

Ecological Monitoring 2013  
**rare** Charitable Research Reserve



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### **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
IV	Importance Value
SARA	Species at Risk Act
SARO	Species at Risk in Ontario
ADR	Annual Decay Rate

## 1.0 INTRODUCTION

Prepared by: Jenna Quinn & Amy Reinert

### 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 (Figure A.1).

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 2013 monitoring year are reported and discussed.

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## 2.0 BUTTERFLY MONITORING

Prepared by: Amy Reinert

### 2.1 Introduction

#### 2.1.1 *Lepidoptera Taxonomy*

The order Lepidoptera consists of butterflies and moths, which differ from most other insects by having scales over most or all of their wings. Moths and butterflies are distinguished from each other by their body structure and habits. Butterflies are primarily diurnal, tend to rest with their wings folded vertically and have clubbed antennae, while moths mostly fly at night, tend to fold their wings flat when at rest and lack antennal clubs (Pyle 1981). Butterflies consist of five true butterfly families (*Papilionidae*, *Pieridae*, *Lycaenidae*, *Nymphalidae*, and *Riodinidae*) and the skipper family (*Hesperiidae*). True butterflies tend to have narrow bodies, long antennae and brightly coloured wings, while the skippers are often stocky, compact and hairy (Pyle 1981).

#### 2.1.2 *Why Monitor Butterflies?*

The long term monitoring of butterfly populations can provide valuable information about overall ecosystem health and environmental change for many reasons. The short lifespan of butterflies results in a relatively rapid response to environmental change (Thomas 2005; van Swaay et al. 2006) and also allows for the observation of several generations in a relatively short period of time. In addition, their large size and differentiating colours make butterflies easy to observe and identify, and therefore monitor. Butterflies also tend to invoke a positive response from the general public, which allows for easier recruitment of volunteers for butterfly monitoring programs (De Heer et al. 2005; Thomas 2005).

Because many butterfly species require specific host plants for mating, oviposition, and feeding as both larva and adults, they have specific habitat requirements throughout their various life stages. Many female butterflies only oviposit on a specific plant species and the absence of this species may be detrimental to the production of a succeeding butterfly generation. This dependence on habitat type and quality suggests that an observed change in butterfly populations can be indicative of a change in their habitat. Habitat loss and the replacement of native plants with exotic species can contribute to declining butterfly populations by altering the availability of specific host and food plants.

In addition to habitat, butterflies are also sensitive to both the local weather conditions (Wikstrom et al. 2009) and the global climate (Warren et al. 2001). In order to fly, butterflies require a wing temperature of at least 25°C, which is most often achieved during warm, clear weather. As a result, harsh weather events and unusually cold or rainy weather can impact the overall health, development, and reproduction of butterfly populations. On a larger scale, increasing global temperatures may lead to an expansion or shift of the traditional range of some butterfly species (Parmesan 2006). Therefore, determining the distribution of butterfly species in Canada can provide information on environmental changes across the country.

#### 2.1.3 *Butterfly Monitoring at rare Charitable Research Reserve*

The EMAN standardized protocol for long term butterfly monitoring was developed and piloted at **rare** in 2006 and led to the implementation of a butterfly monitoring program on the property. This program aims to record and identify long term trends in butterfly abundance and diversity. The standardized collection of data allows for accurate comparison of data over time, which can lead to the identification of long term population trends and can act as an early warning signal for environmental change (van Swaay et al. 2006).

Baseline data for the monitoring program was collected during the pilot season in 2006 and monitoring has continued yearly from 2009 to 2013. In 2006, monitoring occurred over a five week period on the Cliffs and Alvares and the South Field/Sparrow Field transects. The 2009 monitoring season expanded to a period of thirteen weeks and occurred on three transects, including the newly developed Thompson Tract transect. Monitoring from 2010 to 2013 took place over a fourteen week period and included all four transects, including the Blair Flats transect that was established in 2010.

## 2.2 Methods

### 2.2.1 Transect Descriptions

Butterfly monitoring occurred weekly along four established transects at the **rare Charitable Research Reserve**. Property maps, transect locations and detailed transect descriptions can be found in Appendix A.

The Cliffs and Alvares transect is 3.5 km and follows the Riverside and Grand Trunk trails. It primarily consists of mature hardwood stands that are dominated by American Beech (*Fagus grandifolia*) and Sugar Maple (*Acer saccharum*). It also contains deciduous swamps, limestone cliffs, open alvar habitats, and passes through an extensive floodplain.

The South Field/Sparrow Field transect is 3.4 km and runs along the edge of agricultural fields, hedgerows, and the south east perimeter of Indian Woods. Several fields in this area are in agricultural production, with a soybean crop in South Field East and a wheat clover crop in South Field West in 2013. As a result of several ongoing research projects, Sparrow field has gradually been removed from agricultural production and restored to native vegetation. South Field West and South Field East are located at the southern boundary of the **rare** property.

The Thompson Tract transect is 2.2 km and follows established trails through meadows, forest plantations, and lowland and upland forests that are 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 follows the boundary of a restored tallgrass prairie field. This field was previously in agricultural production but was planted as a tallgrass prairie in 2010 as part of a long term restoration study. This transect begins at the large Bur Oak (*Quercus macrocarpa*) near Blair Road and continues north, stopping at the regenerating portion of the field. It then runs west toward the **rare** property boundary, continues south towards the road and then follows the road back to the starting point. This area was dominated by Tansy (*Tanacetum vulgare*), Queen Anne's Lace (*Daucus carota*), Black Eyed Susan (*Rudbeckia hirta*), and various grass species during the monitoring season.

### 2.2.2 Monitoring Protocol

Butterfly monitoring at the **rare Charitable Research Reserve** is conducted using the Transect Walk Method (Pollard 1977; Pollard & Yates 1993). This method is one of the most commonly used monitoring methods around the world (Pollard & Yates 1993; Grealey 2006; Van Swaay et al. 2008). Unlike most butterfly monitoring methods, it does not disturb or influence butterfly behaviour, or require a lot of time and effort. The Transect Walk Method involves walking an established route, or transect, at a uniform pace over a specified period of time while recording the butterflies observed within a given radius (Pollard 1977).

The most effective butterfly monitoring programs would occur over a twenty six week period from April to September (Layberry et al. 1998) but as a result of time and monetary constraints, the monitoring period at **rare** has been reduced. The monitoring that occurred in 2010 and in all subsequent



years occurred over a fourteen week period from mid-May to mid-August. Monitoring begins on the third Monday of May, however the spring weather conditions can either advance or delay the monitoring period by one week if there are particularly warm or cold conditions. Butterfly monitoring should take place during the warmest part of the day, between 10:00am and 3:00pm, when butterflies are the most active (Grealey 2006). In addition, the ideal temperature for monitoring is 13°C or greater if there is at least 60% sunshine, or more than 17°C if overcast but not raining (UK Butterfly Monitoring Scheme; Butterfly Monitoring Scheme Germany) and the wind speed should be less than a force of five on the Beaufort Wind Scale (Grealey 2006).

Butterfly monitoring took place on four transects located throughout the *rare* property. Each transect is broken into sections based on changes in habitat, which are described in Appendix A. Prior to the monitoring season, the observer walked all transects and used flagging tape to mark section breaks as required. Monitoring began on the third Monday in May and each transect was walked once weekly for a total of fourteen consecutive weeks. During the monitoring period, observations were recorded during optimal weather conditions but in the absence of rain, the data collected during suboptimal weather conditions would be more valuable than not collecting data. In order to minimize observer bias, all monitoring was conducted by the same individual with occasional assistance from volunteers.

At the beginning and end of each transect the time and air temperature, which was determined with a hand-held Kestrel 3000© (Nielson-Kellerman, Boothwyn, PA, USA), were recorded. Each transect was walked once a week at a uniform pace, with the observer recording all butterflies observed within a 10 m radius. Ten minute stops for stationary observations occurred at the half way point of each section within each transect. Coordinates for section stops can be found in Appendix A. At each section stop, the percent cloud cover (0-100%) was estimated and the wind speed was determined based on the Beaufort Wind Scale using the Kestrel 3000©. While walking, temporary stops were permitted to catch butterflies to aid in identification. When stops were made, recording continued from where the stop initially occurred. Butterflies were visually identified in the field and species that could not be identified were photographed and sent to local experts for identification. In the absence of experts or photographs, the unknown butterfly was recorded as the most common of all possible species. The observer carried a butterfly net and digital camera to aid in identification at all times. A suggested list of field equipment and a sample data sheet can be found in Appendix B.

### *2.2.3 Data Analysis*

All data analysis was conducted using SPSS Statistics Version 21.0. Due to the varying length and habitat type of each transect, direct analysis between transects would not produce an accurate comparison of butterfly populations. Therefore, butterfly abundance was compared across years within transects to detect trends over time. Within transects, the number of individuals observed were analyzed using a negative binomial log link generalized linear model. Pairwise comparisons were performed following significant results to determine which years were significantly different.

In addition, the Shannon Diversity Index and species evenness value were calculated for each transect in 2013 (Figure 2.1). The Shannon Diversity Index takes into account both the number of species in a population and the evenness with which individuals are distributed among those species. A value of zero indicates low diversity, a value of four indicates high diversity, and real world values are typically between 1.5 and 3.5.

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

**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 species.

**Figure 2.1:** Formulas used for calculating the Shannon Diversity Index and species evenness value.

## 2.3 Results

### 2.3.1 Overall Abundance and Diversity

During the 2013 monitoring season, a total of 46 species and 5260 individual butterflies were observed on all four transects at the **rare Charitable Research Reserve**. The most abundant species observed in 2013 were the Cabbage White (n=1909), Common Wood-Nymph (n=557), Clouded Sulphur (n=473), Inornate Ringlet (n=435), and Little Wood-Satyr (n=263). The Cabbage White, Clouded Sulphur, and Inornate Ringlet were also considered dominant species during the 2012 monitoring season. The total number of observations of the Common Wood-Nymph had increased by 139% compared to the 2012 monitoring season and reached the highest abundance ever recorded. In contrast, the total number of observations of the Monarch had decreased by 96% compared to the 2012 monitoring season and reached the lowest abundance ever recorded during the monitoring program. Observations of the Red Admiral had also decreased by 96% compared to the 2012 monitoring season.

Table 2.1 compares the number of individuals observed, species richness, Shannon Diversity Index and species evenness value for each transect and monitoring season since 2009. Table 2.2 displays all butterfly observations for each transect during the 2013 monitoring season with the Waterloo Regional Status for each species.

**Table 2.1:** Comparison of butterfly observations for each transect and every 14 week monitoring season since 2009. The total number of individuals and species observed during the monitoring period, the calculated Shannon Diversity Index and calculated species evenness values are included.

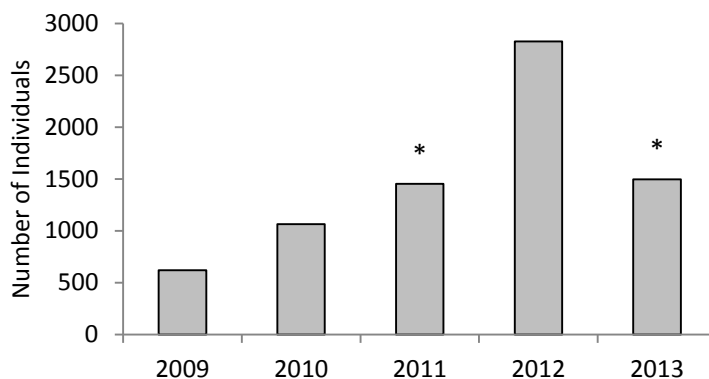
Measures	Transect One					Transect Two					Transect Three				Transect Four			
	2009	2010	2011	2012	2013	2009	2010	2011	2012	2013	2010	2011	2012	2013	2010	2011	2012	2013
Number of Individuals (n)	620	1063	1453	2826	1494	717	1778	1146	2427	1751	938	911	2116	1636	270	298	497	381
Species Richness (S)	25	33	35	46	43	24	26	30	37	35	30	35	38	36	14	20	35	21
Species Evenness (S)	0.59	0.59	0.50	0.57	0.65	0.52	0.44	1.47	0.49	0.57	0.70	0.72	0.71	0.71	0.49	0.42	0.60	0.63
Shannon-Diversity Index (H)	1.90	2.07	1.77	2.19	2.45	1.65	1.42	1.60	1.76	2.02	2.37	2.56	2.56	2.55	1.30	1.26	2.12	1.93

**Table 2.2:** Summary of butterfly observations for each transect during the 2013 monitoring season. The Waterloo Regional Status of each observed species is included (Grealey et al. 2010).

Species	Transect				Total	Regional Status
	1	2	3	4		
American Lady	1	2	1	0	4	Common
Appalachian Brown	7	2	0	0	9	Uncommon
Arctic Skipper	2	0	1	0	3	Rare
Banded Hairstreak	1	2	1	0	4	Uncommon
Black Dash	2	0	0	0	2	Uncommon
Black Swallowtail	5	40	4	4	53	Very Common
Broad-Winged Skipper	6	0	0	0	6	Common
Cabbage White	591	842	287	189	1909	Very Common
Clouded Sulphur	53	273	123	24	473	Very Common
Common Sootywing	0	7	0	0	7	Rare
Common Wood-Nymph	158	39	316	44	557	Very Common
Delaware Skipper	7	23	4	19	53	Common
Dun Skipper	25	8	12	3	48	Very Common
Eastern Comma	5	4	6	0	15	Very Common
Eastern Tailed Blue	4	13	8	5	30	Uncommon
Eastern Tiger Swallowtail	18	27	17	3	65	Very Common
European Skipper	118	63	52	25	258	Very Common
Eyed Brown	50	0	0	1	51	Very Common
Giant Swallowtail	18	13	5	5	41	Uncommon
Great Spangled Fritillary	7	18	35	3	63	Very Common
Hobomok Skipper	7	6	1	0	14	Common
Inornate Ringlet	59	144	217	15	435	Common
Juvenal's Duskywing	24	0	37	0	61	Rare
Least Skipper	23	5	0	0	28	Uncommon
Little Glassywing	8	0	1	0	9	Uncommon
Little Wood-Satyr	109	8	146	0	263	Very Common
Long Dash Skipper	3	0	0	0	3	Uncommon
Monarch	1	4	12	0	17	Very Common
Mourning Cloak	12	14	18	1	45	Very Common
Northern Crescent	55	59	139	5	258	Uncommon
Northern Pearly-Eye	11	9	68	0	88	Common
Orange Sulphur	11	62	11	8	92	Very Common
Painted Lady	2	4	4	0	10	Common
Pearl Crescent	17	33	41	15	106	Common
Peck's Skipper	11	0	3	0	14	Very Common
Question Mark	2	1	2	0	5	Very Common
Red Admiral	5	1	0	1	7	Very Common
Red-Spotted Purple	20	8	17	0	45	Common
Silver-Bordered Fritillary	0	0	1	0	1	Rare
Silver-Spotted Skipper	2	1	24	0	27	Uncommon
Spring Azure	13	4	6	0	23	Common
Striped Hairstreak	2	0	1	0	3	Uncommon
Summer Azure	14	9	9	2	34	Very Common
Tawny Emperor	0	1	0	1	2	Uncommon
Tawny-Edged Skipper	2	0	0	0	2	Common
Viceroy	5	2	7	6	20	Very Common
<b>TOTAL</b>	<b>1496</b>	<b>1751</b>	<b>1637</b>	<b>379</b>	<b>5263</b>	

### 2.3.2 Transect One: Cliffs and Alvares

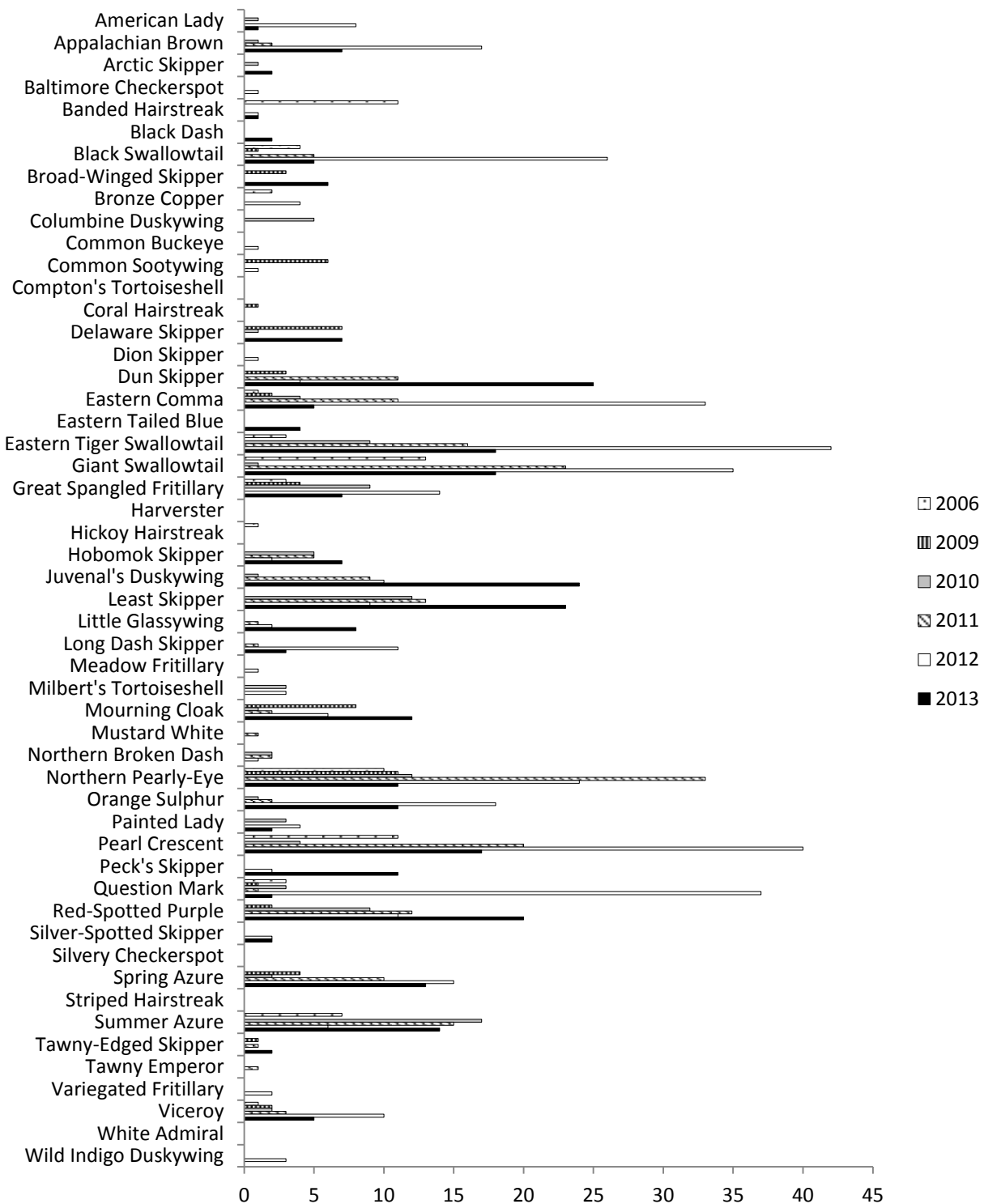
During the 2013 monitoring season, 1494 individuals and 43 species were observed in Transect One (Table 2.1). The total number of individuals observed in Transect One has been significantly increasing each year of the monitoring program, except for the 2013 monitoring season ( $\chi^2 = 446.976$ ,  $df = 4$ ,  $p < 0.001$ ). The total number of individuals observed in 2013 had decreased by 47% from 2012 and was not significantly different from the number of individuals observed during the 2011 monitoring season ( $p = 0.677$ ). All other pairwise comparisons of butterfly abundance each year were significantly different ( $p < 0.001$ ). The total number of individuals observed on this transect has increased by 2.4 times since the beginning of the monitoring program. Figure 2.2 compares the butterfly abundance observed during each year of the monitoring program on Transect One.



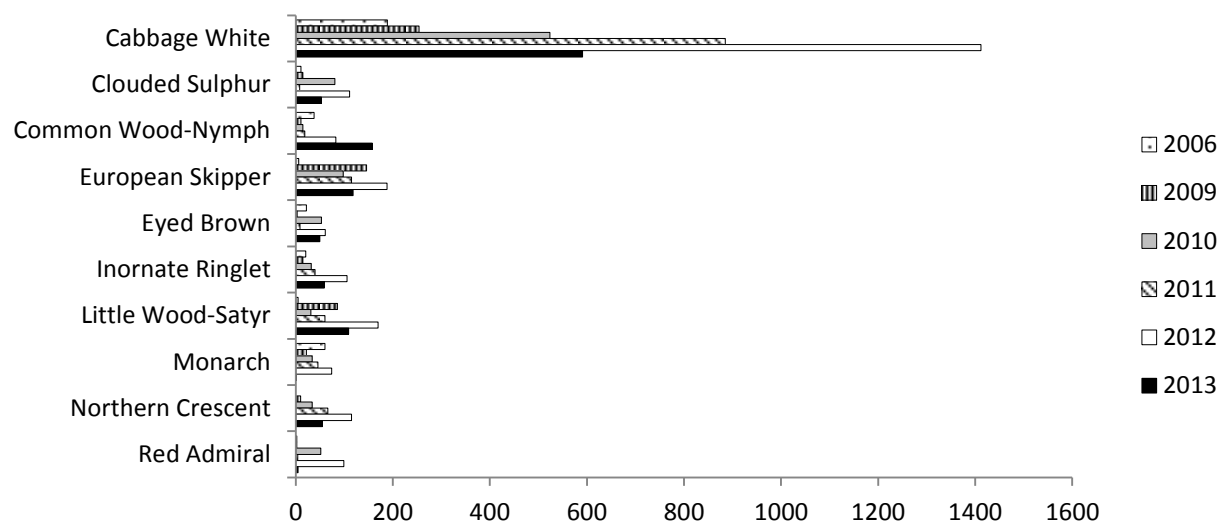
**Figure 2.2:** Comparison of the number of individuals observed each year on Transect One for all monitoring seasons. Years with butterfly abundances that are not significantly different are indicated by an asterisk. The butterfly abundances observed in all other years are significantly different from each other ( $p < 0.001$ ).

The most abundant species observed in this transect were the Cabbage White ( $n=591$ ), Common Wood-Nymph ( $n=158$ ), European Skipper ( $n=118$ ), Little Wood-Satyr ( $n=109$ ), and Inornate Ringlet ( $n=51$ ). The consistently dominant species from the previous monitoring season were the Cabbage White, European Skipper and Little Wood-Satyr. The Cabbage White and European Skipper have been dominant species in Transect One during all previous monitoring periods. During the 2013 monitoring season, the Common Wood-Nymph contributed 10% of the total observations in Transect One and observations of this species had increased by 90% from the 2012 monitoring season. Figure 2.3 compares the total observations of butterfly species with less than 50 observations in Transect One for all monitoring seasons, while Figure 2.4 displays the comparison of species with more than 50 observations.

The Shannon Diversity Index for Transect One during the 2013 monitoring season was 2.45, the highest value calculated of all previous monitoring years. Diversity on this transect has been increasing throughout the monitoring program, and greatly increased from the 2011 to 2012 monitoring season and from the 2012 to 2013 monitoring season. The Species Evenness value was 0.65, a slight increase from all previous monitoring years (Table 2.1).



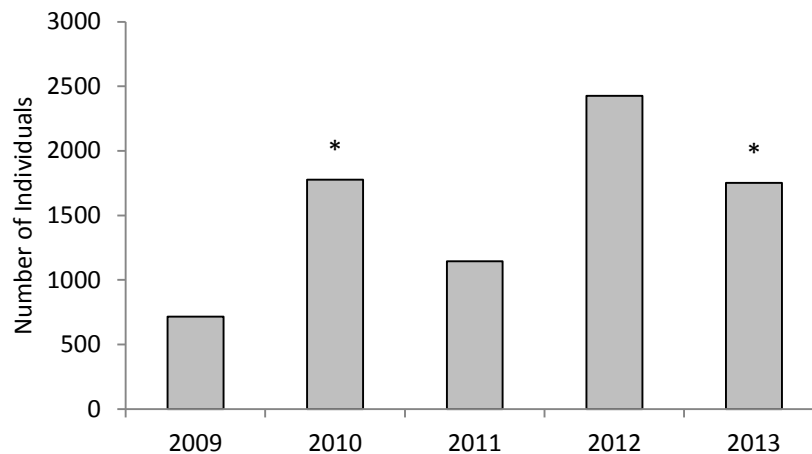
**Figure 2.3:** Comparison of species observations on Transect One during all monitoring seasons. Only species with less than 50 observations are shown. The 2006 monitoring season occurred over a period of five weeks, the 2009 and 2010 monitoring periods occurred over thirteen weeks and the 2011-2013 monitoring periods occurred over fourteen weeks.



**Figure 2.4:** Comparison of species observations on Transect One during all monitoring seasons. Only species with more than 50 observations are shown. The 2006 monitoring season occurred over a period of five weeks, the 2009 and 2010 monitoring periods occurred over thirteen weeks and the 2011-2013 monitoring periods occurred over fourteen weeks.

### 2.3.3 Transect Two: South Field/Sparrow Field

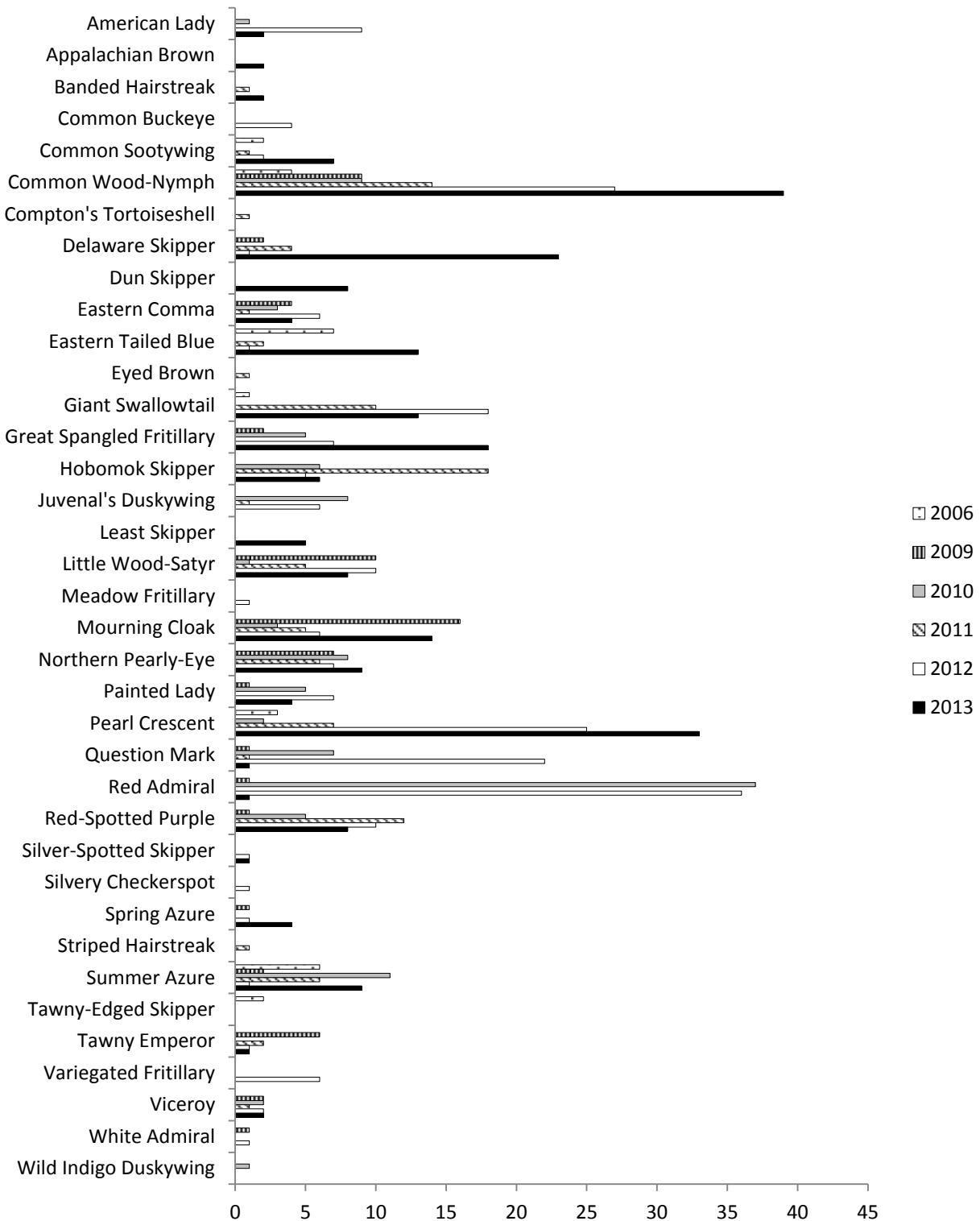
During the 2013 monitoring season, 1751 individuals and 35 species were observed in Transect Two (Table 2.1). The total number of individuals observed in Transect Two has been fluctuating throughout the monitoring program ( $\chi^2 = 391.733$ ,  $df = 4$ ,  $p < 0.001$ ). The total number of individuals observed during the 2013 monitoring season was significantly lower than in 2012 ( $p < 0.001$ ), significantly greater than in 2011 ( $p < 0.001$ ) and not significantly different from 2010 ( $p = 0.844$ ). All other pairwise comparisons of butterfly abundance each year were significantly different ( $p < 0.001$ ). The total number of individuals observed on this transect has increased by 2.4 times since the beginning of the monitoring program. Figure 2.5 compares the butterfly abundance observed during each year of the monitoring program on Transect Two.



**Figure 2.5:** Comparison of the number of individuals observed each year on Transect Two for all monitoring seasons. Years with butterfly abundances that are not significantly different are indicated by an asterisk. The butterfly abundances observed in all other years are significantly different from each other ( $p < 0.001$ ).

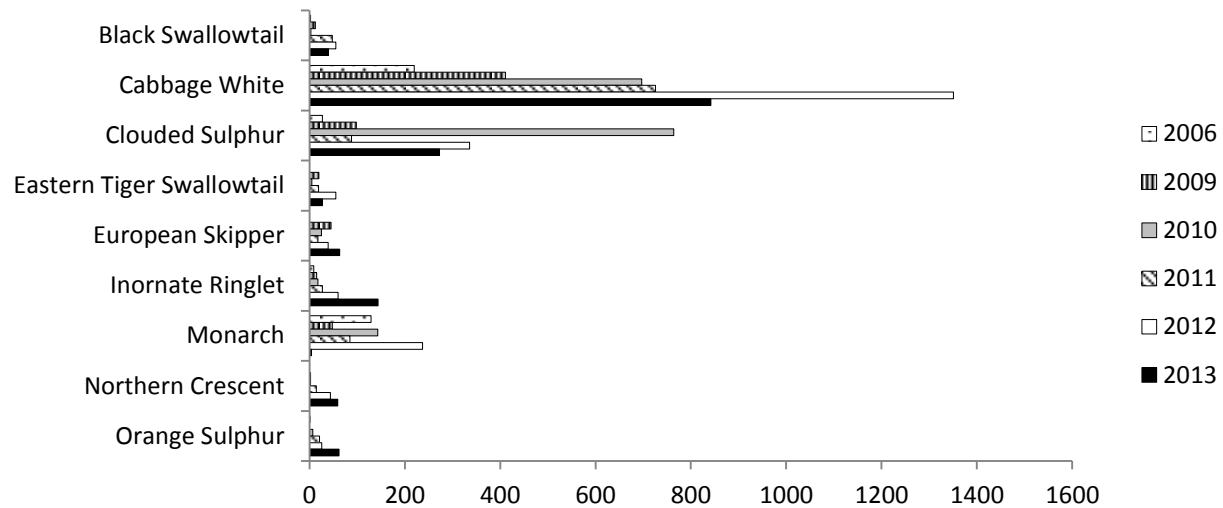
The most abundant species observed in this transect were the Cabbage White ( $n=842$ ), Clouded Sulphur ( $n=273$ ), Inornate Ringlet ( $n=144$ ), European Skipper ( $n=63$ ) and Orange Sulphur ( $n=62$ ). The Cabbage White, Clouded Sulphur and Monarch have consistently been the most abundant species observed in Transect Two during the 2009-2012 monitoring seasons. Observations of the Monarch on Transect Two in 2013 decreased by 98% compared to the number of individuals observed during the 2012 monitoring season. Figure 2.6 compares the total observations of butterfly species with less than 50 observations in Transect Three from all previous monitoring seasons, while Figure 2.7 displays the comparison of species with more than 50 observations.

The Shannon Diversity Index for Transect Two during the 2013 monitoring season was 2.02, the highest value calculated of all previous monitoring years. Diversity has been increasing throughout the monitoring program, and the 2013 value had greatly increased from all other monitoring seasons. The species evenness value was 0.57, a slight increase from the 2012 monitoring period but not the highest calculated value of previous monitoring seasons (Table 2.1).



**Figure 2.6:** Comparison of species observations on Transect Two during all monitoring seasons. Only species with less than 50 observations are shown. The 2006 monitoring season occurred over a period of five weeks, the 2009 and 2010 monitoring periods occurred over thirteen weeks and the 2011-2013 monitoring periods occurred over fourteen weeks.

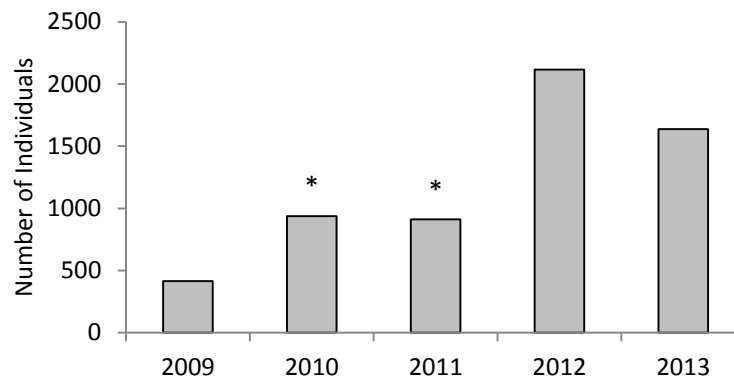




**Figure 2.7:** Comparison of species observations on Transect Two during all monitoring seasons. Only species with more than 50 observations are shown. The 2006 monitoring season occurred over a period of five weeks, the 2009 and 2010 monitoring periods occurred over thirteen weeks and the 2011-2013 monitoring periods occurred over fourteen weeks.

