

Ecological Monitoring 2020

rare Charitable Research Reserve



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The **rare Charitable Research Reserve** acknowledges and is grateful to all of the original stewards of the land in which **rare** resides, within the Haldimand Tract, spanning six miles on either side of the Grand River from source to mouth. Understanding that this land has been rich in diverse Indigenous presence since time immemorial, there are several Indigenous Nations that we would like to mention. We would like to honor and respect the sovereignty of both First Nations in our area: the Haudenosaunee Peoples of Six Nations of the Grand River and the Anishinaabe Peoples of Mississaugas of the New Credit First Nation. Nia:wen and Miigwech to these Nations who share their lands with us. We would also like to acknowledge the Neutral Peoples (and their ancestors) and the Indigenous Paleo hunters that we have archeological evidence for dating back 1,200 and 10,500 years, respectively. Lastly, we would like to acknowledge those Indigenous Peoples who currently live, work, and learn in the urban landscape around us such as other self-identified and status First Nations, Métis, and Inuit.

Cover Photography by Jordan Wrobel. Clockwise from top right; Viceroy butterfly, American Beech, Mourning Cloak butterfly, and Yellow-spotted Salamander.

Ecological Monitoring Report Executive Summary

The **rare Charitable Research Reserve** is an urban land trust and environmental institute in Waterloo Region/Wellington protecting more than 1,000 acres of highly sensitive lands along the Grand and Eramosa Rivers. In 2006, **rare** joined Environment Canada's Ecological Monitoring and Assessment Network (EMAN) to establish long-term ecological monitoring programs for the property with the objective of determining the status of **rare's** ecosystems and tracking how they change over time. Since 2006, several ongoing monitoring programs have been established at **rare** and have been carried out in subsequent years. In 2020, ecological monitoring programs occurred for butterflies, plethodontid salamanders, forest health, and soil humus decay rates.

Butterfly Monitoring

Butterfly monitoring at **rare** occurs across four separate transects for fourteen weeks during the late spring and summer. Butterfly monitoring in 2020 aligns closely with the total overall average from the past eleven monitoring years, with 4,950 individuals observed from 52 species.

The most abundant butterfly species were Cabbage White, followed by Clouded Sulphur, and Inornate Ringlet. Two butterfly species were observed during monitoring for the first time in 2020; the Acadian Hairstreak and Hackberry Emperor. Observations of Monarch butterflies continue to be low during monitoring at **rare** with comparable trends to the overwintering colonies in Mexico.

Weather conditions (temperature, wind speed, and sun availability) during monitoring had significant affects on the number of butterflies observed and species richness. Observations were highest during warm and bright days with low winds.

Despite annual fluctuations, no significant directional trend in butterfly abundances has been observed over the last eleven monitoring years. Continued long-term monitoring is recommended as a means of early detection for significant environmental change.

Plethodontid Salamander Monitoring

Monitoring of lungless (Plethodontid) salamanders occurs at **rare** by turning over pre-placed wooden cover boards in Indian Woods and the Hogsback once a week for consecutive nine-weeks each fall. Eastern Red-backed Salamanders (*Plethodon cinereus*) were the most abundant species found in both Indian Woods and Hogsback in all monitoring years. Observed salamander abundances in both plots have fallen outside threshold levels, and species diversity was low relative to previous years. Abundance decreases were particularly apparent in Hogsback, where, for the first time in 2020, only one species of salamander was observed. The ratio of Eastern Red-backed salamander lead-backed morphs and red-backed morphs have not changed over time, and no significant change in size class has occurred indicating the factors driving these fluctuations are not limited to one life stage.

These changes are likely tied to low moisture levels, air temperature, and disturbance in plots during monitoring, in combination with a variety of other environmental conditions. However the cause of declines observed in **rare** forests are unknown. Increasing the number of environmental parameters monitored, such as downed woody debris and vernal pool characteristics will enhance our understanding on the relationship between salamanders and environmental factors at **rare**. As the salamander monitoring program acts as a warning sign for

environmental change, falling numbers coupled with ongoing human pressures from agriculture, development projects, and the potential for accumulative effects from aggregate extraction highlight the need for continued salamander monitoring at **rare** and continued protection of **rare's** forests.

Forest Canopy and Tree Biodiversity Monitoring

The forest canopy and tree biodiversity monitoring program at **rare** occurs in all three major forest areas; the Hogsback, Indian Woods, and the Cliffs and Alvars. Three permanent plots are set-up within each area to track changes in the health of the trees within these forests. American Beech and Sugar Maple dominate **rare** forests, with all new tree recruits belonging to these two shade tolerant species. Forest diversity, health, and size class distribution have been fairly consistent across monitoring years with the exception of the loss of most ash trees from forest plots. As prevalence of Emerald Ash Borer and Beech Bark Disease continues to be observed within forest stands, it is important that targeted monitoring protocols be developed and implemented in addition to general forest health monitoring.

Soil Humus Decay Rate Monitoring

Decay rate monitoring occurs in late October and early November around one of the permanent forest canopy plots in each forest area at **rare**. Decay rates are measured by burying wooden tongue depressors below the soil surface and comparing their mass lost over a period of a year to those left on the soil surface. Decay rates in 2019 and 2020 monitoring are significantly lower than several previous monitoring years. All three forest areas sampled in the last two monitoring years are below the established baseline range. Multiple factors could be lowering decay rates at **rare** including temperature, moisture, soil microfauna, and changes in chemical composition from local anthropogenic pollution.

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1.0 Introduction

1.1 Ecological Monitoring

Ecological monitoring involves measuring a set of environmental variables at regular intervals over a long period of time (Vaughan et al. 2001; Lindenmayer and Likens 2018). The consistent monitoring of these abiotic or biotic environmental variables can provide information about the environmental changes that are occurring within an ecosystem (Lindenmayer and Likens 2018). 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 (Butler et al. 2012).

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 (Lovett et al. 2007; Hamilton 2015; Kupschus et al. 2016; Reynolds et al. 2016). The results of monitoring programs should be considered during policy development in order to create suitable strategies for mitigating and responding to environmental changes (Noss 1990; Beever 2006; Lovett et al. 2007; Lindenmayer and Linkens 2018).

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 (Siddig et al. 2016).

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). Although the EMAN coordinating office was closed in September 2010, the 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 original Blair property at *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.

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. 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 2020 monitoring year are reported and discussed.

Lists of Acronyms

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

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2.0 Butterfly Monitoring

2.1 Introduction

2.1.1 *Lepidoptera Taxonomy*

The order Lepidoptera, meaning “scaled wings”, is comprised of butterflies and moths, and there are approximately 20,000 species of butterflies worldwide (Lavinia et al. 2017). There are six families of butterflies, including five true families (*Papilionidae*, *Nymphalidae*, *Pieridae*, *Lycaenidae*, and *Riodinidae*) and the skipper family (*Hesperiidae*). There are general rules that can be used to distinguish moths and butterflies from one another. Butterflies are predominately diurnal, have clubbed antennae, and fold their wings vertically over their body while at rest, whereas moths are predominately nocturnal, have feathered or tapering antennae, and hold their wings out flat when resting (Pyle 1981).

2.1.2 *Why Monitor Butterflies?*

Long term monitoring of butterfly populations can provide valuable insight into the overall health of ecosystems and environmental change. Butterflies have short life spans, and thus respond quickly to various ecological pressures, both locally and on a broader scale (Fleischmann and Murphy 2009). Weather conditions play a role in regulating butterfly populations, as fitness can decline when they are exposed to temperatures outside their thermal tolerance or changes in precipitation (Boucher-Lalonde et al. 2014; Mills et al. 2017; van Bergen et al. 2020). Further, global climate change can result in an extension or shift of butterfly populations outside of their typical ranges (Roy et al. 2001). Climate warming is expected to allow butterflies to expand their ranges to higher elevations and latitudes, particularly along the geographic margins of their current ranges (Oliver et al. 2012; Mills et al. 2017). However, this ability to increase geographic range is largely species specific, with species with high dispersal ability or those able to use a range of host plants appearing to best exploit warmer temperatures (Kallioniemi 2013). Therefore, the presence or absence of butterfly species within geographic regions could provide useful information to better understand environmental change, as well as adaptability of different species of butterflies.

Throughout their life cycle, butterflies have specific host plants they require for egg laying and feeding as both caterpillars and adults. Natural and human-caused disturbances, such as climate change, may alter not only butterfly populations but their host plants. Climate change may modify the lifespan, nutrient requirement, reproductive schedule, or habitat range of butterflies; and the flowering period or food quality of host plants (Lawson et al. 2013; Kharouba et al. 2014; Navarro-Cano et al. 2015; Fenberg et al. 2016). This may result in the potential disruption of the delicate but mutually beneficial relationship between butterflies and their host plants (Schweiger et al. 2008; Singer and Parmesan 2010). However, some native insects have demonstrated an ability to expand host plant preferences and gain new feeding resources and reproduction sites from other related native or invasive plants (Bezemer et al. 2014; Masters and Emery 2015). Butterfly species that are able to expand their host plant range may be less impacted by climate change than other species not able to adapt as quickly. Overall, variations in butterfly-plant interactions is another perilous consequence of climate change (Sunny et al. 2015), and monitoring butterflies will improve our understanding on the vegetative community and how important pairings that facilitate pollination are changing.

In addition to their rapid responses to ecological change and close interaction with the local plant community, butterflies are good indicator species because of the ease in which they can be monitored (Wepprich et al. 2019). Their size and the colourful distinctions between species make observation and identification relatively simple for most butterfly families, and their reputation as a charismatic species that invokes a positive response from the public allows for recruitment of volunteers and the promotion of community science programs. Charismatic species can act as important symbols for conservation, and also inspire the public's intrinsic desire to protect them and their habitat.

2.1.3 Importance of Butterflies

Butterflies are important components of ecosystems and human practices. For example, butterflies are important members of the food chain, supplying nutrients to many animals such as bats, frogs, birds, and mice. A decrease in butterfly availability as a food item may result in effects at higher trophic levels. Butterflies are also important pollinators of natural and anthropogenic vegetation (Faegri and Pijl, 1997; Ghazoul 2006). In agriculture, approximately 75 percent of crops used for human consumption depend on pollinators (Klein et al. 2007; Potts 2010), with honeybee pollination valued at approximately \$900 million per year in Ontario (OMAFRA 2020). As honeybee populations around the world continue to decline (Sluijs et al. 2013), the importance of alternative pollinators such as butterflies continues to grow.

