*Libytheana carinenta*
Appalachian Brown	*Satyrodes appalachia*
Arctic Skipper	*Carterocephalus palaemon*
Baltimore Checkerspot	*Euphydryas phaeton*
Banded Hairsteak	*Satyrium calanus*
Black Dash	*Euphyes conspicua*
Black Swallowtail	*Papilio polyxenes*
Broad-Winged Skipper	*Poanes viator*
Bronze Copper	*Lycaena hyllus*
Cabbage White	*Pieris rapae*
Clouded Sulphur	*Colias philodice*
Columbine Duskywing	*Erynnis lucilius*
Common Buckeye	*Junonia coenia*
Common Sooty Wing	*Philodice catullus*
Common Wood-Nymph	*Cercyonis pegala*
Compton Tortoiseshell	*Nymphalis vaualbum*
Coral Hairstreak	*Satyirum titus*
Crossline Skipper	*Polites origines*
Delaware Skipper	*Anatrytone logan*
Dion Skipper	*Euphyes dion*

Common Name	Scientific Name
Dreamy Duskywing*	*Erynnis icelus*
Dun Skipper	*Euphyes vestris*
Eastern Comma	*Polygonia comma*
Eastern Pine Elfin	*Callophrys niphon*
Eastern Tailed Blue	*Cupido comyntas*
Eastern Tiger Swallowtail	*Papilio glaucus*
European Skipper	*Thymelicus lineola*
Eyed Brown	*Satyrodes Eurydice*
Giant Swallowtail	*Papilio cresphontes*
Grey Comma	*Polygonia progne*
Great Spangled Fritillary	*Speyeria Cybele*
Harvester	*Feniseca tarquinius*
Hickory Hairstreak	*Satyrium caryaevorum*
Hobomok Skipper	*Poanes hobomok*
Indian Skipper*	*Hesperia sassacus*
Inornate Ringlet	*Coenonympha tullia*
Juvenal's duskywing	*Erynnis juvenalis*
Least Skipper	*Ancloxypha numitor*
Leonard's Skipper*	*Hesperia leonardus*
Little Glassywing	*Pompeius verna*
Little Wood-Satyr	*Megisto cymela*
Little Yellow†	*Eurema lisa*
Long Dash	*Polites mystic*

Meadow Fritillary	*Boloria bellona*
Milbert's Tortoiseshell	*Nymphalis milberti*
Monarch	*Danaus plexippus*
Mourning Cloak	*Nymphalis antiopa*
Mulberry Wing†	*Poanes massasoit*
Mustard White	*Pieris oleracea*
Northern Broken-Dash	*Wallengrenia egeremet*
Northern Crescent	*Phyciodes cocyta*
Northern Pearly-Eye	*Enodia anthedon*
Ocola Skipper*	*Panoquina ocola*
Orange Sulphur	*Colias eurytheme*
Painted Lady	*Vanessa cardui*
Pearl Crescent	*Phyciodes tharos*
Peck's Skipper	*Polites peckius*
Question Mark	*Polygonia interrogationis*
Red Admiral	*Vanessa atalanta*
Red-Spotted Purple	*Limenitis arthemis astyanax*
Sachem*	*Atalopedes campestris*
Silver-Bordered Fritillary	*Boloria selene*
Silvery Blue	*Glaucopsyche lygdamus*
Silvery Checkerspot	*Chlosyne nycteis*
Silver-Spotted Skipper	*Epargyreus clarus*
Spring Azure	*Celastrina ladon*
Striped Hairstreak	*Satyrium liparops*
'Summer' Spring Azure	*Celastrina neglecta*
Tawny-Edged Skipper	*Polites themistocles*
Tawny Emperor	*Asterocampa clyton*
Variegated Fritillary	*Euptoieta claudia*
Viceroy	*Limenitis archippus*
White Admiral	*Limenitis arthemis arthemis*
Wild Indigo Duskywing	*Erynnis baptisiae*

†Denotes observation only seen during annual butterfly count
*Denotes incidental observation outside of monitoring or annual count

Table D.2: The earliest record of observation for each butterfly species historically observed at the ***rare* Charitable Research Reserve**. The first date of observation is noted for each previous monitoring year and each annual butterfly count, as well as the overall earliest observation.

Species	**Earliest Record By Year**									Annual Butterfly Counts	Earliest Record at ***rare***
	2006	2009	2010	2011	2012	2013	2014	2015	2016		
Aphrodite Fritillary										July 17 (2016)	July 17 (2016)
Acadian Hairstreak										July 13 (2008)	July 13 (2008)
American Lady			May-20		May-15	May-22	Jul-17	May-20	May-17	July 10 (2010)	May 15 (2012)
American Snout					Jul-11					July 10 (2010)	July 10 (2010)
Appalachian Brown				Jul-06	Jun-18	Jul-02	Jul-03			July 2 (2011)	June 18 (2012)
Arctic Skipper			Jun-03			Jun-04	Jun-03		June-10	July 10 (2010)	June 3 (2010)
Baltimore Checkerspot					Jun-26					July 3 (2011)	June 26 (2012)
Banded Hairstreak	Jul-18	Jul-16		Jul-12	Jun-25	Jul-15	Jul-03	Jun-29	Jul-18	July 2 (2011)	June 25 (2012)
Black Dash			Jun-08		Jul-14	Jul-30	Jul-30	Jul-28	Aug-4	July 10 (2010)	June 8 (2010)
Black Swallowtail	Jul-21	May-20	May-04	May-30	May-14	May-22	May-23	May-20	May-17	July 10 (2010)	May 4 (2010)
Broad-Winged		Jul-24			Jul-14	Jul-12	Jul-18			July 10 (2010)	July 10 (2010)
Bronze Copper	Aug-18				Jun-06		Jun-20	Jun-24	Jun-14	July 2 (2011)	June 6 (2012)
Cabbage White	Jul-18	May-12	May-03	May-19	May-14	May-21	May-21	May-20	May-17	July 2 (2011)	May 3 (2010)
Clouded Sulphur	Jul-18	May-22	May-04	May-31	May-14	May-21	May-24	May-19	May-17	July 10 (2010)	May 4 (2010)
Columbine Duskywing			May-19		May-31			May-19			May 19 (2010)
Common Buckeye				Sep-15	Jun-06						June 6 (2012)
Common Sooty Wing	Jul-21	Jun-02		Aug-04	Jun-07	May-22	Jun-06	May-26	Jul-11	July 10 (2010)	May 22 (2013)
Common Wood-	Jul-18	Jun-16	Jun-25	Jun-14	Jun-18	Jun-13	Jun-19		Jun-29	July 2 (2011)	June 13 (2013)
Compton Tortoiseshell				Jul-12							July 12 (2011)
Coral Hairstreak		Jul-16						Jul-08		July 2 (2011)	July 2 (2011)
Crossline Skipper								Jul-15	Jul-12	July 2 (2011)	July 2 (2011)
Delaware Skipper		Jun-02	May-24	Jul-11	Jul-09	Jul-04	Jul-10	Jul-06	Jul-7	July 10 (2010)	June 2 (2009)
Dion Skipper					Jul-14					July 13 (2008)	July 13 (2008)
Dun Skipper		Jul-24		Jul-06	Jun-26	Jul-12	Jul-04	Jul-08	Jul-12	July 10 (2010)	June 26 (2012)
Eastern Comma	Aug-02	Jun-30	May-14	Jun-01	May-15	May-27	Jun-19	Jun-16	May-19	July 10 (2010)	May 14 (2010)