#### 2.3.4 Transect Three: Thompson Tract

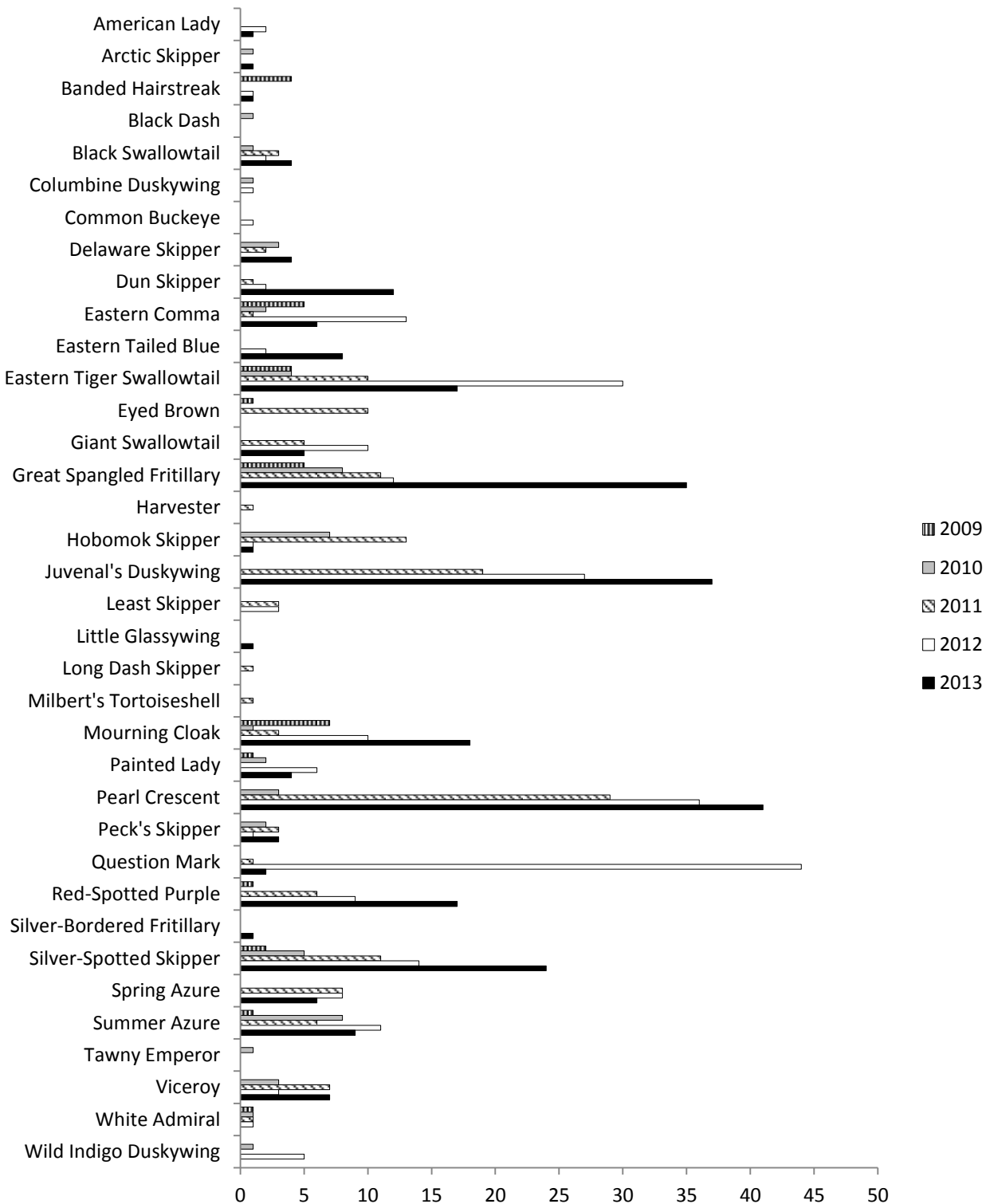
During the 2013 monitoring season, 1636 individuals and 36 species were observed in Transect Three (Table 2.1). The total number of individuals observed in Transect Three has been fluctuating throughout the monitoring program ( $\chi^2 = 472.936$ ,  $df = 4$ ,  $p < 0.001$ ). The number of individuals observed in 2013 was significantly lower than in 2012 ( $p < 0.001$ ) and significantly greater than in 2010 and 2011 ( $p < 0.001$ ). The total number of individuals observed during the 2010 and 2011 monitoring seasons were not significantly different ( $p = 0.703$ ). All other pairwise comparisons of butterfly abundance each year were significantly different ( $p < 0.001$ ). The total number of individuals observed on this transect has increased 1.7 times since the beginning of the monitoring program. Figure 2.8 compares the butterfly abundance observed during each year of the monitoring program on Transect Three.



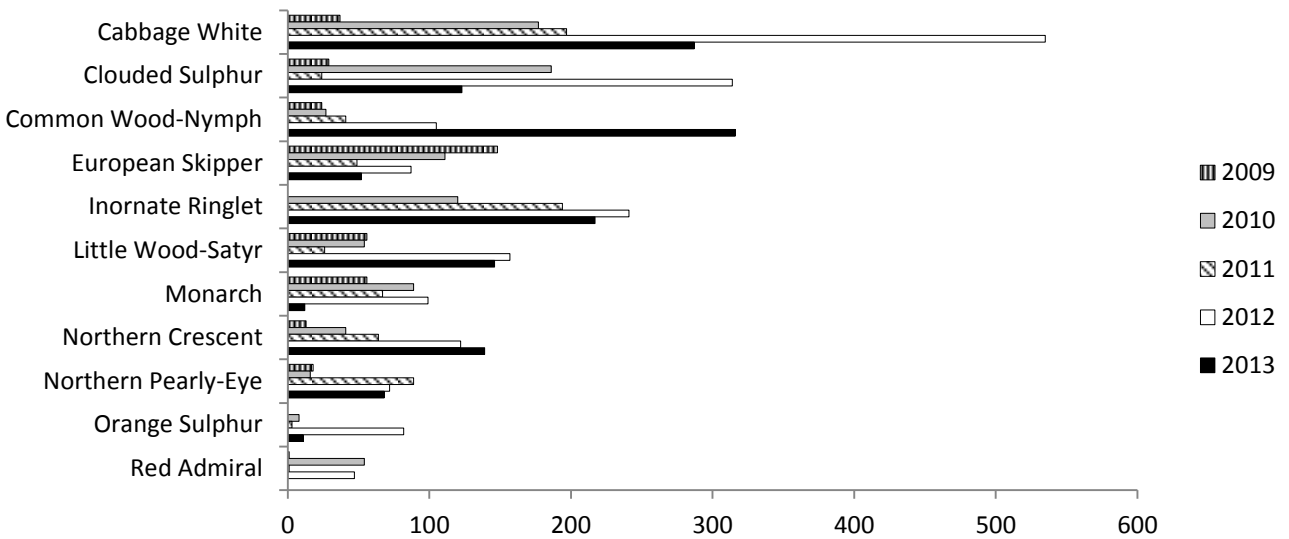
**Figure 2.8:** Comparison of the number of individuals observed each year on Transect Three for all monitoring seasons. Years with butterfly abundances that are not significantly different are indicated by an asterisk. The butterfly abundances observed in all other years are significantly different from each other ( $p < 0.001$ ). Monitoring on this transect in 2009 occurred over a seven week period while all other monitoring periods consisted of fourteen weeks.

The most abundant species observed in this transect were the Common Wood-Nymph ( $n=316$ ), Cabbage White ( $n=287$ ), Inornate Ringlet ( $n=217$ ), Little Wood-Satyr ( $n=146$ ) and Northern Crescent ( $n=139$ ). The Cabbage White, Inornate Ringlet and Northern Crescent were all previously dominant species during the 2011-2013 monitoring periods. The number of Common Wood-Nymph individuals observed on Transect Three was 200% greater during the 2013 monitoring season than in 2012 and was the only species that outnumbered the Cabbage White for an individual transect. Figure 2.9 compares the total observations of butterfly species with less than 50 observations in Transect Three from all previous monitoring seasons, while Figure 2.10 displays the comparison of species with more than 50 observations.

The Shannon Diversity Index for Transect Three during the 2013 monitoring season was 2.55, a similar value to all previous monitoring periods. The species evenness value was 0.71, also a similar value to the previous monitoring periods (Table 2.1).



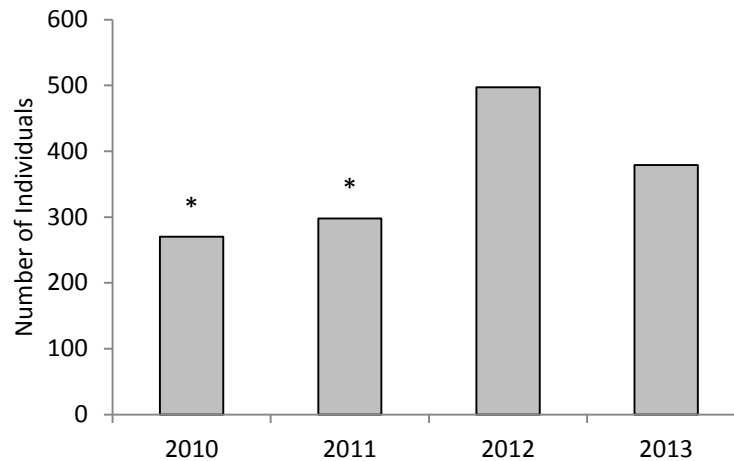
**Figure 2.9:** Comparison of species observations on Transect Three during all monitoring seasons. Only species with less than 50 observations are shown. The 2009 monitoring period occurred over seven weeks, the 2010 monitoring period occurred over thirteen weeks and the 2011-2013 monitoring periods occurred over fourteen weeks.



**Figure 2.10:** Comparison of species observations on Transect Three during all monitoring seasons. Only species with more than 50 observations are shown. The 2009 monitoring period occurred over seven weeks, the 2010 monitoring period occurred over thirteen weeks and the 2011-2013 monitoring periods occurred over fourteen weeks.

### 2.3.5 Transect Four: Blair Flats

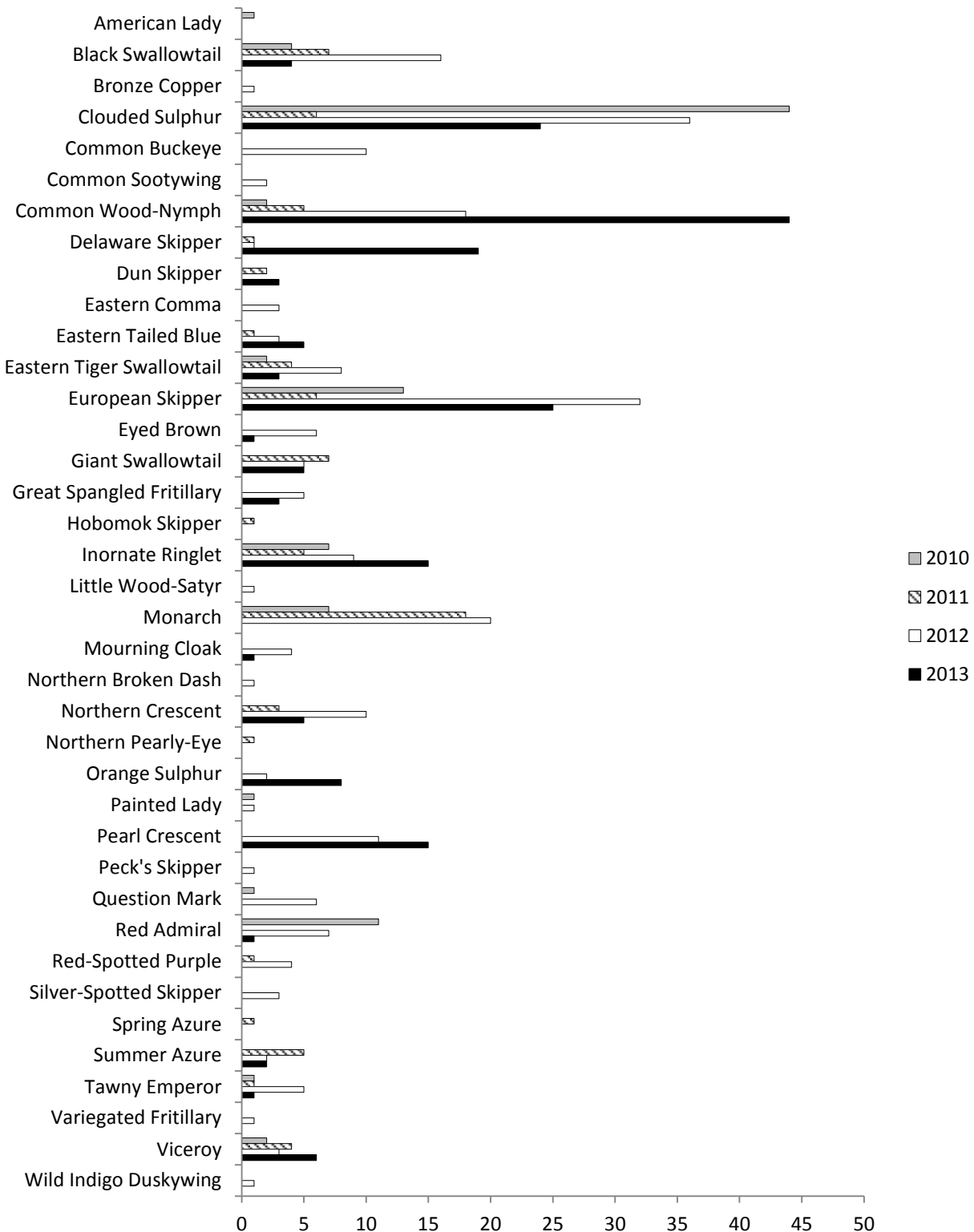
During the 2013 monitoring season, 379 individuals and 21 species were observed in Transect Four (Table 2.1). The total number of individuals observed in Transect Four has generally been increasing throughout the monitoring program ( $\chi^2 = 25.959$ ,  $df = 3$ ,  $p < 0.001$ ). There was no significant difference between the number of individuals observed during the 2010 and 2011 monitoring periods ( $p = 0.459$ ). The number of individuals observed significantly increased from 2010 and 2011 to 2012 ( $p < 0.001$ ) and significantly decreased from 2012 to 2013 ( $p = 0.031$ ). The number of individuals observed during the 2013 monitoring season was significantly greater than the number of individuals observed in 2010 ( $p = 0.007$ ) and 2011 ( $p = 0.047$ ), the first two seasons of the monitoring program for this transect. The total number of observed individuals on this transect has increased by 1.4 times since the beginning of the monitoring program. All other pairwise comparisons of butterfly abundance each year were significantly different ( $p < 0.001$ ). Figure 2.11 compares the butterfly abundance observed during each year of the monitoring program.



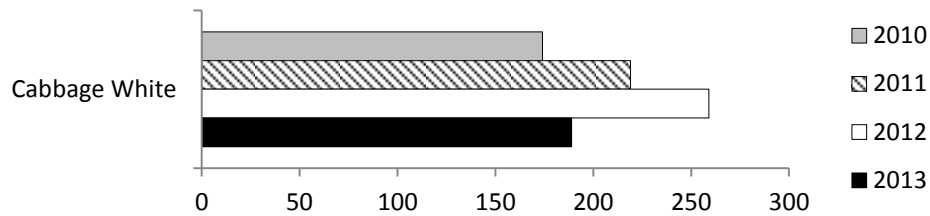
**Figure 2.11:** Comparison of the number of individuals observed each year on Transect Four for all monitoring seasons. Years with butterfly abundances that are not significantly different are indicated by an asterisk. The butterfly abundances observed in all other years are significantly different from each other ( $p < 0.001$ ).

The most abundant species observed in this transect were the Cabbage White ( $n=189$ ), Common Wood-Nymph ( $n=44$ ), European Skipper ( $n=25$ ), Clouded Sulphur ( $n=24$ ) and Delaware Skipper ( $n=19$ ). The Cabbage White, Monarch and Clouded Sulphur have been consistently dominant during the 2010-2012 monitoring periods. The Monarch was not observed on Transect Four during the 2013 monitoring season, while 20 individuals were observed on this transect during the 2012 monitoring season. Figure 2.12 compares the total observations of butterfly species with less than 50 observations in Transect Four from all previous monitoring seasons, while Figure 2.13 displays the comparison of species with more than 50 observations.

The Shannon Diversity Index for Transect Four during the 2013 monitoring season was 1.93, a lower value than the 2012 monitoring season but an increase from the 2010 and 2011 monitoring seasons. The species evenness value was 0.63, a similar value to the 2012 monitoring season but an increase from the 2010 and 2011 monitoring seasons (Table 2.1).



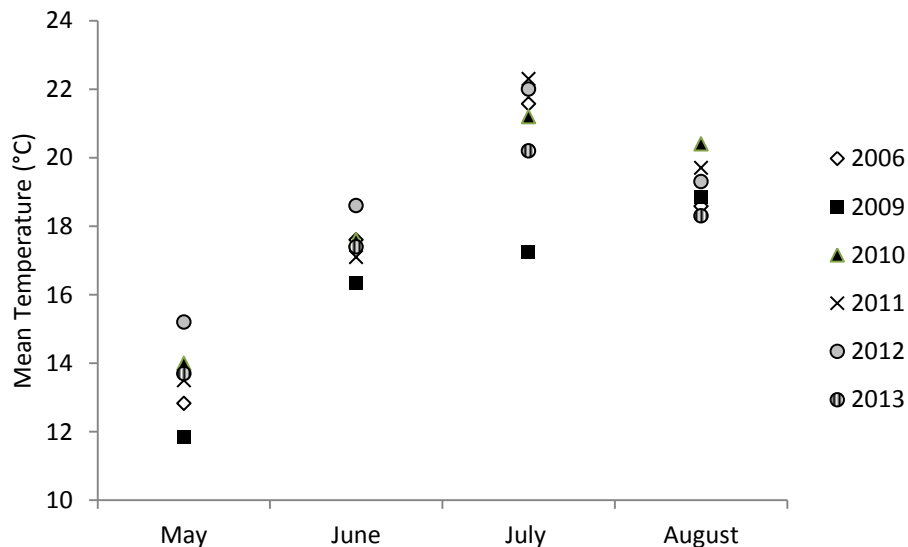
**Figure 2.12:** Comparison of species observations on Transect Four during all monitoring seasons. Only species with less than 50 observations are shown. The 2010 monitoring period occurred over thirteen weeks and the 2011-2013 monitoring periods occurred over fourteen weeks.



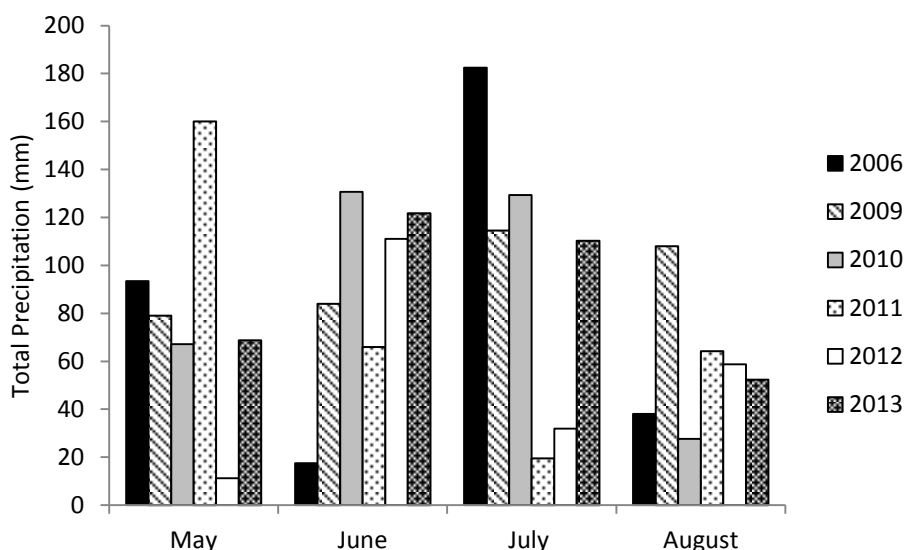
**Figure 2.13:** Comparison of species observations on Transect Four during all monitoring seasons. Only species with more than 50 observations are shown. The 2010 monitoring period occurred over thirteen weeks and the 2011-2013 monitoring periods occurred over fourteen weeks.

### 2.3.6 Weather Conditions

The 2013 monitoring season was characterized by relatively cooler temperatures and higher rainfall. The mean monthly temperatures during the 2013 monitoring season were significantly lower than the temperatures experienced during the 2012 monitoring season. The mean temperatures in May and June were similar for 2011 and 2013, while the temperatures in July and August were significantly higher in 2011 than in 2013 (Figure 2.14). Total monthly precipitation was relatively high during the 2013 monitoring season, with the highest amounts of precipitation occurring in June and July (Figure 2.15). Total precipitation in 2013 was significantly greater than precipitation in 2012 and slightly greater than in 2011.



**Figure 2.14:** Mean monthly temperature during the 2013 monitoring season. Weather data for 2006 and 2009 are from the Waterloo International Airport Weather Station, while the data for 2010-2013 are from the Kitchener-Waterloo Weather Station (Environment Canada 2012).



**Figure 2.15:** Total monthly precipitation during the 2013 monitoring season. Weather data for 2006 and 2009 are from the Waterloo International Airport Weather Station while the data for 2010-2013 are from the Kitchener-Waterloo Weather Station (Environment Canada 2012).

### 2.3.7 Annual Butterfly Count

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. Results of the 2013 count can be found below and results from previous years can be found in Appendix E.

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.

## 2.4 Discussion

### 2.4.1 Overall Abundance and Diversity

During the 2013 monitoring season, a total of 5260 individuals and 46 species were observed on all four transects at the **rare Charitable Research Reserve**. The number of individuals observed on each transect has significantly decreased compared to the 2012 monitoring season and significantly increased compared to the 2011 monitoring season. These fluctuations in butterfly populations are most likely influenced by multiple interacting factors, but the weather conditions experienced each season have a strong influence (Roy et al. 2001). The 2010 and 2012 monitoring seasons experienced ideal weather conditions, while the 2011 and 2013 monitoring seasons experienced relatively cooler temperatures and



higher precipitation, which is unfavourable for butterfly populations. Roy et al. (2001) suggests that there is a strong association between the current summer temperature and butterfly abundance, which explains the high abundances observed during the warmer monitoring seasons of 2010 and 2012. Warmer summer temperatures may benefit butterflies by increasing their rate of development during the egg and larval stages, as well as improving reproductive success (Roy et al. 2001).

A total of 46 species were observed during the 2013 monitoring season, which represents 65% of the total number of species that have been historically observed on *rare* property since the beginning of the monitoring program in 2006. Overall species richness in 2013 had decreased from 2012 but was similar to the richness observed during the 2011 and 2010 monitoring seasons. The mild winter and hot start to spring in 2012 may have increased the number of migrant butterfly species observed at *rare* that year, while the cooler temperatures in 2011 and 2013 may have resulted in fewer observations of migrants and therefore a lower species richness.

The non-native Cabbage White (*Pieris rapae*) has consistently been the most dominant species observed during all monitoring years. 1909 individuals were observed during the 2013 monitoring season, which represents 36% of the total number of butterfly observations. The Cabbage White was first introduced to Quebec in the 1860s and spread rapidly across Canada over the next few decades (Layberry 1998). The host plants of the Cabbage White are members of the Mustard (*Brassicaceae*) family, including the invasive Garlic Mustard (*Alliaria petiolata*), which is abundant on the *rare* property (*rare* Draft Environmental Management Plan 2012).

#### 2.4.2 Transect One: Cliffs and Alvars

Transect One is the longest of the four monitoring transects and therefore it is not surprising that it has experienced consistently high abundance, species richness and diversity throughout the monitoring program. Abundance in Transect One has been increasing each year, except for 2013 which experienced a 50% decrease in abundance compared to 2012. The ideal weather conditions experienced during the 2012 monitoring season resulted in an anomaly year of dramatically high butterfly observations. Therefore, abundance on all transects has significantly decreased from 2012. Although Transect One is the longest of the four monitoring transects, it had relatively low butterfly abundance during the 2013 monitoring season compared to other transects. A large portion of this transect is characterised as forested habitat, which contains fewer food and host plants and therefore may attract fewer butterflies than meadow habitats. When considering butterfly observations on a per distance travelled basis, 427 butterflies were observed per kilometer on Transect One, making this the third most abundant transect.

During the 2013 monitoring season, this transect had the highest species richness and the second highest Shannon Diversity Index value. Because this transect has the most diverse habitat compared to other transects, it attracts a wide variety of butterfly species. The 2013 Shannon Diversity Index and species evenness values for Transect One have both increased compared to all previous monitoring years, suggesting that diversity is increasing throughout the monitoring program and the relative abundance of species is shifting towards a more equal abundance of species. Two butterfly species that are considered rare in the Region of Waterloo were observed on this transect in 2013, the Arctic Skipper and Juvenal's Duskywing.

#### 2.4.3 Transect Two: South Field/Sparrow Field

Abundance in Transect Two has been fluctuating each year throughout the monitoring program. In 2013, butterfly abundance had significantly decreased from 2012, significantly increased from 2011 and was similar to abundance in 2010. This may be a result of the ideal weather conditions that were

experienced during the 2012 and 2010 monitoring seasons. Transect Two was the most abundant transect during the 2013 monitoring season, which is most likely a result of the large populations of the Cabbage White and Clouded Sulphur that were observed on this transect. When considering butterfly observations on a per distance travelled basis, 515 butterflies were observed per kilometer in Transect Two making this the second most abundant transect when considering transect length.

Transect Two is slightly shorter than Transect One but has less of a variety in butterfly habitats and therefore it typically exhibits a lower species richness and diversity. Low diversity may be a result of the large size and homogeneity of agricultural monocultures that have fragmented the natural landscape in this area. Habitat fragmentation has been shown to limit butterfly movement (Avirona et al. 2007) and monoculture cropping can reduce food plant diversity and reduce the number of butterfly species that an area can support. The highest number of individuals were observed on this transect, which is most likely a result of the high numbers of the Cabbage White and Clouded Sulphur. The Cabbage White has consistently contributed to approximately 50% of the total butterfly observations on this transect throughout the monitoring program, and in 2013 they contributed to 48% of total observations. The Cabbage White is a generalist species and seems to be able to best exploit the resources in an agricultural landscape. The second most abundant species on this transect was the Clouded Sulphur, whose larvae feed on various plants such as soybean, alfalfa and various species of clover, all of which were present in surrounding agricultural fields in 2013. This transect also had the lowest species evenness value of all transects because 80% of observations on this transect were of the five most abundant species. One species that is considered rare in the Region of Waterloo was observed on this transect in 2013, the Common Sootywing.

The 2013 calculated Shannon Diversity Index for Transect Two has increased compared to all previous monitoring years, suggesting that diversity in this transect has been increasing over time. This increase in butterfly diversity may be influenced by the regeneration that has occurred in this area. Several fields have been gradually removed from agricultural production from 2004-2012 and have been left to naturally regenerate, resulting in an increase in the diversity of food and host plants for butterflies.

#### *2.4.4 Transect Three: Thompson Tract*

Butterfly abundance on Transect Three has been fluctuating throughout the monitoring seasons and has significantly decreased from the 2012 to 2013 monitoring seasons. Weather conditions may account for much of this variation in butterfly abundance during each monitoring season. Transect Three was the second most abundant transect during the 2013 monitoring season, even though it has had a relatively low abundance in previous years. This may be a result of the dramatic increase in the Common Wood-Nymph population, which had increased by 139% compared to the 2012 monitoring season and reached the highest total abundance ever recorded during the monitoring program at **rare**. The Common Wood-Nymph prefers large, sunny, grassy areas such as meadows, as the larvae feed on various grass species and therefore, it was most likely more dominant on Transect Three than any other transect due to the grassy meadows that constitute a main portion of the habitat on this transect. When considering butterfly observations on a per distance travelled basis, 744 butterflies were observed per kilometer in Transect Three, making it the most abundant transect when accounting for distance.

Transect Three is one of the more diverse transects, consisting of various meadow and forest habitats, which has resulted in the highest Shannon Diversity Index of all transects and the second highest species richness. Three species that are considered rare in the Region of Waterloo were observed on this transect in 2013, the Arctic Skipper, Juvenal's Duskywing and Silver-Bordered Fritillary. This transect has consistently had the highest butterfly diversity of all transects for all previous monitoring years and has also had relatively high species evenness values. Diversity may be influenced

by the large, grassy meadow sections of this transect that can be utilized by a wide variety of butterfly species. In addition, there are several other habitat types that provide more diverse resources for both generalist and specialist butterfly species. Butterfly diversity and species evenness values have been relatively consistent for this transect over all previous monitoring periods, indicating that butterfly populations have been fairly stable in this area. The stable butterfly populations may be influenced by the stable surrounding environment, as no major landscape changes have occurred on Transect Three throughout the monitoring program.

#### 2.4.5 Transect Four: Blair Flats

Transect Four had the lowest butterfly abundance of all transects during the 2013 monitoring season, which is consistent with all previous monitoring years. This transect was planted as a tall grass prairie in 2010, resulting in a landscape that has been transitioning throughout the course of the monitoring program. Low butterfly abundance may be a result of low plant diversity or quality, both of which would not provide butterflies with the resources they require. This transitioning landscape has resulted in changing conditions and changes in plant composition throughout the monitoring program, which may have influenced changes in butterfly abundance and diversity. Abundance has been generally increasing over the course of the monitoring program, suggesting that this landscape is becoming more habitable for butterflies over the course of its restoration. When considering butterfly observations on a per distance travelled basis, 291 butterflies were observed per kilometer on Transect Four, making it the least abundant transect.

The homogeneity of this prairie habitat provides butterflies with little variability in food and host plants, making this the transect with the lowest Shannon Diversity Index and species richness for 2013. Transect Four has consistently had the lowest species richness for all previous monitoring seasons and the lowest Shannon Diversity Index for all previous monitoring seasons except 2012. The Shannon Diversity Index and species richness on this transect have generally been increasing over time, suggesting that the restored land is becoming more diverse and is now capable of supporting more butterfly species. No species that are considered rare in the Region of Waterloo were observed on this transect in 2013. Continued regeneration and restoration of this area may increase the diversity of resources available for butterflies and produce a more diverse butterfly population.

#### 2.4.6 Noteworthy Observations for the 2013 Monitoring Season

During the 2013 monitoring season, thirty of the species observed are considered either common or very common based on their Waterloo Regional Status and four species are considered rare (Table 2.2).

Two Arctic Skippers (*Carterocephalus palaemon*) were observed on Transect One and one was observed on Transect Three during the 2013 monitoring season. Although this species is considered common throughout its Canadian range, it has only been observed at seven sites within the Region of Waterloo and is therefore considered rare in the Region (Grealey et al. 2010). The Arctic Skipper has previously only been observed on **rare** property during the 2010 monitoring season and the 2010 annual butterfly count.

Seven Common Sootywings (*Pholisora catullus*) were observed on Transect Two during the 2013 monitoring season. This species can be locally common in Southern Ontario, but is considered provincially vulnerable and rare in the Region of Waterloo (Grealey et al. 2010). The Common Sootywing has been observed several times throughout the monitoring program at **rare**.

Many Juvenal's Duskywings (*Erynnis juvenalis*) were observed on Transect One and Three in 2013. This species was not observed in the Region of Waterloo since the 1960s until 2010 and has been

observed during **rare** monitoring in all subsequent years. This species is common throughout its Southern Ontario range, but is considered regionally rare (Grealey et al. 2010). Due to their early flight season, which occurs early May to late June, this species may be overlooked during monitoring programs that occur later in the season.

A Silver-Bordered Fritillary (*Boloria selene*) was observed for the first time during the monitoring program on Transect Three during the 2013 monitoring season. Historically, this species was frequently observed in the Region of Waterloo until the 1960s when a dramatic decrease in observations occurred (Grealey et al. 2010). This species had previously only been observed at **rare** during the annual butterfly count in 2011.

#### 2.4.7 Species Population Trends for the 2013 Monitoring Season

Several common species had experienced dramatic changes in abundance during the 2013 monitoring season. The Common Wood-Nymph (*Cercyonis pegala*) population had increased by 139% compared to the 2012 monitoring season, reaching the highest total abundance ever recorded during the monitoring program at **rare**. Butterfly populations can experience dramatic population fluctuations over time and are known to undergo population explosions once every 5 to 10 years, which may have occurred for the Common Wood-Nymph population in 2013.

The Red Admiral (*Vanessa atalanta*) population has been oscillating each year at **rare** and this trend has continued into the 2013 monitoring season. During the 2009, 2011 and 2013 monitoring seasons, the abundance of the Red Admiral was less than 10 observations (Moore 2009; Dodds 2011) and during the 2010 and 2012 monitoring seasons, abundance was over 150 observations (Moore 2010; Quinn 2012). The weather experienced each season may have strongly influenced the number of Red Admirals that successfully completed their migration. The mild winter and warm spring that occurred in 2010 and 2012 may have allowed the butterflies to migrate farther north with few obstacles and arrive in Southern Ontario in higher abundances than usual. In addition, during a warm summer this species can breed continuously and can complete more generations in one season, resulting in higher abundances (Pollard 1988).

The Monarch (*Danaus plexippus*) was noticeably absent from the 2013 monitoring season and had the lowest observed abundance of all previous monitoring seasons. This trend was consistent with observations from across the breeding range in eastern North America. The hot temperatures and drought that occurred in several areas of Ontario during the summer of 2012 may have resulted in low survival of Monarch larvae and eggs, as consistently high temperatures and dry conditions can be detrimental to Monarch development (York & Oberhauser 2002). This decreased the size of the succeeding generation and resulted in fewer Monarchs migrating to Mexico during the fall of 2012. The World Wildlife Fund and Mexico's National Commission of Protected Areas determined that the forested area occupied by Monarchs while overwintering in Mexico during the 2012/2013 winter reached a twenty year low and a 59% decrease from the previous winter. In addition, the cooler spring temperatures that occurred in the southern United States in 2013 resulted in a lack of mature milkweed when the Monarchs arrived. This absence of host plants may have reduced the number of Monarchs that were able to successfully complete their spring migration to Ontario, resulting in lower abundances at **rare** in 2013.

The dramatic decline in this years Monarch population highlights the importance of protecting their host plant. Because the caterpillar feeds solely on milkweed plants, the Monarch population is strongly dependent on the quality and abundance of this plant. Therefore, a decline in milkweed populations, as seen in the Southern United States during the 2013 spring, can have a dramatic effect on Monarch populations. Agricultural intensification, development of rural land, and the use of mowing and herbicides to control roadside vegetation have all reduced the abundance of milkweed in the

landscape. Because the Monarch is listed as a species of Special Concern both provincially and nationally, the North American Monarch Conservation Plan (Commission for Environmental Cooperation 2008) suggests that the planting of regionally appropriate native milkweed species should occur in order to address this loss in the Monarch breeding habitat and conserve the population.

The future response of the Monarch population to this year's decline is unknown. It has been suggested that they have the potential to rebound, and the species isn't expected to become extinct (Spears 2013). A productive breeding season this summer would have assisted the population's recovery but low observations this summer indicated an unproductive breeding season. Monarchs, like other insects, reproduce rapidly and the population will most likely recover if left alone. However, it has been suggested that if their current 2.94 acre wintering ground in Mexico drops below 2.50 acres, bouncing back could be difficult (Wines 2013). The Monarch butterfly represents one of the world's greatest migrations and is one of the few butterfly species that is well recognized by the public, making its recovery a conservation priority.

#### *2.4.8 Overall Trends for the 5 Year Monitoring Period at **rare Charitable Research Reserve***

The butterfly population at **rare** has a Shannon Diversity Index value of 2.33, indicating that it is relatively stable and diverse. Values typically vary from 1 which indicates extremely low diversity, to 5 which indicates extremely high diversity (Gering et al. 2003). The species evenness value for the butterfly population is 0.56, which indicates a moderately even distribution. Evenness values range from near 0 when most individuals belong to a few species, to 1 when species are equally abundant (Smith & Wilson 1996).

Butterfly abundance and species richness on all four transects has been generally increasing throughout the monitoring program. In addition, the Shannon Diversity Index is also increasing over time on all transects except for transect three, where it has remained consistent. The species evenness value has also been slightly increasing over time on all transects and has been significantly increasing on Transect Four. These trends suggest that the butterfly communities at **rare** are becoming more diverse and stable over time, which indicates that butterflies are able to acquire the necessary resources from a healthy and diverse habitat. Several studies have identified a correlation between butterfly abundance and restoration treatments, suggesting that as their habitat reaches a more natural state, butterfly abundance will increase in response to the availability of diverse resources (Waltz and Covington 1999). It has also been suggested that butterfly diversity increases along a successional gradient (Bowman et al. 1990), indicating that the ecosystems on the **rare** property are becoming more stable over time. Therefore, the trends observed in butterfly populations have indicated that since the beginning of the butterfly monitoring program, **rare's** management of the property has facilitated the increase in diversity, stability and environmental health of ecosystems.