Over the past few decades, populations of butterflies have declined at an alarming rate in many parts of the world (Lister and Garcia 2018; Wepprich et al. 2019). Habitat loss, pesticide use, habitat degradation, climate change, and fragmentation are just some of the proposed causes of these declines (Merckx et al. 2013; Wepprich et al. 2019). This decrease in butterfly populations may be indicative of broader scale ecosystem changes. Continued pressure from these sources and the response from butterfly populations highlight the urgent need for monitoring efforts to help make conservation management decisions to protect these charismatic and important species.

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

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

In 2006, two transects were established: one located in the Cliffs and Alvares and one in South Field/Sparrow Field. Baseline data were collected over a five week period during the initial pilot study. Butterfly monitoring at **rare** resumed in 2009 and two more transects were established: Thompson Tract (2009), and Blair Flats (2010). Monitoring took place over thirteen weeks in 2009, and fourteen weeks in 2010 through to 2020. It is important to note that due to a change in property boundaries, the South Field/Sparrow Field transect had to be slightly altered in 2014; these changes are described below (Section 2.2.2).

2.2 Methods

2.2.1 Monitoring Protocol

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

Ideally, butterfly monitoring programs should take place over a 26 week period, from April to September (Layberry et al. 1998). At **rare**, this time period has been reduced due to time and monetary constraints. Monitoring typically begins on the third Monday of May; however, this may be either advanced or delayed, depending on weather conditions (i.e. particularly cold or warm local temperatures). Butterflies are most active during the warmest part of the day, and thus monitoring is completed between the hours of 10am and 3pm (Grealey 2006). In late June and July, monitoring can extend to 4pm should weather conditions be suitable to do so. Monitoring is completed on sunny days, when the temperature is above 13°C. If it is overcast, the temperature must be at least 17°C (UK Butterfly Monitoring Scheme; Butterfly Monitoring Scheme Germany). Wind should also be less than five on the Beaufort Wind Scale (refer to table C.1. in Appendix C).

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

In 2020, monitoring began on May 20th and four transects were each walked once weekly for fourteen weeks. A recommended list of field equipment can be found in Appendix B, List B.1. At the start and end of each transect, the start time and air temperature were recorded. Transects were walked at a uniform pace and all butterflies observed within a ten metre radius were recorded. Approximately halfway through each section, a ten minute stop was made at a predetermined location and all butterflies observed within a ten metre radius were recorded. At each stop, the percent of blue sky was estimated (0-100; where 100 = no clouds) and the average wind speed was recorded. Butterflies were identified in the field and caught with a net when necessary to aid in identification. Unknown species were photographed and sent to local experts for identification. If identification was not possible, the individual was recorded as the most common possibility or as an appropriate higher taxonomy. While walking each transect, occasional stops were permitted to properly identify butterflies. All observations were recorded in a standard field form (Appendix C and on the **rare** server).

2.2.2 Transect Descriptions

Butterfly monitoring occurred across the following transects at the **rare Charitable Research Reserve**.

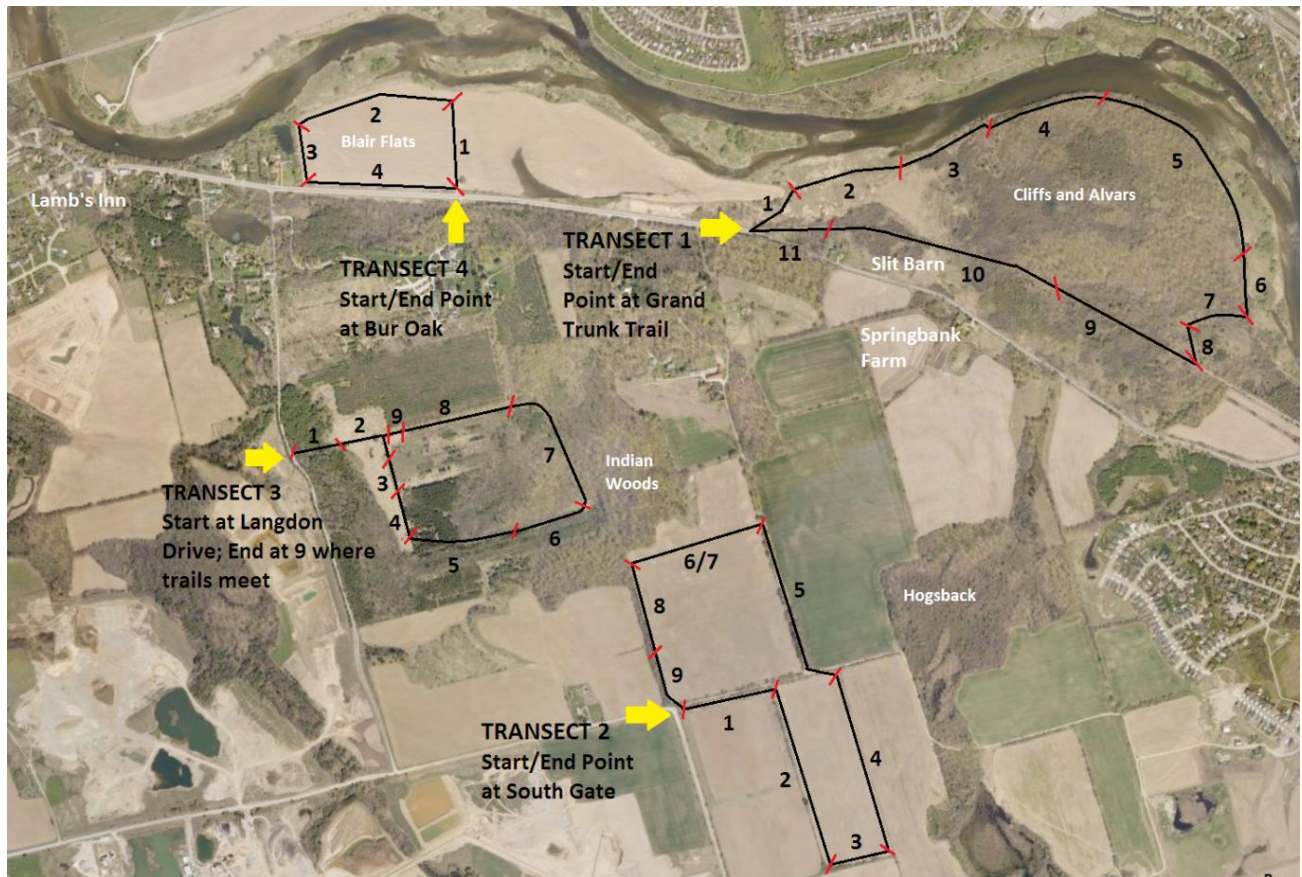


Figure 2.1: Location of the four butterfly monitoring transects at the **rare Charitable Research Reserve** with start/end points and section breaks.

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

The **South Field/Sparrow Field** transect is 2.9 km, running along the edge of agricultural fields, hedgerows, restored meadow, and an experimental research field largely maintained as tallgrass prairie. Nearby fields are currently in agricultural production, primarily hay. Sparrow Field has gradually been removed from agricultural production and is being restored to native vegetation with an approximately 20-hectare portion involved in tallgrass prairie restoration research. Prior to the 2014 monitoring year, this transect traveled along the south-eastern perimeter of Indian Woods. However, due to a change in the **rare** property boundary in early 2014, this part of the transect (formerly section 6 and 7) was eliminated and an alternative route was used. To minimize the effects of this change, the new section is referred to as 6/7.

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

The **Blair Flats** transect is a 1.3 km loop that walks the perimeter of a restored tallgrass prairie. Prior to 2010, Blair Flats was in agricultural production. As part of a long term study, the area was restored to a tallgrass prairie. In 2015, Blair Flats was burned as part of a prescribed management regime, which intends to encourage and promote native prairie plants and overall tallgrass prairie ecosystem health. Beginning at the large Bur Oak (*Quercus macrocarpa*) just off of Blair Road, the transect heads north towards the river, turns west and runs parallel to the river at the margin of the tallgrass field, then turns south and follows the property boundary, and finally travels eastward, parallel to the road and ending at the Bur Oak.

2.2.3 Data Analysis

Data analysis was conducted using R, version 4.0.2 (R Development Core Team 2020) and Microsoft Excel 2020. All transects were pooled in global analyses to represent the property as a whole. Data before the 2010 monitoring season were excluded in the analysis because of unequal sampling effort. Due to variations in transect lengths and habitats, additional analyses consider each transect individually over time.

The Shannon diversity index and species evenness were used to measure global butterfly diversity for each monitoring year at *rare*, as well as diversity within each transect. Species evenness refers to the relative abundance of individuals of different species while the Shannon diversity index considers both the evenness and total number of species to calculate a value commonly ranging from 0-4. Zero indicates low diversity, while four represents high diversity; typically values fall between 1.5 and 3.5 (Magurran 2004; Ortiz-Burgos 2016).

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

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

To analyse how butterfly abundance was affected by temporal (year, week) and environmental (habitat, wind speed, temperature, sun availability) variables a generalized linear mixed model was used to incorporate fixed-effects parameters and random effects. This model was run with a poisson error distribution, and the nAGQ=0 parameter optimizer was utilized (Bolker 2019). To improve the fit of the data a negative binomial model was used to correct the

over-dispersed count variables. Measurements taken were repeated within all four transects, therefore transect was considered a repeated factor in each week and year. The significance of the main effects was determined with a chi-squared test. Due to the complexity of these data, interactions could not be tested, however correlation between variables was assessed prior to analyses. This analysis was also used to understand the changes in species richness at **rare**.

To test whether there has been a significant change in abundance across monitoring years (i.e. whether butterfly populations varied over time) for specific noteworthy species, a generalized linear model was run with transformed data when necessary to meet the assumptions of normal distribution for analysis. The number of observed individuals was the response variable, and year was the independent continuous variable. The significance was tested with an iterative F-test (Crawley 2007). An alpha value of 0.05 was used to determine significance. This continuous analysis was included due to the importance of identifying directional global abundance trends occurring over all monitoring years.