Species	Earliest Record By Year									Annual Butterfly Counts	Earliest Record at ***rare***
	2006	2009	2010	2011	2012	2013	2014	2015	2016		
Eastern Pine Elfin								May-20			May 20 (2015)
Eastern Tailed Blue	Aug-18			Jul-27	Jul-14	Jul-15	Aug-06	Jun-26	May-30	July 11 (2006)	July 11 (2006)
Eastern Tiger	Jul-18	May-21	May-19	Jun-01	May-14	May-22	Jun-06	May-21	May-24	July 2 (2011)	May 14 (2012)
European Skipper	Jul-18	Jun-24	May-24	Jun-14	May-15	May-30	Jun-10	May-19	Jun-9	July 2 (2011)	May 15 (2012)
Eyed Brown	Aug-02	Jul-16	Jun-15	Jul-05	Jun-08	Jun-25	Jun-20	Jun-16	Jun-9	July 2 (2011)	June 8 (2012)
Giant Swallowtail	Jul-24			Jun-08	May-15	May-30	Jun-10	May-27	May-24	July 11 (2006)	May 15 (2012)
Grey Comma								Jun-22	Aug-2	July 19 (2009)	July 19 (2009)
Great Spangled	Jul-18	Jul-24	Jun-21	Jul-11	Jun-18	Jul-02	Jun-25	Jun-29	Jun-27	July 10 (2010)	June 18 (2012)
Harvester				Aug-19			Jun-21	Jun-22			Aug 19 (2011)
Hickory Hairstreak	Jul-18									July 11 (2006)	July 11 (2006)
Hobomok Skipper			May-26	Jun-01	May-30	Jun-04	Jun-06	May-27	May-31	July 2 (2011)	May 26 (2010)
Inornate Ringlet	Aug-02	Jun-02	May-19	Jun-06	May-14	May-21	Jun-02	May-26	May-24	July 2 (2011)	May 14 (2012)
Juvenal's Duskywing			May-26	May-25	May-14	May-21	May-23	May-20	May-17		May 14 (2012)
Least Skipper				Aug-05	May-28	Jul-30	Jun-19	Jun-23	Jun-14	July 19 (2009)	May 28 (2012)
Leonard's Skipper											Aug 14 (2016)
Little Glassywing				Jul-06	Jul-10	Jul-12	Jun-30	Jul-03	July-18	July 2 (2011)	July 2 (2011)
Little Wood-Satyr	Jul-18	Jun-10	Jun-03	Jun-08	May-30	Jun-04	Jun-10	May-28	Jun-06	July 2 (2011)	May 28 (2015)
Little Yellow										July 11 (2006)	July 11 (2006)
Long Dash				Jun-14	May-28	Jul-04	Jun-27	Jun-17		July 2 (2011)	May 28 (2012)
Meadow Fritillary					Jul-18		Jul-22			July 10 (2010)	July 10 (2010)
Milbert's Tortoiseshell			Jun-21	Jul-19	Jun-11		Jun-16	May-21	Jun-14		June 11 (2012)
Monarch	Jul-18	Jun-22	Jun-25	May-30	May-14	Jun-19	May-30	Jun-11	Jun-09	July 2 (2011)	May 14 (2012)
Mourning Cloak		May-25	May-04	Jun-07	May-14	May-21	May-24	Jun-22	May-17	July 10 (2010)	May 4 (2010)
Mulberry Wing										July 20 (2013)	July 20 (2013)
Mustard White				Aug-12							Aug 12 (2011)
Northern Broken-					Jun-26		Jul-04	Jul-03	Jul-11	July 10 (2010)	June 26 (2012)
Northern Crescent		May-21	Jun-03	Jun-07	Jun-04	Jun-12	Jun-03	Jun-02	Jun-09	July 10 (2010)	May 21 (2009)

Species	Earliest Record By Year									Annual Butterfly Counts	First Record at ***rare***
	2006	2009	2010	2011	2012	2013	2014	2015	2016		
Northern Pearly-Eye	Jul-18	Jun-30	Jun-03	Jun-20	Jun-11	Jun-13	Jun-19	Jun-10	Jul-17	July 10 (2010)	June 3 (2010)
Orange Sulphur	Aug-24		Jun-30	Jul-19	May-14	Jun-04	May-30	Jun-02	Jun-10	July 10 (2010)	May 14 (2012)
Painted Lady		Jun-04	May-04		May-15	May-21	Jul-07	May-19	May-17		May 4 (2010)
Pearl Crescent	Jul-18			May-25	May-14	May-22	May-26	May-19	May-17	July 2 (2011)	May 14 (2012)
Peck's Skipper				Jul-11	Jun-18	Jul-06	Jul-03	Jun-22	July-12	July 2 (2011)	June 18 (2012)
Question Mark	Jul-18	Jun-10	May-19	Jun-07	May-17	Jun-14	Jul-21	May-28	May-19	July 10 (2010)	May 17 (2012)
Red Admiral	Aug-18	May-14	May-03	May-25	May-14	Jun-04	May-21	May-19	May-17	July 10 (2010)	May 3 (2010)
Red-Spotted Purple		Jun-16	Jun-01	Jun-14	May-25	Jun-04	Jun-19	Jun-02	Jun-14	July 10 (2010)	May 25 (2012)
Silver-Bordered Fritillary						Jun-03		May-27	May-16	July 2 (2011)	May 27 (2015)
Silver-Spotted		Jul-30	Jun-08	Jun-20	Jun-25	Jun-13	Jun-13	Jun-11	May-31	July 10 (2010)	June 8 (2010)
Silvery Blue								Jun-02	May-31		June 2 (2015)
Silvery Checkerspot					Jun-20		Jul-18				June 20 (2012)
Spring Azure		May-13	May-04	May-20	May-15	May-21	May-21	May-20	May-17		May 4 (2010)
Striped Hairstreak				Jul-26		Jul-12	Jul-18	Jul-02	July-12	July 11 (2006)	July 02 (2015
Summer Azure	Aug-02	Jul-22	Jun-08	Jul-05	Jun-11	Jun-13	Jun-13	Jun-11	Jun-09	July 2 (2011)	June 8 (2010)
Tawny Emperor	Jul-21	Jul-30		Aug-04	Jul-17	Jul-25	Jul-28	Jul-16	Jul-06	July 10 (2010)	July 16 (2015)
Tawny-Edged		Jul-16		Jul-22		Jul-16	Jul-17	Jun-16	July-05	July 2 (2011)	June 16 (2015
Variegated Fritillary					Jul-05						July 5 (2012)
Viceroy	Aug-02	Jun-10	Jun-08	Jun-20	May-25	Jun-04	May-28	May-26	Jun-21	July 10 (2010)	May 25 (2012)
White Admiral		Jul-14		Jun-14	Aug-01		Aug-18	Jun-22		July 11 (2006)	June 14 (2011)
Wild Indigo Duskywing			May-17		Jul-11		Jul-28	May-28	Jun-09	July 2 (2011)	May 17 (2010)

Table D.3: Common and scientific names with shorthand abbreviations of all salamander species observed at ***rare*** **Charitable Research Reserve** since 2006. The Eastern Red-backed salamander has two colour phases, red- and lead-backed, which are distinguished during sampling.

Common Name	**Scientific Name**	**Abbreviation**
Yellow-spotted Salamander	*Ambystoma maculatum*	YESA
Blue-spotted Salamander	*Ambystoma laterale*	BLSA
Four-toed Salamander	*Hemidactylium scutatum*	FOSA
Eastern Red-backed Salamander*	*Plethodon cinereus*	RESA/LESA

Table D.4: Common and scientific names with shorthand abbreviations of all tree species observed in forest canopy biodiversity monitoring plots at ***rare* Charitable Research Reserve** since 2009.

Common Name	Scientific Name	Abbreviation
American Beech	*Fagus grandifolia*	FAGUGRAN
Black Ash	*Fraxinus nigra*	FRAXNIGR
Black Cherry	*Prunus serotina*	PRUNSERO
Butternut	*Juglans cinerea*	JUGLCINE
Green Ash	*Fraxinus pennsilvanica*	FRAXPENN
Hophornbeam	*Ostrya virginiana*	OSTRVIRG
Red Maple	*Acer rubrum*	ACERRUBR
Red Oak	*Quercus rubra*	QUERRUBR
Sugar Maple	*Acer saccharum*	ACERSACC
White Ash	*Fraxinus americana*	FRAXAMER
White Oak	*Quercus alba*	QUERALBA
White Pine	*Pinus strobus*	PINUSTRO
Yellow Birch	*Betula alleghaniensis*	BETUALLE

APPENDIX E: Annual Butterfly Count Results

Note: 2007 and 2014 Annual Counts were cancelled due to inclement weather. Data from 2006 is missing.