Restoration efforts have taken place on Transect Two and Transect Four. General increases in butterfly abundance, species richness, species evenness and the Shannon Diversity Index over time have been observed on both of these transects, indicating an overall trend towards increasing diversity and stability of butterfly populations. Monitoring the response of butterfly populations to an environmental change can facilitate the evaluation of these restoration efforts. The increased diversity and abundance of butterflies observed in these restored areas indicates that habitat quality and diversity has been increasing throughout the course of the regeneration.

The butterfly monitoring program at **rare** has now collected five full years of data on the abundance and diversity of butterflies on the property. This data suggests that yearly variations in butterfly populations can be expected as butterfly abundance and diversity are strongly influenced by the weather conditions experienced each year. Weather conditions can impact butterfly development and reproduction, resulting in fluctuations in abundance and can also influence the number of

butterflies that successfully complete their migration, resulting in fluctuations in species richness. The information from previous monitoring years can act as a baseline dataset that indicates the expected yearly variation in butterfly populations due to the weather conditions. Future monitoring data will be compared to this baseline dataset in order to identify changes in butterfly populations that exceed this expected range. Unusual population fluctuations would indicate an environmental change that has either improved or negatively impacted the environmental health of the *rare* property. While an increase in butterfly abundance would generally indicate an improvement in habitat, an extreme increase may not accurately represent the health of the property. For example, the 2012 monitoring season was the highest year for butterfly abundance, but the high temperatures and dry conditions that contributed to this increase resulted in poor health of the property.

Table 2.3 displays the average abundance and species richness, with standard deviation, for each transect over the past monitoring periods from 2009-2013. These values will act as a baseline average to use for comparison with data from future monitoring years. Due to changing weather conditions and yearly variability, population increases up to 150% and decreases up to 40% can be expected each year. During future monitoring years, fluctuations beyond this range may be indicative of an environmental change that has either improved or negatively impacted the environmental health of the *rare* property. This range was estimated using the largest yearly population increase and decrease of all transects over the course of the monitoring program. However, data from 2012 were excluded due to the exceptional weather conditions that produced unusual butterfly population fluctuations.

**Table 2.3:** Average butterfly abundance and species richness, with standard deviations, for the past five monitoring seasons from 2009-2013. Values will act as a baseline dataset to be used for comparison with future monitoring years.

Transect	Number of Individuals		Species Richness	
	Average	Standard Deviation	Average	Standard Deviation
Transect One	1491	+/- 825	36	+/- 8
Transect Two	1563	+/- 655	30	+/- 6
Transect Three	1203	+/- 670	35	+/- 3
Transect Four	361	+/- 101	23	+/- 9

## 2.5 Conclusions and Recommendations

The restored Blair Flats transect has exhibited a general increasing trend in butterfly abundance and diversity throughout the monitoring program. Long term monitoring on this transect should continue in order to evaluate the apparent success of the restoration efforts that took place in this area. Transect Two has consistently had relatively low species diversity and it is recommended that increased regeneration of the agricultural land in this area occur in order to decrease fragmentation of butterfly habitat and increase food and host plant diversity, which may eventually lead to increased butterfly diversity. It is also recommended that the annual butterfly count continue in the future, as it can provide information on butterfly abundance and diversity for the entire *rare* property, unlike the monitoring

program which focuses on specific areas. This provides us with the opportunity to observe species with specialized niches that may not be found along the monitoring transects.

It is essential that the butterfly monitoring program continue on all four transects for the full fourteen week period every year. The data collected can provide *rare* with information that can be used to evaluate the success of restoration efforts, like the restored Blair Flats area, and also provide information that can be used to guide management decisions. The *rare* property faces many external threats that have the potential to influence the natural ecosystems. Local threats such as the surrounding aggregate pits, conventionally farmed agricultural fields, and residential development are a result of being located in a densely populated urban area. Global threats, such as climate change, have the potential to alter ecosystems by shifting species ranges and influencing migration patterns. The data collected from continued butterfly monitoring will document the effect of these external threats on the natural ecosystems at *rare* over time.

The consecutive monitoring of butterfly populations is required in order to provide accurate information on population trends. Because butterfly populations are expected to fluctuate each year as a result of the weather conditions, it is important that these fluctuations are fully documented in order to reduce the risk of misinterpreting a possible trend. In addition, the short lifespan of butterflies and their ability to respond rapidly to environmental change suggests that a gap in monitoring data may result in the misinterpretation of data or an undocumented population change. Therefore, the butterfly monitoring program at *rare* should continue every year in order to fully document all changes in butterfly populations. The collection of this data will facilitate the evaluation of the environmental health of the property, and continue to compliment a wider data set that represents the health of ecosystems within the Region of Waterloo.

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### 3.0 SALAMANDER MONITORING

Prepared by: Jenna Quinn

#### 3.1 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 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**. Potentially, **rare** may also be home to a population of Jefferson salamanders (*A. jeffersonian*) however this has yet to be investigated.

The lungless salamanders (plethodontids) 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 Salamanders are 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 completely grey.

##### 2.1.2 Global Amphibian Decline

It is estimated that one-third of all amphibian species worldwide are endangered or threatened with extinction (Stuart et al. 2004). 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). Alford and Richards (1999) suggest decline of amphibian populations are a global problem with local complex 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). Given the difficulty in neutralizing or reversing these threats, the outlook for amphibians globally seems 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 a forested ecosystem (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<sup>2</sup> for males and juveniles and 24m<sup>2</sup> 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. Their role, therefore, 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 important effects on decomposition and nutrient cycling on the forest floor, plethodontid salamanders help in managing these important ecosystem roles.

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 influencing their micro-climate and water and air quality. Potential stresses include both human activities (development, pollution, etc.) and 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 salamanders 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. Since populations remain relatively stable, population trends can still be detected with small sample sizes (Zorn et al. 2004).

### 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 collective 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. It was established in 2006 with the installation of twenty-nine ACOs in the 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 the Indian Woods, bringing the total of ACOs in that plot to thirty-two and increasing the length of monitoring in the Hogsback to the full nine weeks. Monitoring has therefore been ongoing with consistent sampling effort each fall since 2009 in both sites, making this fall the fifth consecutive year of data collection.

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* to 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 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 expands approximately 20 acres and contains trees as old as 240 years. The Indian Woods salamander monitoring plot is located on the east side of the 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 Bared 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 thirty-two ACOs are distributed in a large square made up of four lines of eight ACOs each (Appendix A). Boards five, six, and seven were missing prior to 2009.

The Hogsback (HB) 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 is accessed through South Gate, off of Whistle Bare Road, and heading east along the lane to where it turns at the edge of the Hogsback. On foot, keep left and walk north and then east along the edge of the forest, finally heading south into the stand at the 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). 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

One month 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. Indian Woods and the Hogsback were monitored for only five weeks in their pilot years, 2006 and 2008 respectively.

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). Additionally, the precipitation from the 24hrs prior to monitoring was recorded using the data collected by the Environment Canada Weather Office. In the 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. 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 respectively. 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). A complete list of required equipment is available in Appendix B, List B.2.

**Table 3.1:** Weather stations and the artificial cover objects (ACO) associated with them in the 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

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. Three samples were collected from a depth of 10cm from the ground adjacent to the ACO weather station. Samples were brought back to the office and left open to dry for one week prior to pH testing. A Hellige-Truog Soil pH Tester Kit (Forestry Supplies Inc., Jackson, MS, USA) was used to determine the pH for each sample, and the three samples from each weather station were averaged to give a mean pH per weather station. A complete list of required equipment is available in Appendix B, List B.4.

### 3.2.3 Data Analysis

Data were analysed using Microsoft Excel 14.0.6 (Microsoft 2010) and PASW Statistics 17.0 (SPSS Inc.) for Windows. Prior to analysis, assumptions of parametric testing were examined. When transformation was required, the appropriate transformation to decouple variance and mean was determined using Taylor's Power Law (Perry 1981). Otherwise, the best transformation was applied and the most robust tests were used, followed by cautious interpretation of results. 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.

Since 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. 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.

A univariate analysis of variance (ANOVA) with one recoded combination fixed factor (plot and year) was used to look for differences in salamander abundance represented by CPUE. A two-way ANOVA split by plot was used to investigate weekly differences in salamander abundance, with week and year as independent variables. A two-way ANOVA split by plot was used to examine differences in species composition across all years. When interactions occurred data were either split (Zar 1999) or variables were combined and recoded into plot/year combination variables (Leech et al. 2008) depending on the question of interest. This was followed by Bonferroni post hoc testing to determine where the differences between the levels occurred.

Only Eastern Red-backed Salamanders (both colour phases) were considered in a size class comparison. Individuals were classified as either an 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. 2006 and 2008 (Indian Woods) were eliminated from this analysis since its sampling effort varied from other years. To determine which environmental factors (year, soil temperature, soil moisture, soil pH, pond depth, precipitation, sky and wind codes, wind speed, relative humidity, and air temperature) affected total salamander abundance, multiple linear regressions were used. Preliminary assessments indicate that only year affected abundance in the Hogsback, and in the Indian Woods soil pH, pond depth, precipitation, sky and wind codes, wind speed, and relative humidity did not affect abundance. Further analysis therefore focused only on these significant variables in the Indian Woods, considering soil temperature, soil moisture, air temperature, and year. Hierarchical multiple regressions followed with total abundance as the dependent variable and related parameters as the independent variables. Variables were entered into models based on their inherent relationship with salamanders (i.e. since salamanders live in the soil, soil factors were likely important). 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 Results

#### 3.3.1 Total Observations

A total of 330 salamanders were observed between September 4 and October 31 at the **rare Charitable Research Reserve** in 2013. In Indian Woods, 117 salamanders were observed and in the Hogsback, 213 salamanders were observed.

Eastern Red-backed Salamanders represented 96.9% of detections; 83.3% were the red-backed form, 13.6% were the lead-backed form of the same species. The remaining 3.1% of salamanders found under ACOs were comprised of seven Yellow-spotted Salamander observations, one Blue-spotted Salamander observation, and two observations of a Four-toed Salamander. Using age classes outlined in Zorn et al. (2004), 71.9% of the total detections of the Red-backed Salamanders were adults. There are 52 instances with two salamanders under one board, and 27 instances of three or more.

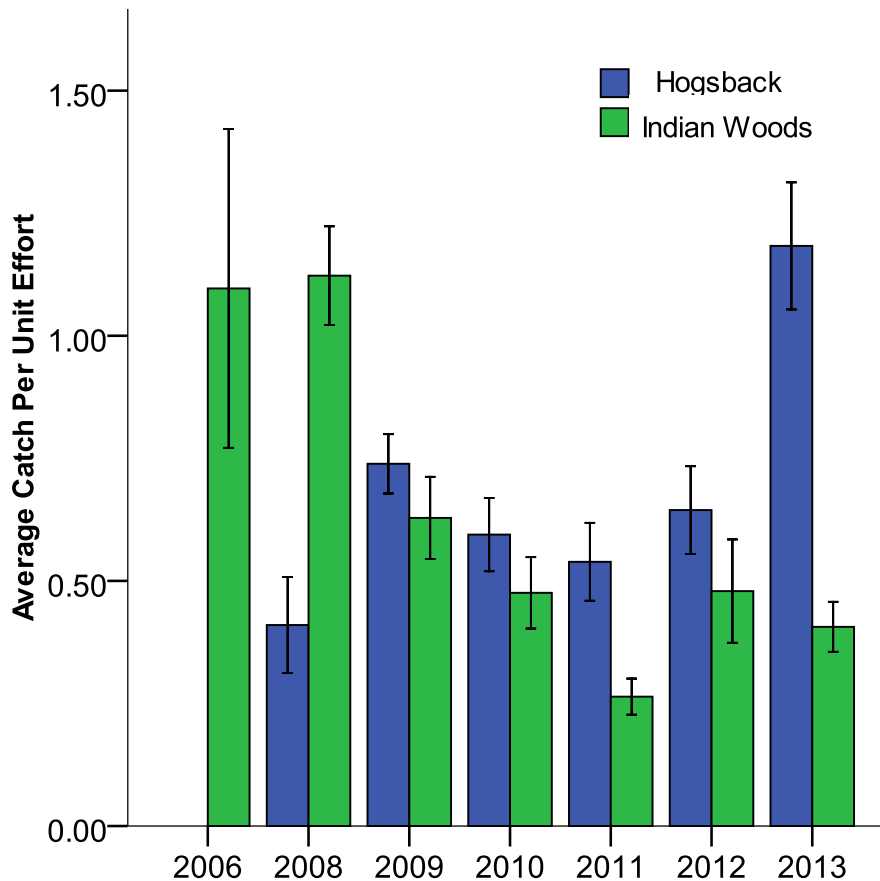
#### 3.3.2 Salamander Abundance

Plot differences varied with years (interaction  $F_{5,96}=10.28$ ,  $p<0.001$ ), so both factors were considered simultaneously in an thirteen-level combination variable of plots and years (Leech *et al.* 2008), and significant differences occurred between these levels (ANOVA  $F_{12,96}=8.569$ ,  $p<0.001$ ). Within years, CPUE at each plot generally did not significantly differ, although largely more observations were documented in the Hogsback (Figure 3.1). Two exceptions exist. In 2008, there was a significantly higher



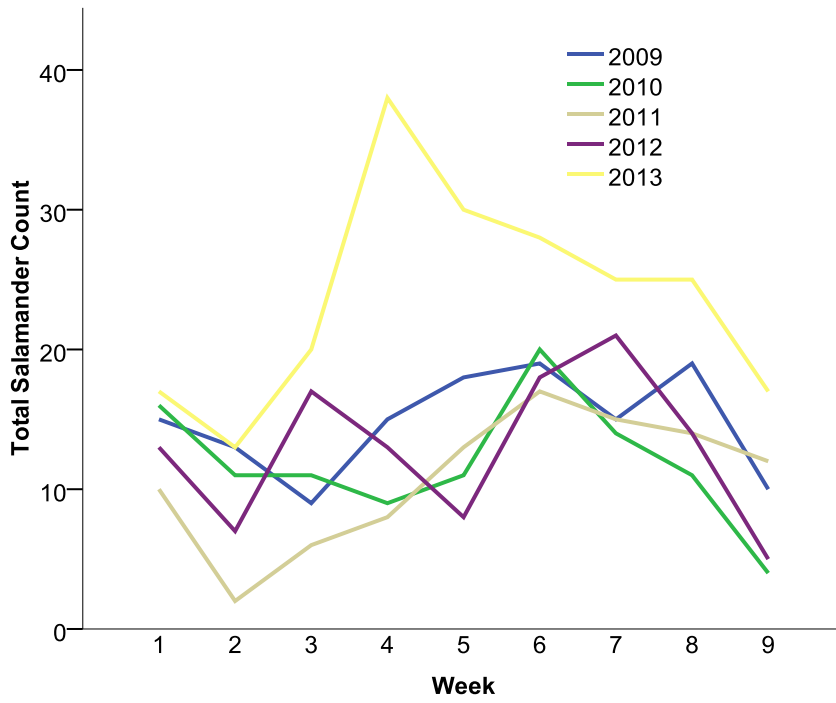
CPUE in Indian Woods (post hoc  $p=0.002$ ), and in 2013 the reverse is true with a significantly greater CPUE observed in the Hogsback (post hoc  $p<0.001$ ). CPUE in 2008 at Indian Woods is the highest on record for that site, and significantly differs from several subsequent years at both sites (post hoc  $p<0.050$ ). 2013 in the Hogsback saw the highest CPUE ever recorded throughout monitoring, and significantly differs from all years of monitoring at the site except 2009 (post hoc  $p<0.010$ ), and all years of monitoring at Indian Woods, post 2008 (post-hoc  $p<0.007$ ). CPUE in 2011 at Indian Woods is the lowest on record, and significantly differs from the first two years of Indian Woods sampling (post hoc  $p<0.001$ ) as well as CPUE in the Hogsback in 2013 (post hoc  $p<0.001$ ).

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 when sampling efforts differed. Since number of ACOs in each plot differed, Indian Woods and Hogsback were examined independent of one another. No significant differences occurred between weeks at either plot (IN:  $F_{8,433}=1.329$ ,  $p=0.227$ ; HO:  $F_{8,397}=1.321$ ,  $p=0.231$ ).

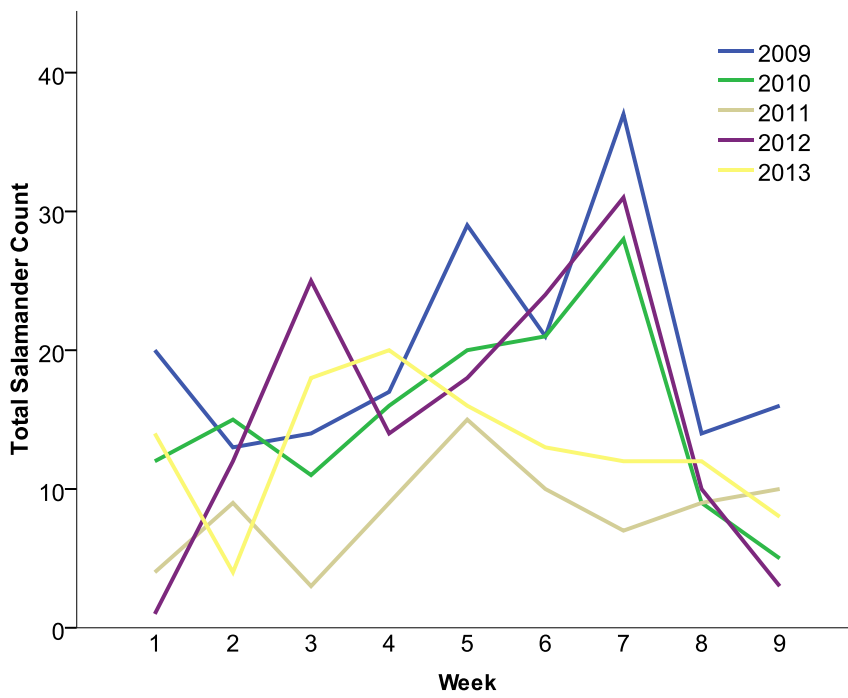


**Figure 3.1:** Average weekly salamander observation per artificial cover object (ACO) (Catch per Unit Effort) for both Indian Woods and Hogsback throughout monitoring, 2006, 2008-2013. Error bars represent +/- one standard error.

A)



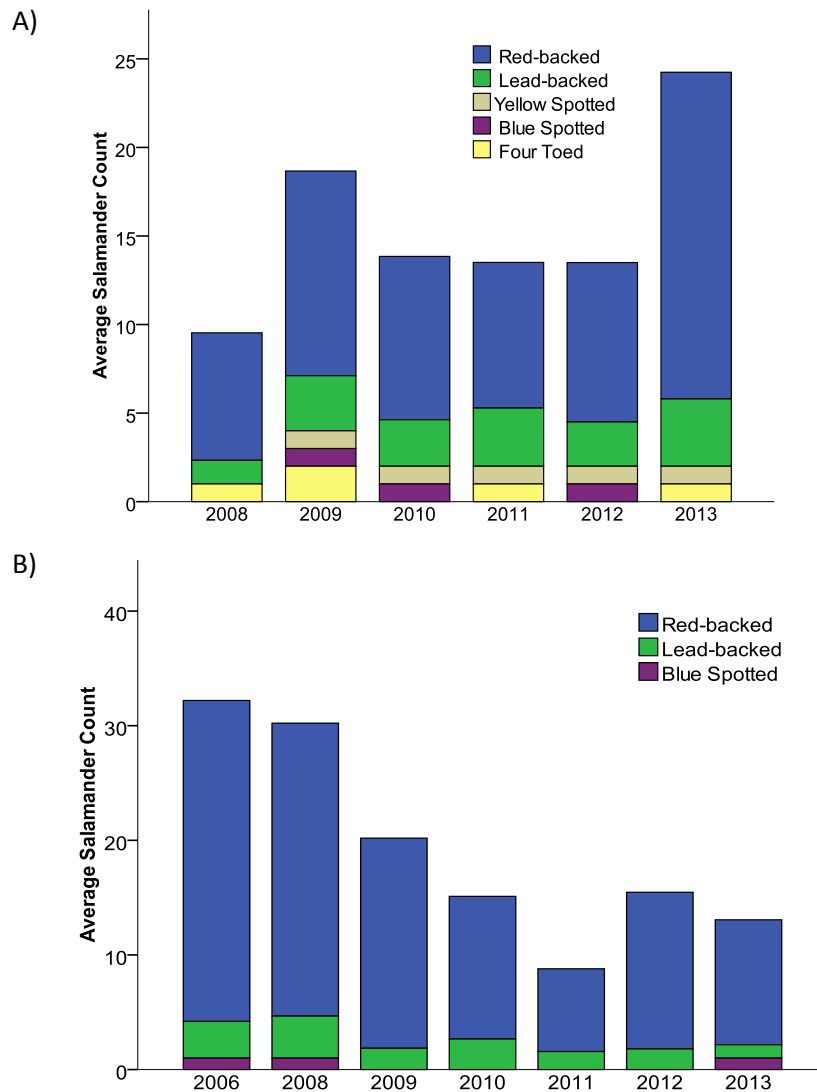
B)



**Figure 3.2:** Total weekly salamander counts in A) the Hogsback and B) Indian Woods from 2009 to 2013.

### 3.3.3 Salamander Species Composition

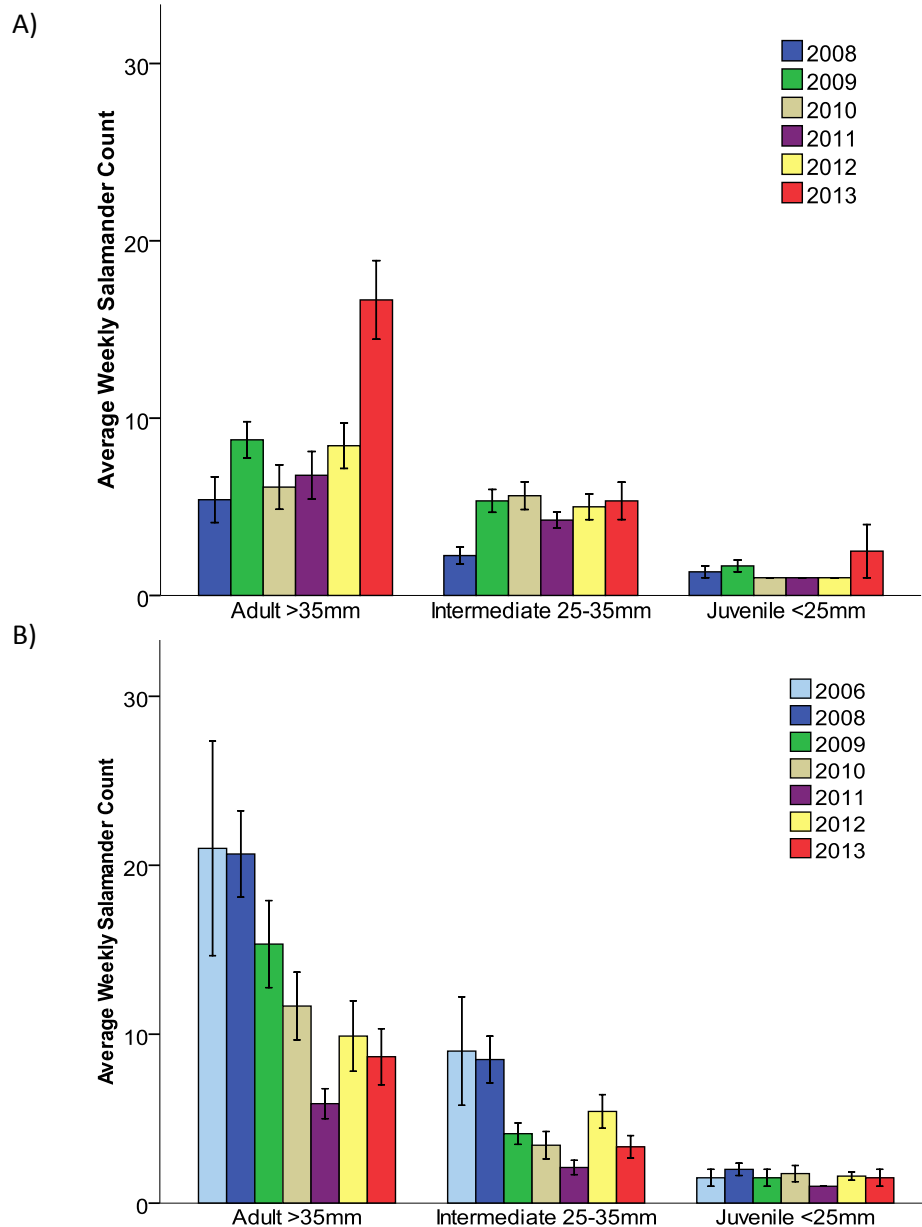
Plot differences varied with species (interaction  $F_{1,191}=7.251$ ,  $p=0.008$ ) and year (interaction  $F_{5,191}=6.036$ ,  $p<0.001$ ), so data were split by plot (Zar 1999) and Indian Woods and Hogsback were each considered independently of one another. In both plots, significant differences occurred between species ( $p<0.001$ ), with significantly more Eastern Red-backed Salamanders occurring than any other species regardless of year (post hoc  $p<0.001$ ). Red-backed and Lead-backed salamanders are two colourmorphs of the same species, the Eastern Red-backed salamander. 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).



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

### 3.3.4 Eastern Red-Backed Salamander Size Class Distribution

Plot interacted with size class ( $F_{2,216}=3.621$ ,  $p=0.028$ ) and year ( $F_{5,216}=6.263$ ,  $p<0.001$ ) so data were spit by plot and Indian Woods and Hogsback were investigated separately. In both plots total weekly salamander observations significantly differed by size class (Indian Woods:  $F_{2,123}=88.939$ ,  $p<0.001$ ; Hogsback:  $F_{2,93}=53.073$ ,  $p<0.001$ ), regardless of year (no interaction Indian Woods:  $F_{12,123}=1.066$ ,  $p=0.395$ ; Hogsback:  $F_{10,93}=1.801$ ,  $p=0.071$ ). All size classes significantly differed from one another in both plots (post hoc  $p<0.001$ ; Figure 3.4).



**Figure 3.4:** Average size distribution of salamanders observed weekly during monitoring in both A) Hogsback and B) Indian Woods from 2006 to 2013. Error bars represent +/- one standard error.

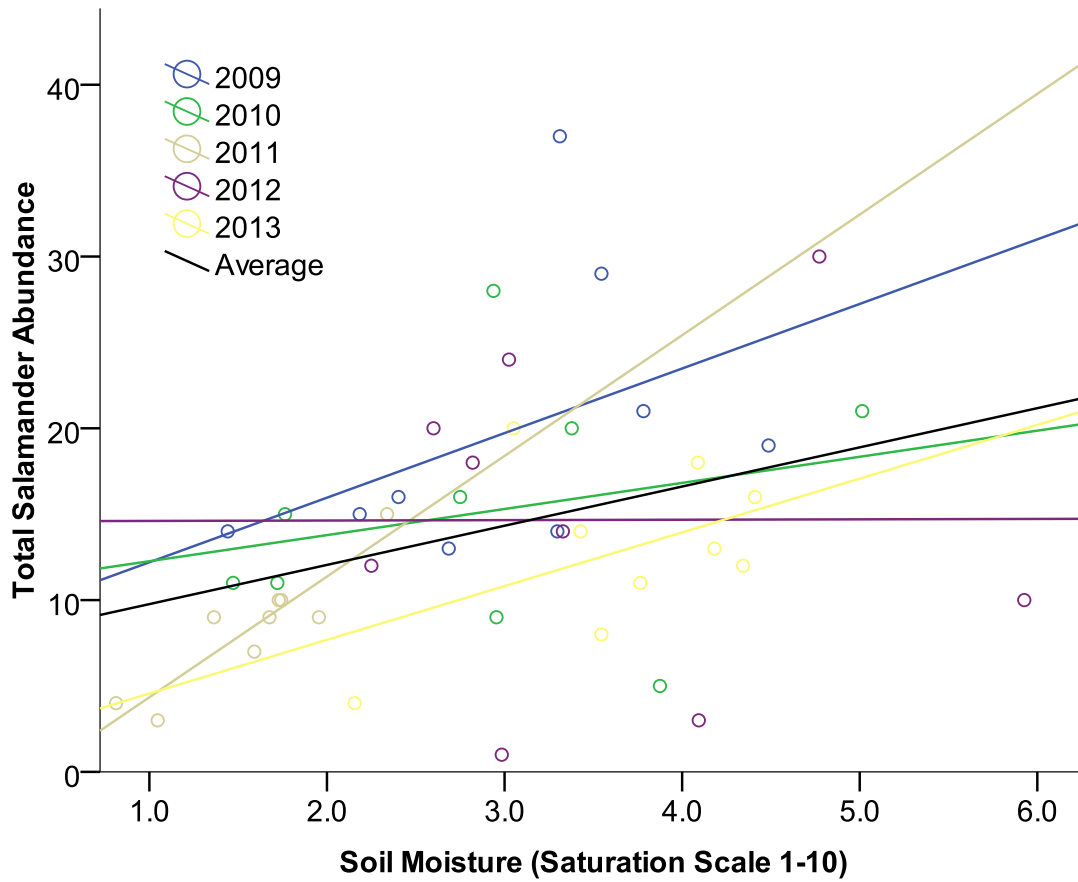
### 3.3.5 Environmental Parameters

Since air temperature and soil temperature were highly correlated ( $r=0.881$ ;  $p<0.001$ ), only one could be included in the analysis and air temperature was therefore removed. It should be noted that while week of sampling was not a significant predictor of salamander abundance in the preliminary analysis, week is highly correlated with both air temperature and soil temperature. The best models predicting salamander abundance in the Indian Woods included soil moisture and year ( $F_{2,44}=8.434$ ,  $p=0.001$ ,  $r^2=0.223$ )(Table 3.3). Salamander abundance had an overall positive relationship with soil moisture ( $r^2=0.120$ ), particularly high in 2009 and 2011 and low 2012 (Figure 3.5). In the Hogsback, only year significantly affected salamander abundance ( $F_{1,49}=9.745$ ,  $p=0.003$ ) and therefore no additional models were explored.

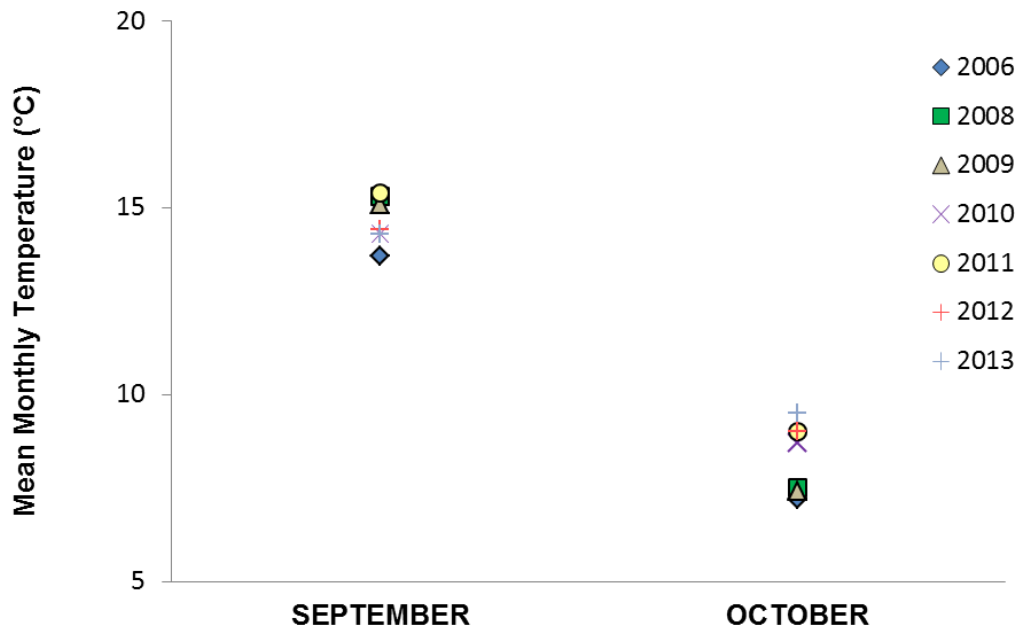
Monthly temperatures during the 2013 monitoring seasons were similar to previous years, although average monthly temperature in October was the highest on record (Figure 3.6). Precipitation levels were slightly lower than previous years, particularly in September (Figure 3.7).

**Table 3.3:** Results of hierarchical multiple regressions and AIC model selection using year, soil moisture, and soil temperature to predict salamander abundance in Indian Woods from 2009 to 2013. Plots were examined separately due to differing sample effort. Independent variables in Indian Woods were log-transformed to meet parametric assumptions. The magnitude and direction of each independent variable's influence is represented by the standardized beta coefficient ( $\beta$ ).  $\Delta AIC$  is the difference in Akaike's Information Criterion from the above model and  $AIC_w$  is the likelihood of a model being the best model, with the best model indicated in bold.

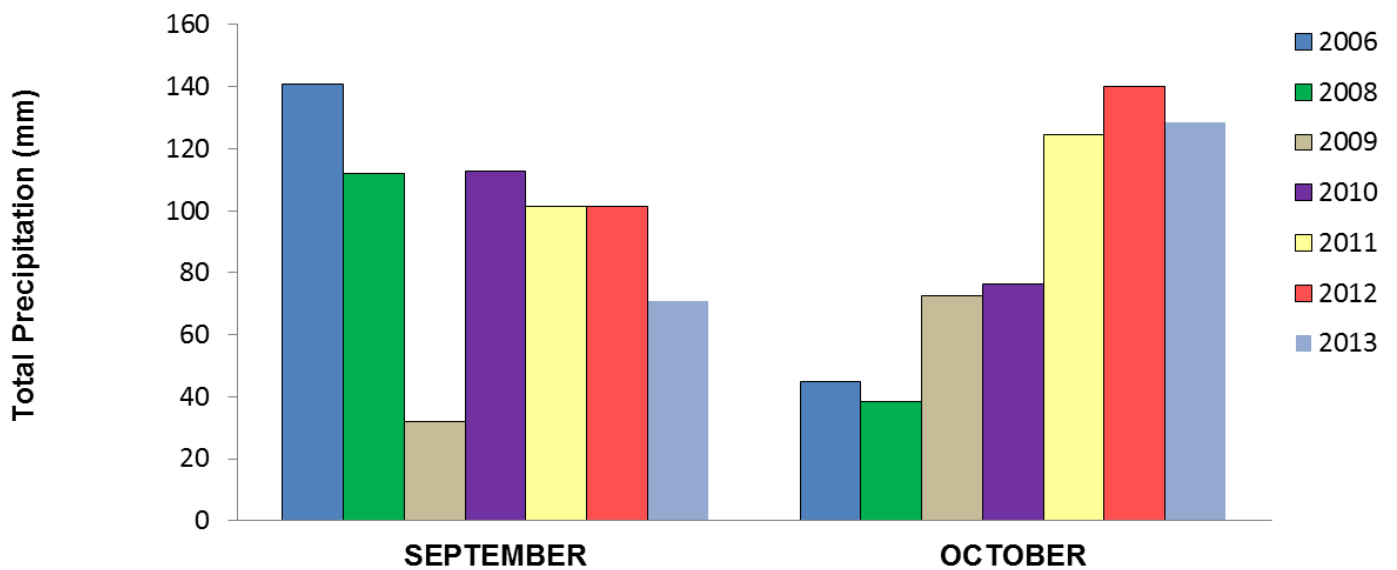
Plot	Model	F	R <sup>2</sup>	P-value	$\beta$ Soil Moisture	$\beta$ Soil Temperature	$\beta$ Year	$\Delta AIC$	$AIC_w$
IN	Soil Moisture	7.694	0.132	0.008	0.390			5.786	0.037
	Soil Moisture + Soil Temperature	3.945	0.118	0.027	0.363	-0.084		7.447	0.016
	Soil Moisture + Soil Temperature + Year	5.580	0.238	0.003	0.45	-0.061	-0.374	1.787	0.272
	<b>Soil Moisture + Year</b>	<b>8.434</b>	<b>0.223</b>	<b>0.001</b>	<b>0.476</b>		<b>-0.377</b>	<b>0</b>	<b>0.665</b>
	Year	3.313	0.050	0.076			-0.67	9.853	0.005
	Year + Soil Temperature	2.653	0.070	0.082		-0.202	-0.271	9.840	0.005



**Figure 3.5:** Relationship between total salamander abundance at Indian Woods and measured soil moisture for 2009 to 2013.