2.3 Results

2.3.1 Overall Abundance and Diversity

Across all transects at **rare Charitable Research Reserve**, 4,950 individuals were recorded belonging to 52 butterfly species during the 2020 monitoring season. This aligns closely with the total overall average of 4,946 across all years of monitoring. From the last eleven monitoring years, 2012 was the most abundant year for total butterfly observations and 2019 had the lowest recorded total (Table 2.1).

Species richness ($S=52$) for 2020 was mid-range for all monitoring years, whereas the Shannon diversity index ($H=2.41$) and species evenness ($E_H=0.61$) were both the fourth lowest (Table 2.1). From the 4,950 observed butterflies in 2020, 50 could not be identified to species and were categorized as unknown. These observations were excluded from the above calculations.

The most abundant species observed during monitoring were Cabbage White ($N=1,676$), Clouded Sulphur ($N=1,1075$), Inornate Ringlet ($N=273$), Common Wood Nymph ($N=256$), and European Skipper ($N=278$). Cabbage White and Clouded Sulphur have been the two most abundant species since the beginning of monitoring. Abundances of each butterfly species as well as global abundances are presented in Table 2.2, and a visual comparison can be seen in Figure 2.2 & 2.3.

Table 2.1: A summary of all transects for species richness, the Shannon diversity index, and species evenness excluding unknown observations, and the number of observed individuals from 2010 to 2020.

	Number of Individuals (n)	Species Richness (S)	Species Evenness (E _H)	Shannon-Diversity Index (H)
2010	4,049	41	0.54	2.00
2011	3,808	46	0.54	2.06
2012	7,866	52	0.58	2.28
2013	5,262	45	0.65	2.47
2014	4,105	53	0.69	2.73
2015	4,931	55	0.72	2.90
2016	5,820	51	0.65	2.56
2017	4,355	53	0.72	2.85
2018	6,627	55	0.63	2.52
2019	2,589	53	0.73	2.90
2020	4,950	52	0.61	2.41

Table 2.2: Summary of observed butterflies during 2020 monitoring season at the **rare Charitable Research Reserve**. The Waterloo Regional Status for each of the observed species is also included from Linton (2012).

Species	Transect				Total	Regional Status
	1	2	3	4		
Acadian Hairstreak	0	0	1	0	1	Uncommon
American Lady	1	0	0	1	2	Common
Appalachian Brown	9	0	3	0	12	Uncommon
Azure sp.	33	8	47	0	88	Common
Baltimore Checkerspot	3	0	1	0	4	Rare
Banded Hairstreak	1	0	10	0	11	Uncommon
Black Dash	6	0	0	0	6	Uncommon
Black Swallowtail	12	41	3	3	59	Very Common
Bronze Copper	9	0	0	1	10	Very Common
Cabbage White	960	425	178	113	1676	Very Common
Clouded Sulphur	67	887	67	54	1075	Very Common
Common Sootywing	1	1	0	2	4	Rare
Common Wood-Nymph	43	95	78	40	256	Very Common
Coral Hairstreak	1	0	0	0	1	Uncommon
Crossline Skipper	0	1	0	0	1	Rare
Delaware Skipper	3	1	5	26	35	Common
Dion Skipper	1	0	0	0	1	Rare
Dun Skipper	9	0	2	4	15	Very Common
Eastern Comma	3	2	6	0	11	Very Common
Eastern Pine Elfin	0	0	1	0	1	Rare

Eastern Tailed Blue	1	18	7	8	34	Uncommon
Eastern Tiger Swallowtail	15	11	12	1	39	Very Common
European Skipper	95	22	64	2	183	Very Common
Eyed Brown	24	0	0	0	24	Very Common
Giant Swallowtail	15	14	7	2	38	Uncommon
Great Spangled Fritillary	9	1	9	3	22	Very Common
Grey Comma	1	1	5	0	7	Uncommon
Hackberry Emperor	1	0	0	0	1	Rare
Hobomok Skipper	3	7	0	0	10	Common
Inornate Ringlet	30	147	48	48	273	Common
Juvenal's Duskywing	0	3	13	0	16	Rare
Least Skipper	18	0	2	2	22	Uncommon
Little Wood-Satyr	45	15	104	0	164	Very Common
Milbert's Tortoiseshell	2	0	0	0	2	Uncommon
Monarch	37	60	31	11	139	Very Common
Mourning Cloak	6	7	13	2	28	Very Common
Northern Broken-Dash	0	0	3	1	4	Common
Northern Crescent	41	23	102	10	176	Uncommon
Northern Pearly-Eye	14	29	100	0	143	Common
Orange Sulphur	1	22	0	1	24	Very Common
Painted Lady	1	0	0	0	1	Common
Pearl Crescent	4	49	24	3	80	Common
Question Mark	3	1	1	0	5	Very Common
Red Admiral	34	9	7	13	63	Very Common
Red-Spotted Purple	12	10	11	0	33	Common
Silver-Spotted Skipper	7	1	24	6	38	Uncommon
Silvery Blue	1	2	7	1	11	Uncommon
Striped Hairstreak	1	0	0	0	1	Uncommon
Tawny Emperor	3	3	9	4	19	Uncommon
Tawny-edged Skipper	12	1	3	1	17	Common
Viceroy	2	4	0	1	7	Very Common
Wild Indigo Duskywing	2	3	1	1	7	Common
Unknown Species	24	8	14	4	50	
Total	1626	1932	1023	369	4950	

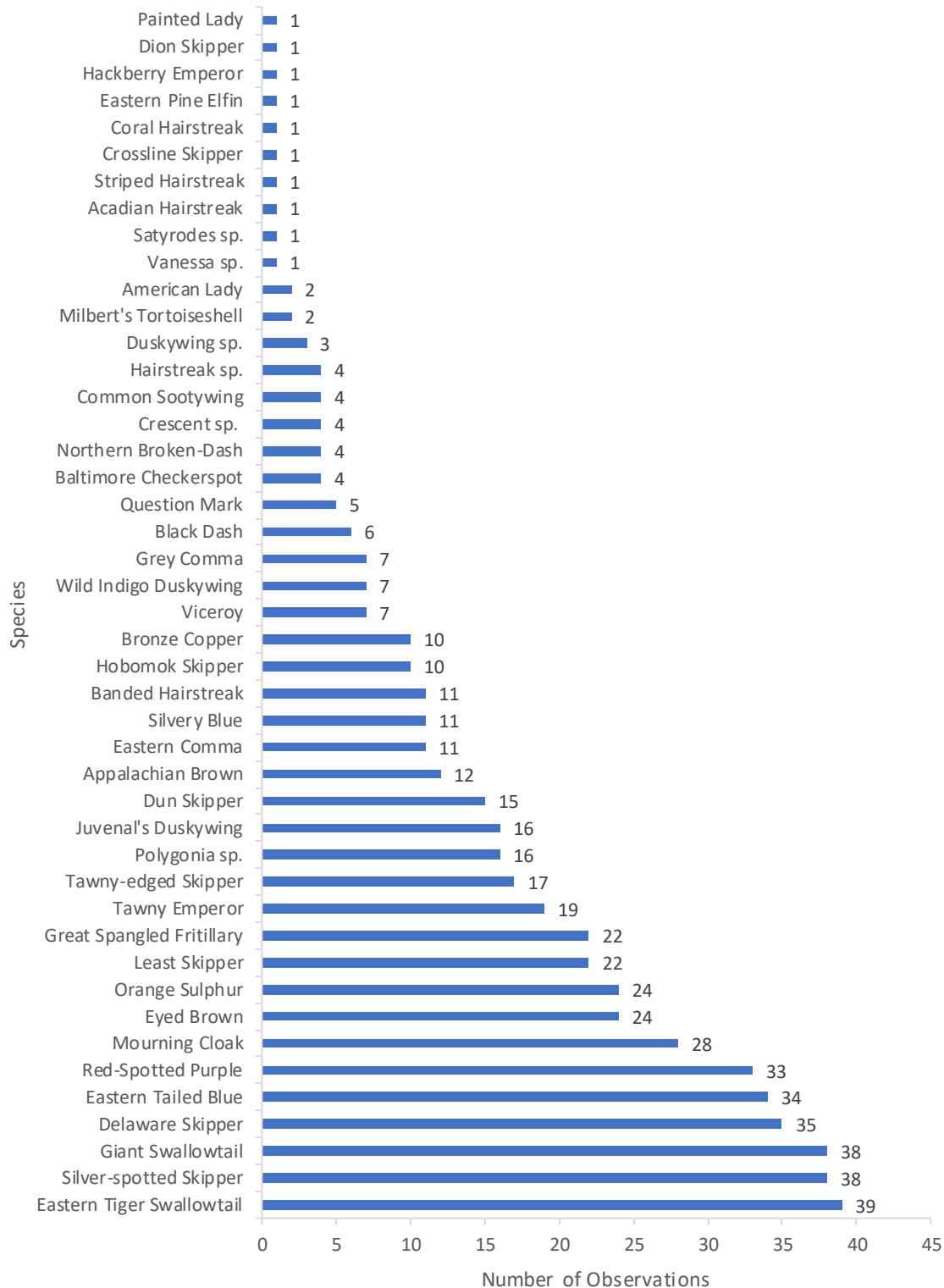


Figure 2.2: Comparison of species observed on *rare charitable research reserve* in 2020, only species with less than 50 observations are shown.