List E.1: Results from Annual Butterfly Count 2015.

The 10th Annual Butterfly Count was held at ***rare*** on July 11, 2015. A total of 31 species and 883 individuals were observed. Data collected from the count was submitted to the North American Butterfly Association and results from the count can be found below.

Black Swallowtail 14, E. Tiger Sw. 3, Cabbage White 247, Clouded Sulphur 162, Coral Hairstreak 1, Banded Ha. 1, E. Tailed-Blue 3, 'Summer' Spring Azure 7, Gr. Spangled Fritillary 20, Pearl Crescent 12, N. Cr. 28, Question Mark 2, Mourning Cloak 8, Red Admiral 13, Red-spotted Admiral 3, N. Pearly-eye 15, Eyed Brown 1, Little Wood-Satyr 24, Com. Wood-Nymph 212, Monarch 5, Silver-spotted Skipper 3, Least Sk. 1, European Sk. 46, Peck's Sk. 10, Tawny-edged Sk. 13, N. Broken-Dash 12, Little Glassywing 1, Delaware Sk. 4, Mulberry Wing 2, Dun Sk. 7. **Unidentified:** Polygonia spp. 3. **Field Notes:** Immatures: Giant Sw. 1 caterpillar on Northern Prickly Ash (recently predated)

List E.2: Results from Annual Butterfly Count 2013.

An annual butterfly count for the North American Butterfly Association was held at ***rare*** on July 20, 2013. A total of 39 species and 429 individuals were observed. The annual butterfly count has occurred yearly on the ***rare*** property since 2006, with the exception of 2007.

Black Swallowtail 7, E. Tiger Sw. 3, Cabbage White 104, Clouded Sulphur 39, Orange Su. 5, Acadian Hairstreak 1, Banded Ha. 1, E. Tailed-Blue 3, 'Summer' Spring Azure 2, Gr. Spangled Fritillary 6, Pearl Crescent 29, N. Cr. 4, Question Mark 1, Mourning Cloak 2, Red Admiral 2, Red-spotted Purple 3, Viceroy 5, Tawny Emperor 4, N. Pearly-eye 2, Eyed Brown 3, Little Wood-Satyr 15, Com. Wood-Nymph 111, Monarch 5, Silver-spotted Skipper 3, Wild Indigo Duskywing 9, European Sk. 2, Peck's Sk. 1, Tawny-edged Sk. 2, N. Broken-Dash 2, Little Glassywing 1, Delaware Sk. 16, Mulberry Wing 3, Hobomok Sk. 1, Broad-winged Sk. 5, Dion Sk. 2, Black Da. 8, Dun Sk. 14. **Unidentified:** skipper species 1, Polygonia species 2. **Total:** 39 species, 429 individuals. **Field Notes:** The previous evening to the count (July 19), the area experienced a large storm. Winds up to 200 mph, significant amounts of rain, thunder, lightning, etc. A good amount of damage was done to trees in the area.

List E.3: Results from Annual Butterfly Count 2012.

Cambridge (*rare* Charitable Research Reserve), ON. Yr. 6, 43.3817°, -80.355°, center at N of Blair Rd. about 1.7 mi E of jct. of Blair Rd. and Fountain St. in Cambridge. See 2006 report for habitats. Imminent threats to habitat: None. Habitat changes since last year: Researchers have planted one area previously which was active agriculture with tall grass prairie. This will be an improvement to habitat. **14 July 2012;** 0900-1500 hrs; sun AM 10%, PM 10%; 82-89°F; wind 2-2 mi/hr. 14 observers in 5 parties. **Total party-hours** 12; **total party-miles on foot** 9. **Observers:** J. Guenther, M. Hulme, S. Hulme, Jessica Linton (245 Rodney Street, Waterloo, ON, N2J 1G7; jlinton@nrsi.on.ca), A. MacNaughton, J. Quinn, G. Richardson, S. Shiplo, A. Turchin, E. Turchin, J. Turchin, B. Wilson, B. Woodman, E. Woodman.

Black Swallowtail 40, Giant Sw. 6, E. Tiger Sw. 18, Cabbage White 169, Clouded Sulphur 39, Orange Su. 29, E. Tailed-Blue 1, 'Summer' Spring Azure 1, Am. Snout 1, Variegated Fritillary 1, Gr. Spangled Fr. 3, Pearl Crescent 7, N. Cr. 2, Question Mark 1, Mourning Cloak 1, Am. Lady 1, Painted La. 4, Red Admiral 12, Com. Buckeye 1, Red-spotted

Purple 4, Viceroy 8, Tawny Emperor 1, N. Pearly-eye 1, Eyed Brown 2, Appalachian Brown 5, Little Wood-Satyr 2, 'Inornate' Com. Ringlet 2, Com. Wood-Nymph 29, Monarch 61, Silver-spotted Skipper 3, Wild Indigo Duskywing 12, European Sk. 1, Peck's Sk. 1, N. Broken-Dash 2, Broad-winged Sk. 2, Dion Sk. 2, Black Da. 12, Dun Sk. 11. **Unidentified:** Skipper Species 3. **Total** 39 species, 501 individuals. **Immatures:** Black Sw. 15 eggs; Am. Snout 1 caterpillar. **Field Notes:** 2012 has been exceptionally dry and hot in southern Ontario.

List E.4: Results from Annual Butterfly Count 2011.

Cambridge (*rare* Charitable Research Reserve), ON. Yr. 5, 43.3817°, -80.355°, center at N of Blair Rd. about 1.7 mi E of jct. of Blair Rd. and Fountain St. in Cambridge. See 2006 report for habitats. **03 July 2011;** 0930-1530 hrs; sun AM 76-100%, PM 76-100%; 24-26°F; wind 7-34 mi/hr. 6 observers in 3 parties. **Total party-hours** 10; **total party-miles on foot** 7. **Observers:** E. Damstra, H. Dodds, B. Foell, Jessica Grealey (709 Keatswood Crescent, Waterloo, ON, N2T 2R6), P. Raspberry, G. Richardson.

E. Tiger Swallowtail 1, Cabbage White 95, Bronze Copper 4, Coral Hairstreak 2, Banded Ha. 3, 'Summer' Spring Azure 3, Silver-bordered Fritillary 2, Pearl Crescent 3, N. Cr. 26, Baltimore Checkerspot 12, Red-spotted Admiral 3, Viceroy 1, Tawny Emperor 2, N. Pearly-eye 13, Eyed Brown 62, Appalachian Brown 3, Little Wood-Satyr 13, Com. Ringlet 4, Com. Wood-Nymph 3, Monarch 10, Wild Indigo Duskywing 1, European Skipper 196, Peck's Sk. 2, Tawny-edged Sk. 5, Crossline Sk. 3, Long Dash 2, Little Glassywing 5, Hobomok Sk. 8. **Total** 28 species, 487 individuals.

List E.5: Results from Annual Butterfly Count 2010.