**Figure 3.6:** Mean monthly temperatures for Waterloo Region during the salamander monitoring season in 2006, 2008-2013 (Environment Canada- 2006, 2008-2009 data from Waterloo International Airport Weather Station, and 2010-2013 data from Kitchener-Waterloo Weather Station).



**Figure 3.7:** Total monthly rainfall for Waterloo Region during the salamander monitoring season in 2006, 2008-2013 (Environment Canada- 2006/2008-2009 data from Waterloo International Airport Weather Station, and 2010-2013 data from Kitchener-Waterloo Weather Station).



### 3.4 Discussion

#### 3.4.1 Eastern Red-Backed Salamander Abundance

Given their importance in food web dynamics and 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 could 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 will be completed in 2013. Information gathered on salamander populations in the inaugural years does not contribute to the EMAN protocol for testing monitoring thresholds as it is suggested that the ACOs weather in situ for a winter prior to monitoring to avoid skewing abundance estimates due to the disturbance of plot establishment. Regardless, all data surrounding salamander abundance and diversity is of great value to *rare*, and we can compare the yearly salamander abundance data collected to date.

In Indian Woods, the first two years of monitoring had the highest abundances on record, followed by a steady decline culminating at the lowest abundance recorded in 2011 (Figure 3.1). 2012 and 2013 abundances rebounded to levels similar to 2009 and 2010. Original establishment of ACOs in the Indian Woods may have impacted the observed abundances by providing additional cover, acting as an artefact in attracting salamanders in early years and levelling out as ACOs became weathered and established over time (Van Wieren 2003). Studies on this topic are varied, with some reporting salamanders almost immediately making use of cover boards (Ballantyne 2004; Bennett et al. 2003; Monti et al. 2000) and others suggesting boards must be left for a year to weather before data collected is valid (Zorn et al. 2004; Droege et al. 1997). It may be dependent on other factors, as in Ballantyne (2004), where excess precipitation just prior to and at the start of monitoring may have sped the weathering process, making the boards more appealing to salamanders. The low abundance observed in 2011 may be attributable to the high precipitation levels. Jaeger (1972, 1980) reports that cover objects become more important during dry periods, acting as a moisture refuge for salamanders. Given this, salamanders may be less dependent on cover boards in wetter years, having more moist spaces to use for foraging, and thus lower abundances may be observed under ACOs (Van Wieren 2003). However fall precipitation in 2012 and 2013 were similar to 2011 and salamander abundances observed were higher in 2011, so again many factors including temperature, moisture, and available cover can be having an impact on abundances (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). Winter 2010-2011 had the lowest recorded temperatures through the monitoring years, and so the low abundances recorded in fall 2011 could be the result of low survivorship through the previous winter. The major winter strategy of Eastern Red-Backed Salamanders is avoidance of subzero temperatures by retreating into the soil column (Storey and Storey 1986), having been observed as deep as one meter in the soil (Grizzell 1949; Hoff and Hoff 1977). While no significant differences were found between weekly abundances, there was variation between weeks with generally a peak at the seventh week and a rapid decline in the two weeks following (Figure 3.2). From 2009 to 2013, there are no significant differences in the abundance of

salamanders, which may imply a constant trend after the initial settling of the ACOs, the exception being the significantly higher abundance of salamanders observed in the Hogsback in 2013.

In the Hogsback, 2013 abundances differ from all other years, with the exception of 2009 (Figure 3.1). Contrary to the establishment years in the Indian Woods, the original monitoring session in the Hogsback is the lowest of all years, suggesting perhaps acclimatization of the newly placed ACOs was taking place (Zorn et al. 2004; Droege et al. 1997). The relatively consistent observed abundances following the first year of monitoring in the Hogsback are an encouraging trend, reflecting a likely stable population. In 2013, a large population spike occurred, with salamander observations greater or the same than previous years in every week. In particular, week four saw an extremely high abundance when salamanders were found under seventeen of the twenty boards. In week eight, nine salamanders were found under a single board with representatives from three different species. It is unknown what caused this large increase in observations. While precipitation was lower in September and October than in previous years, the annual rainfall was higher and so conditions may have been well suited for populations to thrive. This certainly appears true when considering the soil moisture levels measured throughout monitoring. 2013 in the Hogsback has the highest recorded moisture levels (Table 3.4), indicating that the ACOs (as the reading was taken within the microclimate created by the ACO) were an ideal location for salamander. The average monthly temperature in October was higher than that in previous years, which may have prevented the typical drop-off in observations that has been previously observed nearing the end of the monitoring season, thus inflating observation numbers. Salamanders retreat underground to avoid winter temperatures. As can be observed in Figure 3.1 and Figure 3.4, the increase in salamanders observed was primarily observations of adult Eastern Red-Backed Salamanders.

**Table 3.4:** Average soil moisture levels during the salamander monitoring season in 2009-2013 at Indian Woods and the Hogsback.

Plot	Year	Mean Soil Moisture Level
Indian Woods	2009	3.02+/-0.93
	2010	2.87+/-1.14
	<b>2011</b>	<b>1.58+/-0.460</b>
	2012	3.53+/-1.18
	2013	3.68+/-1.91
Hogsback	2009	4.87+/-11.07
	2010	5.47+/-1.42
	2011	3.65+/-0.912
	2012	4.63+/-1.37
	2013	<b>5.63+/-2.34</b>

### 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. Between 2009 and 2012 in Indian

Woods, only Red-backed Salamanders were observed with the red-backed colour phase being dominant (Figure 3.3B). In 2013, a Blue-Spotted Salamander was observed in Indian Woods for the first time since 2008, possibly connected to the vernal pond adjacent to the plots which could be used by this species for breeding. While this pond has been established since the beginning of monitoring, it was almost completely dry in 2012. Mole salamanders are more easily found in the spring (Whitford and Vinegar 1966) and therefore their presence may be an abnormality as opposed to their absence. In the future, expanding monitoring to include the spring season may allow for a more complete representation of the salamander species diversity at *rare*.

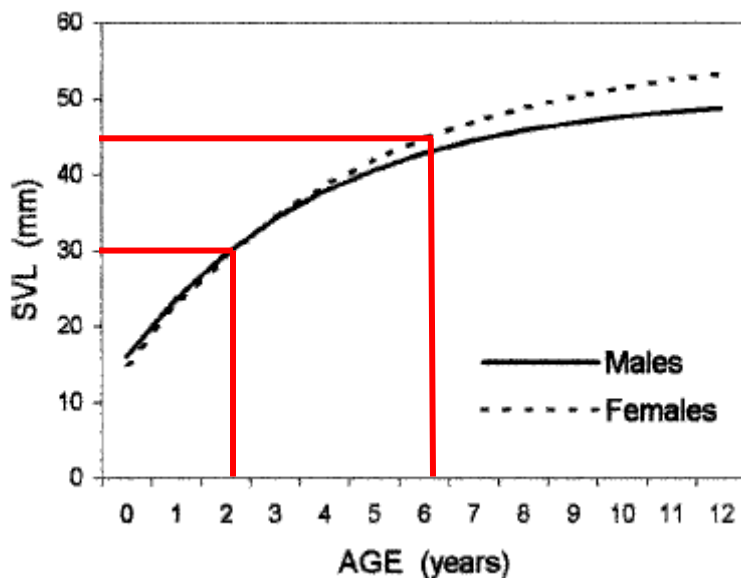
In the Hogsback, Red-backed Salamanders are again dominant with the red-back phase more abundant than the lead-back phase (Figure 3.3A). This is unsurprising, as the lead-backed phase experience preferential predation pressures (Moreno 1989; Venesky and Anthony 2007) and the red-backed phase is known 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 individuals to lead-backed ones in temperate areas. Continued monitoring of Eastern Red-Backed Salamanders and the ratio of the varying colour morphs could therefore be indicative of important global temperature changes affecting the entire forest ecosystem.

Species diversity is higher in the Hogsback than Indian Woods (Figure 3.3). Four-toed Salamanders, another member of the plethodontid family, have been observed in 2008, 2009, 2011, and 2013. It is 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 2009, 2010, 2012, and 2013 and consecutive observations of Yellow-spotted Salamanders from 2009 to 2013. Likely the same individual was repeatedly observed from 2009 to 2012, which has always been observed under the same board. This suggests salamanders may exhibit fidelity to ACOs. In 2013, a new smaller Yellow-spotted Salamander was observed. Expanding monitoring efforts at *rare* to include gender and individual identification may be of benefit.

### 3.4.3 Eastern Red-backed Salamander Size Class Distribution

In both Indian Woods and the Hogsback, the greatest proportion of Eastern Red-backed Salamanders in 2013 fell within the snout-vent length range of 35mm-45mm. Salamanders measured in the Hogsback were on average slightly longer (mean SVL: 37.85+/-5.19) and heavier (mean weight: 0.994+/-0.318) than those in the Indian Woods (mean SVL: 37.01+/-6.22; mean weight: 0.954+/-0.355). 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 3.4). 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 approximate ages of two and six (Figure 3.8). If other size class distinctions had been used to categorize salamanders at *rare*, such as those outlined in

Saylor (1966) and subsequently used in additional studies (Brooks 1999; Ballantyne 2004), data would have been 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.



**Figure 3.8:** Growth in length (SVL) of Red-Backed Salamanders modified from LeClair et al. 2006. Red lines bound the dominant size range observed at *rare* plots.

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). Of twenty occasions in the Indian Woods where multiple Red-backed Salamanders were found under the same ACO, no occasions involved juveniles. Similarly in the Hogsback, of fifty-six occasions with multiple salamanders located under a single ACO, only three involved juveniles. Red-backed Salamanders have 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) however this seems to be occurring minimally, if at all, during the fall months at *rare*. Territoriality of boards in connection to mating may be part of the cause for the underrepresentation of juveniles in this study.

Another likely hypothesis 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. ACOs used in this study may therefore 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. In 2013, canopy cover was monitored by assigning each board into one of three categories: complete, incomplete or no canopy cover. This was found to have no relationship with salamander abundance.

After five consecutive years of consistent monitoring, we are able to establish baseline ranges for salamander populations within Indian Woods and the Hogsback within which we would expect populations to fluctuate within (Indian Woods:  $130^{+/-39}$ ; Hogsback:  $137^{+/-45}$ ). Observations of

salamander abundances outside of these ranges may be indicative of some environmental stress or success, depending on the direction in which the population fluctuates. Only by continuing long-term monitoring, can *rare* best monitor the impact of land management decisions both on an adjacent to the property. Given the upcoming developmental pressures around the Hogsback, and the ongoing aggregate extraction near the Indian Woods, monitoring remains an essential practice at *rare*.

#### 3.4.4 Environmental Parameters

Many factors have been shown to impact plethodontid salamanders including temperature (Spotila 1972; Feder & Pough 1975), moisture (Grover 1998; Feder & Londos 1984), soil pH (Wyman and Hawksley-Lescault 1987; Sugalski and Claussen 1997; Moore and Wyman 2010) and others (Heatwole 1962; Feder 1983; DeMaynadier and Hunter 1998; Jaeger 1972, 1980). This study found that the location of the plot had a large effect on whether or not environmental or temporal variables had an impact on abundance. In the Hogsback, a forest-wetland complex with a thick canopy, no relationship was found between salamander abundance and any tested environmental variable (soil temperature, soil moisture, soil pH, precipitation, sky and wind codes, wind speed, relative humidity, and air temperature). In the Indian Woods, a remnant old-growth forest with a thin, sparse canopy in areas, total abundance was most related to the moisture level in the soil and year of monitoring.

The significance of the temporal variable year is interesting (Table 3.3), since plethodontid salamander populations typically have high stability (Welsh and Droege 2001; Zorn et al. 2004). Some form of population cycling could possibly be accountable for this observed effect, or perhaps this can be attributed to predator-prey cycling, since plethodontids are known to be aggressive predators of soil invertebrates (Wyman 1988) and can significantly reduce soil detritivore numbers (Wyman 1998). Alternatively, this could be a reflection of extremely variable yearly conditions which may be more influential in the more exposed Indian Woods.

The significance of soil moisture on salamander abundance is not surprising (Table 3.3). Plethodontid salamanders require moist skin to facilitate gas exchange across their cutaneous membrane for respiration (Behler and King 1979; Welsh and Droege 2001). These salamanders are therefore highly dependent on receiving moisture from their micro-environment and are most likely to reside in damp wooded areas (Froom 1982). Plethodontid behaviours, such as foraging and reproduction, can be altered depending on the moisture available in their microhabitats. During cool, moist weather they can disperse across the forest floor, while in drier conditions they would be confined to moist microhabitats or spend very little time in dry exposed areas (Jaeger 1972, 1980; Feder 1983; Droege et al 1997). The relationship between soil moisture and total abundance in the Indian Woods is overall a strong positive one, where more salamanders are observed where the soil moisture is higher (Figure 6). This relationship was particularly strong in 2011, a year where soil moisture measurements were lower than any other year (Table 3.4). Average air temperature was higher than other years, and humidity was lower so the boards may have been providing crucial moisture refuges for salamanders when moist microhabitat was limited (Jaeger 1972; Van Wieren 2003). This relationship was additionally strong in 2009, a year when precipitation in the fall, particularly September, was especially low (Figure 3.7). Likely a similar moisture refuge was occurring. The relationship is weakest in 2012 when an

abundance of rainfall (Figure 3.7) and high average soil moisture (Table 3.4) may have made a ubiquitously wet environment, reducing dependency on cover boards to provide a moist microhabitat.

Sugalski and Claussen (1997) found soil pH to be the more influential factor on salamander distribution, more so than soil moisture. This is not the case in this study, likely because soil conditions in both forest stands fall within or close to their preferred pH range of 6.0 to 6.8 (Heatwole 1962), and it is suggested that plethodontid salamanders avoid soil with a pH of 3.8 or less (Wyman and Hawksley-Lescault 1987; Wyman 1988). In future monitoring years, if pH becomes a more accurate predictor of salamander abundance, it may be an early warning sign of soil acidification.

### 3.5 Conclusions and recommendations

After five years of consistent and consecutive monitoring, this program has established baseline data of expected salamander population in both the Indian Woods and Hogsback for future years to be compared to. This program acts as an early warning sign for environmental change in two of *rare's* forest stands. It is therefore recommended that a full nine week monitoring program continue at both plots. In the future, the addition of a spring monitoring session would be an asset to *rare*, when it is feasible to run long-term. This will allow *rare* to gather a better understanding of the true biodiversity of species on the property, and may tease apart some of trends relating to the monitoring season.

The addition of Mirrored Research salamander boards into the Cliffs and Alvars forest will continue to expand the program to include all three forest stands on *rare* property, and allow for a more complete analysis of ecological health.

### 3.6 Literature Cited

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### 4.1 Introduction

#### 4.1.1 Forest Biodiversity 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 of flora and fauna, providing habitats for numerous ecosystems (Butt 2011). They are also an integral part of soil conservation, water cycling, and air quality mediation (Butt 2011). Globally, initiatives establishing policy and protocol related to the safeguarding of forests are a 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 a change in land use, forest species have been removed and land cover has been significantly altered. What remains are forests that are highly fragmented and smaller in size than what were historically present (Waldron 2003). These forests face significant pressures from both biotic and abiotic factors.

Establishing long-term monitoring across a network of sites can aid in developing an improved understanding of baseline levels of variability and health in natural systems (Gardner 2011). Monitoring crown conditions and stem defects is essential in providing an early warning system to recognise changes in tree health of Canadian forests and urban areas (Environment Canada and Canadian Forest Service 2004). Records of tree damage will help to identify the cause and effect of tree and forest decline. Information on population or species decline can be used as a platform to launch conservation initiatives (Gardner 2011), and may influence management objectives when considering human-impact on forest tracts. The age, diversity, and overall health of a forest stand can be derived from canopy tree monitoring however it says little about its likely successional trajectory. 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 & Gillepsie 1999). Shrub and small tree monitoring can give valuable insight into the successional direction of a forest stand by observing saplings that may eventually replace canopy species. Historical records can aid in understanding a forest's past dynamics and structure while long-term monitoring of both canopy tree and shrub and 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 designed 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 termed Indian Woods harbouring 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 rare habitat to countless plants and animals that require mature forest interior (Ontario Ministry of Natural Resources 1999).

These 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 conditions at the *rare* forests and continuing long-term monitoring, changes in the forest ecosystem may be detected early, and 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 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 species, location, 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 wooded 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 completely monitored in 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 monitoring program specific to this forest stratum has been developed to better accommodate the needs and resources of *rare*.

## 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 houses 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, Figure A.4). To access these plots, walk from the ECO Centre to the Grand Trunk Trail. Follow the 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. The plots are located to the left and right of this trail past the large fallen trees. Plot corner are clearly 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, Figure A.4). The plots are approximately 30m apart and the flagging tape on the corners of each plot should be visible from the adjacent plot.

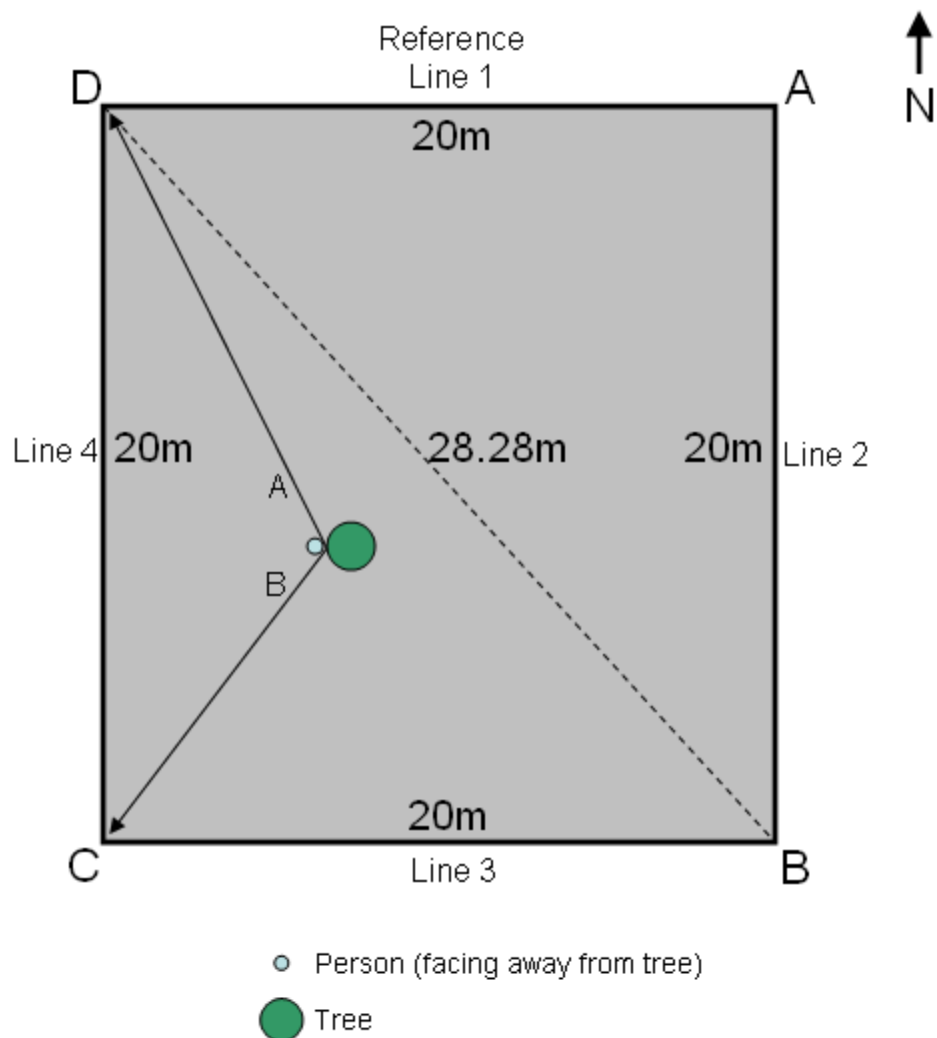
**Hogsback:** Located at the south-west corner of the property, the Hogsback is bisected by Cruickston Creek and bordered by the Newman Drive subdivision to the west. The Hogsback is a mixed swamp forest with upland ridges dominated by White Pine, Red Maple, American Beech, and Sugar Maple. The three 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 plot and can be reached by driving east down South Gate Road to edge of the forest stand, and following the hedgerow around the forest (north, east, north, east), until heading south into the forest at part of fence lowered with a fallen log, marked by pink flagging tape. This entry point is at the southern edge of Hogsback Field (303). 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, Figure A.4).

### 4.2.2 Monitoring Protocol: 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 20m x 20m permanent plots location 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 so 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, the 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 tagging.

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 of the plot. The line number was recorded, and the “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.



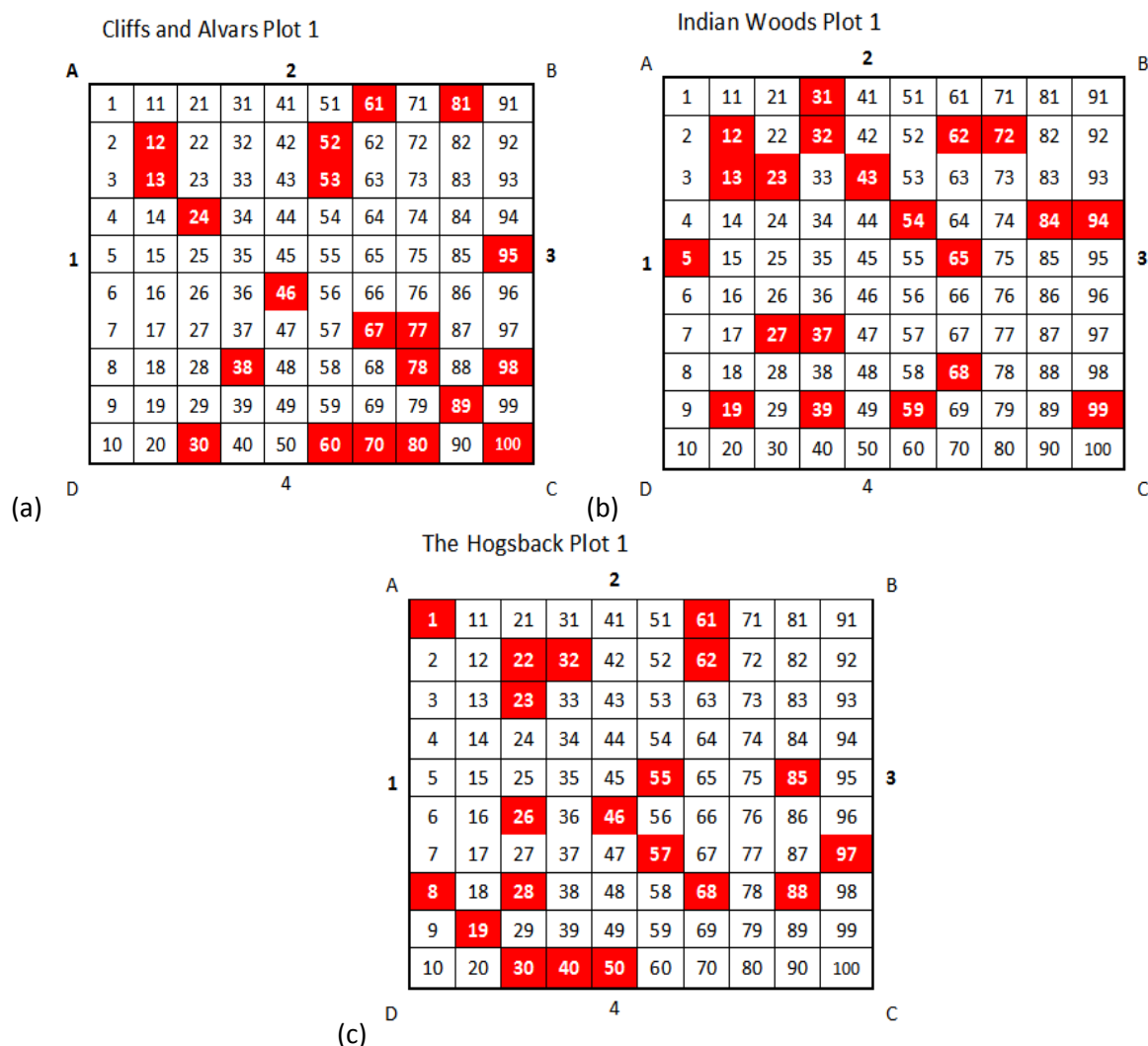
**Figure 4.1:** Diagram of an EMAN forest canopy tree biodiversity plots from McCarter 2009. The A and B distances are used to map the position of the tree within the plot. The A distance is measured from the tree to the corner to the right of the observer standing facing the reference line. The B distance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

To conduct the shrub and small tree pilot study, one existing canopy tree forest plot was selected in each of the three forest stands on *rare* property. In order to limit disturbance with other ongoing monitoring programs at *rare* (i.e. Plethodontid Salamander Monitoring), Forest Plot 1 was selected in all forests.

The shrub and small tree monitoring pilot study was formulated according to the recommendations laid out by EMAN terrestrial vegetation monitoring protocol (Roberts-Pichette & Gillespie 1999). In each forest stand, Forest Plot 1 was first subdivided into one hundred 2 x 2 metre sections and a random number generator was used to select twenty nested quadrats for sampling (Figure 4.2). A 2 x 2m quadrat was used in this pilot study because of the dense understory of the Indian Woods, and allowed for comparable results between the three forest stands. Smaller plot sizes are also a more efficient use of time and resources (Roberts-Pichette & Gillespie 1999). Forest plots were



delineated by attaching twine to the steel pigtails marking the corners of the canopy tree monitoring plots. If the corner stakes had shifted or were missing, the pigtail stakes were adjusted or replaced to the correct distance as set out by the tree canopy monitoring protocol. Along the twine outlining the plot perimeter, flagging tape was tied at two metre intervals to help the observer better visualize a grid over the entire plot. Because the shrub and small tree monitoring pilot study was nested within existing monitoring plots, corners were oriented along the same cardinal directions as canopy tree plots (Figure 4.1) with nested quadrat number one located at corner 'A'. A steel pigtail marked with flagging tape was placed at the centre with a tag identifying the nested quadrat number (i.e. Q70). The data collected from the twenty 2 x 2m nested quadrats was used to establish a species accumulation curve for each plot, which was used as an indication of adequate sampling effort.



**Figure 4.2:** Nested quadrat layout for shrub and small tree monitoring pilot study for (a) Cliffs and Alvars Forest Plot 1, (b) Indian Woods Forest Plot 1, and (c) Hogsback Forest Plot 1. Squares highlighted in red represent nested quadrats sampled in the pilot study.

3.2.3 Monitoring Protocol: Procedure

### 4.2.3 Canopy-Tree Monitoring

Each plot should be visited once annually, ideally in the summer or when leaves are still present on trees for ease of identification. In 2013, forest plots were visited July 29<sup>th</sup>, August 1<sup>st</sup>, and August 16<sup>th</sup> (Cliffs and Alvars), August 6<sup>th</sup>, August 9<sup>th</sup>, and August 23<sup>rd</sup> (Indian Woods), and August 27<sup>th</sup> and August 28<sup>th</sup> (Hogsback). 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), tree height (Haglöf Electronic Clinometer & Mastercraft® Fibre glass measuring tape), 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). Trees that had newly met minimum requirements were tagged in a manner consistent with their plot and measured into the plot using distance from adjacent corners as described above. All trees were plotted into BioMon (BioMon for Windows Suite Version 2), a biodiversity monitoring software package, to generate tree species maps for each forest plot (Appendix A, Figure A.6-A.14).

**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
Codominant: 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 codominant 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

#### 4.2.4 Shrub and Small Tree Monitoring

Shrub and small tree monitoring at each of the three plots should be performed at least once every five years. In 2013, plots were visited on August 16<sup>th</sup> 2013 (Cliffs and Alvars), August 23<sup>rd</sup> 2013 (Indian Woods), and August 28<sup>th</sup> 2013 (Hogsback). At each monitoring visit, individual shrubs and saplings were sampled if their dbh was or exceeded 4cm and/or they were at least one meter tall. If either condition was satisfied, the following data were collected: species, number of stems, shrub and small tree distinction, dbh, and general condition. Individuals that had an exceedingly small dbh (less than 2 cm) were ascribed the value “<2 cm” to facilitate quick sampling. General condition of sampled individuals was ascribed using the same coding system as provided by EMAN (Table 4.1).

EMAN shrub and small tree protocols recommend measuring the height of the tree from the ground to the lowest living branch, which allows for the calculation of the live canopy. The size of the live canopy of a tree can give an indication as to the vigor of an individual (Lhotka n.d). For the monitoring purposes of *rare* this measurement was omitted due to limited time and resources. In the future these measurements could be added to the data collection procedure however the above listed variables were deemed sufficient for the needs of the pilot study.

#### 4.2.5 Data Analysis

Data were analysed using Microsoft Excel 14.0.6 (Microsoft 2010) and PASW Statistics 20.0 (SPSS Inc.) for MacIntosh. Prior to analysis, assumptions of parametric testing were examined. When transformation was required, the appropriate transformation to decouple variance and mean was determined using Taylor's Power Law (Perry 1981).

For each forest stand, summary statistics were calculated by combining the data from the three plots which represent the same stand. Within each stand, the number of species present, the number of trees present, the mean diameter at breast height for included trees, and the total basal area were all recorded from the three tree plots combined. Basal area was calculated as the cross sectional area of all tagged tree stems in the plot and was determined using the dbh data. In addition to the summary statistics, a univariate analysis of variance (ANOVA) with two fixed factors, year and a recoded size class and location combination variable, was used to investigate differences between size classes at each location, using the abundance of trees in each of eight size classes from 0.1m at dbh to 0.8+m at dbh. Data compiled from the past four years of forest health monitoring (2009-2013) was used to conduct the ANOVA. Due to an interaction between size class and location factors, these variables were combined into a combination variable (Leech et al. 2008) in order to effectively answer the question of interest. Only relevant comparisons were considered in the subsequent Bonferroni post hoc testing. Only living trees were included in this analysis.

Mean stem dbh and standard deviation were calculated for each forest stand, and the species diversity and evenness were calculated using the Shannon-Wiener Diversity Index (Shannon H) (Figure 4.3). Additionally, the relative density (Figure 4.4), relative frequency (Figure 4.5), relative dominance (Figure 4.6), and importance value (Figure 4.7) were calculated for each species (Roberts-Pichette & Gillespie 1999). Only living trees were included in these calculations.

Shannon index:

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

Where  $p_i$  is the proportion of individuals belonging to the  $i$ th species

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

Where  $H$  is the Shannon index and  $S$  is the number of species

**Figure 4.3:** Shannon-Wiener Diversity Index and Evenness formulas.

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

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

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

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

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

$$\text{Relative Dominance} = \frac{\text{Basal area of species A (m}^2\text{)}}{\text{Total basal area of all species in the plots (m}^2\text{)}} \times 100$$

**Figure 4.6:** Formula for calculating the relative dominance of tree species in a forest stand, with all three plots per stand combined.

$\text{Importance Value} = \text{Relative Density} + \text{Relative Frequency} + \text{Relative Dominance}$
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**Figure 4.7:** Formula for calculating the importance value of each tree species in a forest stand.

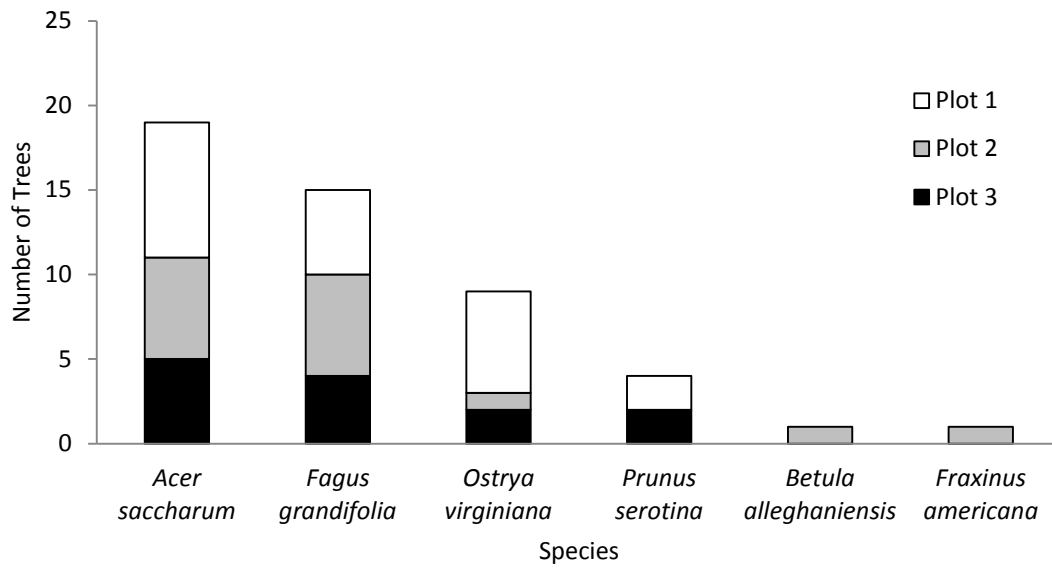
Data analysis for the shrub and small tree monitoring pilot study consisted of creating a species accumulation curve for each of the three forest plots to determine adequate sampling effort. Shannon-H and  $E_H$  values were also calculated for the three plots selected for the pilot study using the equations in Figure 4.3.