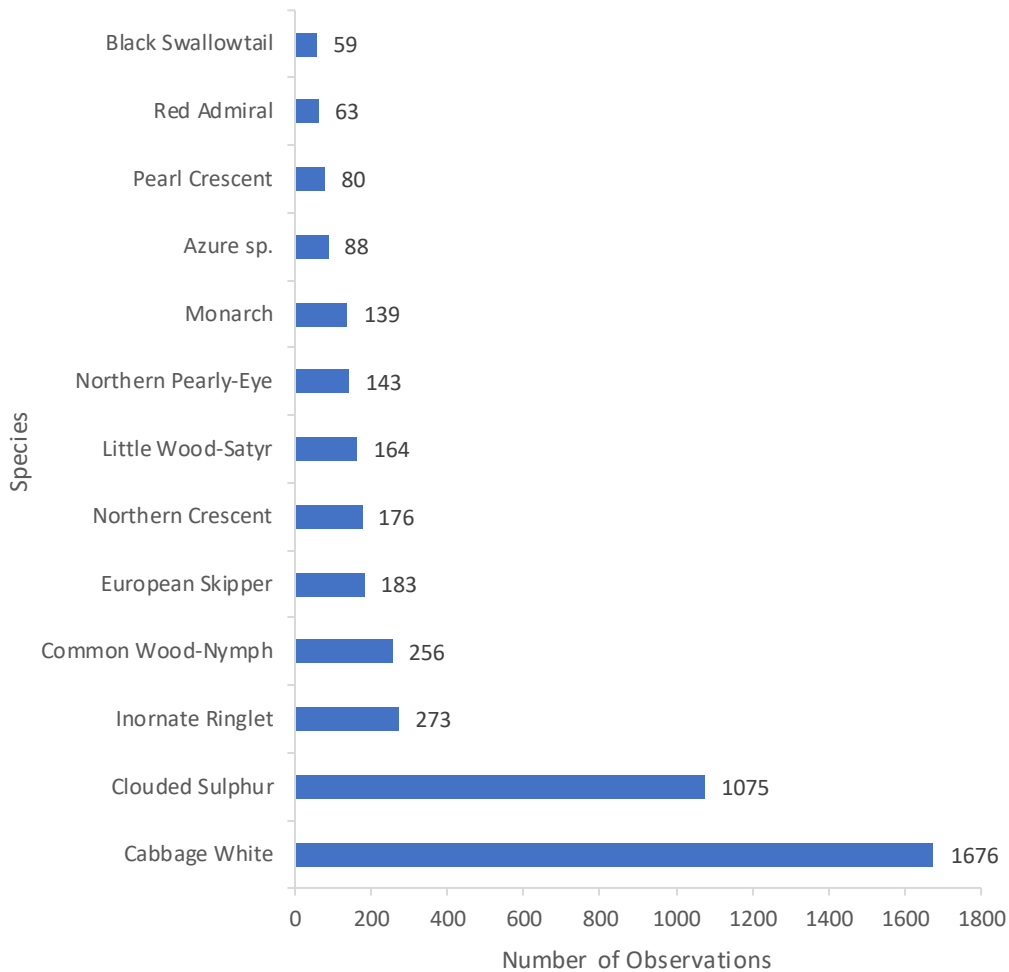


Figure 2.3: Comparison of species observed on **rare charitable research reserve** in 2020, only species exceeding 50 observations are shown.

2.3.2 Global Abundance Trends

Global butterfly abundances and richness at **rare** have not changed significantly over time ($p > 0.05$). However, comparison between years in a given transect indicate differences in abundance between monitoring years (Figure 2.4). This variation between years appears to be driven by Transect One and Two (e.g. compare 2018 and 2019 and note the larger variations in One and Two compared to Three and Four). This information can be useful to compare to other variables such as weather and restoration efforts to infer possible explanations of population trends. The earliest record of observation for each butterfly historically observed at **rare** can be found in Appendix D (Table D.1).

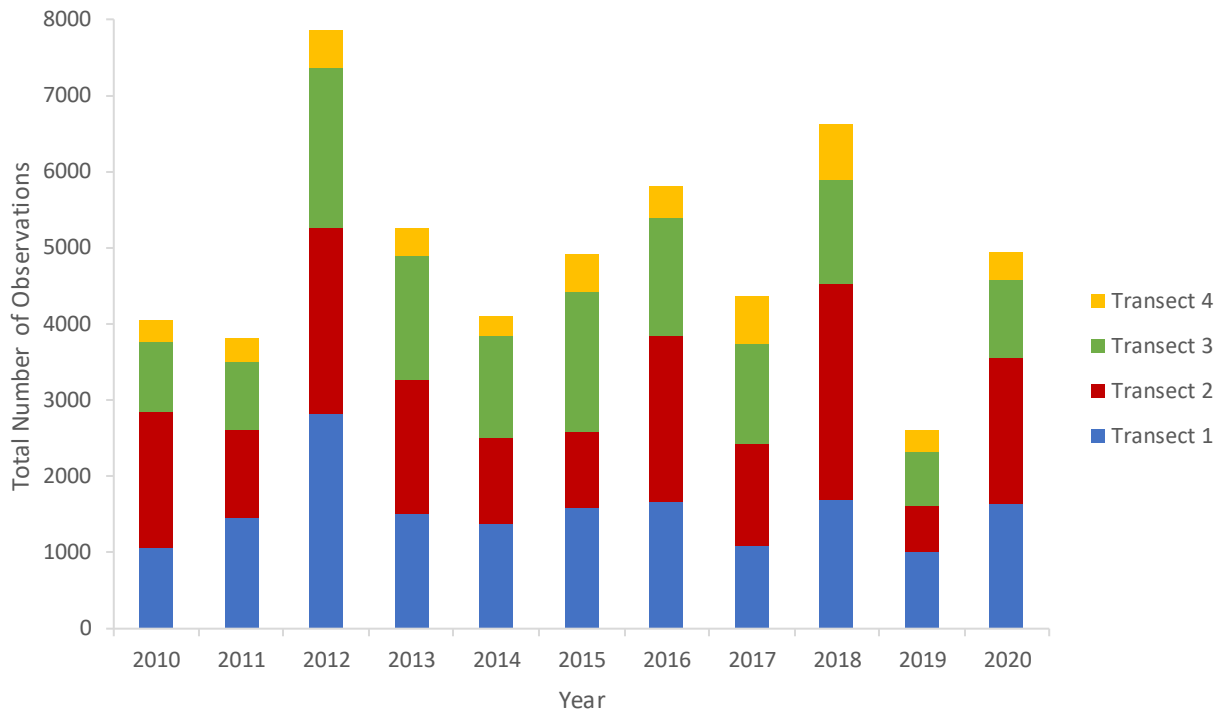


Figure 2.4: Combined transect abundance of butterflies across monitoring years.

2.3.3 Within Transect Comparisons

Transect One: Cliffs and Alvars

A total of 1,602 butterflies were observed from 47 species in Transect One during the 2020 monitoring period. The total number of observations made in Transect One this year was the fourth highest after 2012, 2018, and 2016. Species evenness ($E_H=0.50$) and the Shannon diversity index ($H=1.91$) were the lowest recorded values since 2011 (Table 2.3). The average number of butterflies observed per kilometer this monitoring season was 465 (11-year average 438, SD +/- 142) in Transect One. A visual comparison of abundance within Transect One reveals the number of observed individuals remains relatively stable between the majority of years, with the exceptions of a large number of observations occurring in 2012. (Figure 2.5).

The species with the highest number of observed individuals in Transect One in 2020 were Cabbage White (960), European Skipper (95), Clouded Sulphur (67), Little-Wood Satyr (45), and Common Wood-Nymph (43), accounting for 75.5 percent of all observations. These species were also the five most abundant species observed in Transect One from the accumulated data of 2010-2020. One species, the Hackberry Emperor, was observed for the first-time during monitoring in 2020. Additionally, two species were observed in 2020 that have not been seen in multiple years in this transect; the Dion Skipper had not been observed since 2012 and the Stripped Hairstreak has not been observed since 2016.

Table 2.3: A summary of Species Richness, the Shannon Diversity Index, and Species Evenness excluding unknown observations, and the number of observed individuals from 2010 to 2020

	Number of Individuals (n)	Species Richness (S)	Species Evenness (E_H)	Shannon-Diversity Index (H)
2010	1,061	32	0.60	2.06
2011	1,454	35	0.50	1.76
2012	2,828	45	0.58	2.19
2013	1,505	42	0.66	2.45
2014	1,365	47	0.70	2.71
2015	1,590	42	0.76	2.82
2016	1,652	45	0.64	2.43
2017	1,068	47	0.74	2.85
2018	1,699	48	0.60	2.31
2019	1,001	42	0.69	2.59
2020	1,602	47	0.50	1.91

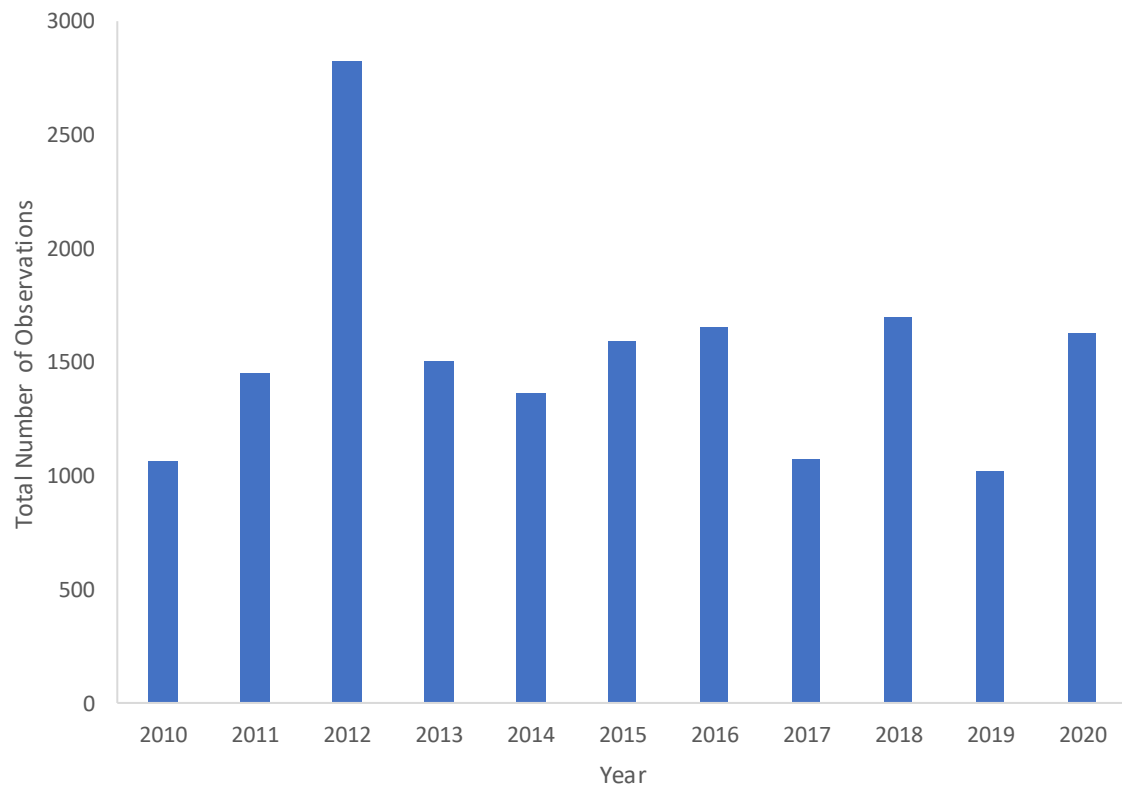


Figure 2.5: Total number of butterfly observations made during each monitoring year at Transect One.