Cambridge (*rare* Charitable Research Reserve), ON. Yr. 4, 43.3817°, -80.355°, center at N of Blair Rd. about 1.7 mi E of jct. of Blair Rd. and Fountain St. in Cambridge. Floodplain; riparian; agricultural field and hedgerow; open meadow; wet meadow; forested; thicket; alvar; gravel trail; marsh. Habitat changes since last year: A large area has been seeded this year for a tall grass prairie restoration project. This will no doubt increase and improve butterfly habitat within the reserve. **10 July 2010;** 0930-1530 hrs; sun AM 76-100%, PM 76-100%; 68-83°F; wind 2-2 mi/hr. 19 observers in 6 parties. **Total party-hours** 25; **total party-miles on foot** 9. **Observers:** R. Beaubien, T. Beaubien, E. Damstra, S. Fogo, G. Grainge, Jessica Grealey (709 Keatswood Cresent, waterloo, ON, N2T 2R6; jgrealey@nrsi.on.ca), J. Grealey, K. Hodder, L. Lamb, A. MacNaughton, G. Michalenko, C. Moore, G. Richardson, B. Snider, E. Snider, E. Turchin, J. Turchin, W. Watson, M. Wolosinecky.

Black Swallowtail 27, E. Tiger Sw. 6, Cabbage White 187, Clouded Sulphur 93, Orange Su. 3, 'Summer' Spring Azure 2, Am. Snout 1, Gr. Spangled Fritillary 5, Meadow Fr. 1, Pearl Crescent 1, N. Cr. 2, Question Mark 8, E. Comma 2, Mourning Cloak 1, Am. Lady 5, Red Admiral 78, Red-spotted Purple 1, Viceroy 2, Tawny Emperor 4, N. Pearly-eye 18, Eyed Brown 7, Appalachian Brown 2, Little Wood-Satyr 8, Com. Wood-Nymph 73, Monarch 70, Silver-spotted Skipper 1, [1]**Wild Indigo Duskywing 9**, Com. Sootywing 1, Arctic Sk. 1, European Sk. 18, Peck's Sk. 1, Tawny-edged Sk. 6, N. Broken-Dash 1, Little Glassywing 2, Delaware Sk. 3, Broad-winged Sk. 1, [2]**Black Da. 24**, Dun Sk. 5. **Unidentified:** Polygonia sp. 3. **Total** 39 species, 683 individuals. **Field Notes:** [1]This species is widespread in Waterloo Region for the first time in 2010. Previously very rare. [2]Local population known from this area but uncommon in the Region of Waterloo.

List E.6: Results from Annual Butterfly Count 2009.

Cambridge (*rare* Charitable Research Reserve), ON. Yr. 3, 43°22.9'N, 80°21.3'W, center at N of Blair Rd. about 1.7 mi E of jct. of Blair Rd. and Fountain St. in Cambridge. Floodplain; agricultural; old field; cliffs & alvars; hedgerows; old growth forest; early successional; roadside. **19 July 2009;** 1030-1530 hrs; sun AM 11-25%, PM 11-25%; 64-70°F; wind 13-24 mi/hr. 16 observers in 5 parties. **Total party-hours** 24; **total party-miles on foot** 9. **Observers:** E. Damstra, G. Grainge, Jessica Grealey (709 Keatswood Cresent, WAterloo, ON, N2T 2R6), K. Hodder, L. Lamb, C. Moore, I. Moore, S. O'Neil, C. Pomeroy, G. Richardson, J. Shea, V. Slocombe, B. Snider, C. Snider, E. Snider, W. Watson.

Black Swallowtail 1, E. Tiger Sw. 1, Cabbage White 151, Clouded Sulphur 25, Orange Su. 3, Coral Hairstreak 1, Banded Ha. 8, Gr. Spangled Fritillary 4, Pearl Crescent 12, N. Cr. 2, E. Comma 3, Gray Comma 1, Red Admiral 1, Red-spotted Admiral 1, Tawny Emperor 2, N. Pearly-eye 20, Eyed Brown 24, Appalachian Brown 11, Little Wood-Satyr 20, Com. Wood-Nymph 75, Monarch 11, Least Skipper 1, European Sk. 62, Peck's Sk. 1, Tawny-edged Sk. 2, Delaware Sk. 6, Broad-winged Sk. 1, Black Dash 1, Dun Sk. 12. **Total** 29 species, 463 individuals. **Field Notes:** Count originally scheduled for July 18th but was re-scheduled for the 19th. Conditions were not ideal (cool, overcast) but were consistent with the unusually cool and rainy weather experienced in southern Ontario this summer. On average, temperatures are 6 degrees Celsius cooler.

List E.7: Results from Annual Butterfly Count 2008.

Cambridge (*rare* Charitable Research Reserve), ON. Yr. 2, 43°22.9'N 80°21.3'W, center at center N of Blair Rd. about 1.7 mi E of jct. of Blair Rd. and Fountain St. in Cambridge. See 2006 report for habitats. Elevation: 928-928 ft. **13 July 2008**; 0930-1500 hrs; sun AM 76-100%, PM 51-75%; 15-28°F; wind 13-17 mi/hr. 14 observers in 5 parties. **Total party-hours** 6; **total party-miles on foot** 9. **Observers:** E. Barkley, M. Burrell, M. Cassidy, Jessica Grealey (709 Keatswood Cresent, Waterloo, ON N2T 2R6), S. Hentsch, C. Humphrey, K. Jackson, L. Lamb, G. Michalenko, M. Muir, G. Richardson, J. Turchin, M. Wolosinecky, L. Work.

Black Swallowtail 4, E. Tiger Sw. 19, Cabbage White 816, Clouded Sulphur 85, Orange Su. 10, Coral Hairstreak 15, Acadian Ha. 4, Banded Ha. 59, Hickory Ha. 1, Striped Ha. 20, E. Tailed-Blue 2, 'Summer' Spring Azure 2, Am. Snout 2, Gr. Spangled Fritillary 8, Meadow Fr. 2, Pearl Crescent 3, N. Cr. 12, Question Mark 2, E. Comma 1, Mourning Cloak 29, Am. Lady 4, Red Admiral 4, Red-spotted Admiral 12, Viceroy 1, Tawny Emperor 1, N. Pearly-eye 23, Eyed Brown 25, Appalachian Brown 3, Little Wood-Satyr 63, Com. Wood-Nymph 154, Monarch 14, Silver-spotted Skipper 2, European Sk. 127, Peck's Sk. 1, Tawny-edged Sk. 24, Long Dash 1, N. Broken-Da. 3, Delaware Sk. 15, Dion Sk. 2, Black Da. 6, Dun Sk. 8, Polygonia sp. 1. **Total** 42 species, 1,590 individuals. **Note**: Giant Swallowtail butterfly observed at Springbank garden during the summer of 2008

APPENDIX F: Milkweed Monitoring

A description of the protocol can be found on the ***rare*** server: Z:\LEVEL4\RESEARCH & MONITORING\ECOLOGICAL MONITORING\MILKWEED MONITORING

Figure F.1: Map of ***rare*** property showing the four study sites delineated in red. The sites are as follows; 1 - Northern portion of Blair Flats, 2 - The Butterfly Meadow in Thompson Tract, 3 – The field adjacent to the Community Gardens, 4 – Field adjacent to ECO Centre.

		2015		2016	
Study Site	Potential Milkweed Area (ha)	Milkweed Stem Density (#individuals/m²)	Estimated Total Stems at Site	Milkweed Stem Density (#individuals/m²)	Estimated Total Stems at Site
Blair Flats	1.91	0.25	4775	0.14	2674
Thompson Tract	1.63	5	81500	2.93	47759
Community Gardens	2.08	0.18	3744	0.13	2704
ECO Centre	0.79	0.76	6004	0.34	2686

Table F.1: Summary of total potential area milkweed was found, stem densities, and estimates of total number of Milkweed stems for each of the four study areas in 2015 and 2016