## 4.3 Results

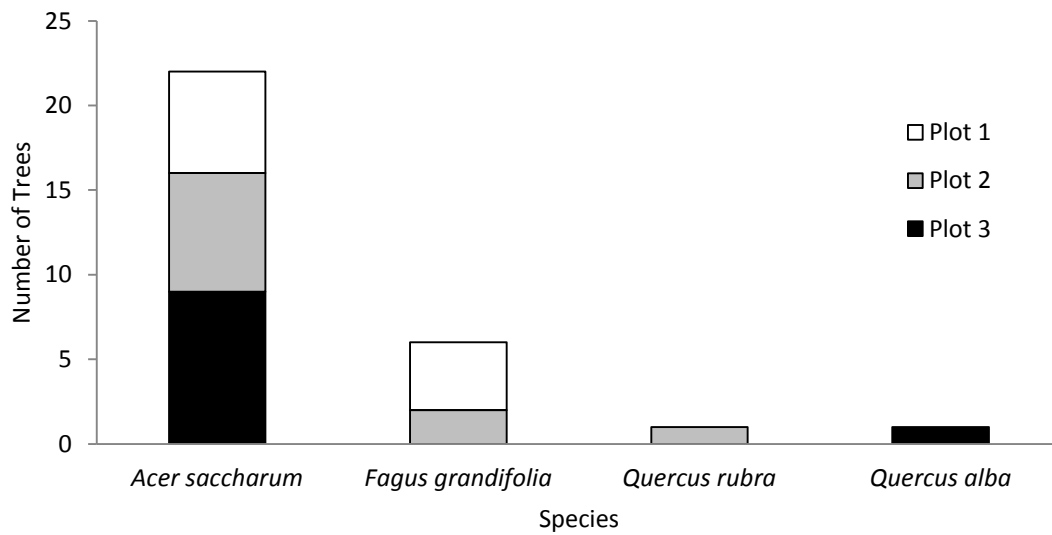
### 4.3.1 Tree Species Diversity

#### Canopy Tree Monitoring

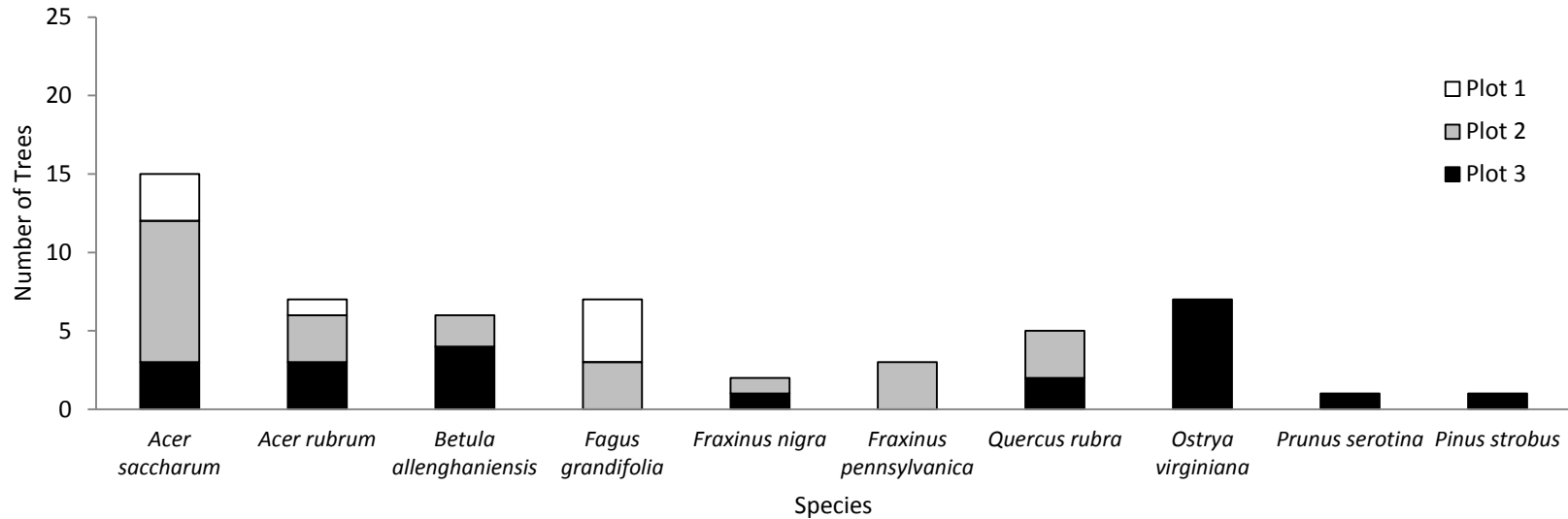
In 2013, the Cliffs and Alvars forest plots contained six living species which represented six different families (Figure 4.8). The forest monitoring plots allow us to make inferences about the forest stand as a whole, with regards to dominant species and age. The Cliffs and Alvars forest stand remains dominated by Sugar Maple and American Beech). As no new tree mortalities or recruitments occurred, the Shannon-H (1.394) and  $E_H$  (0.778) remained the same as was calculated during the 2012 monitoring season (Table 4.3). Indian Woods was found to have four living species representing two families (Figure 4.9) and exhibited the lowest species diversity in comparison with the other forest stands on the property (Table 4.3). The calculated Shannon H (0.761) and  $E_H$  (0.549) both showed a decrease from previous monitoring years, despite suffering no mortalities and gaining one new recruit between the 2012 and 2013 monitoring seasons. The increased abundance of individuals within Indian Woods is a result of a new Sugar Maple recruit in forest plot 2 as well as the reclassification of an individual that was incorrectly labelled as dead. The new recruit gained between the 2012 and 2013 monitoring seasons does not increase the Shannon H value because Sugar Maple is already the dominant species within the stand. Furthermore, it lowers the calculated evenness value as it further contributes to the dominance of Sugar Maple. The Hogsback forest plots continued to represent the highest species diversity of the three monitored forests on *rare* property, despite a decrease in both the Shannon H (2.054) and  $E_H$  (0.892) values from previous years of monitoring (Table 4.3). This stand recruited trees for the first time since monitoring began. Forest plots were mainly dominated by Sugar Maple and American Beech with forest plot three being dominated by Hop hornbeam (*Ostrya virginiana*) (Figure 4.10).



**Figure 4.8:** Tree species composition and abundance from three forest plots in the Cliffs and Alvars.



**Figure 4.9:** Tree species composition and abundance from three forest plots in the Indian Woods.



**Figure 4.10:** Tree species composition and abundance from the three forest plots in the Hogsback.

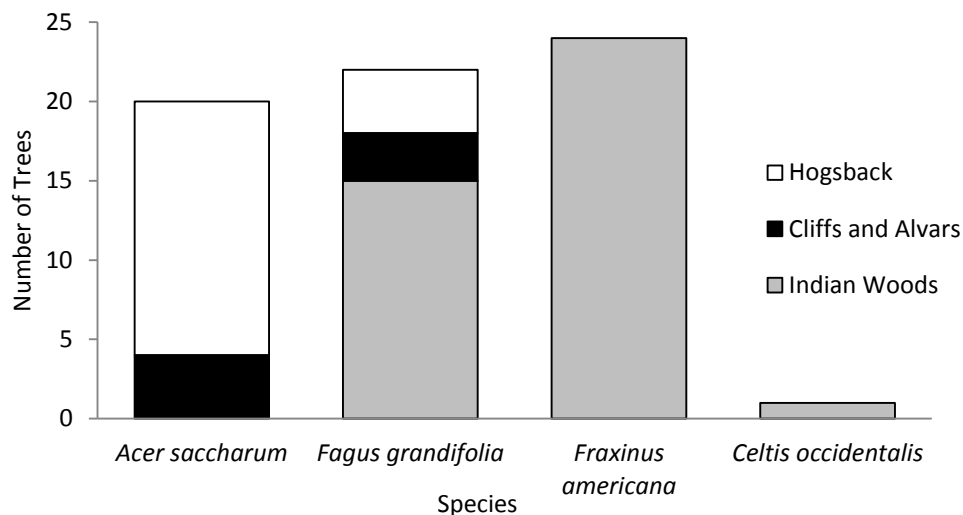
**Table 4.3:** Summary of forest monitoring plot observations with Shannon-Wiener Diversity Index and evenness value for each forest stand, with three plots per stand pooled to calculate values.

Measures	Indian Woods					Cliffs and Alvars					Hogsback			
	2009	2010	2011	2012	2013	2009	2010	2011	2012	2013	2010	2011	2012	2013
Number of Live Trees	34	32	32	29	31	49	52	51	51	51	55	55	55	57
Number of Dead Trees	4	7	7	10	10	8	8	9	10	10	6	6	6	6
Number of Species	5	4	4	4	4	7	7	7	6	6	10	10	10	10
Mean Stem DBH (cm)	32.97	32.11	32.30	33.10	32.90	23.07	23.34	23.30	23.40	23.40	24.92	25.10	24.49	25.30
Shannon-Weiner Diversity Index	0.843	0.746	0.746	0.792	0.761	1.503	1.509	1.469	1.394	1.394	2.078	2.078	2.078	2.054
Evenness	0.524	0.538	0.538	0.571	0.549	0.772	0.775	0.755	0.778	0.778	0.903	0.903	0.903	0.892



## Shrub and Small Tree Monitoring

This pilot study intended to monitor both shrubs and small trees, however no shrubs were found that met the inclusion requirements. The species composition of the three forest plots monitored during the shrub and small tree pilot study is given in Figure 4.11. Three of the four species found were consistent with those recorded in canopy tree forest monitoring (*A. saccharum*, *F. grandifolia*, and *F. americana*). The plot with the highest species richness and abundance was found in Indian Woods, and this is reflected in the high Shannon-Wiener Diversity value (Table 4.4). The Cliffs and Alvares forest plot exhibited the lowest abundance of observed individuals, of which nearly half were found to be dead.



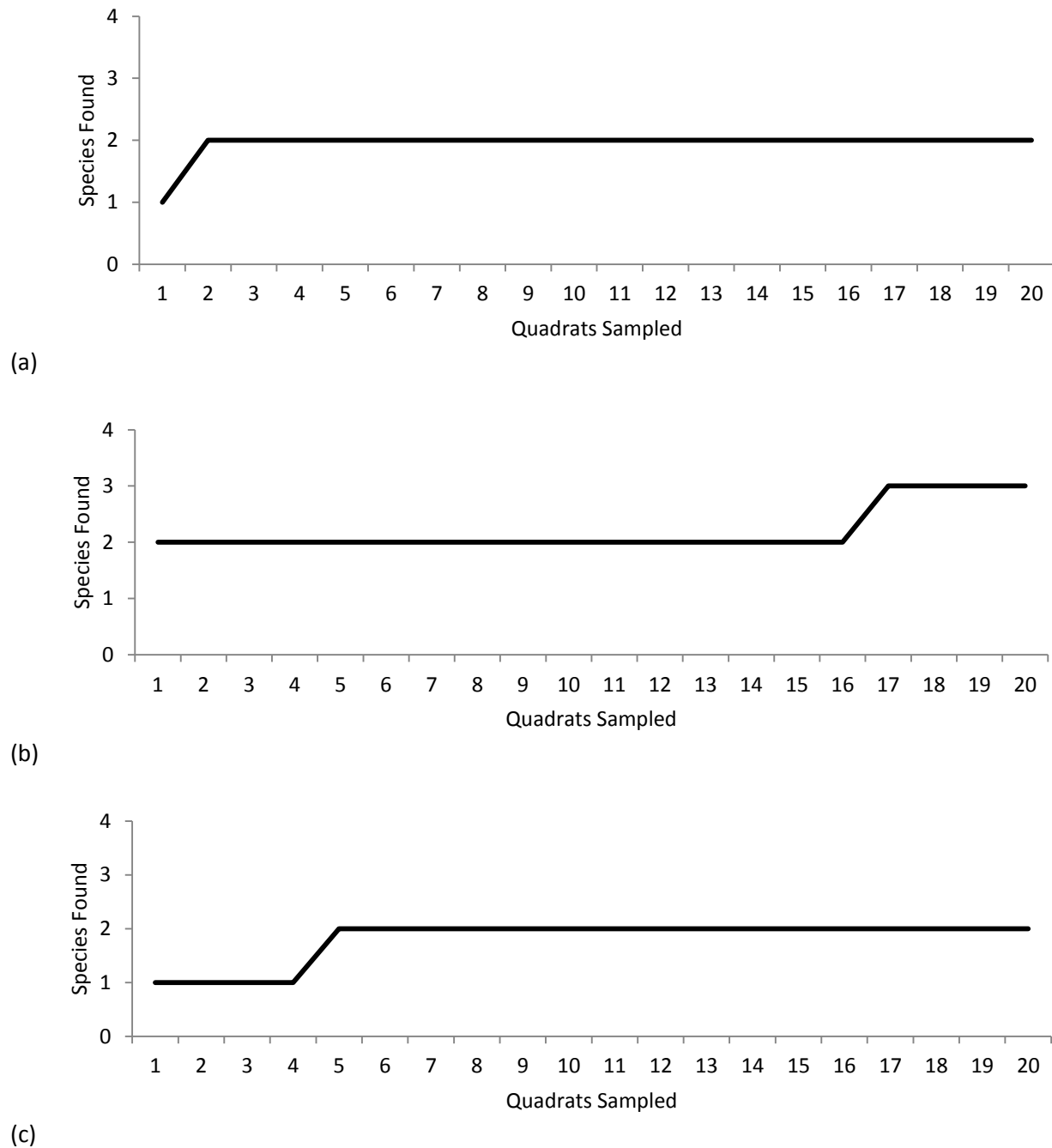
**Figure 4.11:** Trees species composition and abundance from monitored shrub and small tree quadrats

**Table 4.4:** Summary of shrub and small tree monitoring observations with Shannon-Wiener Diversity Index and evenness value for each selected forest plot.

Measures	Cliffs & Alvares	Indian Woods	Hogsback
Number of Live Trees	7	40	20
Number of Dead Trees	6	3	7
Number of Species	2	3	2
Shannon-Wiener Diversity Index	0.683	0.767	0.500
Evenness Measure	0.985	0.698	0.455

The principle goal of the shrub and small tree monitoring pilot study was to create a species accumulation curve for each of the plots included in the study (Figure 4.12). As each additional quadrat was sampled, the number of new species was plotted, which yields a curve similar to those shown in Figure 4.12: an initial rise of species found followed by a plateau as each additional species becomes increasingly rare (Ugland et al. 2003). The species accumulation curves for the shrub and small tree

monitoring pilot study give a visual representation of the sampling effort required to adequately capture the species diversity within each plot. The shallow curves observed are indicative of relatively small species diversity; indeed only four living species were observed in all the monitoring plots included in this pilot study (Figure 4.11).



**Figure 4.12:** Species accumulation curves for shrub and small tree monitoring pilot study for (a) Cliffs and Alvares Forest Plot 1, (b) Indian Woods Forest Plot 1, and (c) Hogsback Forest Plot 1.

#### 4.3.2 Stand Characteristics and Size Class

Size class differences varied with location (interaction,  $F_{14, 698} = 3.130$ ,  $p < 0.001$ ) so both factors were considered simultaneously in a combination variable of size class and location (Leech et al. 2008), and significant differences occurred between these levels ( $F_{23, 698} = 14.072$ ,  $p < 0.001$ ) (Figure 4.13). No differences existing between monitoring years ( $F_{4, 698} = 0.464$ ,  $p = 0.762$ ).

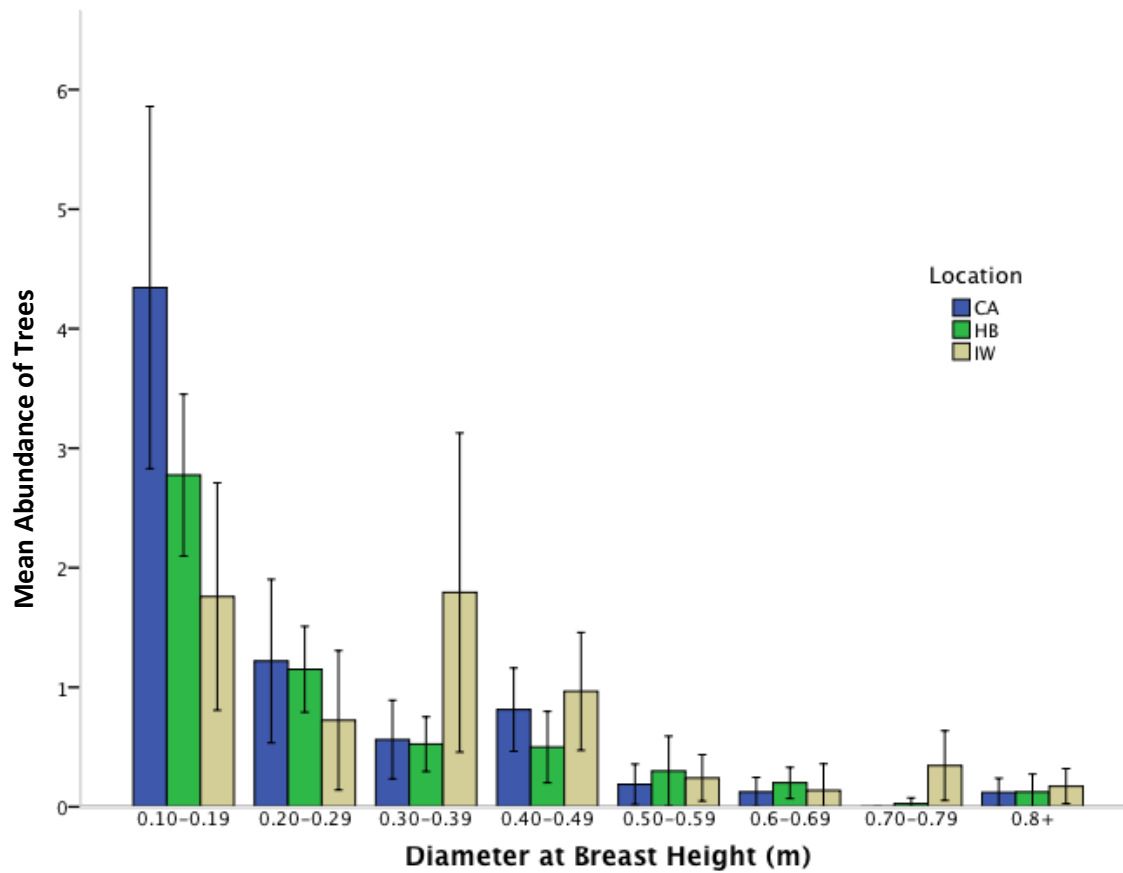
The Cliffs and Alvars and Hogsback forests both housed a significantly greater abundance of the smallest size class (0.10-0.19m dbh) than any other size class included in this monitoring program (post hoc  $p < 0.001$ ). The majority of trees in all three forest stands on *rare* property were found to be within the smallest size class (0.1-0.19 m dbh), with the greatest abundance of trees of this size being in the Cliffs and Alvars. The second size class (0.2-0.29m at dbh) in the Cliffs and Alvars was additionally significantly different from the size class with the least abundance of trees, 0.7-0.79m at dbh (Figure 4.13).

In the Hogsback forest, the smallest size class (0.1-0.19m at dbh) differed significantly from all other size classes with the exception of the second smallest (0.2-0.29 m at dbh) ( $p < 0.001$ ). Similar to the Cliffs and Alvars forest, a greater proportion of trees found in the Hogsback forest are housed within the first four size classes.

The Indian Woods had comparatively the lowest abundance of trees of the three monitored forests, but those trees making up that stand were spread out amongst the size classes, particularly the first four (Figure 4.13). The Indian Woods showed no significant differences between size classes within the forest (post hoc  $p < 0.001$ ).

In 2013, three trees were added to the monitoring plots (Indian Woods and The Hogsback) that newly achieved the minimum threshold for diameter measurement. In all three of the forest stands, no mortalities were recorded. The Hogsback has yet to suffer any mortalities in any of the three monitored forest plots and, in 2013, two new recruits were recorded for the first time since monitoring in the forest stand began in 2009.

The yearly growth per forest stand is documented in Table 4.3, indicating the mean dbh of trees within the forest plots at each location. Abundance, basal area, relative density, relative frequency, relative dominance, and importance value for each species are shown in Table 4.5 for each location in 2012. In all three stands, Sugar Maple was found to have the highest importance value.



**Figure 4.13:** Tree trunk size distribution measured at breast height from 2009 to 2013 forest health monitoring at *rare*, CA= Cliffs and Alvars, HB= Hogsback, IW= Indian Woods. Error bars represent Confidence Intervals at 95%.

**Table 4.5:** Tree species composition and summary statistics for the three monitored forest stands at *rare* in 2013.

Location	Species name	Abundance	Basal Area (m <sup>2</sup> )	Relative Density	Relative Frequency	Relative Dominance	Importance Value
Indian Woods	<i>Acer saccharum</i>	23	2.59	74.19	42.86	72.24	189.29
	<i>Fagus grandifolia</i>	6	0.21	19.35	28.57	5.92	53.84
	<i>Quercus alba</i>	1	0.17	3.23	14.29	4.67	22.18
	<i>Quercus rubra</i>	1	0.62	3.23	14.29	17.18	34.69
Cliffs and Alvars	<i>Acer saccharum</i>	19	1.55	37.25	23.08	45.59	108.92
	<i>Betula alleghaniensis</i>	1	0.15	1.96	7.69	4.76	14.42
	<i>Fagus grandifolia</i>	17	1.13	33.33	23.08	35.39	91.80
	<i>Fraxinus americana</i>	1	0.02	1.96	7.69	0.48	10.13
	<i>Ostrya virginiana</i>	9	0.10	17.65	23.08	3.18	43.90
	<i>Prunus serotina</i>	4	0.24	7.84	15.38	7.61	30.83
Hogsback	<i>Acer rubrum</i>	7	1.14	12.28	15.00	28.05	55.33
	<i>Acer saccharum</i>	15	0.75	26.32	15.00	18.52	59.83
	<i>Betula alleghaniensis</i>	6	0.32	10.53	10.00	7.88	28.40
	<i>Fagus grandifolia</i>	9	1.07	15.79	15.00	26.27	57.06
	<i>Fraxinus nigra</i>	2	0.17	3.51	10.00	4.10	17.61
	<i>Fraxinus pennsylvanica</i>	4	0.15	7.02	10.00	3.77	20.78
	<i>Ostrya virginiana</i>	7	0.11	12.28	5.00	2.74	20.02
	<i>Pinus strobus</i>	1	0.01	1.75	5.00	0.27	7.03
	<i>Prunus serotina</i>	1	0.06	1.75	5.00	1.45	8.20
	<i>Quercus rubra</i>	5	0.28	8.77	10.00	6.96	25.73

## 4.4 Discussion

### 3.4.1 Tree Species Diversity

With the addition of a shrub and small tree monitoring program, a more detailed analysis of the forests at *rare* can be added to the baseline data collected since the beginning of monitoring with respect to present and near future characteristics. Each of the three forest stands at *rare* included in this monitoring program exhibit individual characteristics and are subject to different influences. Changes in forest structure and species composition can be inferred from the species assemblages of the understory, which can ultimately aid in the development of suitable management strategies that can meet the challenges of each unique forest.

Two species that were not only present in all three forests but also dominant in each were Sugar Maple and American Beech, which is a typical species composition in North American late-successional forests (Takahashi & Lechowicz 2008). The species recorded in the shrub and small tree pilot study reflect a similar composition to that of the canopy tree monitoring program at *rare*. Sugar Maple and American Beech constitute as much as 63% of the total number of individuals recorded in the shrub and small tree pilot study and as much as 64% in canopy tree monitoring.

The Cliffs and Alvars forest is a mature stand co-dominated by Sugar Maple and American Beech, which together make up 69.4% of the trees in the three monitoring plots. Most species found here prefer well drained upland habitats and are tolerant of shade (Laird Farrar 1995), performing well in the complete canopy. In exception is the Yellow Birch (*Betula allaghaniensis*), which favours moist soils but commonly occurs in mixed woods with Sugar Maple and American Beech, and Black Cherry (*Prunus serotina*) which is intolerant of shade and found in canopy gaps (Laird Farrar 1995). The species composition of the shrub and small tree stratum reflects a similar dominance of Sugar Maple and American Beech. Both of these species are very shade-tolerant, and can thrive as saplings beneath the canopy through their individual growth strategies (Laird Farrar 1995; Takahasi & Lechowicz 2008). Based upon the results of the canopy tree and shrub and small tree monitoring programs, the Cliffs and Alvars forest appears to be trending towards a late successional community, where canopy species are replicating in forest strata below in the next generation of individuals.

Indian Woods is an eastern deciduous remnant old-growth forest dominated by Sugar Maple; an ecosystem that is rare in the region and to southwestern Ontario (Robson et al. 2012). The Shannon H and  $E_H$  values were found to be the lowest in Indian Woods out of the three forest stands at *rare* (Table 4.3), which is indicative of the variable abundances of each species found within the monitoring plots. Old-growth forests like Indian Woods are often considered to have reached a climax community stage, wherein the community structure will continue in a state of dynamic equilibrium in the prevailing environmental conditions (Krebs 2011). Typically climax communities are found to be self-replicating, in that seedling and sapling species are congruent with dominant canopy species as they create favorable conditions for the propagation of their species (Ostergren & Vale 1997). The Indian Woods shrub and small tree monitoring plot was found to have the greatest abundance of individuals and the highest Shannon-Wiener Diversity index (0.767) across all plots, although this could be falsely inflated by the single Hackberry (*Celtis occidentalis*) found in the second last nested quadrat. This species is also absent from the canopy stratum in the forest monitoring plots in Indian Woods and could be considered to be

anomalous to the species richness. In Indian Woods, the majority of individuals observed in the shrub and small tree pilot study were White Ash (Figure 4.11) which is commonly associated in well-drained soils with other broadleaf species and, like the lone Hackberry is moderately shade-tolerant (Laird Farrar 1995). It is possible that these species have been observed in the understory of Indian Woods because of the greater amount of canopy gaps present, thereby increasing the amount of light reaching the shrub and small tree stratum. This could also account for the overall higher abundance of individuals observed in the pilot study. Canopy gaps and closures, otherwise known as canopy dynamics, are known to influence the regeneration and growth rates of forests, as well as species composition (Weiskittel & Hix 2003). Forest canopies are in a constant flux of openings created by disturbance and closures from canopy growth of individuals capitalizing on canopy gaps. For this reason, the canopy dynamics of a forest continue to be the major influence on forest microhabitat (Jennings et al. 1999). As succession progresses and the canopy closes, the composition of canopy trees shifts toward more shade tolerant species like Sugar Maple and American Beech (constituting 74.1% and 19.3% of the individuals in Indian Woods respectively) in eastern deciduous forests (Fox 1977). These species are able to grow suppressed in the understory and exploit canopy gaps when they occur, outcompeting other shade-sensitive species (Weiskittel & Hix 2003). Based upon the findings of the shrub and small tree monitoring pilot study, it would appear that American Beech will continue to constitute a large portion of the canopy in subsequent years. However, it should be noted that a dense seedling layer of Sugar Maple was observed throughout Indian Woods that did not meet the requirements for inclusion in the pilot study. Sugar Maple is a very shade-tolerant species that readily capitalizes on the increased nutrients from canopy gaps (Laird Farrar 1995) and will likely continue to be a major constituent of the Indian Woods canopy in the future.

The Hogsback forest is a forest-wetland complex and as such offers a greater diversity of habitats than the other two forest stands monitored at *rare*. Each of the plots in the Hogsback forest is dominated by a different species: American Beech (Plot 1), Sugar Maple (Plot 2), and Hop Hornbeam (Plot 3). The Hogsback forest also exhibited the highest diversity and was calculated to be the most even of the forest monitoring plots (Table 4.3). The wet margins of the Hogsback plots are likely a source of increased diversity, as Yellow Birch, Black Ash (*Fraxinus nigra*), Green Ash (*Fraxinus pennsylvanica*), and Red Maple (*Acer rubrum*) all thrive in wet soils (Sibley, 2009). The Hogsback forest surprisingly exhibited the lowest Shannon H (0.500) and Evenness (0.455) values of all the plots included in this pilot study (Table 4.4), in contrast to the findings of canopy tree monitoring. One possible reason for this could be the thick canopy layer that covers the Hogsback forest for much of the growing season. This would make it much more difficult for species less shade-tolerant than Sugar Maple and American Beech (i.e. Black Cherry) to survive long enough to capitalize on a canopy gap when it occurs (Laird Ferrar 1995; Kershaw 2001). Indeed, the only identified species in the pilot study were Sugar Maple and American Beech individuals, likely because they are shade-tolerant enough to withstand limited light penetration through the canopy. Given the species composition from the pilot study, it could be inferred that the Hogsback forest is trending towards a Sugar Maple and American Beech dominated forest as seen in Forest Monitoring Plots 1 and 2 (Figure 4.11).

An important consideration is the effect of below-ground competition on sapling and seedling growth and establishment. Understory vegetation can create below ground competition for seedlings by filling root gaps (Pecot et al. 2007). The Hogsback forest supports a multitude of understory species,

such as Skunk Cabbage (*Lysichiton americanus*), Spotted Touch-Me-Not (*Impatiens capensis*), and Poison Ivy (*Toxicodendron radicans*). Invasive species may also influence the Shannon H and  $E_H$  values for the shrub and small tree stratum through outcompeting native species for resources. Indeed, Japanese Barberry (*Berberis thunbergii*) was observed to be in the vicinity of the forest monitoring plots in the Hogsback. Invasive species may have a greater impact on these diversity measures in the future and invasions should be monitored closely in following years.

As of 2013, the Hogsback remains a portion of the *rare* property that is not open for public use and therefore is subject to fewer disturbances. In the future, trails may be developed in this forest stand for public use which may result in a decline in the species diversity and evenness in the Hogsback. Recreational use of forests (i.e. trails) increases the amount of traffic within the forest community, and can result in soil compaction, trampling, and erosion (Jordan 2000). Moreover, increased traffic and habitat fragmentation from trail establishment and use creates vectors of invasion by alien invasive species that can jeopardize the health and integrity of a community (Jordan 2000; Marzano & Dandy 2012). Invasive species are of particular concern because many species are capable of establishing dense monocultures that can outcompete and displace native species. The impact of trail use is magnified with repetition (Marzano & Dandy 2012) and its effects should be monitored closely. If trails are to be created within the Hogsback forest, it is strongly recommended that impacts be measured and recorded with further studies so that changes in the community structure and forest health can be identified as early as possible.

Although the sample size of the shrub and small tree monitoring pilot study was relatively small in comparison with the canopy tree monitoring program, the Shannon H and E statistics are still considered to be valid analyses of biodiversity because they are minimally impacted by sample size (Elsevier 2008). This lower number of species and individuals encountered however is indicative of a lower required sampling effort in the future, as depicted by the species accumulation curves (Figure 4.12), which means that the current design of a shrub and small tree monitoring program could be scaled back. A suggested monitoring timeline and protocol can be found in Appendix F.

### 3.4.2 Stand Characteristics and Size Class

The tree dbh distributions of the three forests were plotted in Figure 4.13 to give a visual representation of the size-class composition of the stands. This information is useful baseline data to which monitoring data from future years may be compared to examine recruitment and replacement patterns of each stand (Forrester & Runkle, 2000; Parker, 2003). The dbh distribution of trees can be used to estimate the age of a forest stand, and in conjunction with height and species composition, can help characterize a forest structure. Both Cliffs and Alvars and the Hogsback have significantly more trees in the smallest size class ( $p < 0.001$ ), which is indicative of a forest comprised of young trees. The Hogsback appears to exhibit the classic distribution of trunk size in a young forest stand with fewer trees in bigger size classes, and tree sizes progressing in a right skewed manner. The Cliffs and Alvars have a similar distribution with one size class exception, the 0.4-0.49 group. Historically, this forest stand was grazed by cattle in the early twentieth century and this past use of the land could account for this increased number of trees in the 0.4-0.49m dbh size class. Trees that are now in the 0.4-0.49m category could have possibly been large enough at the time of this grazing to not be striped by cattle. Conversely,



those trees in the 0.3-0.39m grouping were more likely to be targets of grazing and thus succumbed to this hazard. Alternative to both Cliffs and Alvars and the Hogsback, the Indian Woods has a more even distribution across dbh categories, particularly the first four groupings. The low species richness and more even distribution indicate that while there is regeneration occurring in this forest stand, it is settling as a climax community forest where dominant trees are stable in the understory for many years using a series of gaps to reach the canopy (Forrester & Runkle 2000). It is likely that this forest will continue to be dominated by Sugar Maple and American Beech trees, both shade tolerant and able to withstand years under a complete canopy. Preliminary results from the shrub and small tree pilot study support this hypothesis as American Beech constituted nearly 40% of individuals recorded in Indian Woods. Continued long term monitoring of these plots is essential.

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 & Gillispie 1999). This relative measure is calculated for each species in each plot, by incorporating the relative density, relative frequency, and relative dominance of each species (Table 4.5). The IV therefore comparatively looks at species within plots considering how common and abundant that species is as well as the total amount of forest area that species occupies within each plot. Since we are using relative values, the maximum IV is 300 (Figure 4.7). From a management perspective, the IV is indicative of the overall influence of a particular species in the community structure and may contribute to defining a community based upon its species assemblage.

Despite the apparent difference in forest stand age between Indian Woods, Cliffs and Alvars and the Hogsback forests, Sugar Maple was found to be the species with the highest IV, and was typically followed by American Beech. This combination is commonly associated with late-successional northern hardwood forests, and is typical of the Carolinian forest region (Takahasi & Lechowicz 2008). In the Cliffs and Alvars, Sugar Maple and American Beech are relatively close in IV, however the greater abundance and basal area of Sugar Maple gives it its greater value. The difference between these two species is more striking in Indian Woods where Sugar Maple has an exceedingly high IV in comparison with other species, although this was to be expected because of its greater abundance in the forest monitoring plots (Table 4.5). The American Beech present in the plot are few and generally small in size, which is shown in the results of the shrub and small tree Monitoring pilot study (Figure 4.11). There are also singular trees of species that are not found elsewhere in the monitoring plots, Red and White Oak (*Quercus rubra* and *alba*), but their large size inflates their importance values (Table 4.5). In contrast to the findings from monitoring in previous years, Sugar Maple surpassed American Beech in IV in the Hogsback forest. In the case of Sugar Maple, its greater abundance is the most influential factor in its higher IV whereas American Beech has fewer but larger individuals, therefore enhancing its IV. The same is true for the Red Maple, which has an importance value closely ranked with that of Sugar Maple and American Beech, but fewer individual trees. This indicates that a fewer number of Red Maples have a strong influence on the forest community. Red Maple can thrive in a wider range of soil types than any other forest species in North America (Walters and Yawney 1990) and grows on diverse sites including dry upland ridges and slopes and swamps- both habitats located in the Hogsback forest. While generally Red Maple tends to give way to the more shade tolerant Sugar Maple and American Beech in a mature forest, in wet areas that reach an edaphic climax, Red Maple may be able to maintain a dominant status (Walters and Yawney 1990).

Prior to the start of the monitoring season in 2013, a severe summer windstorm occurred in late July resulting in several large canopy trees being blown over. The damages incurred from this event were the most evident in Indian Woods, where several large canopy trees came down in plot three. Due to the importance of canopy gap dynamics in forest community succession, the newly opened canopy will likely give the opportunity for saplings in the understorey to take advantage of the increased availability of nutrients (Forrester & Runkle 2000). Indeed, tree fall gaps continue to play a very important role in forest succession and greatly influence community dynamics (Forrester & Runkle 2000). For this reason, the continued monitoring of both canopy and shrub and small tree strata especially in this forest stand is strongly recommended as the dynamics of succession within the newly formed canopy gaps will be most evident in the following years.

#### 4.5 Conclusions and Recommendations

In the 2013 monitoring season, several of the American Beech individuals included in canopy tree monitoring were observed to be covered in Beech Blight Aphids (*Grylloprociphilus imbricator*) which have been speculated to kill or damage small limbs and branches of infested individuals (Childs 2011). While this insect on its own is unlikely to kill or severely harm its host tree, in combination with other stresses (i.e. drought, Beech scale etc.) it may result in tree damage or death (Childs 2011). Since the effects of the Beech Blight Aphid upon American Beech are not fully understood, it is recommended that no control measures be taken to reduce infestations, and further monitoring continue. The great abundance of American Beech in all three of the forests at **rare** could enhance the potential for research of the Beech Blight Aphid in a variety of habitats and stresses. Another concern for American Beech individuals at **rare** is the Beech Bark disease (*Nectria coccinea*). This disease is typically found in association with other insects and pathogens that weaken the tree and provide entry points for infection (Davis & Meyer 2004). Since American Beech constitutes a considerable portion of the overstory in the Cliffs and Alvars and the Hogsback forests, widespread infection by Beech Bark disease could potentially cause significant losses in individuals. This pathogen has already been documented in the Kitchener area in Steckles Woods (Burt 2005). Control measures such as selective cutting and removal of severely infected individuals is recommended within these stands to avoid major losses (Davis & Meyer 2004).