Transect Two: South Field/ Sparrow Field

A total of 1,924 butterflies were observed from 34 different species in Transect Two during the 2020 monitoring period. The total number of individuals observed in Transect Two in 2020 was the fourth highest from the last eleven monitoring years. Species evenness ($E_H=0.54$) and the Shannon diversity index ($H=1.89$) were both mid-range in comparison to previous years, and lower than the most recent monitoring in 2019 (Table 2.4). The average number of butterflies observed per kilometer this monitoring season was 666 (11-year average 535, SD +/- 225) in Transect Two.

Species observed most frequently during monitoring at Transect Two were Clouded Sulphur (887), Cabbage White (425), Inornate Ringlet (147), Common Wood-Nymph (95), and Monarch (60) accounting for 83.9 percent of all observations. The top three observed species are consistent with most abundant species observed in Transect Two from accumulated data of 2010 to 2020.

Three species had the highest observations in Transect Two of all years (Common Wood-Nymph, Little Wood-Satyr, and Northern Pearly-Eye). Additionally, a Crossline Skipper was observed this monitoring year for the first time since 2015.

The butterfly abundance at this transect in the last eleven monitoring years varies greatly (580 in 2019 to 2,826 in 2018). Much of this variation is attributed to the two most common species, Cabbage White and Clouded Sulphur, which vary from 127 to 1,861 in combined total abundance. Years with low total abundance observed coincide with years of a lower percentage of Cabbage White and Clouded Sulphur observations (Figure 2.6). 2019 and 2015 have the lowest recorded total abundance in the last eleven monitoring years and these two years have the lowest percentages of these two species (21.8 and 44 percent of all observations).

Table 2.4: A summary of species richness, the Shannon diversity index, and species evenness excluding unknown observations, and the number of observed individuals from 2010 to 2020.

	Number of Individuals (n)	Species Richness (S)	Species Evenness (E_H)	Shannon-Diversity Index (H)
2010	1,777	26	0.44	1.42
2011	1,145	30	0.47	1.60
2012	2,426	36	0.49	1.75
2013	1,758	34	0.57	2.01
2014	1,130	32	0.61	2.12
2015	989	37	0.67	2.43
2016	2,187	32	0.53	1.84
2017	1,353	34	0.67	2.37
2018	2,826	33	0.50	1.75
2019	580	31	0.73	2.50
2020	1,924	34	0.54	1.89

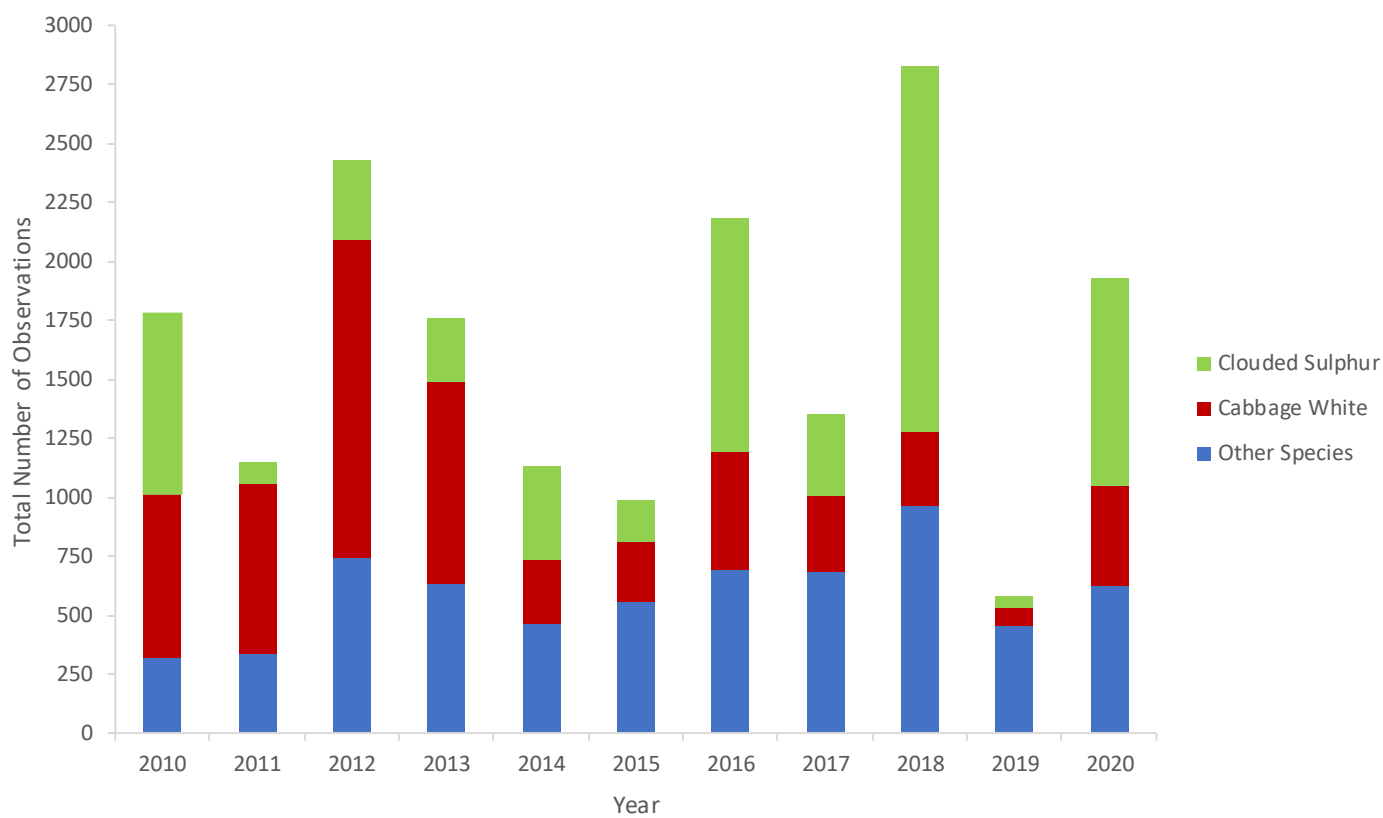


Figure 2.6: Total number of butterfly observations made during each monitoring year divided into three categories (Cabbage White, Clouded Sulphur, and all other species).

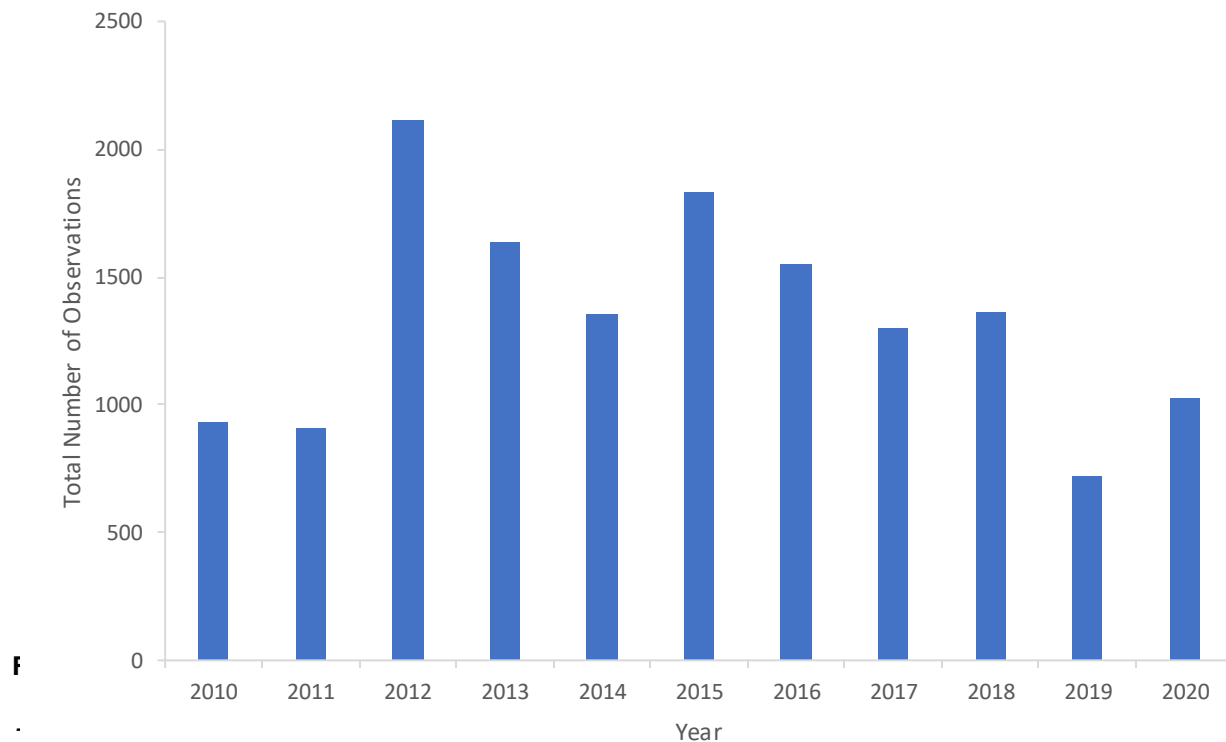
Transect Three: Thompson Tract

A total of 1,009 butterflies were observed from 37 different species in Transect Three during the 2020 monitoring period. Although the number of individuals was in the lower range compared to other years, species richness was mid-range. Species evenness ($E_H=0.78$) and the Shannon diversity index ($H=2.82$) were both third highest recorded values, after 2018 and 2019 (Table 2.5). The average number of butterflies observed per kilometer this monitoring season was 465 (11-year average 609, SD +/- 139) in Transect Three. A visual comparison of abundance within Transect Three reveals no trend in the number of observed individuals over the last eleven monitoring years. (Figure 2.7).