APPENDIX G: Emerald Ash Borer Assessment Protocol and Guides

List G.1: Revised EAB Monitoring Protocol

1. Plot selection should be determined for each site using at largest a 75 by 75 m to produce possible plot locations. Plot locations occur at corners of grid squares, unless corners fall in areas that are not appropriate for sampling (such as plantations, creek beds, and trails), in which case that plot will not be included.
2. GPS coordinates will be used to find plot locations within forest stands. Upon location of plots, place a pigtail stake in the centre.
3. If no ash trees are present within a 10 m radius of the centre point, identify all species within the 10 m radius, and record the number of each species present. DBH measurements need only be taken if there is a question of whether an individual has a DBH>10 cm. No rejuvenation/ invasive species survey is conducted.
4. If ash trees are present within a 10 m radius of the centre point:
    a. A complete forest inventory should be conducted. Within a 10 m radius, identify all live trees ≥ 7 cm DBH (1.30m) by species and record the cardinal orientation from, as well as distance to, the center point. Take DBH measurements of each tree using DBH tape. For trees with a leaning less than 45°, DBH was measured at 1.30 meter stem length instead of height and in case of significant diameter anomalies (e.g. canker), take the measurement at its lowest point). Apply the same procedure to standing coarse woody debris at all degrees of decay, if clearly identifiable as ash.
    b. Complete forest and rejuvenation/invasive species inventories should be conducted. Beginning at the centre point, identify all individuals of a given species (if more than three individuals of a species was present) within a 3 meter radius and grade each individual with a six point height system (1 = 0-20cm; 2 = 21-40cm; 3 = 41-80cm; 4 = 81-130cm; 5 = 131-199cm, 6= >200cm). Estimate canopy cover over plots within a 3 meter radius using 5 % increments.
    c. The pigtail stake at the centre of the plot should be flagged with flagging tape, labelled with plot number, and left in the plot.
5. Based on a protocol for ash trees in urban areas provided by the University of Toronto (Melamed & Zhou, 2012), a variety of criteria were considered as indicators of Emerald Ash Borer presence. Complete a visual scan for these indicators in each forest plot that contains ash trees. Criteria are available in Appendix A, Table 1. An identification guide from the University of Toronto Emerald Ash Borer Management Plan can also be used as reference material (Appendix A, Figure 1).
6. Plots with ash trees should be revisited every two to three years and EAB visual surveys should be conducted.
7. Data sheets can be found in Appendix C, and field equipment list can be found in Appendix D.

Table G.1: Ash condition criteria (crown reduction, unbalanced crown, weak/yellowing foliage, crown defoliation, cracks, exit holes, bird/animal feeding, debarking, bald spots, epicormic shoots and dead crown) – Edited version of Emerald Ash Borer Management Plan (Appendix II) University of Toronto (Melamed & Zhou, 2012).

Crown Reduction

0 There are no signs of permanent crown reduction within the typical shape.
1 Less than 25 percent of the crown volume is missing.
2 25 to 50 percent of the crown volume is missing.

Unbalanced Crown

0 There are no signs of imbalanced or lopsided crown.
1 Crown slightly asymmetrical due to restricted growing space and light conditions.
2 Crown is asymmetrical, unbalanced or lopsided. Between 75 and 90 percent of crown is on one side of the stem.
3 Crown is severely asymmetrical with more than 90 percent of crown volume on one side.

Weak or Yellowing Foliage

0 Leaves are of normal size, color and texture.
1 Leaves appear to be somewhat smaller and/or pale in color.
2 Leaves are significant smaller than what is normal and/or pale foliage. Crown is significantly more transparent than what is expected.
3 Leaves are dramatically smaller than what is normal and/or very pale. Crown is very transparent; the tree appears to be in severe decline.

Crown Defoliation

0 Crown shows no signs of defoliation (expected minor decline excluded)
1 Crown is slightly defoliated with less than 25 percent loss.
2 Crown is moderately defoliated with between 25 and 50 percent loss.
3 Crown is severely defoliated with more than 50 percent loss.

Cracks

0 Tree does not have major cracks on either stem or major branches.
1 One minor crack extends into stem or major branch (less than ½ diameter in debt, but entering the woody part).
2 Two or more minor cracks are located in the same general area of the stem. No other obvious stem defects.
3 One or more major cracks (beyond ½ of diameter in debt), cracks in horizontal direction.

D-shaped Exit Holes

0 Exit holes are not visible at eye height or are not clearly D-shaped.
1 D-shaped exit holes are existing at eye height (photographic record)

Bird/Animal Feeding

0 No signs for animal feeding
1 Clear evidence for vertebrate feeding (e.g. enlarged exit holes, or torn and ragged bark)

Peeling Bark in the Crown

0 There are no signs of peeling bark.
1 Less than 25 percent of branches show signs of peeling or missing bark.
2 Between 25 and 50 percent of branches show signs of peeling or missing bark.
3 More than 50 percent of branches show signs of peeling or missing bark.

Bald Spots on Trunk (If clearly not caused by an animal)

0 Trunk shows no signs of bald spot (some traces are to be expected).
1 Trunk shows traces of bald spot (beyond normal).
2 1/3 of the trunk shows signs of balding.
3 More than 1/3 of the trunk shows signs of balding.

Epicormic Shoots

0 There are no epicormic shoots.
1 Some epicormic shoots are established or freshly grown, but make up less than 10 percent of the total (green) canopy.
2 Epicormic shoots make up 10 to 40 percent of the total (green) canopy.
3 Epicormic shoots make up more than 40 percent of the total (green) canopy.

Dead Crown

0 Crown is typically foliated.
1 Entire crown, or more than 50 percent from the top are dead (completely defoliated)

IDENTIFYING ASH TREES

Take this guide to each tree on your property to identify ash

Ridged Bark:
On mature trees (left), bark is tight and displays patterns of diamond shaped ridges. On young trees (right), bark is relatively smooth.

Compound 'Opposite' Leaves:
Leaves contain 5 to 11 leaflets with smooth or toothed margins (tips). Leaflets are positioned opposite with one at the top.

Seeds:
When present, seeds usually hang in clusters and are dry and oar-shaped.

'Opposite' Branches:
Branches and buds are directly across from each other rather than staggered. However, due to the death and grooming of individual branches, it is possible that not every branch will be opposite.

WHAT IS THE EMERALD ASH BORER?

The Emerald Ash Borer is a metallic green wood-boring beetle of about 1 to 1.5 cm in length that attacks all native species of ash trees, typically killing them in 2 to 3 years. Its larva bore tunnels inside the tree, feeding off the inner bark until the tree dies.

Native to northeastern Asia, the pest was first discovered in Ontario in the Windsor area in 2002. Since then, infested ash trees have been discovered in Essex, Lambton, Elgin and Middlesex Counties, and in the Municipality of Chatham-Kent.

RECOGNIZING INFESTED ASH TREES

Infested ash trees often exhibit the following symptoms

Crown Dieback:
Severely attacked trees may exhibit crown dieback as the canopy dies from the top down. Leaves may wilt or turn yellow during the growing season.

Bark Cracks:
Vertical splits of 7 - 10 cm are often present over larval galleries. These are often more noticeable on young trees that do not already have splits from growth-related expansion.

Woodpeckers:
Woodpeckers feed on the larvae under the bark. Look for increased Woodpecker feedings or signs of their probing in the bark.

Exit Holes:
Once fully mature, the adult beetles emerge through exit holes they chew through the bark. These holes are distinctly D-shaped and are 3.5 to 4 mm across.

Tunnels:
Winding S-shaped larval tunnels snake under the bark where larvae bore channels. Removing the bark exposes larvae and sawdust-filled galleries.

Photos courtesy of Michigan State University, Forestry Images (www.forestryimages.org).

How to Identify Ash Trees, Infested Trees and the Emerald Ash Borer

Figure G.1: Identification guide (Introduction) - Emerald Ash Borer Management Plan (Appendix I) University of Toronto (Melamed & Zhou, 2012].