Invasive species are another significant threat to the overall forest community health within the three forests on **rare** property. As previously mentioned, increased disturbance through pedestrian traffic and recreation can result in the invasion of aggressive invasive alien species that can quickly dominate the community (Marzano & Dandy 2012). With respect to canopy trees, invasive species that pose the greatest threat are typically insects, like the Emerald Ash Borer (*Agrilus planipennis*) which can infest and kill healthy trees (OMNR 2010). Ash species make up a relatively small portion of the canopy trees in the forest health monitoring program; however the results of the shrub and small tree pilot study indicated that White Ash constitutes much of the understory in Indian Woods. These young individuals will likely be lost before they have the opportunity to be a part of the canopy since the Emerald Ash Borer has been detected in Waterloo Region in close proximity to **rare** (Region of Waterloo 2010). Invasive species that pose significant problems for the health of the forests at **rare** should be identified and their progression tracked in conjunction with **rare's** Invasive Species Management Work

Plan .

One of twelve Butternut trees of *rare* property falls within the monitoring plots in the Cliffs and Alvars area. It has been misidentified as dead-standing previously, but was living with severe crown dieback and extensive wounds over the course of monitoring, until it was found dead and broken just below a height of five meters in 2012. Butternut is listed as Endangered by both the Federal Species at Risk Act (SARA) and provincially on the Species at Risk in Ontario (SARO). The decline of Butternut in North America is attributed to Butternut canker caused by a fungal pathogen (*Sirococcus clavigignenti-juglandacearum*) that evidence suggests is a relatively recent introduction to North America (Broders & Boland 2010). Symptoms of the disease are elongated, sunken cankers, which commonly originate at leaf scars, buds, or wounds (Davis & Meyer 1997). There is currently no prevention, control, or treatment for the disease and most Butternut conservation efforts are focused on the detection of resistant individuals for seed banking and grafting (Forest Gene Conservation Association 2010). As no remaining living Butternut are located in any monitoring plots, continued observation of Butternuts on the property should occur outside of this monitoring program, and continued review of new literature and policy should occur to effectively manage this species at risk.

Continued monitoring at all forest plots is recommended. It is suggested that photo evidence of suspected diseased or dying trees be taken to assist in identification and yearly comparisons to protect the health of the forest stands and species therein. It may be beneficial for monitors to undergo more extensive training to better identify tree ailments, particularly for signs of known problems like the Emerald Ash Borer Beetle and Beech Bark Disease.

#### Recommendations for Future Shrub and Small Tree Monitoring

The intended result of the shrub and small tree monitoring pilot study implemented in 2013 was to develop a species accumulation curve for each of the monitored forests such that a future monitoring strategy could be developed to suit the needs and resources of *rare*.

During the pilot study, several of the individuals remained unidentified because they had no distinguishing characteristics (i.e. distinctive bark, leaves etc.). In subsequent years, it could be advantageous to conduct shrub and small tree monitoring earlier in the summer to facilitate easier identification from leaves. There remains a possibility that the individuals that were unidentifiable during the pilot study of 2013 were dead however holding monitoring earlier in the summer may eliminate the risk of misidentifying individuals as dead that have prematurely dropped their leaves.

Based upon the results obtained during this pilot study, it is recommended that monitoring activities be scaled down to a degree that is more appropriate for the species richness encountered in the monitored plots. This could include reducing the overall number of quadrats used in monitoring from twenty to four placed in each corner of the plot and therefore reducing the amount of nested quadrats in each of the selected monitoring plots. The modification of placing the nested quadrats within the corners of the canopy tree monitoring plots is consistent with the recommendations of EMAN (Roberts-Pichette & Gillespie 1999), and would facilitate quicker set up and monitoring in each session. In this way, the protocols of EMAN are still being implemented in monitoring programs while accommodating needs of *rare*. To accommodate for reduced sampling area, each of the four nested quadrats could be increased from 2 to 5 square metres, which follows the recommendations by EMAN for most monitoring situations (Roberts-Pichette & Gillespie 1999). Based upon the species

accumulation curve, it is likely that the reduced number of nested quadrats will still be able to accurately capture the species diversity and thus expansion to other forest plots is not recommended at this time.

Changes in this forest stratum are likely minimal from year to year, because individuals typically remain as understory trees until the canopy opens up and they can capitalize on the increased light availability. For this reason, shrub and small tree monitoring should be conducted at most once every three years and at least every five years. With permanent nested quadrats in the corners of the canopy tree monitoring plots, individuals can be tracked from each monitoring session to the next, in a similar manner to that of the canopy tree monitoring program. This would provide a greater understanding of seedling and sapling recruitment and mortality rates; one of the core tenants of the shrub and small tree monitoring protocol as prescribed by EMAN (Roberts-Pichette & Gillepsie 1999).

Altering the shrub and small tree monitoring program to these specifications would enable long-term monitoring to take place while still limiting disruption of other ongoing monitoring programs at *rare* (i.e. Annual Decay Rate Monitoring). A more detailed description of a proposed protocol for subsequent shrub and small tree monitoring at *rare* is given in Appendix F.

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## 5.0 SOIL HUMUS DECAY RATE MONITORING

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### 5.1 Introduction

#### 5.1.1 Soil Characteristics and Functions

Decomposition is defined as the physical, chemical, and biological breakdown of organic material into simpler matter, and it is a significant producer of carbon dioxide, as well as methane and nitrogen gases (Berg & McClaugherty 2008). Soil humus, the stable organic material remaining after initial decomposition, acts as the reservoir for the carbon that was not released during decay, as well as storage for the nutrients that support plant growth and the microbial and fungal communities of the soil (Berg & McClaugherty 2008). The rate at which decomposition occurs is dependent on many factors, including the composition of the material being decomposed, the ecology (species composition and abundance) of the decomposer organisms available in the soil, and a suite of environmental variables, including soil temperature, moisture, pH and aeration (Tenney & Waksman 1929).

#### 5.1.2 Soil Humus Decay Rate Monitoring at **rare**

In response to concerns that climate change may affect forest carbon budgets, Natural Resources Canada developed the Canadian Intersite Decomposition Experiment (NRC 2007) to examine the long-term litter decomposition rates and nutrient mineralization of forests across Canada. In Canadian forests, large amount of carbon are stored in trees, soils, and decaying plant litter and any change in the balance between the uptake of carbon through photosynthesis and the release of carbon through decay and other activities could have an impact on levels of atmospheric carbon dioxide, an important greenhouse gas linked to global climate change. Thus, warmer temperatures could increase decay rates, which in turn would release carbon stored in the soils and litter and potentially accelerate rises in atmospheric carbon dioxide. The moderate temperature zone of southwestern Ontario was excluded from the NRC long-term decomposition study. As long-term monitoring of soil decay rates can provide valuable information on the relationship between soil decomposition and environmental factors, it may serve to inform forest management decisions at **rare**. For example, the effects that nearby aggregate mining or pesticide application may have on the health of our forest soils are unknown. Decay rate monitoring, together with the other biological monitoring protocols in place at **rare** such as forest tree biodiversity and plethodontid salamander monitoring, can provide us with a greater understanding of the integrity and stability of our forest ecosystems.

The first EMAN soil humus decay rate monitoring plots at **rare** were established on November 9, 2009 at the Cliffs and Alvars forest canopy tree biodiversity plot one. The success of the first monitoring year resulted in an expansion of the study in 2010 by the establishment of monitoring plots in both the Indian Woods and the Hogsback forest stands, within the first tree plot at each location.

At **rare**, the objective of this monitoring procedure is to contribute to the assessment of forest ecosystem functioning by monitoring yearly mass loss in standardized decay sticks as a representation of soil decomposition rates. As per the EMAN soil humus decay rate monitoring protocol (Parks Canada 2006), Annual Decay Rate (ADR) plots were located at the corners of the permanent forest canopy tree biodiversity plots in each forest stand. The information gain from decay monitoring can then be directly



linked to the forest health and productivity data. Decay rates compared over years are expected to remain relatively stable, and soil inserts positioned on the surface of the soil are expected to experience less mean weight loss than those placed below the surface where they are more accessible to soil microorganism responsible for decomposition. A change in decay rates would reflect a change in the physical or biological soil environments.

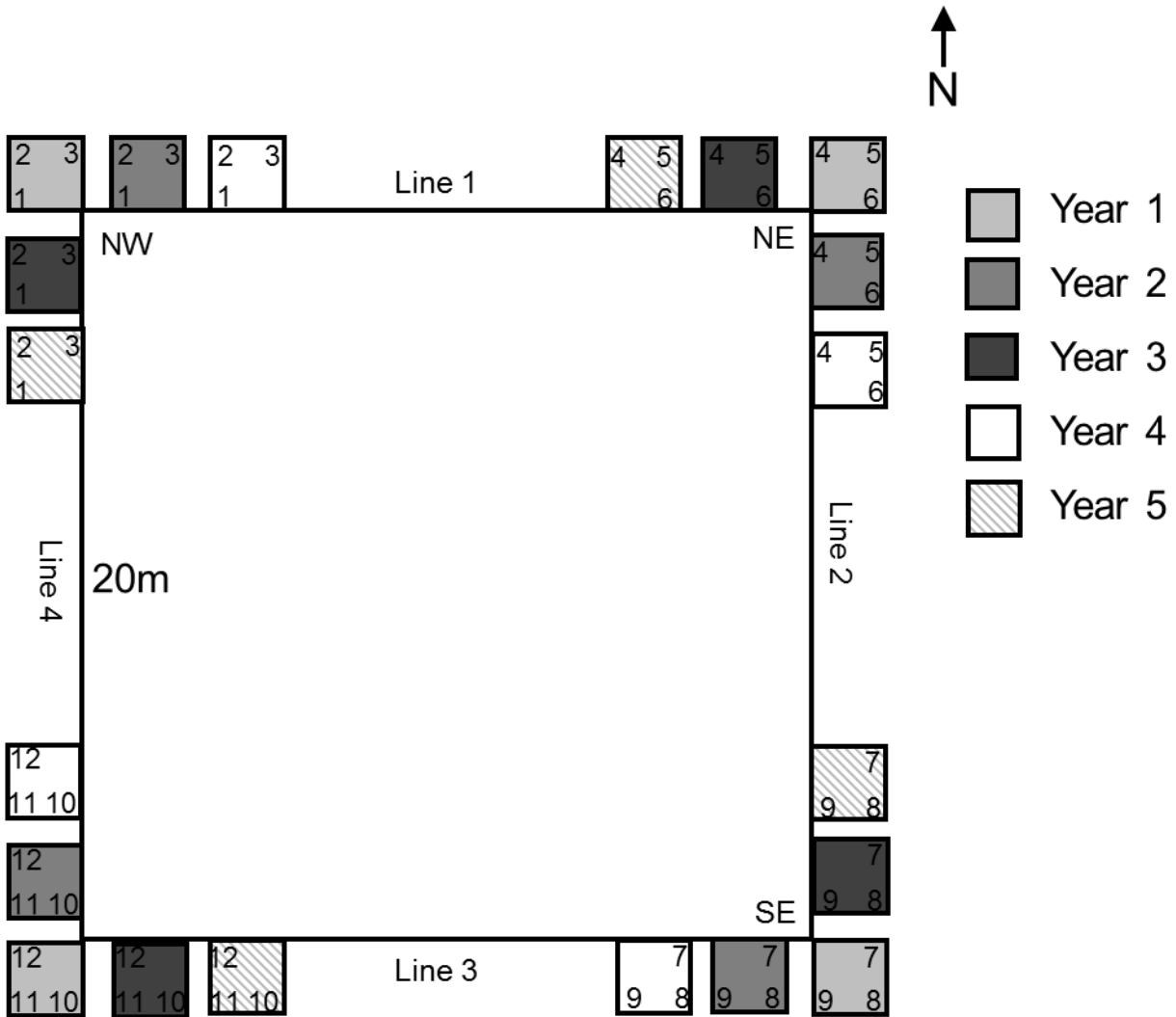
## 5.2 Methods

### *5.2.1 Soil Humus Decay Rate Plot Locations*

ADR plots were established on all four corners of forest canopy biodiversity monitoring plot one in each of the three forest stands (Figure 5.1). Each forest monitoring plot had twelve ADR plots established, with three at each of the four corners. ADR locations must be shifted each year to avoid the use of previously disrupted soil. In 2012, ADRs were located clockwise from 2010 locations (Figure 5.1). Descriptions of forest stands and instructions to access plots can be found in Section 4.2.1 and a map can be found in Appendix A, Figure A.4 with associated GPS coordinates in Table A.2

### *5.2.2 Monitoring Protocol: Decay Stick Installation*

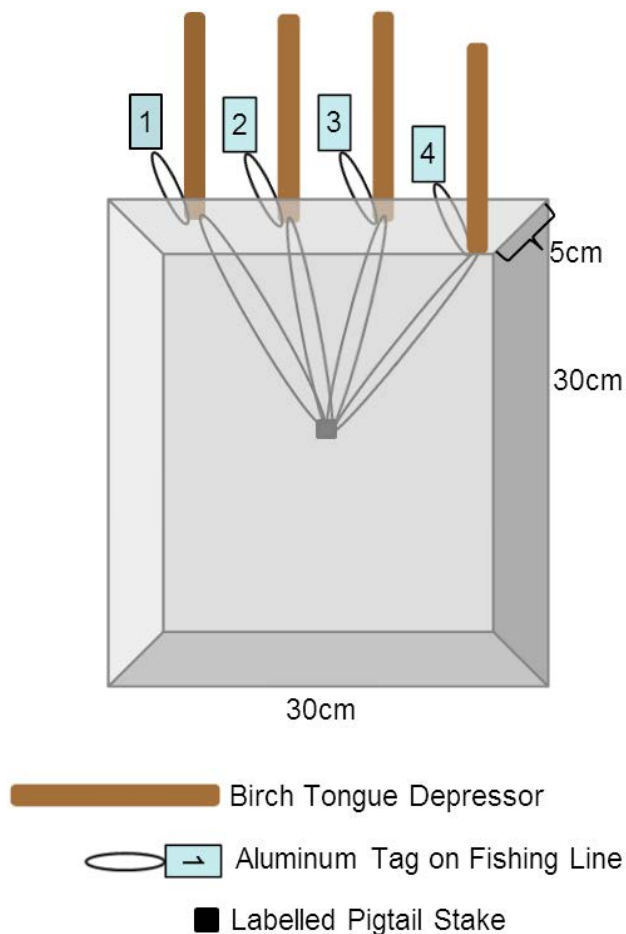
Decay sticks were prepared in-house prior to ground installation. To prepare the tongue depressors (MedPro, 100% natural birch wood, ultra smooth finish) a 2mm hole was drilled at one end of each stick to allow for the attachment of identification tags. While only 144 decay sticks are used during monitoring, it is best to prepare approximately fifteen sticks in excess in case of damage prior to installation. Once drilled, decay sticks were oven-dried at 70°C for 48 hours (Quincy 0Gc-181512 Gravity-Convection Oven). Following this, decay sticks were left for 24 hours at room temperature and then weighed (+/- 0.001g) on a Sartorius 1265MP balance. A sample datasheet to record stick weight pre and post decay can be found in Appendix C, Figure C.6. After recording their mass, decay sticks were tagged with pre-labelled aluminum tags attached with approximately 30cm of extra-strong (40LB) fishing line. With the exception of the initial year of monitoring, decay stick were placed in 100% vinyl mesh bags (dimension: 17cm x 4cm with an approximate pocket size of 16cm x 3cm; hole size: 3mm x 2mm). Vinyl mesh bags were prepared in advance of decay stick placement, with an excess created in case of damage during installation. These bags were an amendment to the monitoring protocol added in 2010 in an attempt to keep all the decay stick's pieces together and increase the number of decay sticks excavated intact. Mesh bags are often used in studies of leaf litter decay rate (Moore et al. 2005; Albers et al. 2004; Gallardo et al. 1995). A complete list of equipment required for installation can be found in Appendix B, List B.5.



**Figure 5.1:** Distribution of annual soil humus decay rate (ADR) plots (numbered 1-12) around a forest canopy tree biodiversity plot. Twelve ADR plots are arranged around the corners of each plot; three located in the originally recommended location of the corner and moved counter-clockwise and clock-wise in alternating years from the original location to avoid previously sampled soil areas. Plots are colour coded by monitoring year.

A 1m<sup>2</sup> quadrat was marked on each corner of the forest plots and three ADR plots were positioned within each quadrat on the corners radiating out from the corner of the forest plot (Figure 5.1). At each ADR plot, a 30cm x 30cm hole was excavated with the soil plug removed intact if possible and placed to the side. Using a knife or chisel, three slots were made parallel to the forest floor on the north wall of the excavated hole. The slots were of large enough size to accommodate the bagged decay sticks snugly. Slots were measured 5cm below the soil surface and were re-measured upon completion with the accurate depth below the surface recorded. The three slots were measured to be approximately 10cm apart. The bagged decay sticks were inserted into the slots, with the pre-labelled

aluminum tags previously attached via fishing line left on the soil surface. A pigtail stake marked with flagging tape labelled with the forest stand and ADR plot number (i.e. CA-ADR 2) was inserted into the centre of the excavated hole. Fishing line was used to attach each bagged decay stick to one another and the centre pigtail stake, with enough excess that they would not be shifted. This fishing line is to be used as a guide to locate the sticks upon excavation and therefore should not be so taut as to affect their movement throughout the year. A fourth bagged decay stick was attached to the centre pigtail stake via fishing line and left on the soil surface (Figure 5.2). The excavated hole was then refilled with the displaced soil and soil plug, and the exposed tags were covered with leaf litter to prevent public or wildlife tampering. In 2013, decay sticks were installed on November 1<sup>st</sup> in the Cliffs and Alvars, November 5<sup>th</sup> in the Indian Woods, and November 7<sup>th</sup> in the Hogsback.



**Figure 5.2:** Diagram of annual soil humus decay rate (ADR) monitoring plot set-up as viewed from above. Decay sticks 1-3 are installed parallel to the soil surface at a depth of 5cm, separated 10cm from each other. Stick 4 is placed on the soil surface, and all decay sticks are tied to the central pigtail stake. Figure from Robson (2010).

### 5.2.3 Monitoring Protocol: Decay Stick Excavation

Decay sticks were excavated one year following their installation. In the event of an early frost and ground freeze, the date of excavation should be moved forward. Using a trowel, soil surrounding the pigtail stake in each ADR, where decay sticks were suspected to be, was slowly removed. As tags and fishing line were uncovered, they were used to help locate the decay sticks and to gently pull the bagged decay sticks from the ground once a hole has been dug. Each decay stick and its associated tag were placed in an individual re-sealable plastic bag. A complete list of equipment required for excavation can be found in Appendix B, List B.5.

Decay sticks were each removed from their vinyl bags and any dirt that adhered to the stick was removed. Each stick was gently brushed with a dry paintbrush and then gently scrubbed with a second paintbrush in water. Decay sticks were placed in individual paper envelopes following cleaning, and each envelope was labelled with the site and tag number. Decay sticks, inside their envelopes, were then oven-dried at 70°C for 48 hours and subsequently let to sit for 24 hours at room temperature before being weighed ( $\pm 0.001\text{g}$ ). Weights were recorded on a datasheet available on the *rare* server and in Appendix C, Figure C.6).

### 5.2.4 Data Analysis

Data were analysed using Microsoft Excel 14.0.6 (Microsoft 2010) and PASW Statistics 17.0 (SPSS Inc.) for Windows. Prior to analysis, assumptions of parametric testing were examined. When transformation was required, the appropriate transformation to decouple variance and mean was determined using Taylor's Power Law (Perry 1981). Otherwise, the best transformation was applied and the most robust tests were used, followed by cautious interpretation of results.

Percent dry weight loss for each decay stick was calculated, as changes in dry weight loss can be examined as a proxy for soil decomposition function. Weight loss was compared across years and sites using a univariate analysis of variance (ANOVA) followed by Bonferroni post hoc testing to determine where the differences occurred.

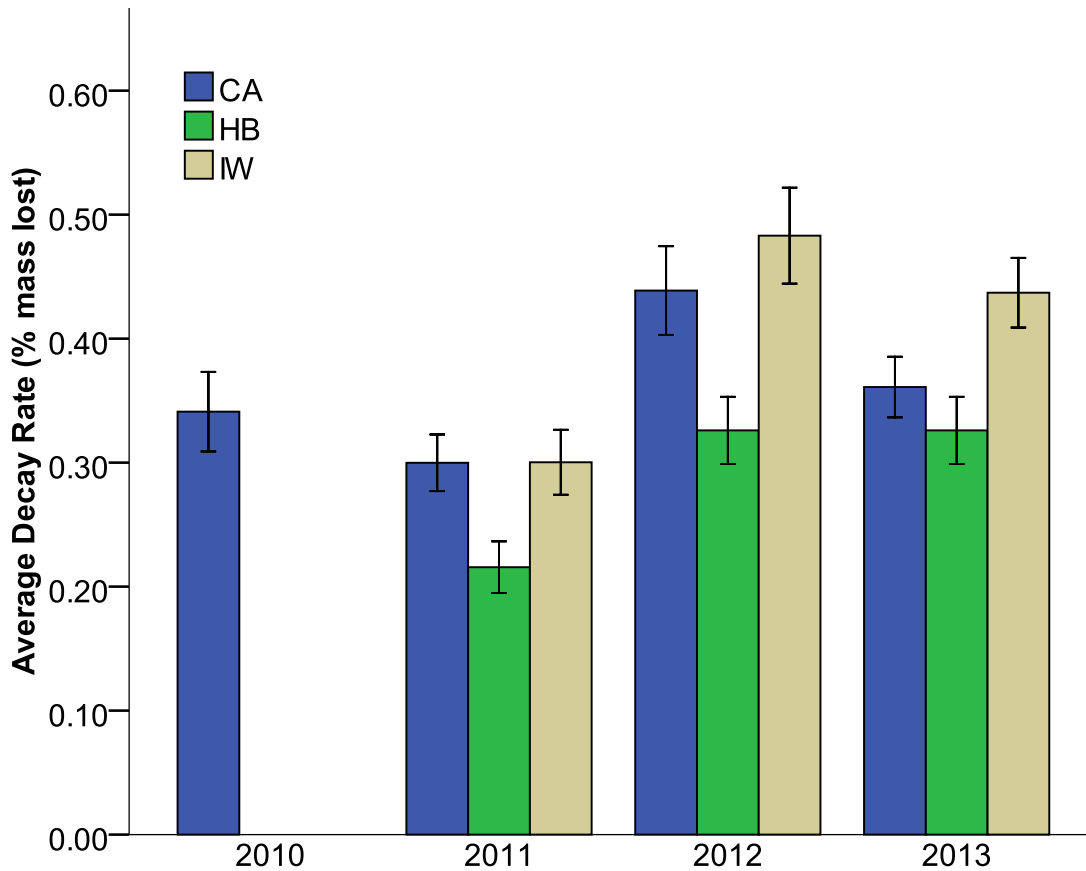
## 5.3 Results

In 2013, a total of 142 decay sticks were recovered from annual decay rate plots. Two decay sticks were lost during the sampling year from the Indian Woods forest stand. As expected, decay sticks positioned below ground were found to have lost significantly more mass than those positioned on the soil surface ( $F_{1,454}=167.08, p<0.001$ ) (Table 5.1).

Significant differences were found in decay rates across years ( $F_{3,464}=13.52, p<0.001$ ) and forest sites ( $F_{2,464}=12.95, p<0.001$ ). Post hoc testing revealed that decay rates in 2011 differed from those recorded in 2012 and 2013 (post hoc  $p<0.001$ ) and both Cliffs and Alvars and Indian Woods significantly differed from decay rates in the Hogsback (post hoc  $p<0.004$ ) (Figure 5.3).

**Table 5.1:** Annual decay rates measured as percent mass loss of decay sticks from Cliffs and Alvars, Indian Woods, and the Hogsback forest stands in all monitoring years. Decay sticks below and above ground had significantly different mass losses, regardless of site or year. SD= Standard Deviation.

	Cliffs and Alvars		Indian Woods		Hogsback	
	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD
All sticks	40.0	20.6	40.6	22.8	29.0	18.1
Sticks below ground	44.2	16.2	46.2	21.2	32.6	17.3
Sticks above ground	11.0	9.00	22.4	18.1	17.7	15.8



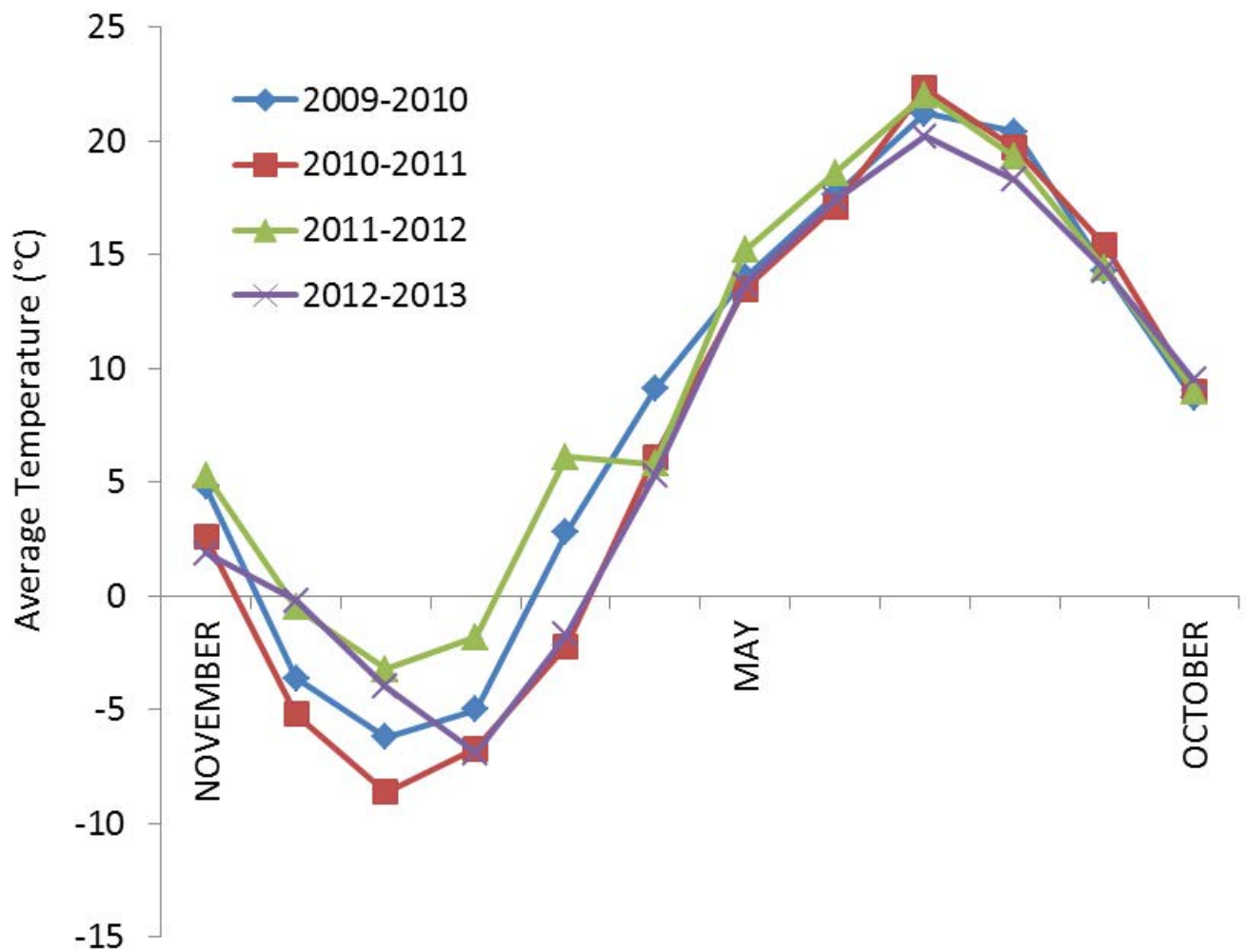
**Figure 5.3:** Average decay rate comparison over monitoring years and sites. Only Cliffs and Alvars was monitored in 2010. Significant differences occur between 2011 and the 2012 and 2013 monitoring years, and between the Hogsback and all other sites. Error bars represent +/- one standard error.

## 5.4 Discussion

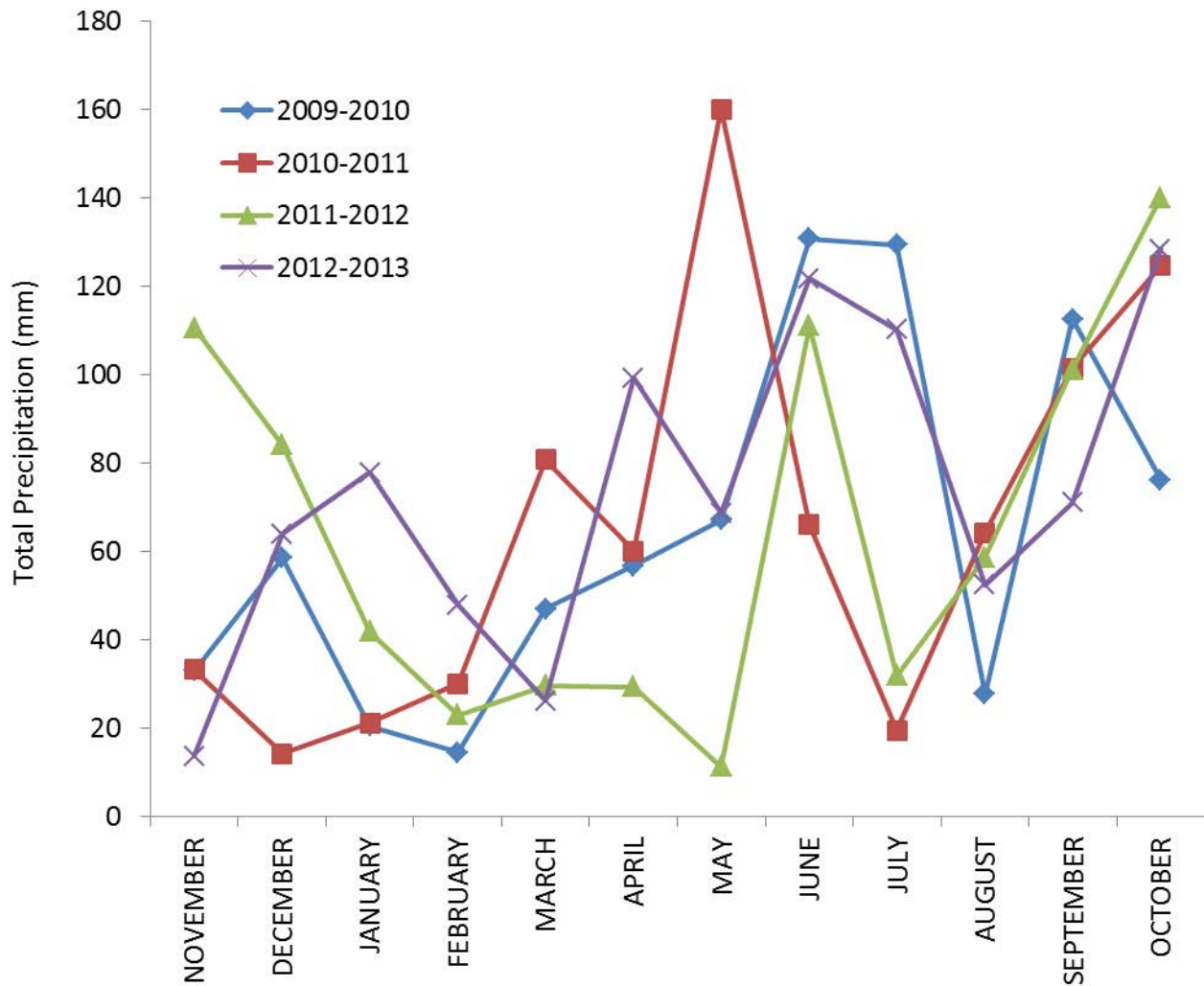
Rates of decay can be influenced by a variety of factors including climate, temperature, substrate type, nutrient concentrations and availability, litter type and size, and soil organisms (Parks Canada 2006). Weight loss associated with decomposition is strongly dependent on aerobic microbial activity (Bunnell et al. 1977). Decay sticks that were placed below ground were more accessible to soil microorganisms, fungi, and moisture, which could explain the higher decay rate observed below ground (Table 5.1).

Moisture and temperature, which vary greatly with local conditions, are the principle factors that affect rate of decay (Singh & Gupta 1977), as they strongly influence microbial activity (Bunnell et al. 1977). Soil decay rates appear to be more strongly impacted by changes in whichever factor, temperature or moisture, is most limiting. Lower moisture contents result in a limited response to temperature changes and lower temperatures result in a limited response to changes in moisture level (Schlentner & Van Cleve 1984). 2011 had significantly lower decay rates than the subsequent monitoring years and also corresponds to the year with the coldest winter of the monitoring program. Temperatures in November through to January in 2011 were the coldest recorded since monitoring began, and could be the factor contributing to the slow decay rates in that year. Higher temperatures generally result in higher decay rates (Olson 1963; Van Cleve 1971; Singh & Gupta 1977), and so the cold temperatures recorded may have slowed decay rates. Additionally, the amount of precipitation may have also played a role as heavy rainfall and a high percentage of rainy days typically speed up decomposition (Singh & Gupta 1977). Based on climate data from the Kitchener-Waterloo Weather Station (Environment Canada), it rained more than 0.5mm on 26% of days in 2011, 31% of 2012, and 33% of 2013. Thus, lower temperature and moisture levels in the 2011 monitoring year than the 2012 and 2013 monitoring years are likely factors associated with the significant change in soil decay rates. As weather is variable, continued monitoring of soil decay rates is important to determine soil health trends in these forest stands.

The Hogsback forest significantly differed from both other stands with lower decay rates. The Hogsback is a forest-wetland complex that has a mixture of upland and lowland areas with swampy features. In particular, one corner of the monitoring plot located here is found within a swamp. If decay sticks are continuously exposed to extremely high moisture levels or are completely submerged in water, decay rates may be slowed by lack of oxygen to support microbial activity (USDA 2007; Schlentner & Van Cleve 1984).



**Figure 5.4:** Temperature data for Waterloo Region by month during soil humus decay monitoring years, where average temperature is the average monthly temperature (Environment Canada- data from Kitchener-Waterloo Weather Station).



**Figure 5.5:** Precipitation data for Waterloo Region by month during soil humus decay monitoring years, where the total precipitation from each month is displayed (Environment Canada- data from Kitchener-Waterloo Weather Station).

## 5.5 Conclusions and Recommendations

The soil humus decay rate monitoring program at *rare* has undergone valuable improvements and expansions in the last four years. It is recommended that the program continue for a minimum of five consecutive years to ensure the establishment of baseline data that can be a measure of soil change beyond weather extremes. Changing decay rates can be indicative of global climate change or local development and agricultural pressures. Only continued monitoring can investigate these potential threats. It would be beneficial to additionally monitor soil moisture content in plots each month during monitoring to allow for a closer comparison of decay rates to average soil moisture than simply regional precipitation. This would require additional and potentially expensive field equipment.