Species with the highest observations were Cabbage White (178), Little Wood-Satyr (104), Northern Crescent (102), Northern Pearly-Eye (100), and Common Wood-Nymph (78) accounting for 55.7 percent of all observations. This is the first year the Inornate Ringlet has not been in the top five highest observed species. Two species had the highest counted observations in Transect Three during 2020 monitoring (Banded Hairstreak and Tawny Emperor), and an Acadian Hairstreak was observed for the first time in this transect in 2020.

Table 2.5: A summary of species richness, the Shannon diversity index, and species evenness excluding unknown observations, and the number of observed individuals from 2010 to 2020.

	Number of Individuals (n)	Species Richness (S)	Species Evenness (E_H)	Shannon-Diversity Index (H)
2010	936	30	0.70	2.37
2011	910	34	0.71	2.52
2012	2,116	36	0.71	2.56
2013	1,636	35	0.72	2.55
2014	1,354	39	0.72	2.63
2015	1,834	43	0.73	2.74
2016	1,551	37	0.71	2.57
2017	1,297	39	0.76	2.78
2018	1,362	40	0.79	2.90
2019	716	33	0.81	2.83
2020	1,009	37	0.78	2.82



A total of 365 butterflies were observed from 29 different species in Transect Four during the 2020 monitoring period. Species richness is third highest after 2012 and 2018. Species evenness and Shannon's diversity index ($E_H=0.69$, $H=2.32$) were both in the mid-range (Table 2.6). A visual comparison of abundance within Transect Four reveals no trend in the number of

observed individuals over the last eleven monitoring years, and the numbers reflect global butterfly abundance trends. (Figure 2.8).

Cabbage White (N=113), Clouded Sulphur (N=54), Inornate Ringlet (N=48), Common Wood-Nymph (40), and Delaware Skipper (26) were the most common species observed in 2020, accounting for 77 percent of all observations. This was the first monitoring year the Delaware Skipper was one of the top five most common species. The top four observed species are consistent with most abundant species observed in Transect Four from accumulated data of 2010-2020. Two skipper species had significant observations in 2020. The Delaware Skipper had its highest recorded number of observations and the Least Skipper was observed for the first-time in Transect Four. The average number of butterflies observed per kilometer this monitoring season was 284 (11-year average 328, SD +/- 122) in Transect Four.

Table 2.6: A summary of species richness, the Shannon diversity index, and species evenness excluding unknown observations, and the number of observed individuals from 2010 to 2020.

	Number of Individuals (n)	Species Richness (S)	Species Evenness (E _H)	Shannon-Diversity Index (H)
2010	270	14	0.49	1.30
2011	298	19	0.43	1.26
2012	497	31	0.60	2.07
2013	374	20	0.64	1.90
2014	256	27	0.74	2.43
2015	518	28	0.68	2.26
2016	431	28	0.70	2.35
2017	637	25	0.78	2.50
2018	740	31	0.71	2.45
2019	292	28	0.76	2.55
2020	365	29	0.69	2.32

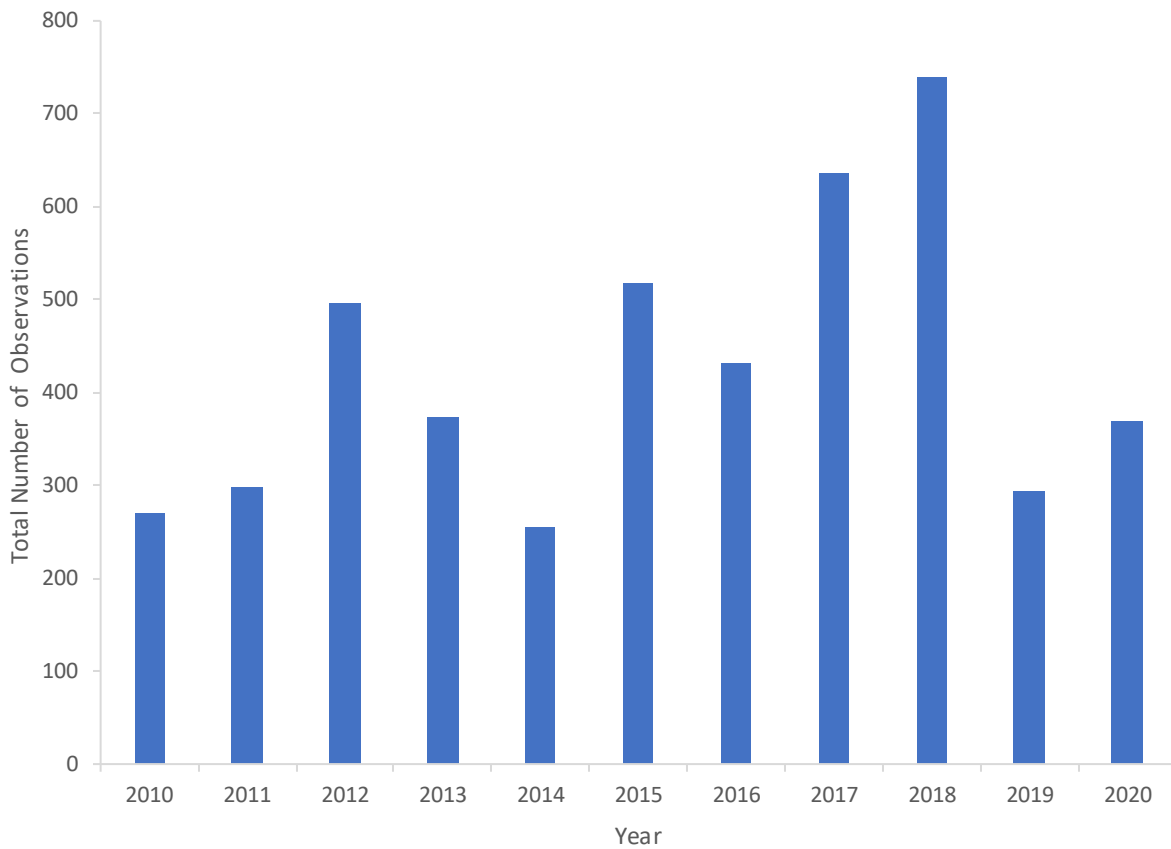


Figure 2.8: Total number of butterfly observations made during each monitoring year at Transect Four.

2.3.4 Noteworthy Species and Species of Special Concern

Global Monarch abundances at **rare** have not changed significantly over time ($p>0.05$). Although there is no significant trend in global abundances over monitoring years, a visual representation of Monarchs suggest they have been increasing in the subsequent monitoring years after a steep decline in 2013. In 2019, 14 percent of total butterfly observations were Monarchs, compared to <1 percent in 2013. This year, 139 Monarchs were observed during monitoring, accounting for only 2.84 percent of all observed individuals, a noticeable decline from 2019. A graphical representation of observed abundances is shown in Figure 2.9.

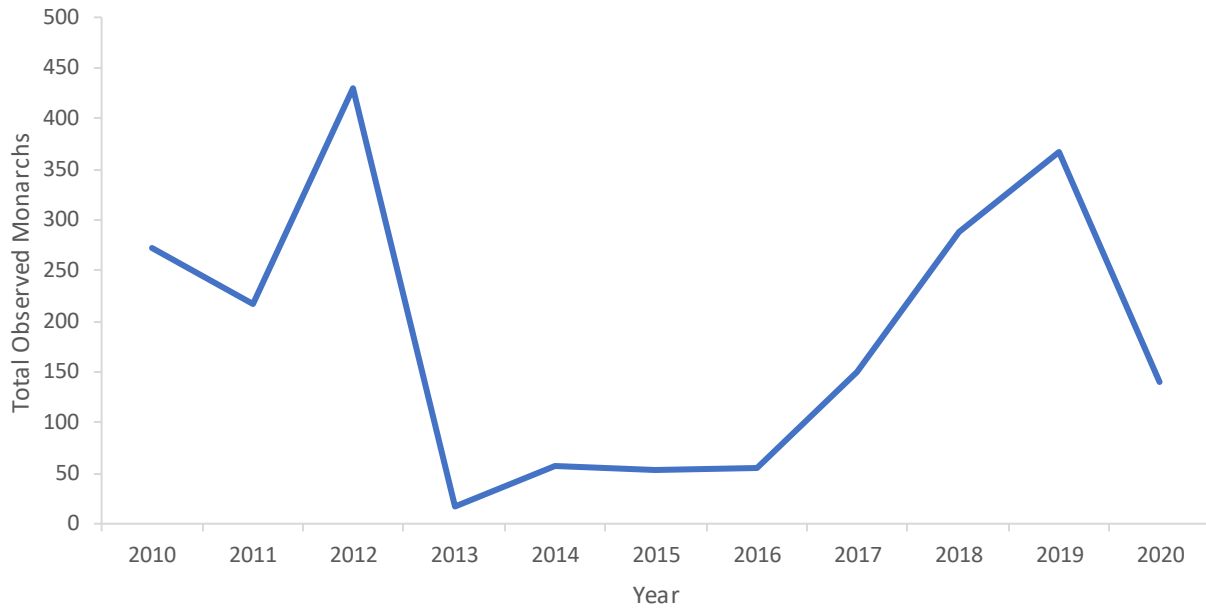


Figure 2.9: Number of observed Monarch individuals recorded for each monitoring year between 2010 and 2020.

At 1,676 individuals observed, Cabbage Whites accounted for 34.2 percent of the total number of individuals observed in 2020 during monitoring. Both numbers had increased from the previous monitoring season and were fourth highest among all years (Figure 2.10). However, there has not been a significant change in Cabbage White abundance over time ($p>0.05$).