APPENDIX H: Additional Data

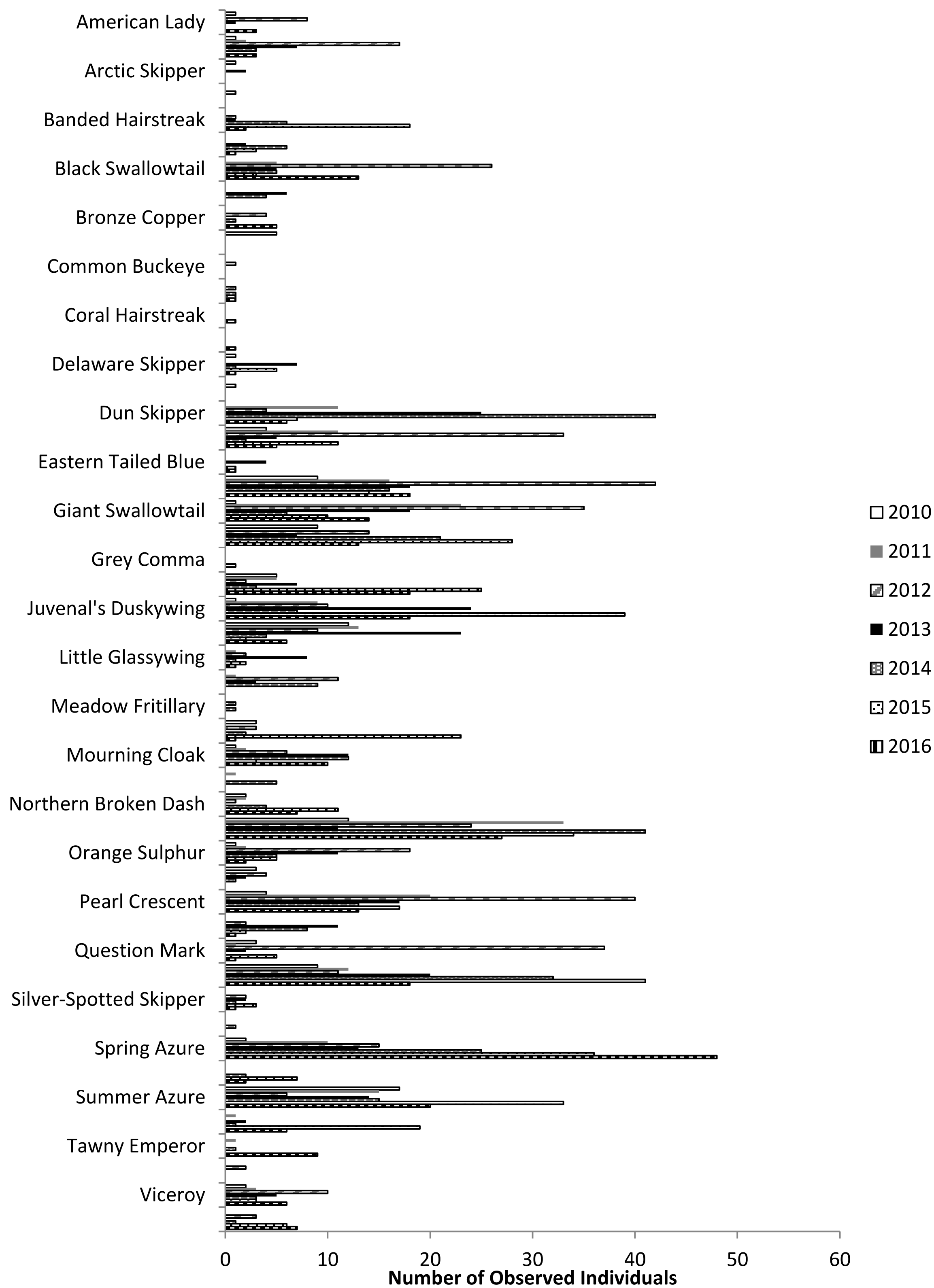


Figure H.1: Comparison of the species observed on Transect One during 2010-2016 monitoring years. Only species with between 1 and 50 observations are shown.

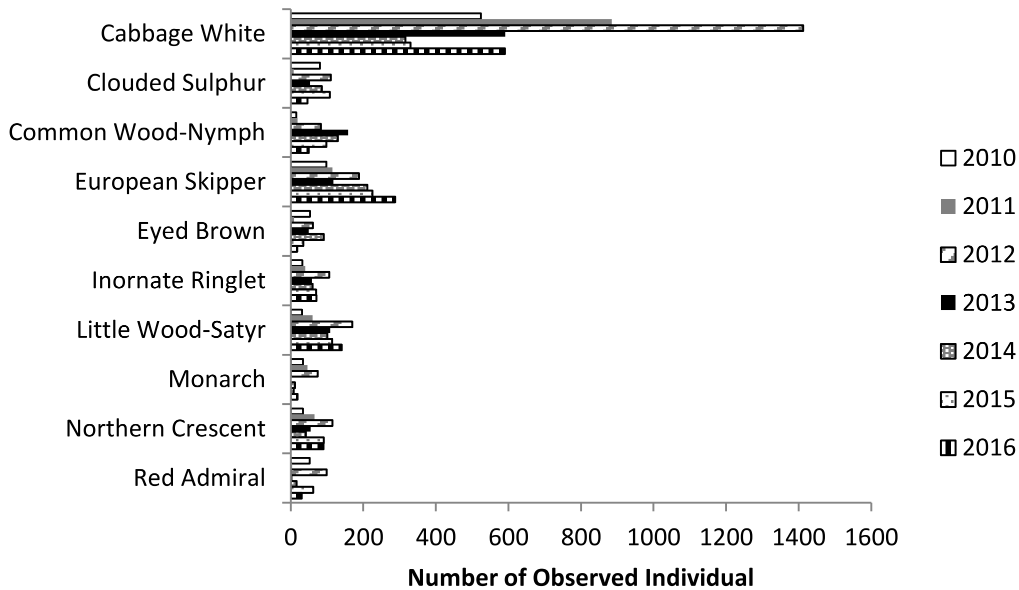


Figure H.2: Comparison of the species observed on Transect One during 2010-2016 monitoring years. Only species which have had more than 50 observations in a given year are shown.