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## APPENDIX A: Maps and Coordinates

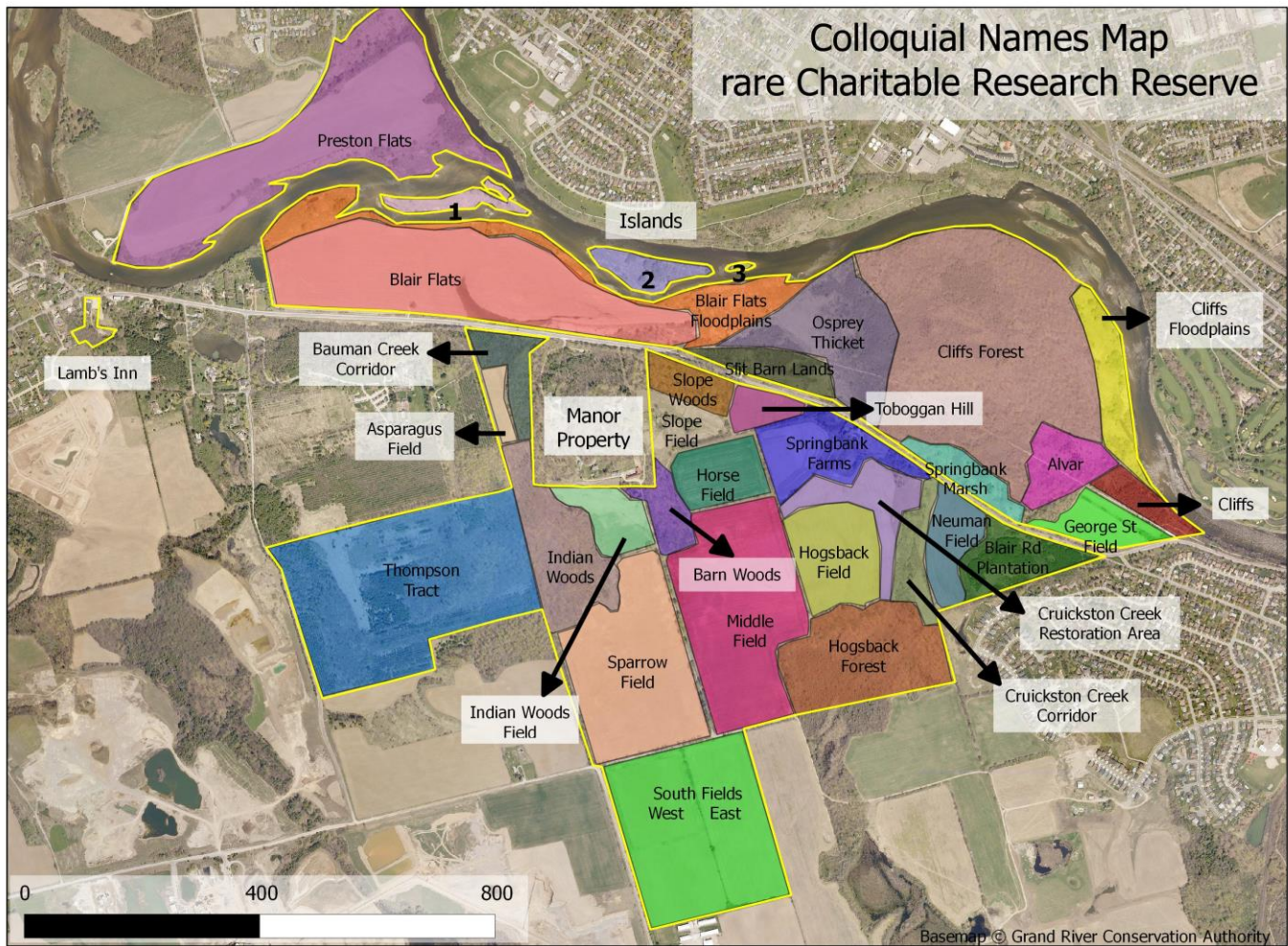
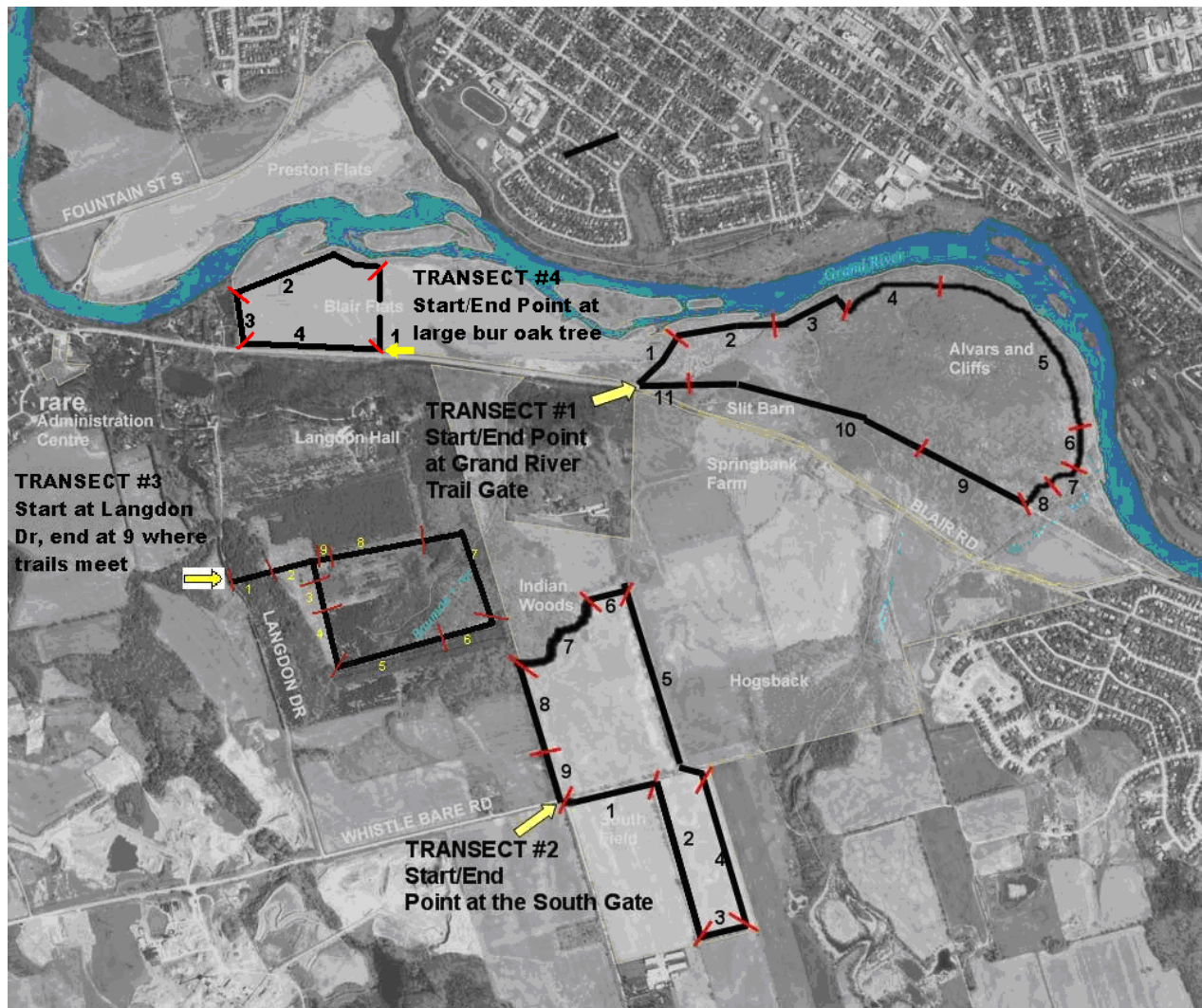
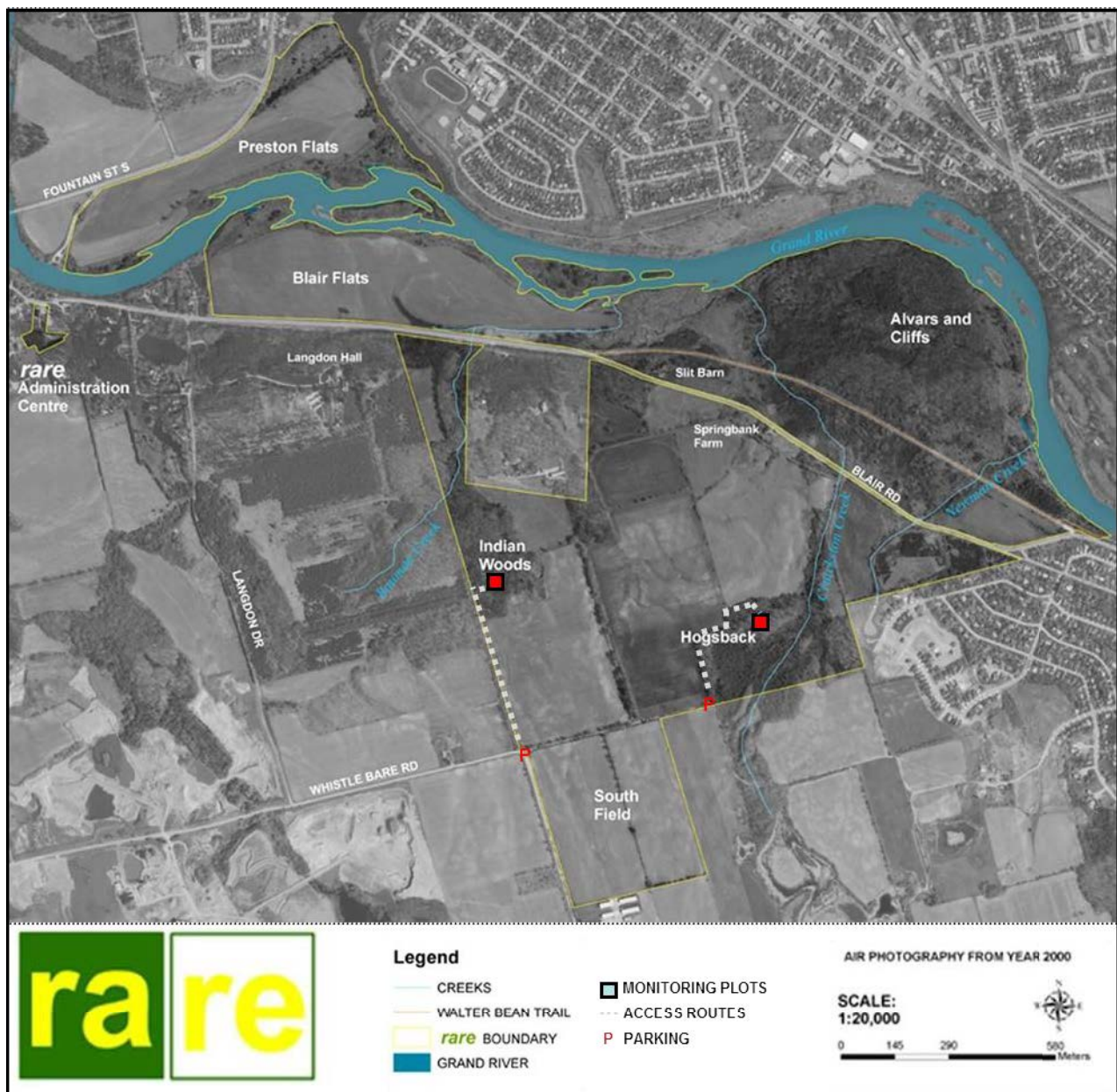


Figure A.1: *rare* Charitable Research Reserve property map.



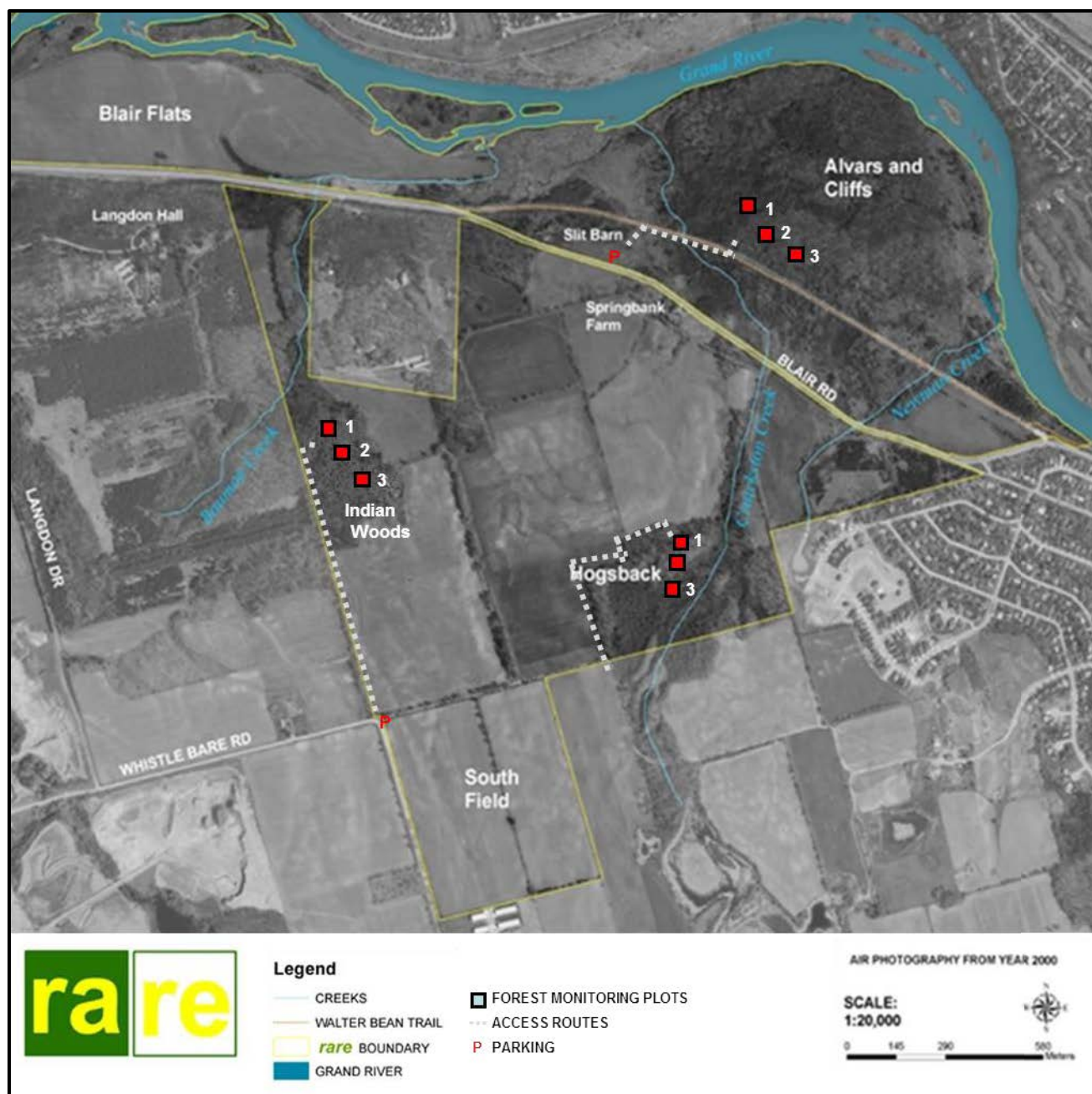


**Figure A.2:** Location of the four butterfly monitoring transects at the *rare* Charitable Research Reserve with section breaks representing habitat changes.



**Figure A.3:** Location of salamander monitoring plots in Indian Woods and Hogsback. Dotted lines indicate walking path to sites, with parking location designated by P.





**Figure A.4:** Location of forest health and soil humus decay rate monitoring plots in Indian Woods, Cliffs and Alvars, and the Hogsback. Each forest stand has three plots for forest health assessment and soil humus decay rate monitoring occurs only in plot one at each stand. Dotted lines indicate walking path to sites, with parking location designated by P.

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

**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
- 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)
- 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
- 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
- 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
- Stop at large alvar, ~10m after tall Oak tree

**Section eight (N 43° 22.749' W 80° 20.734')**

- Open shrub land
- 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 (sedges, cattail, milkweed, goldenrod, purple loosestrife)
- Stop at culvert near Blair Road entrance to Grand Trunk Trail, several Trembling Aspen trees, direct line of sight to stopping point for section one



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

**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° 22.463' W 080° 21.637')**

- Deciduous hedgerow of mostly Oak trees; bordering soybean field on east side and naturalized agricultural field on west side
- Stop halfway down hedgerow at "No Hunting" sign

**Section six (N 43° 22.554' W 080° 21.742')**

- Fenced in corridor/hedgerow at north side of Sparrow Field and south of Cruickston Manor House (deciduous trees, grapevines, tall grasses), bordering soybean field to the north and naturalized agricultural field to the south
- Stop halfway down corridor/hedgerow at solitary Glossy Buckthorn at fence post

**Section seven (N 43° 22.457' W 080° 21.841')**

- Meadow bordered by deciduous trees (Indian Woods) to north and natural regeneration to south
- Stop at fallen tree along old fence, under large Maple trees

**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).

**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

**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, hot tub on west side of transect

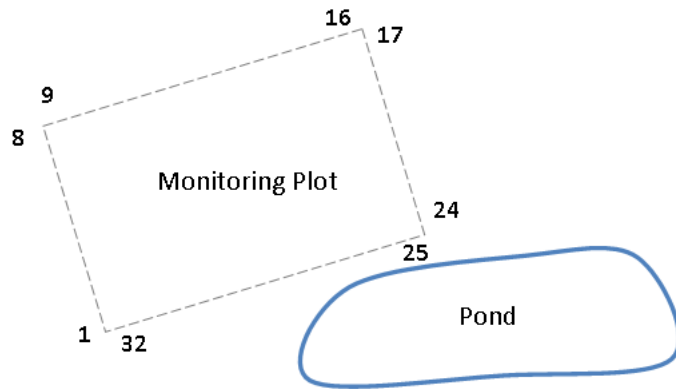
**Section four (N 43° 22.998' W 080° 22.473')**

- 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

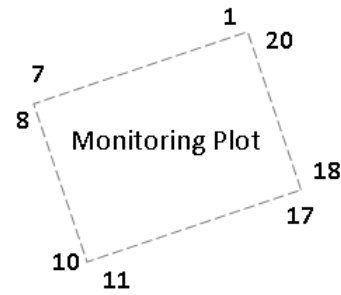
**Table A.1:** GPS coordinates of artificial cover objects (ACO) used for plethodontid salamander monitoring in Indian Woods and the Hogsback (from McCarter 2009).

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

A)



B)

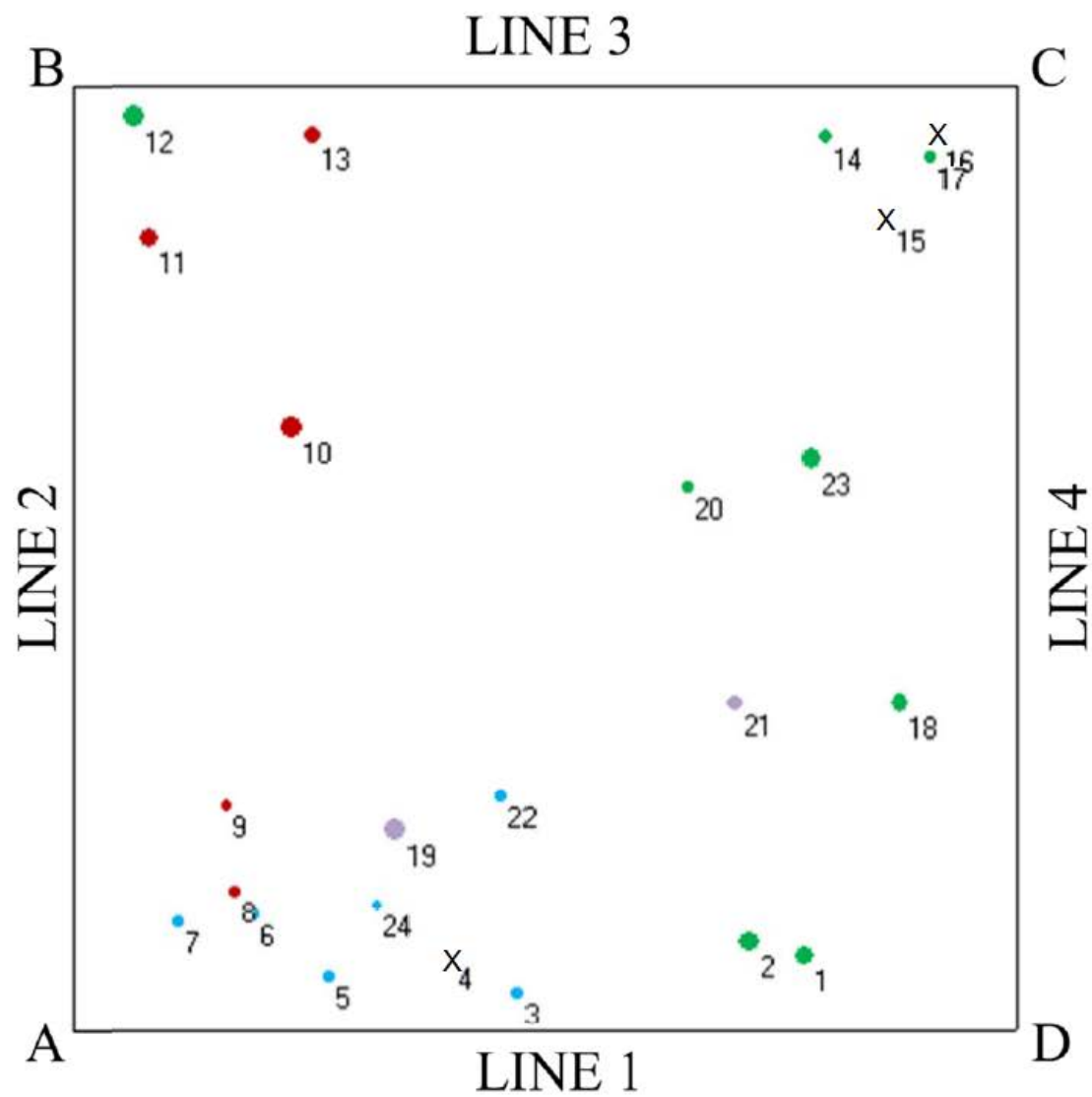


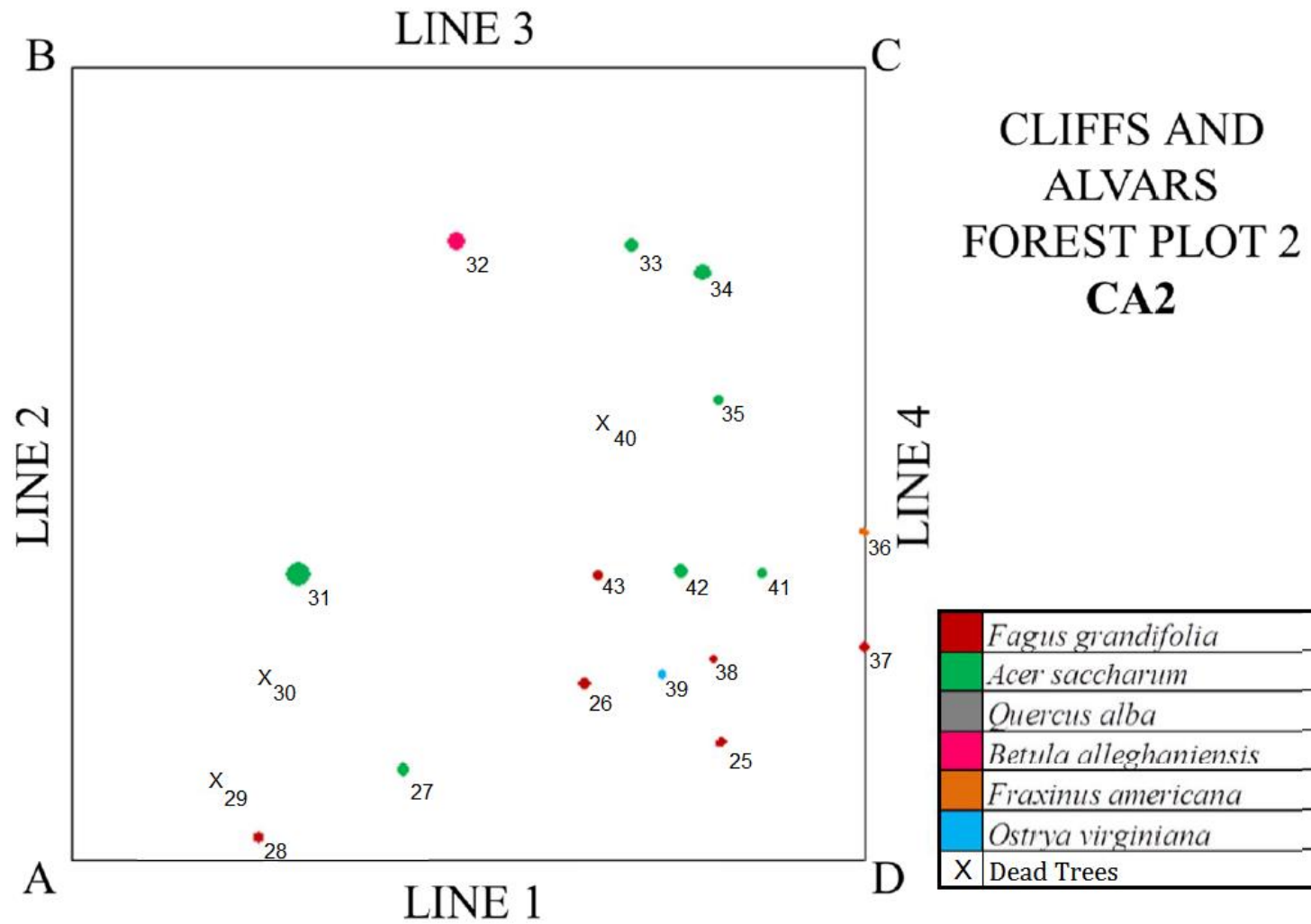
**Figure A.5:** Layout of artificial cover objects (ACOs) on salamander monitoring plots in A) Indian Woods and B) Hogsback.

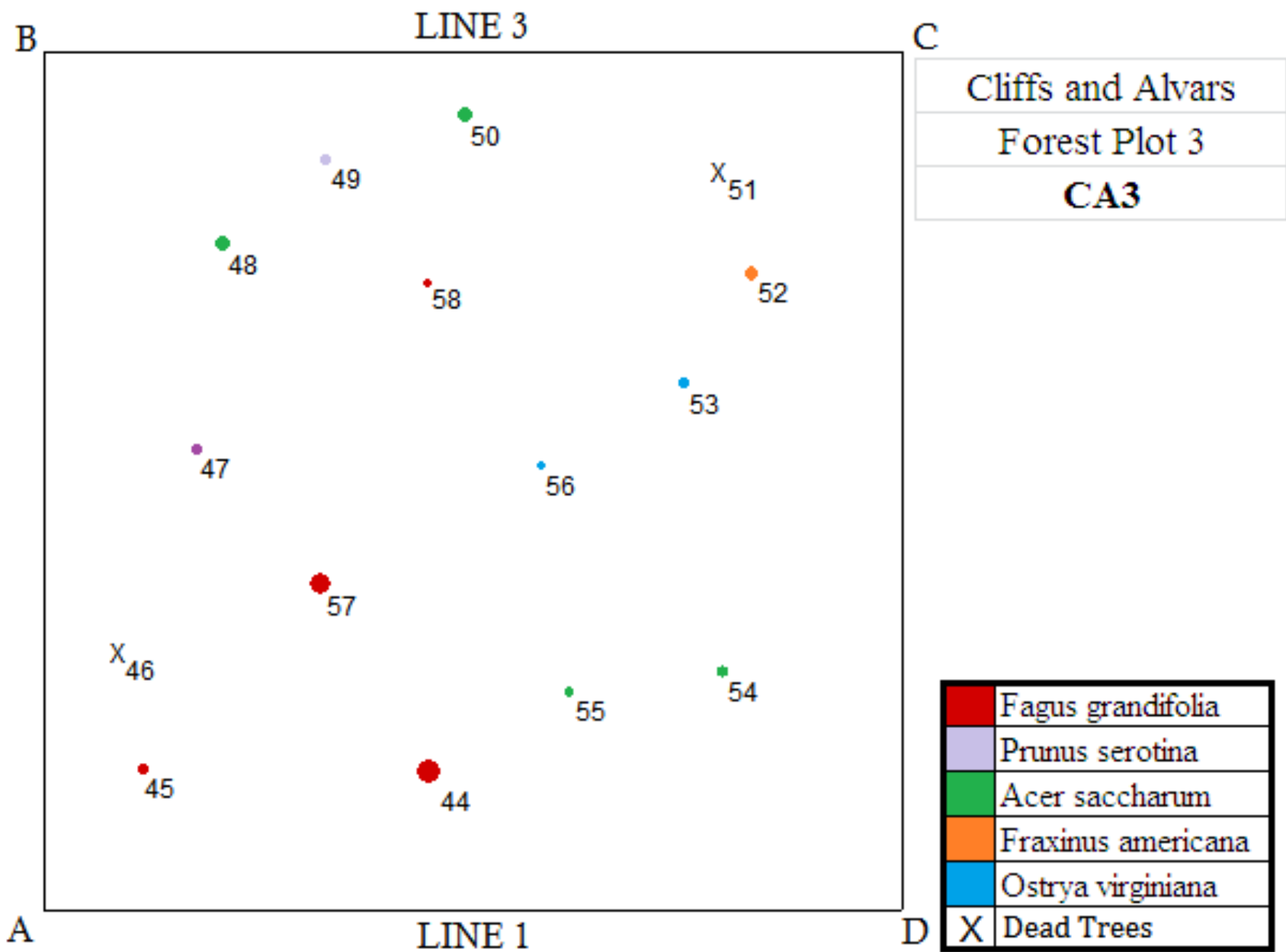
**Table A.2:** GPS coordinates of forest canopy tree biodiversity and health monitoring plots in Cliffs and Alvars, Indian Woods, and the Hogsback (from Robson 2010). The coordinates describe the location of the northwest corner of each plot. The annual soil decay rate monitoring plots are located on all four corners of forest plot one in each stand.

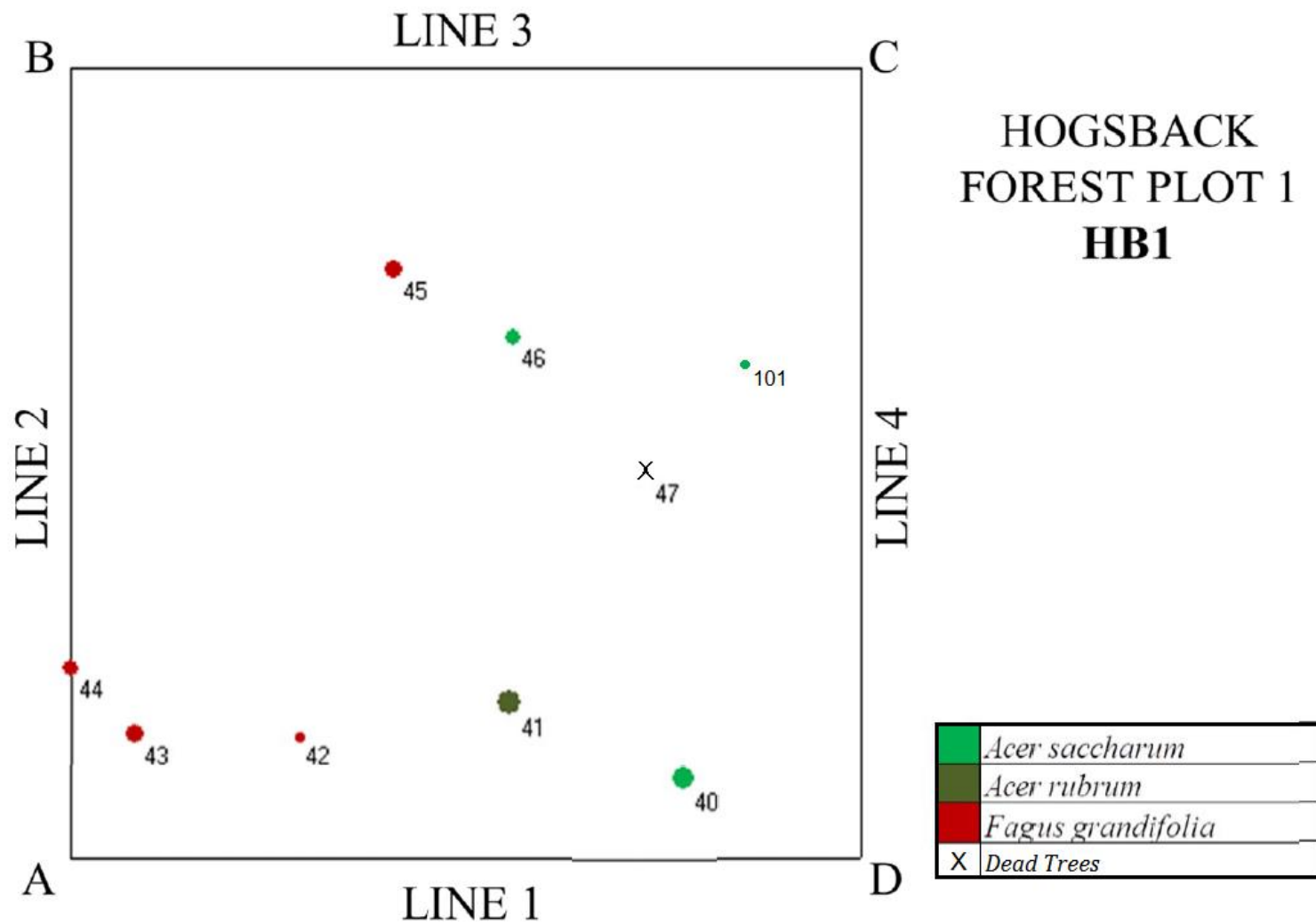
Monitoring Location	Plot	Latitude and Longitude	UTM (zone 17T)
Cliffs and Alvars	1	N43°22'46.30" W80°21'1.34"	552623E 4803167N
	2	N43°22'44.64" W80°21'0.21"	552649E 4803116N
	3	N43°22'43.72" W80°20'57.91"	552701E 4803088N
Indian Woods	1	N43°22'27.27" W80°21'51.45"	551500E 4802571N
	2	N43°22'26.12" W80°21'56.08"	551396E 4802535N
	3	N43°22'23.62" W80°21'54.78"	551426E 4802458N
Hogsback	1	N43°22'24.18" W80°21'11.10"	552409E 4802483N
	2	N43°22'23.28" W80°21'12.66"	552374E 4802455N
	3	N43°22'22.08" W80°21'14.46"	552334E 4802418N