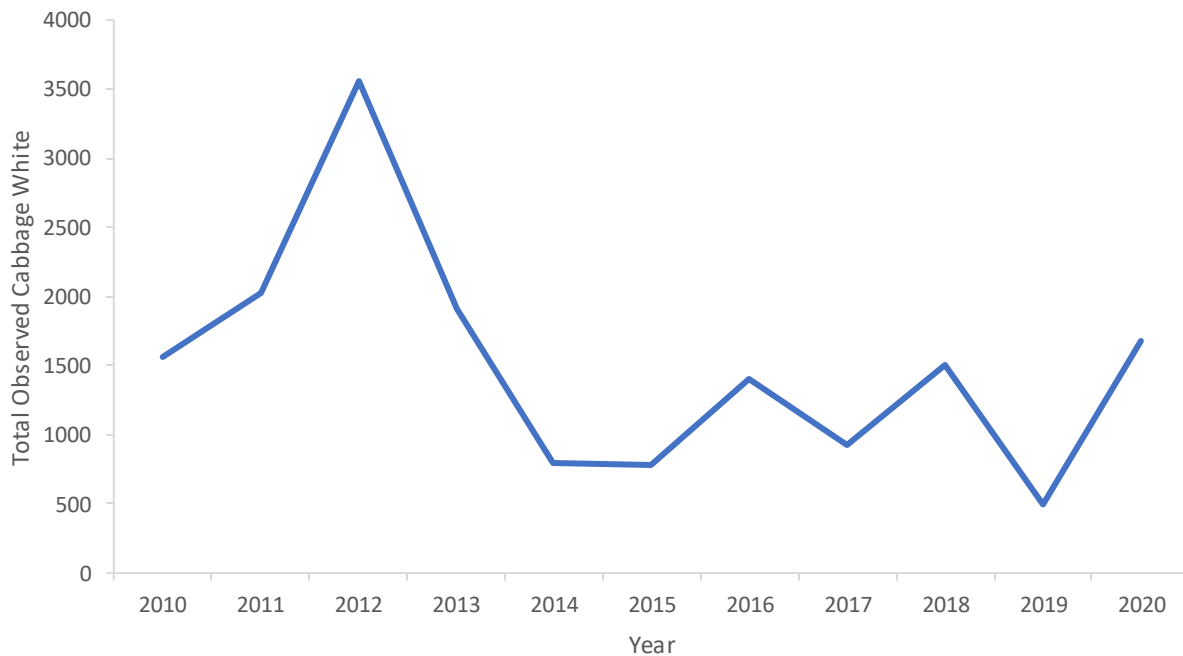


Figure 2.10: Number of observed Cabbage White individuals recorded for each monitoring year between 2010 and 2020.

European Skipper abundance has not changed significantly over time at **rare** ($p>0.05$). European Skippers had generally shown a downward trend in abundance after a spike in 2015 accounting for 13.9 percent of observation. Observations in 2020 were up from the previous year with European Skipper accounting for 3.7 percent of observations, compared to 1.3 percent in 2019, however 2020 had the second lowest number of observed European Skippers in the last eleven years of monitoring (Figure 2.11).

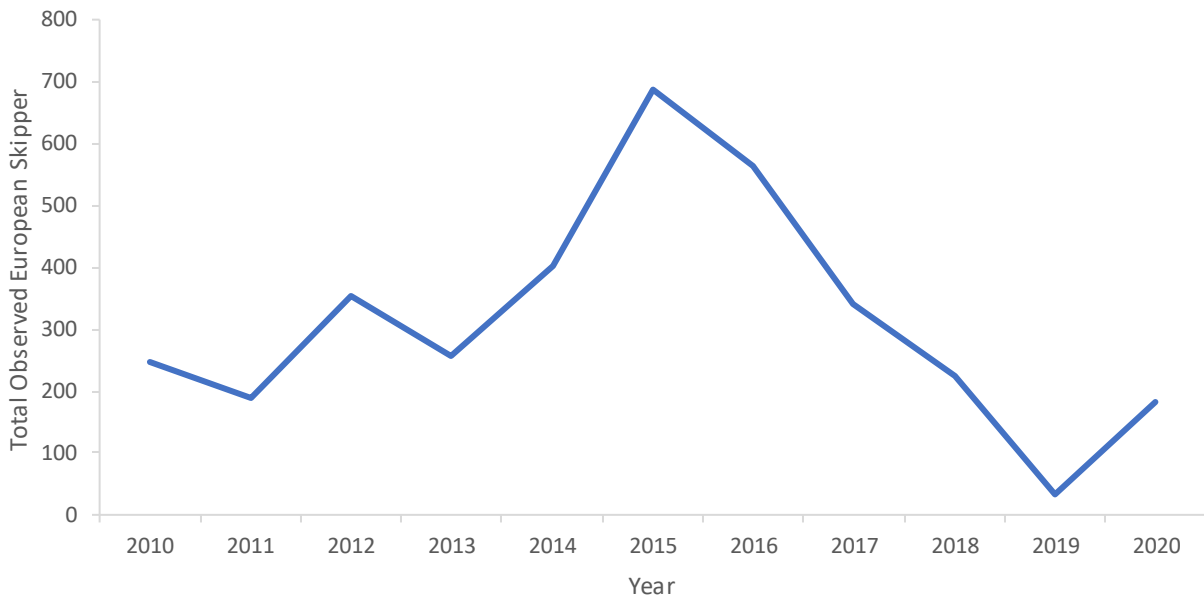


Figure 2.11: Number of observed European Skipper individuals recorded for each monitoring year between 2010 and 2020.

At 20 individuals observed, Tawny Emperor and Hackberry Emperor accounted for 0.4 percent of observations in 2020 during monitoring. Both numbers were the highest among all monitoring years, and a significant upwards trend since 2010 was observed ($P<0.05$, Fig 2.12). It is important to note that the sample size is very small with only 80 total observations over eleven years and this was the first year that a Hackberry Emperor has been observed during monitoring at **rare**.

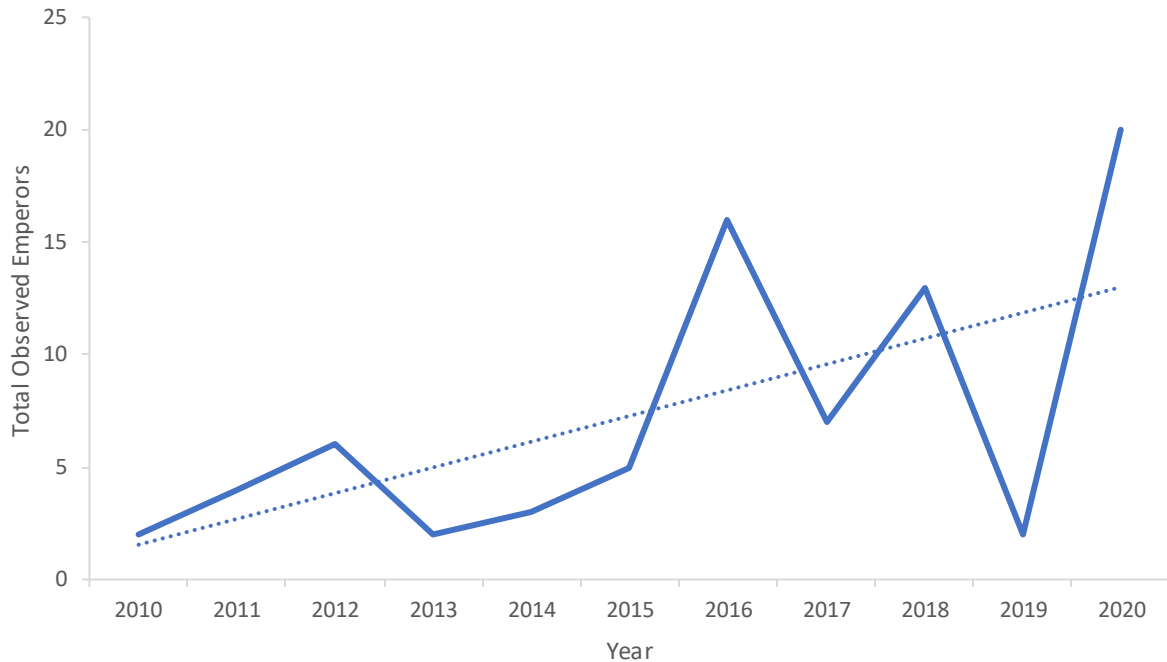


Figure 2.12: Number of observed Tawny Emperor and Hackberry Emperor individuals recorded for each monitoring year between 2010 and 2020. The trendline represent a line of best fit for Emperor data showing increased observations with time ($R^2=0.37$).

2.3.5 Comparison with Baseline Data

EMAN protocol suggests the first five years of monitoring data be used to create a baseline for monitoring programs in order to accurately identify trends and averages for populations (Table 2.7 & 2.8).

Using these data, we can compare subsequent monitoring years to the baseline averages for each transect to determine if monitoring seasons fall within or outside of these averages annually. Values that are outside of the given ranges may indicate environmental change that has had either positive or negative impacts on the populations. A wide range of values are considered acceptable, due to the large variation in observations between years.

The number of individuals observed during the last seven monitoring seasons rarely fell outside the baseline range, only occurring at Transect Two and Transect Four. When comparing species richness to the baseline conditions collected almost every monitoring year has been above the average range for Transect One. Likewise, Transect Three had multiple years above the average range for species richness. Transect Two and Transect Four rarely fell outside the average range from the last seven monitoring seasons.

Table 2.7: Baseline data of average butterfly abundance with standard deviations determined from monitoring seasons 2009-2013 compared to recent monitoring years. Numbers falling outside of baseline range are bolded.

	Number of Individuals								
Transect	Average	Standard Deviation	2020	2019	2018	2017	2016	2015	2014
Transect One	1,491	+/- 825	1,602	1,001	1,699	1,068	1,652	1,590	1,365
Transect Two	1,563	+/- 655	1,924	580	2,826	1,353	2,187	989	1,130
Transect Three	1,203	+/- 670	1,009	716	1,362	1,297	1,551	1,834	1,354
Transect Four	361	+/- 101	365	292	740	637	431	518	256

Table 2.8: Baseline data of species richness with standard deviations determined from monitoring seasons 2009-2013 compared to recent monitoring years. Numbers falling outside of baseline range are bolded.