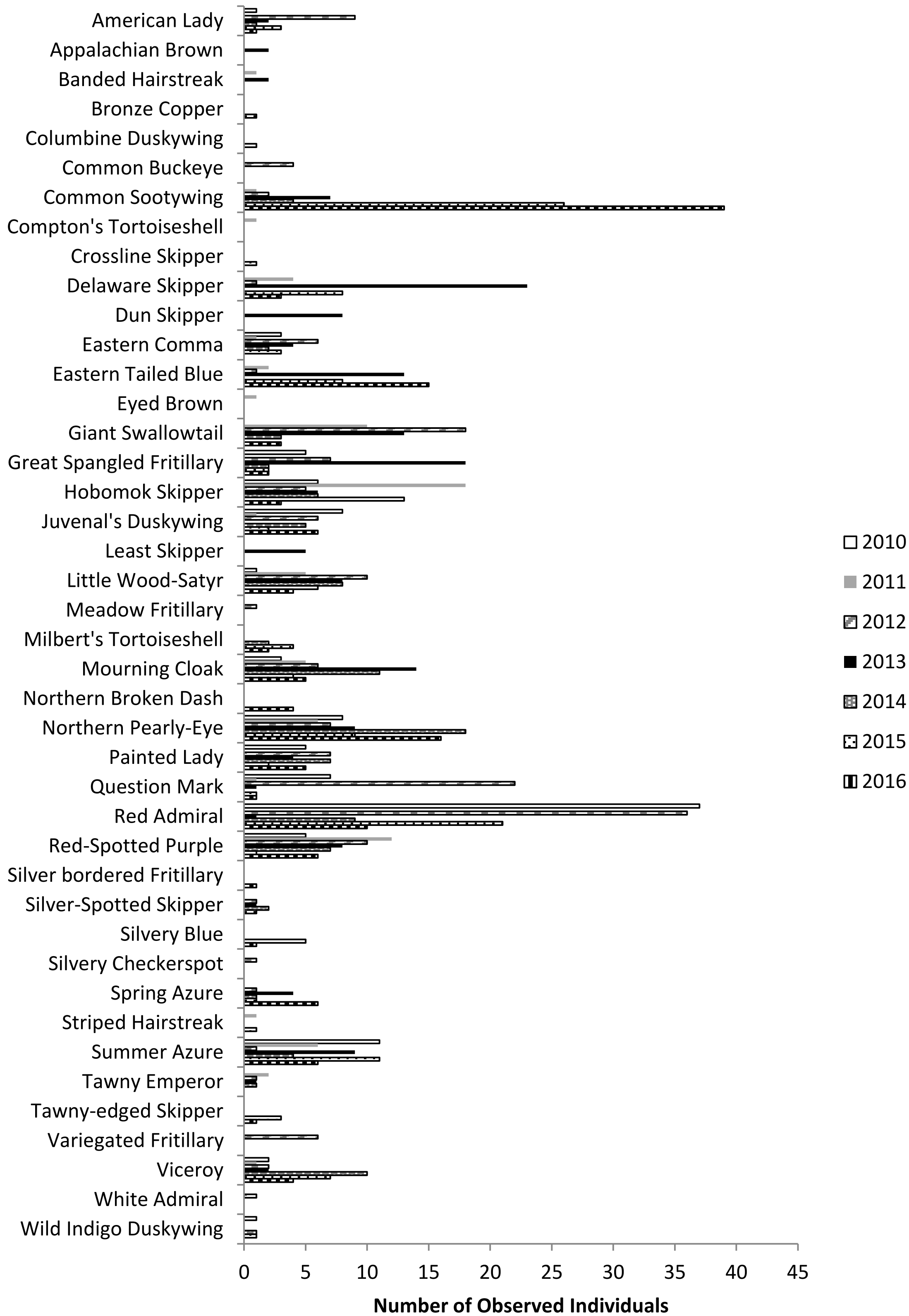


Figure H.3: Comparison of the species observed on Transect Two during 2010-2016 monitoring years. Only species with between 1 and 50 observations are shown.

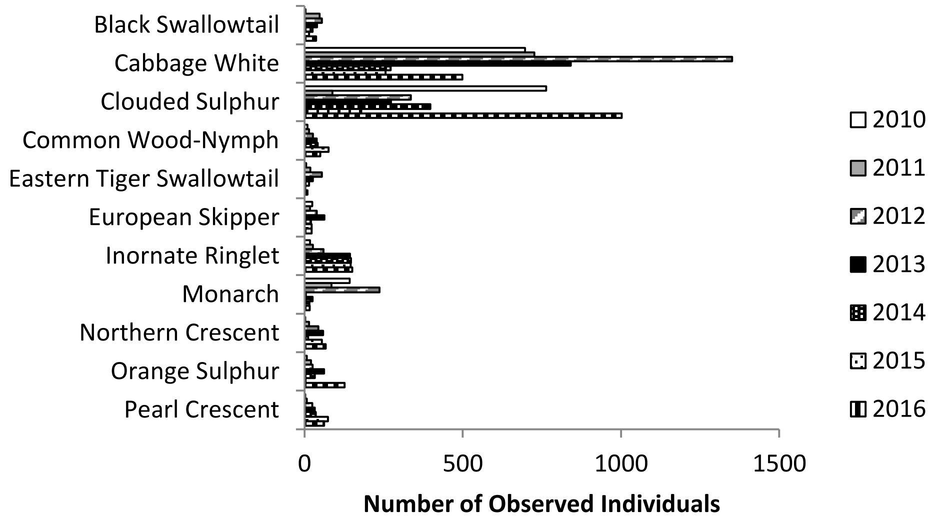


Figure H.4: Comparison of the species observed on Transect Two during 2010-2016 monitoring years. Only species which have had more than 50 observations in a given year are shown.

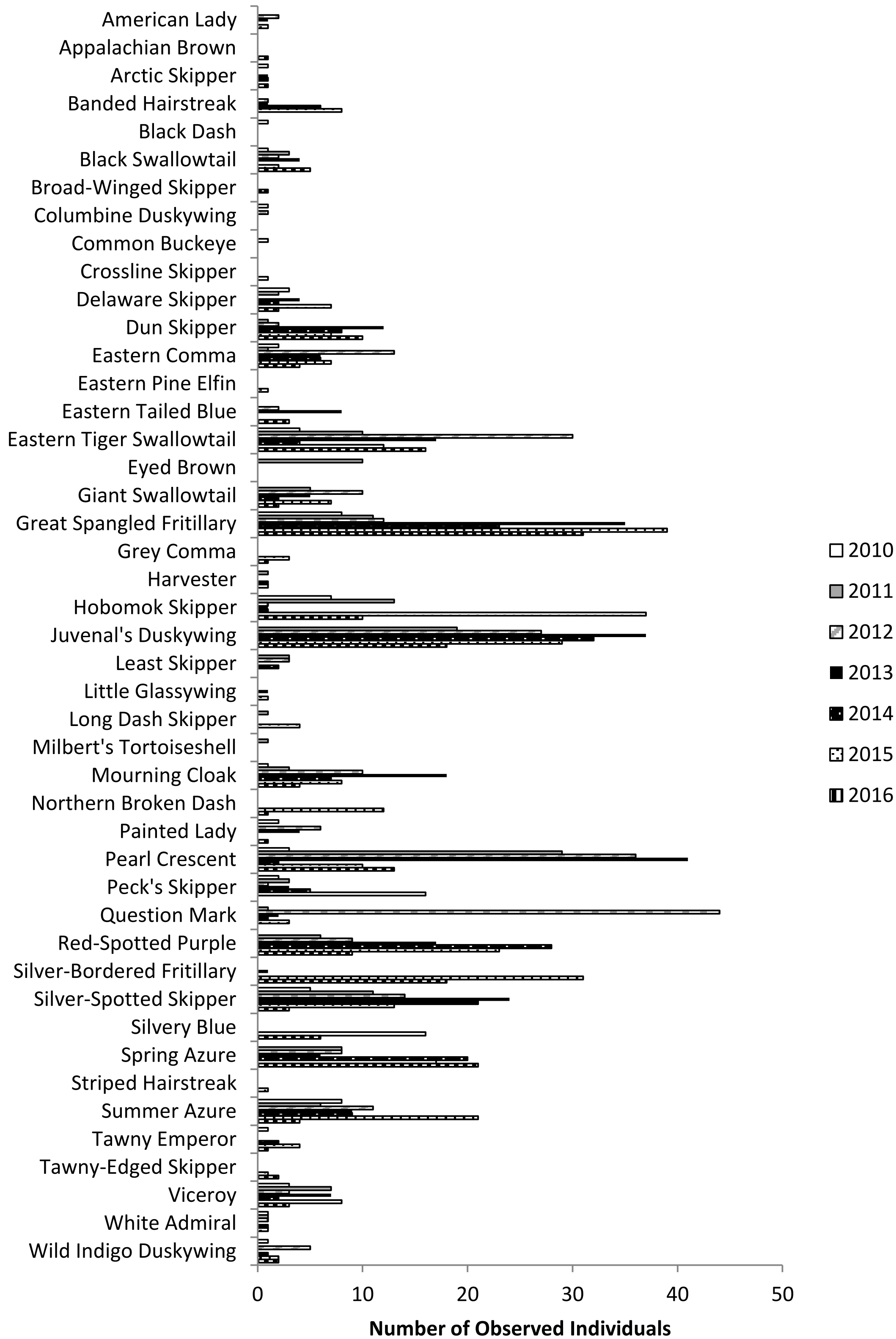


Figure H.5: Comparison of the species observed on Transect Three during 2010-2016 monitoring years. Only species with between 1 and 50 observations are shown.