**Figure A.6- Figure A. 14:** 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 (pages 119-128).

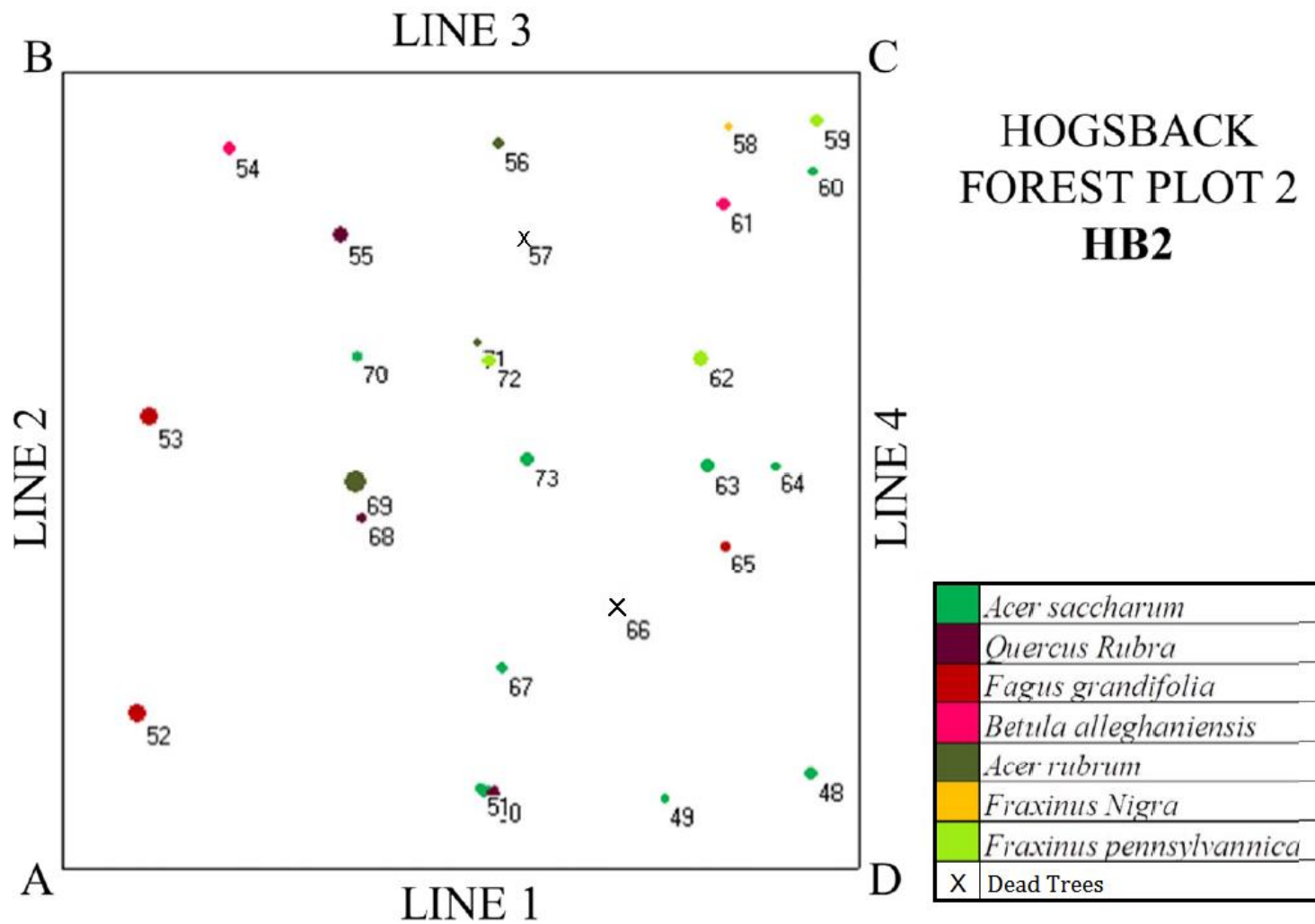


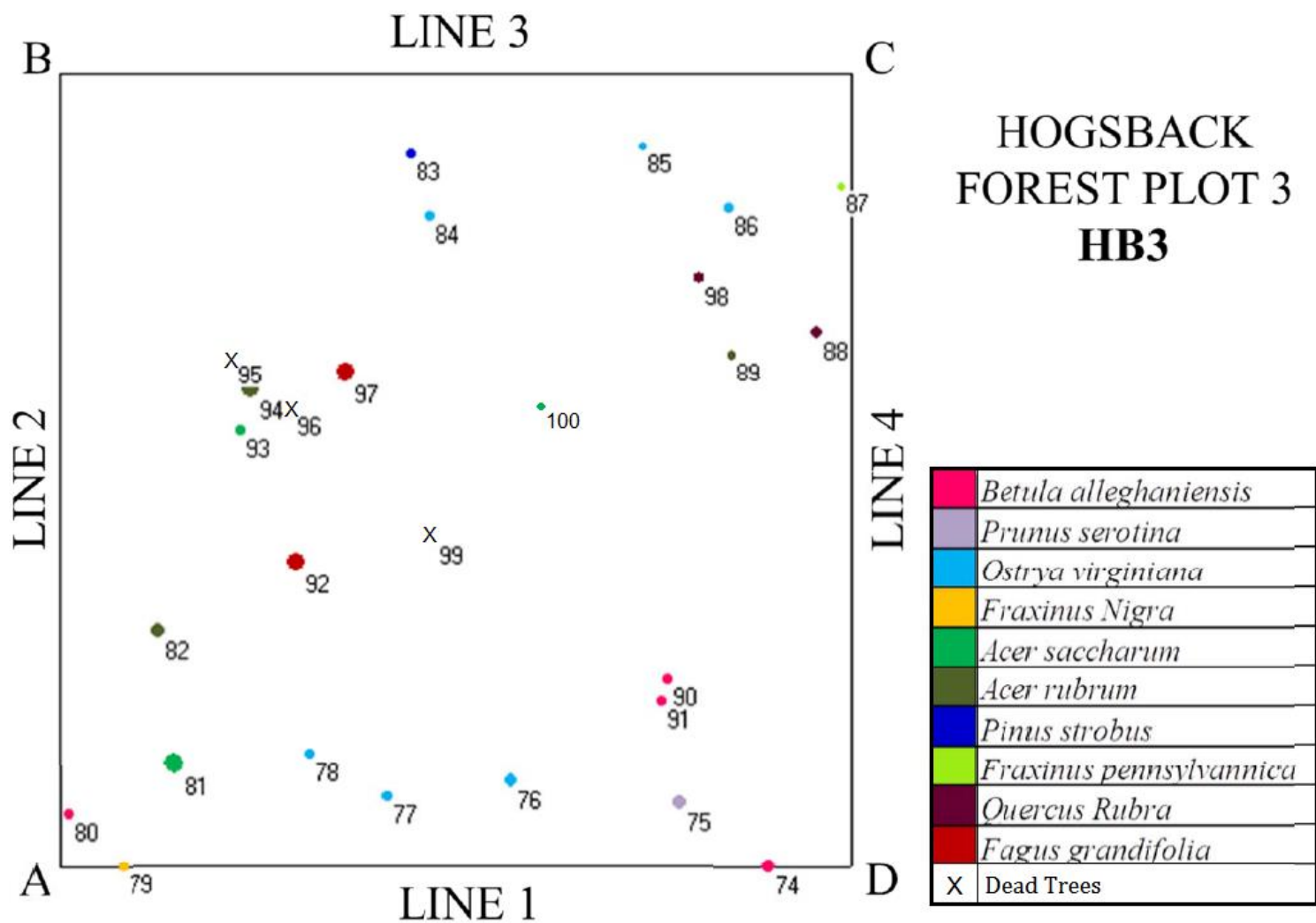


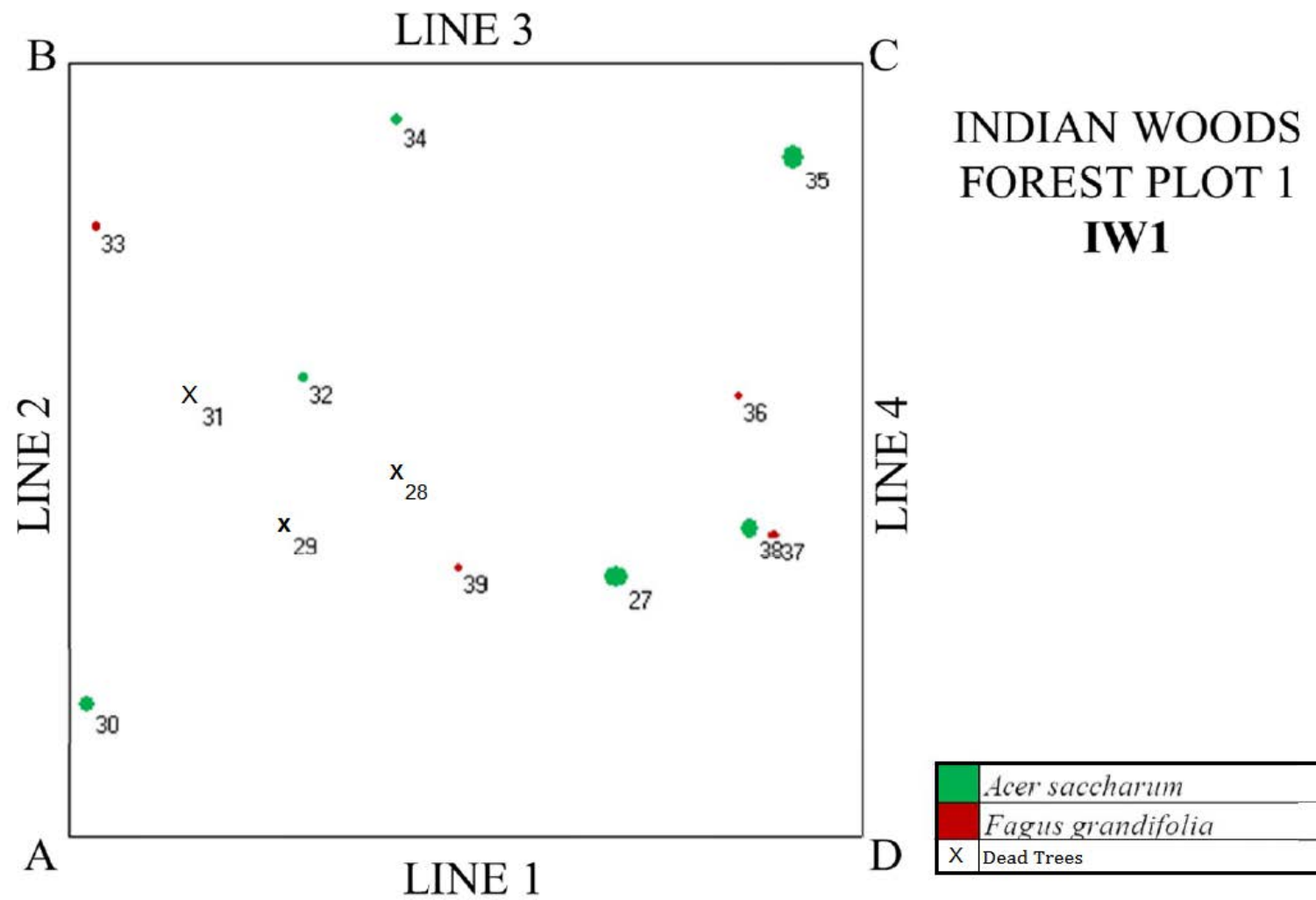


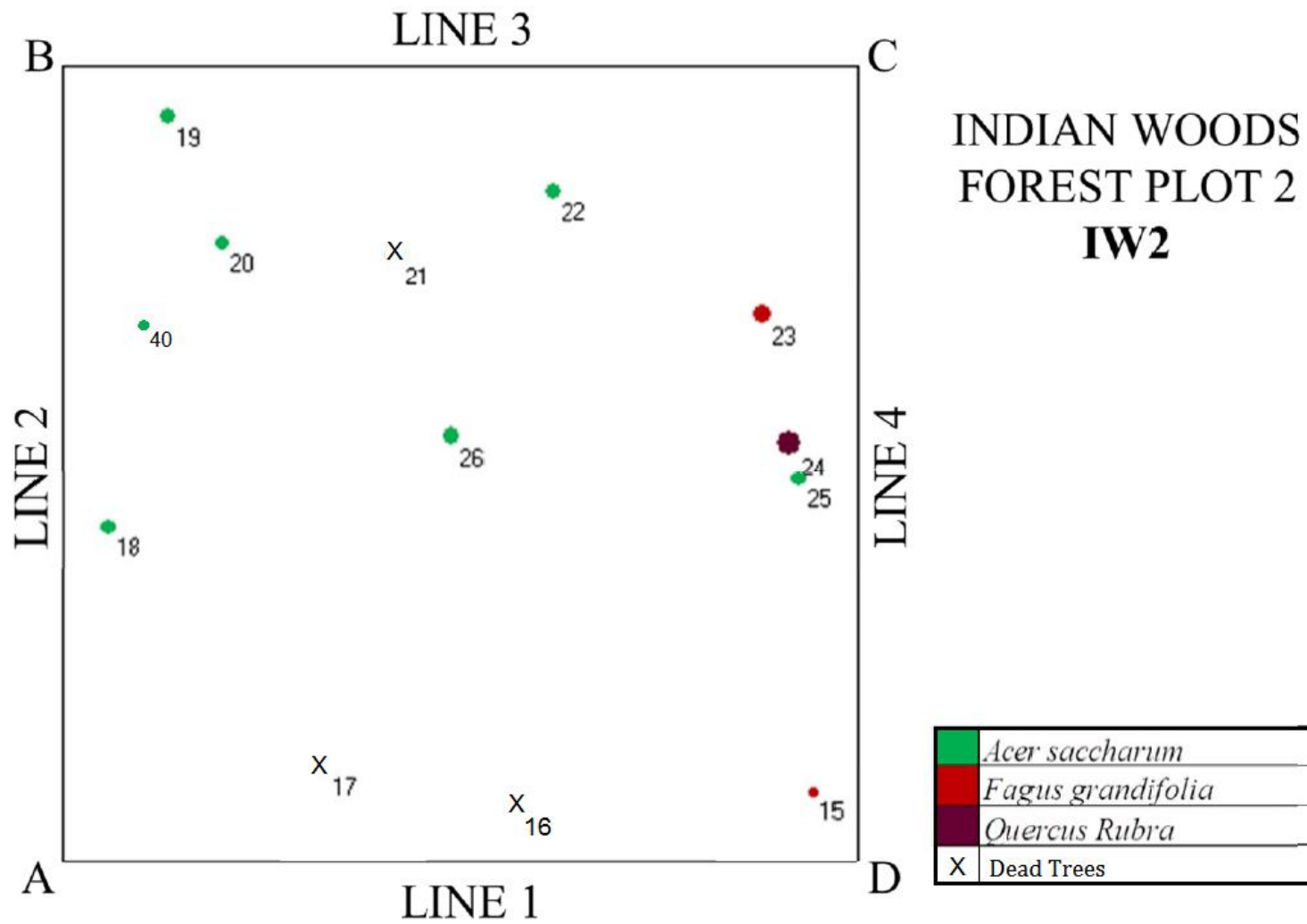


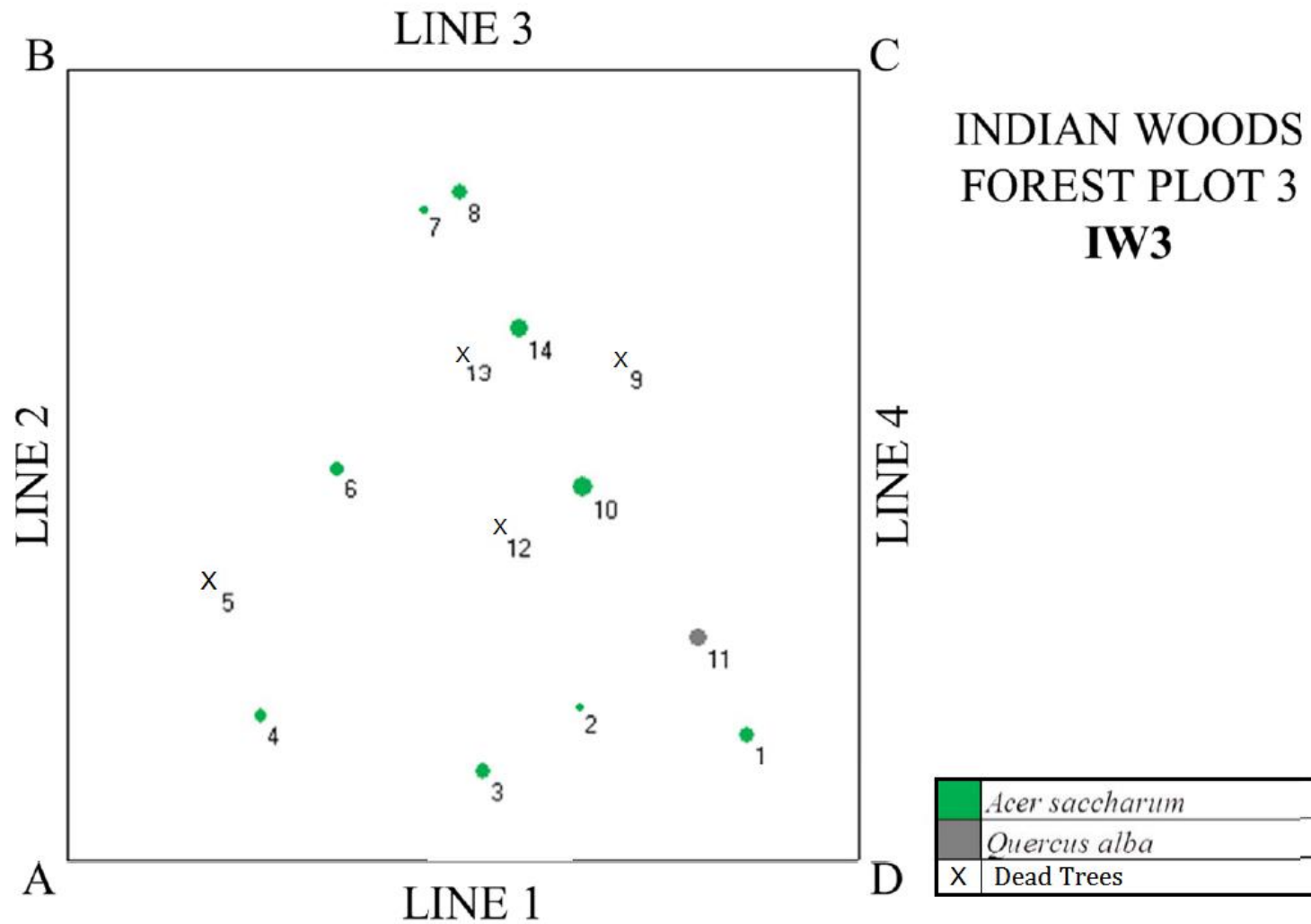












## APPENDIX B: EQUIPMENT LIST

### List B.1: Suggested butterfly monitoring field equipment.

- Field data sheet
- Clipboard
- Pencils
- Stopwatch
- Kestrel 3000©
- Butterfly net
- Binoculars
- 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
- Digital Camera

### List B.2: Salamander monitoring equipment list

- Field data sheets A and B on waterproof paper
- Clipboard
- Pencils
- Nitrile gloves
- Kestrel 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

### 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
- Pre-labelled tags and steel pigtail stakes

### List B.4: Soil pH testing equipment list

- 36 re-sealable plastic bags
- Trowel
- Spoon
- Nitrile gloves
- Permanent marker
- Soil pH testing kit (in lab)

### **List B.5:** Soil humus decay rate monitoring equipment list

#### Installation

- Field data sheet on waterproof paper
- Clipboard
- Pencils
- Nitrile gloves
- Shovel
- Trowel
- Chisel
- Pigtail stakes (12 per plot)
- Tongue depressors (decay sticks), pre-weighed, dried, and labelled
- Pre-prepared mesh bags
- Fishing line

#### Extraction

- Field data sheet on waterproof paper
- Clipboard
- Pencils
- Nitrile gloves
- Trowel
- Scissors
- Utility knife
- Re-sealable plastic bags
- Permanent marker

#### Cleaning

- Nitrile gloves
- Scissors
- Two paint brushes (one wet and one dry)
- Paper envelopes

## APPENDIX C: DATA SHEETS AND CODES

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

Beaufort Scale	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).









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 n°..... OR Stand-alone quadrat n°..... Av. stand height.....  
 Identification manual..... Observer(s).....

[illegible]

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

[illegible]



## 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 70 butterfly species have been observed.

Common Name	Scientific Name	Common Name	Scientific Name
Acadian Hairstreak	<i>Satyrrium acadicum</i>	Juvenal's Duskywing	<i>Erynnis juvenalis</i>
American Lady	<i>Vanessa virginiensis</i>	Least Skipper	<i>Ancloxypha numitor</i>
American Snout	<i>Libytheana carinenta</i>	Little Glassywing	<i>Pompeius verna</i>
Appalachian Brown	<i>Satyrodes appalachia</i>	Little Wood-Satyr	<i>Megisto cymela</i>
Arctic Skipper	<i>Carterocephalus palaemon</i>	Little Yellow	<i>Eurema lisa</i>
Baltimore Checkerspot	<i>Euphydryas phaeton</i>	Long Dash	<i>Polites mystic</i>
Banded Hairstreak	<i>Satyrrium calanus</i>	Meadow Fritillary	<i>Boloria Bellona</i>
Black Dash	<i>Euphyes conspicua</i>	Milbert's Tortoiseshell	<i>Nymphalis milberti</i>
Black Swallowtail	<i>Papilio polyxenes</i>	Monarch	<i>Danaus plexippus</i>
Broad-Winged Skipper	<i>Poanes viator</i>	Mourning Cloak	<i>Nymphalis antiopa</i>
Bronze Copper	<i>Lycaena hyllus</i>	Mulberry Wing	<i>Poanes massasoit</i>
Cabbage White	<i>Pieris rapae</i>	Mustard White	<i>Pieris oleracea</i>
Clouded Sulphur	<i>Colias philodice</i>	Northern Broken-Dash	<i>Wallengrenia egeremet</i>
Columbine Duskywing	<i>Erynnis lucilius</i>	Northern Crescent	<i>Phyciodes cocyta</i>
Common Buckeye	<i>Junonia coenia</i>	Northern Pearly-Eye	<i>Enodia anthedon</i>
Common Sooty Wing	<i>Philodice catullus</i>	Orange Sulphur	<i>Colias eurytheme</i>
Common Wood-Nymph	<i>Cercyonis pegala</i>	Painted Lady	<i>Vanessa cardui</i>
Compton Tortoiseshell	<i>Nymphalis vaualbum</i>	Pearl Crescent	<i>Phyciodes tharos</i>
Coral Hairstreak	<i>Satyrrium titus</i>	Peck's Skipper	<i>Polites peckius</i>
Crossline Skipper	<i>Polites origines</i>	Question Mark	<i>Polygonia interrogationis</i>
Delaware Skipper	<i>Anatrytone logan</i>	Red Admiral	<i>Vanessa atalanta</i>
Dion Skipper	<i>Euphyes dion</i>	Red-Spotted Purple	<i>Limenitis arthemis astyanax</i>
Dun Skipper	<i>Euphyes vestris</i>	Sachem	<i>Atalopedes campestris</i>
Eastern Comma	<i>Polygonia comma</i>	Silver-Bordered Fritillary	<i>Boloria selene</i>
Eastern Tailed Blue	<i>Cupido comyntas</i>	Silvery Checkerspot	<i>Chlosyne nycteis</i>
Eastern Tiger Swallowtail	<i>Papilio glaucus</i>	Silver-Spotted Skipper	<i>Epargyreus clarus</i>
European Skipper	<i>Thymelicus lineola</i>	Spring Azure	<i>Celastrina ladon</i>
Eyed Brown	<i>Satyrodes Eurydice</i>	Striped Hairstreak	<i>Satyrrium liparops</i>
Giant Swallowtail	<i>Papilio cresphontes</i>	'Summer' Spring Azure	<i>Celastrina neglecta</i>
Grey Comma	<i>Polygonia progne</i>	Tawny-Edged Skipper	<i>Polites themistocles</i>
Great Spangled Fritillary	<i>Speyeria Cybele</i>	Tawny Emperor	<i>Asterocampa clyton</i>
Harvester	<i>Feniseca tarquinius</i>	Variegated Fritillary	<i>Euptoietia claudia</i>
Hickory Hairstreak	<i>Satyrrium caryaeorum</i>	Viceroy	<i>Limenitis archippus</i>
Hobomok Skipper	<i>Poanes hobomok</i>	White Admiral	<i>Limenitis arthemis arthemis</i>
Inornate Ringlet	<i>Coenonympha tullia</i>	Wild Indigo Duskywing	<i>Erynnis baptisiae</i>

**Table D.1:** 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 <i>rare</i>
	2006	2009	2010	2011	2012	2013		
Acadian Hairstreak							July 13 (2008)	July 13 (2008)
American Lady			May 20		May 15	May 22	July 10 (2010)	May 15 (2012)
American Snout					July 11		July 10 (2010)	July 10 (2010)
Appalachian Brown				July 6	June 18	July 2	July 2 (2011)	June 18 (2012)
Arctic Skipper			June 3			June 4	July 10 (2010)	June 3 (2010)
Baltimore Checkerspot					June 26		July 3 (2011)	June 26 (2012)
Banded Hairstreak	July 18	July 16		July 12	June 25	July 15	July 2 (2011)	June 25 (2012)
Black Dash			June 8		July 14	July 30	July 10 (2010)	June 8 (2010)
Black Swallowtail	July 21	May 20	May 4	May 30	May 14	May 22	July 10 (2010)	May 4 (2010)
Broad-Winged Skipper		July 24			July 14	July 12	July 10 (2010)	July 10 (2010)
Bronze Copper	Aug 18				June 6		July 2 (2011)	June 6 (2012)
Cabbage White	July 18	May 12	May 3	May 19	May 14	May 21	July 2 (2011)	May 3 (2010)
Clouded Sulphur	July 18	May 22	May 4	May 31	May 14	May 21	July 10 (2010)	May 4 (2010)
Columbine Duskywing			May 19		May 31			May 19 (2010)
Common Buckeye				Sept 15	June 6			June 6 (2012)
Common Sooty Wing	July 21	June 2		Aug 4	June 7	May 22	July 10 (2010)	May 22 (2013)
Common Wood-Nymph	July 18	June 16	June 25	June 14	June 18	Jun 13	July 2 (2011)	June 13 (2013)
Compton Tortoiseshell				July 12				July 12 (2011)
Coral Hairstreak		July 16					July 2 (2011)	July 2 (2011)
Crossline Skipper							July 2 (2011)	July 2 (2011)
Delaware Skipper		June 2	May 24	July 11	July 9	July 4	July 10 (2010)	June 2 (2009)
Dion Skipper					July 14		July 13 (2008)	July 13 (2008)
Dun Skipper		July 24		July 6	June 26	July 12	July 10 (2010)	June 26 (2012)
Eastern Comma	Aug 2	June 30	May 14	June 1	May 15	May 27	July 10 (2010)	May 14 (2010)
Eastern Tailed Blue	Aug 18			July 27	July 14	July 15	July 11 (2006)	July 11 (2006)
Eastern Tiger Swallowtail	July 18	May 21	May 19	June 1	May 14	May 22	July 2 (2011)	May 14 (2012)
European Skipper	July 18	June 24	May 24	June 14	May 15	May 30	July 2 (2011)	May 15 (2012)



Species	Earliest Record By Year						Annual Butterfly Counts	Earliest Record at <i>rare</i>
	2006	2009	2010	2011	2012	2013		
Eyed Brown	Aug 2	July 16	June 15	July 5	June 8	June 25	July 2 (2011)	June 8 (2012)
Giant Swallowtail	July 24			June 8	May 15	May 30	July 11 (2006)	May 15 (2012)
Grey Comma							July 19 (2009)	July 19 (2009)
Great Spangled Fritillary	July 18	July 24	June 21	July 11	June 18	July 2	July 10 (2010)	June 18 (2012)
Harvester				Aug 19				Aug 19 (2011)
Hickory Hairstreak	July 18						July 11 (2006)	July 11 (2006)
Hobomok Skipper			May 26	June 1	May 30	June 4	July 2 (2011)	May 26 (2010)
Inornate Ringlet	Aug 2	June 2	May 19	June 6	May 14	May 21	July 2 (2011)	May 14 (2012)
Juvenal's Duskywing			May 26	May 25	May 14	May 21		May 14 (2012)
Least Skipper				Aug 5	May 28	July 30	July 19 (2009)	May 28 (2012)
Little Glassywing				July 6	July 10	July 12	July 2 (2011)	July 2 (2011)
Little Wood-Satyr	July 18	June 10	June 3	June 8	May 30	June 4	July 2 (2011)	May 30 (2012)
Little Yellow							July 11 (2006)	July 11 (2006)
Long Dash				June 14	May 28	July 4	July 2 (2011)	May 28 (2012)
Meadow Fritillary					July 18		July 10 (2010)	July 10 (2010)
Milbert's Tortoiseshell			June 21	July 19	June 11			June 11 (2012)
Monarch	July 18	June 22	June 25	May 30	May 14	June 19	July 2 (2011)	May 14 (2012)
Mourning Cloak		May 25	May 4	June 7	May 14	May 21	July 10 (2010)	May 4 (2010)
Mulberry Wing							July 20 (2013)	July 20 (2013)
Mustard White				Aug 12				Aug 12 (2011)
Northern Broken-Dash					June 26		July 10 (2010)	June 26 (2012)
Northern Crescent		May 21	June 3	June 7	June 4	June 12	July 10 (2010)	May 21 (2009)
Northern Pearly-Eye	July 18	June 30	June 3	June 20	June 11	June 13	July 10 (2010)	June 3 (2010)
Orange Sulphur	Aug 24		June 30	July 19	May 14	June 4	July 10 (2010)	May 14 (2012)
Painted Lady		June 4	May 4		May 15	May 21		May 4 (2010)
Pearl Crescent	July 18			May 25	May 14	May 22	July 2 (2011)	May 14 (2012)
Peck's Skipper				July 11	June 18	July 6	July 2 (2011)	June 18 (2012)
Question Mark	July 18	June 10	May 19	June 7	May 17	June 14	July 10 (2010)	May 17 (2012)
Red Admiral	Aug 18	May 14	May 3	May 25	May 14	June 4	July 10 (2010)	May 3 (2010)
Red-Spotted Purple		June 16	June 1	June 14	May 25	June 4	July 10 (2010)	May 25 (2012)
Silver-Bordered Fritillary						June 3	July 2 (2011)	June 3 (2013)

Species	Earliest Record By Year						Annual Butterfly Counts	Earliest Record at <i>rare</i>
	2006	2009	2010	2011	2012	2013		
Silver-Spotted Skipper		July 30	June 8	June 20	June 25	June 13	July 10 (2010)	June 8 (2010)
Silvery Checkerspot					June 20			June 20 (2012)
Spring Azure		May 13	May 4	May 20	May 15	May 21		May 4 (2010)
Striped Hairstreak				July 26		July 12	July 11 (2006)	July 11 (2006)
Summer Azure	Aug 2	July 22	June 8	July 5	June 11	June 13	July 2 (2011)	June 8 (2010)
Tawny Emperor	July 21	July 30		Aug 4	July 17	July 25	July 10 (2010)	July 17 (2012)
Tawny-Edged Skipper		July 16		July 22		July 16	July 2 (2011)	July 2 (2011)
Variegated Fritillary					July 5			July 5 (2012)
Viceroy	Aug 2	June 10	June 8	June 20	May 25	June 4	July 10 (2010)	May 25 (2012)
White Admiral		July 14		June 14	Aug 1		July 11 (2006)	June 14 (2011)
Wild Indigo Duskywing			May 17		July 11		July 2 (2011)	May 17 (2010)

**Table D.2:** 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	<i>Ambystoma maculatum</i>	YESA
Blue-spotted Salamander	<i>Ambystoma laterale</i>	BLSA
Four-toed Salamander	<i>Hemidactylium scutatum</i>	FOSA
Eastern Red-backed Salamander*	<i>Plethodon cinereus</i>	RESA/LESA

**Table D.3:** 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	<i>Fagus grandifolia</i>	FAGUGRAN
Black Ash	<i>Fraxinus nigra</i>	FRAXNIGR
Black Cherry	<i>Prunus serotina</i>	PRUNSERO
Butternut	<i>Juglans cinerea</i>	JUGLCINE
Green Ash	<i>Fraxinus pennsylvanica</i>	FRAXPENN
Hophornbeam	<i>Ostrya virginiana</i>	OSTRVIRG
Red Maple	<i>Acer rubrum</i>	ACERRUBR
Red Oak	<i>Quercus rubra</i>	QUERRUBR
Sugar Maple	<i>Acer saccharum</i>	ACERSACC
White Ash	<i>Fraxinus americana</i>	FRAXAMER
White Oak	<i>Quercus alba</i>	QUERALBA
White Pine	<i>Pinus strobus</i>	PINUSTRO
Yellow Birch	<i>Betula alleghaniensis</i>	BETUALLE

## APPENDIX E: ANNUAL BUTTERFLY COUNT RESULTS

### List E.1: 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@nr.si.on.ca](mailto:jlinton@nr.si.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.2: 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.3: 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 Crescent, Waterloo, ON, N2T 2R6; [jgrealey@nr.si.on.ca](mailto:jgrealey@nr.si.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, <sup>1</sup>**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, <sup>2</sup>**Black Da. 24**, Dun Sk. 5. **Unidentified:** Polygonia sp. 3. **Total** 39 species, 683 individuals. **Field Notes:** <sup>1</sup>This species is widespread in Waterloo Region for the first time in 2010. Previously very rare. <sup>2</sup>Local population known from this area but uncommon in the Region of Waterloo.

**List E.4:** 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 Cresnet, 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.5:** 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 Cresnet, 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: SHRUB AND SMALL TREE MONITORING PROTOCOL

Based on the results of the shrub and small tree monitoring pilot study, the adjusted protocol for shrub and small tree monitoring for subsequent years beginning in 2014 is described below. The protocol is based upon the recommendations of the Ecological Monitoring and Assessment Network (EMAN) and is intended to best suit the time and resources available to *rare* for ecological monitoring. Shrub and small tree monitoring is to be done in conjunction with canopy tree monitoring.

### *Equipment List*

- Fiberglass DBH tape and/or Callipers
- 30 metre measuring tape
- Digital Clinometer
- Field guide for species identification
- 6 steel pigtail stakes per Forest Monitoring plot (18 for each forest stand)
- Garden twine (optional)
- Field data sheets
- Pens/pencils

### *Nested Quadrat Establishment*

In each of the canopy tree monitoring plots, two nested 5x5m quadrats will be established in opposite corners for a total of six nested quadrats per forest stand. To select which corners will have a nested quadrat, each was assigned a number from one to four (Corner A = 1 etc.). Using a random number generator, the first corner was selected and its opposing corner was the obligate second nested quadrat. Following EMAN protocols, nested quadrats cannot be placed in adjacent corners within the same canopy tree monitoring plot and thus can only be placed in opposing corners.

Once selected the permanent nested corner quadrats should be set up by measuring 5 m from the desired corner along its adjacent lines. To limit interference with annual canopy tree monitoring, markers (steel pigtails) should be placed at 5m from the corner on either side. The remaining corner (interior) can be marked temporarily by measuring 7.07 m from the canopy tree monitoring forest plot corner. If necessary, a steel pigtail can be placed as a marker however this should be removed once shrub and small tree data collection is completed.

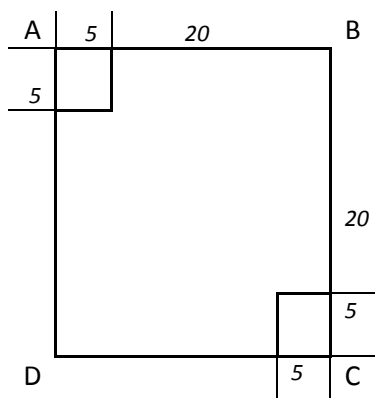
### *Data Collection*

In accordance with EMAN protocols, individuals that are to be included in the data collection for this forest stratum are:

**Table 1** Requirements for inclusion in shrub and small tree monitoring program

Small Tree:	Usually one-stemmed, woody species at least 4cm DBH but less than 10cm DBH, 1 m tall
Shrub:	Usually Multi-stemmed, woody species >4cm DBH; Saplings >4cm DBH are counted as Shrubs, 1 m tall

For the individuals that meet the above requirements, the following data will be collected: shrub or small tree distinction, species, DBH, height, general health (see tree health monitoring for canopy tree monitoring). Further variables may be added to this list in future.



**Figure 1** Sample of nested quadrat for shrub and small tree monitoring within canopy tree monitoring forest plots. Measurements are given in metres

Individuals recorded in shrub and small tree monitoring need not be mapped until they meet the requirements for inclusion for canopy tree monitoring ( $dbh \geq 10$  cm).

Using a random number generator, the following corners will be included in the shrub and small tree monitoring program:

	Cliffs and Alvars	Hogsback	Indian Woods
Forest Plot 1	B, D	A, C	A, C
Forest Plot 2	A, C	B, D	A, C
Forest Plot 3	A, C	A, C	A, C