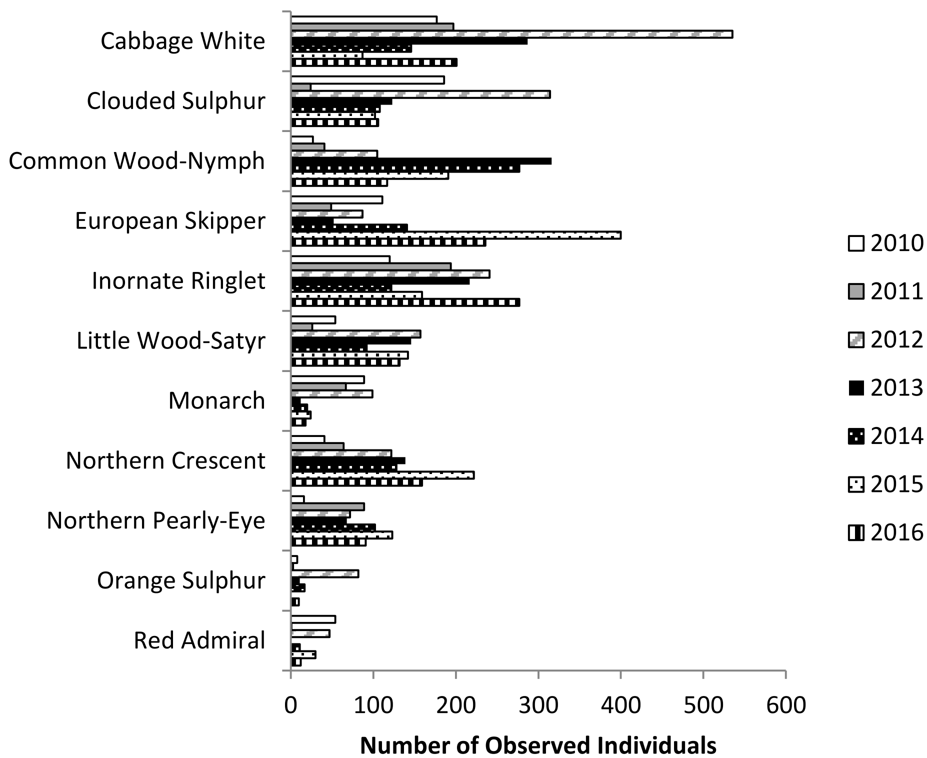


Figure H.6: Comparison of the species observed on Transect Three during 2010-2016 monitoring years. Only species which have had more than 50 observations in a given year are shown.

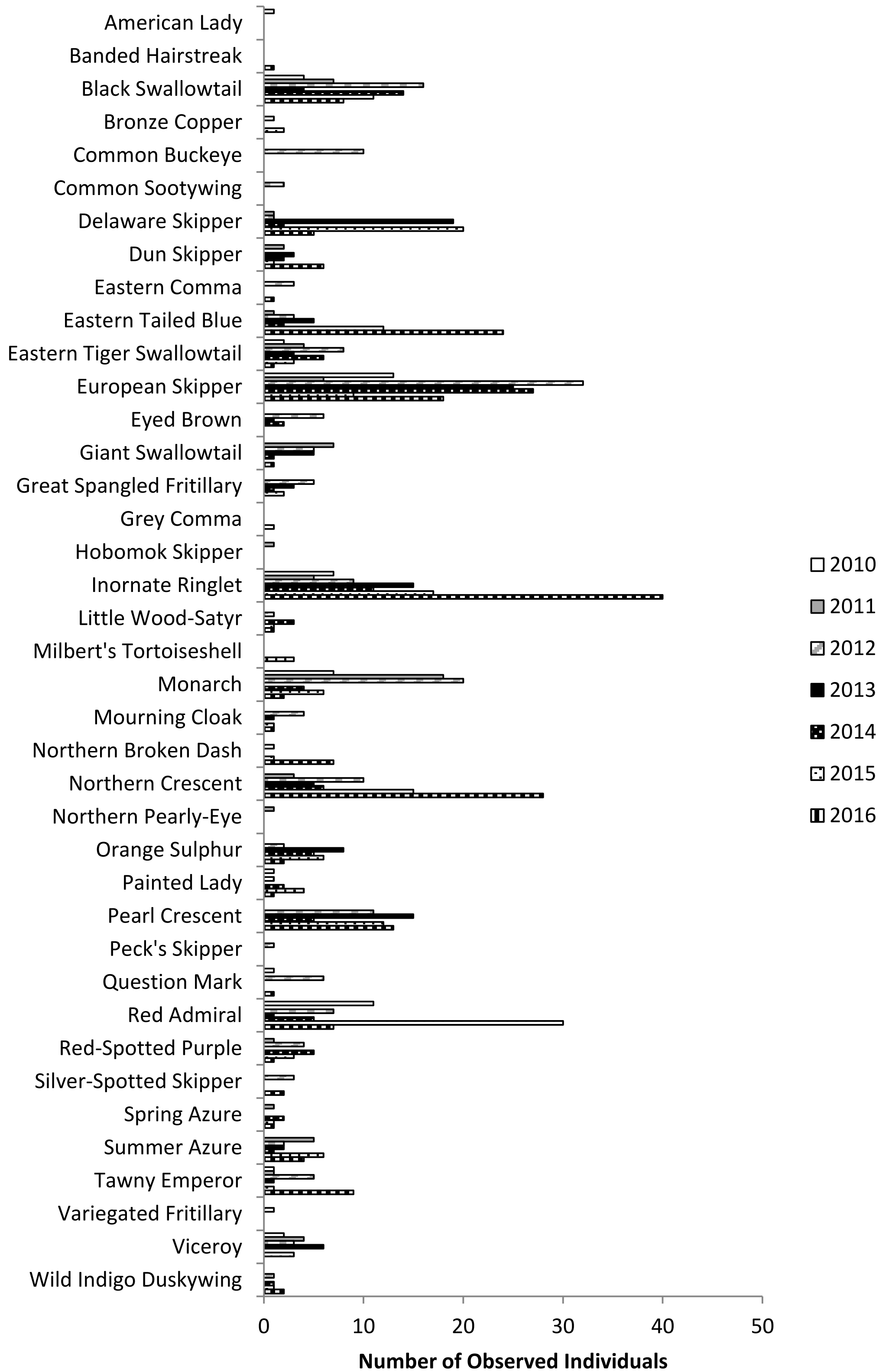


Figure H.7: Comparison of the species observed on Transect Four during 2010-2016 monitoring years. Only species with between 1 and 50 observations are shown.

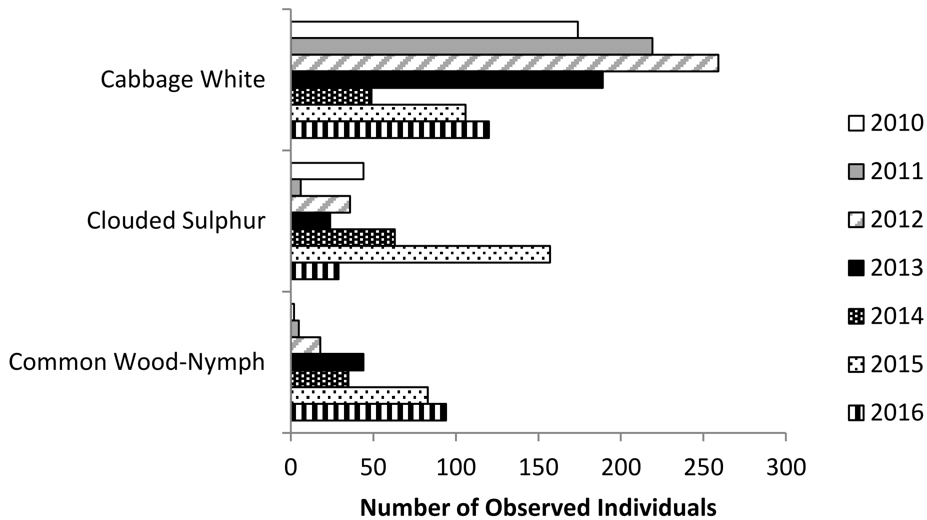


Figure H.8: Comparison of the species observed on Transect Four during 2010-2016 monitoring years. Only species which have had more than 50 observations in a given year are shown.