	Species Richness								
Transect	Average	Standard Deviation	2020	2019	2018	2017	2016	2015	2014
Transect One	36	+/- 8	47	42	48	47	45	42	47
Transect Two	30	+/- 6	34	31	33	34	32	37	32
Transect Three	35	+/- 3	37	33	40	39	37	43	39
Transect Four	23	+/- 9	29	28	31	25	28	28	27

2.3.6 Weather Conditions

Although mean temperature (17.8°C) for 2020 was mid-range in comparison to the previous ten years, the season started cool in contrast to other monitoring years. The mean temperature this May (11°C) was the coldest of all years, but the weather quickly changed as June became the second warmest on record. Additionally, July's mean temperature was the warmest on record for all monitoring years (Table 2.9).

Rainfall was recorded on a total of 44 days during monitoring in 2020, which was the average from the combined monitoring seasons since 2010 (Table 2.10). It consistently rained 10-12 days each monitoring month, however this was only minimally disruptive, with rain interrupting monitoring only once in 2020.

2017 had the highest total number of days with recorded rainfall at 60 days and 2016, the lowest at 37 days. The highest recorded butterfly abundance over the last eleven years occurred in 2012 when there was below average precipitation during the start of monitoring, only seven days in May had rainfall.

The number of butterfly observations made during monitoring were significantly affected by the weather conditions. Abundance and richness both increased with higher temperatures

($p < 0.01$), likewise both measurements significantly increased as sun availability increased ($p < 0.01$). Furthermore, wind speed significantly affected butterfly presence ($p < 0.01$), with higher abundances and richness occurring at lower wind speeds.

Table 2.9: Average monthly temperatures for Kitchener/Waterloo for all months of all butterfly monitoring seasons at the **rare Charitable Research Reserve**. All data from 2010-2020 are from the Kitchener Waterloo Weather Station (Accessed from Environment and Climate Change Canada 2020).

Year	Average Daily Temperature of May	Average Daily Temperature of June	Average Daily Temperature of July	Average Daily Temperature of August	Total Average
2010	14	17.6	21.2	20.4	18.3
2011	13.5	17.1	22.3	19.7	18.15
2012	15.2	18.6	22	19.3	18.88
2013	13.7	17.4	20.4	18.3	17.45
2014	12.8	18.2	17.8	17.8	16.65
2015	15.2	16.6	19.2	18.6	17.4
2016	13	17.4	21.2	21.3	18.23
2017	11.2	17.9	19.8	17.8	16.68
2018	16	18	20.8	20.8	18.9
2019	11.3	16.7	21.3	19	17.08
2020	11	18.2	22.5	19.5	17.8
Average	13.4	17.55	20.77	19.3	17.77

Table 2.10: The monthly number of days with rain for Kitchener/Waterloo for all months of all butterfly monitoring seasons at the **rare Charitable Research Reserve**. All data from 2010-2020 are from the Kitchener Waterloo Weather Station (Accessed from Environment and Climate Change Canada 2020).

Year	Days with Precipitation in May	Days with Precipitation in June	Days with Precipitation in July	Days with Precipitation in August	Total Number of Days with Precipitation
2010	9	16	11	7	43
2011	17	10	7	13	47
2012	7	13	9	11	40
2013	13	14	13	10	50
2014	5	10	14	13	42
2015	9	17	2	10	38
2016	9	8	9	11	37
2017	14	15	14	17	60
2018	10	9	10	12	41
2019	15	10	11	10	46
2020	11	11	10	12	44
Average	10.8	12.1	10.2	11.5	44.4

2.3.7 Observations Per Week

Historical data indicates there are two periods during monitoring when abundance typically spikes each year, weeks 7-8 (beginning of July) and weeks 13-14 (mid to late August) (Figure 2.13). These spikes in flight times for adult butterflies are more prominent in years with high numbers of observed individuals such as 2012 and 2016. Butterfly abundance is significantly affected by week ($p < 0.05$), with weeks having a positive affected on abundance.

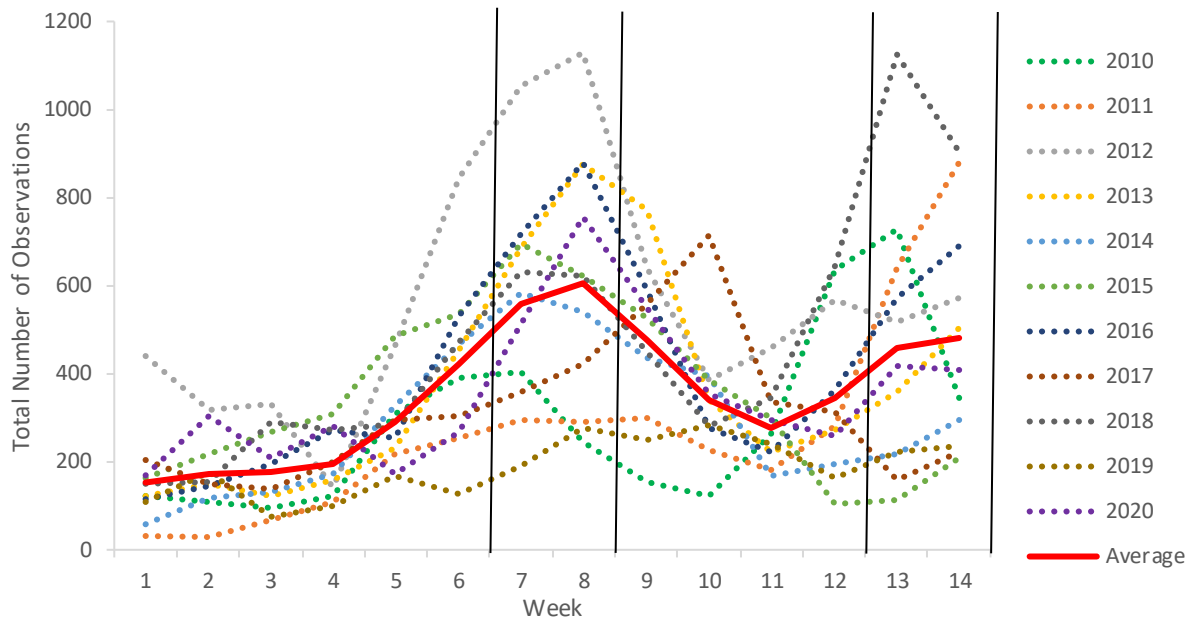


Figure 2.13: Number of total butterfly observations made during the fourteen monitoring weeks from 2010 to 2020. Peak observation windows are delineated with vertical lines.

When butterfly flight times are categorized by the number of generations (life cycles) a species has in one monitoring season, it is clear some species fluctuations coincide with the weekly variation (Figure 2.14). Butterfly species with one generation and those with a minimum of three generations have a spike in flight times that match with the largest combined spike during week 7-8. The less significant peak in weeks 13-14 also coincides with a spike in butterfly species with three or more generations.

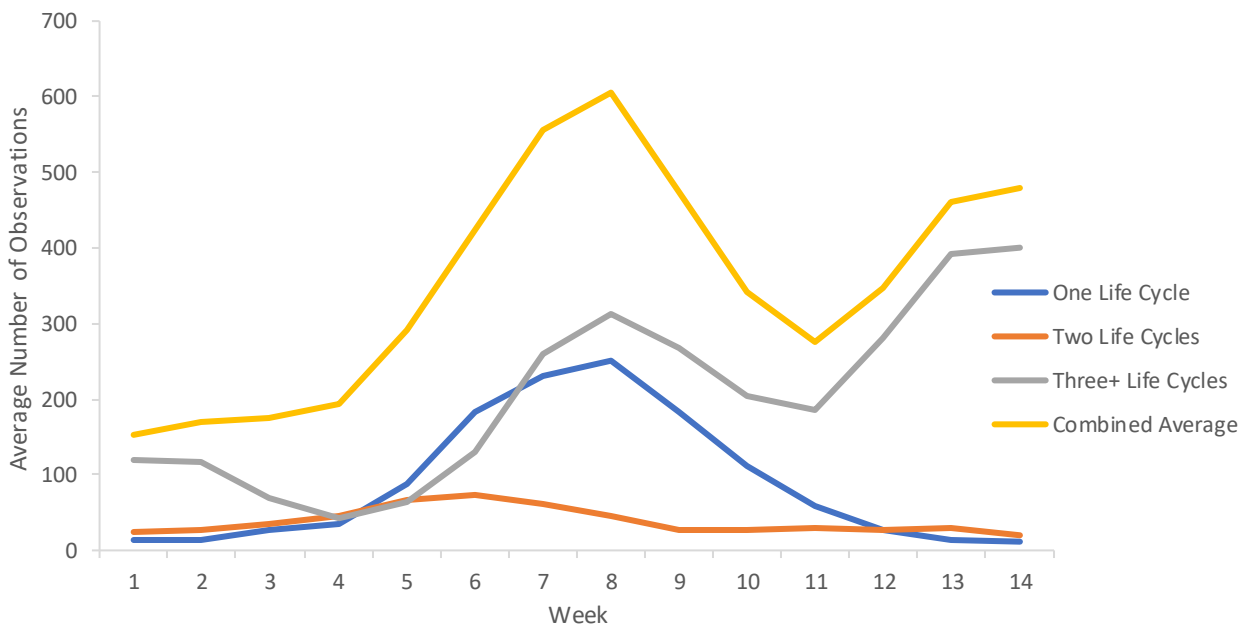


Figure 2.14: The average number of observations during the fourteen monitoring weeks from 2010 to 2020, categorized by the number of life cycles (generations) of butterflies per monitoring season.

2.3.8 Open vs Closed Habitats

When comparing results from open and closed habitats in 2020, butterfly abundance is significantly greater in open environments ($p < 0.01$, Fig 2.15). When comparing abundance from the last eleven monitoring years more variability exists in open compared to closed habitats, and open habitat fluctuation appears to coincide with global variation in abundance (e.g. 2012 and 2018).

Species richness significantly differs between open and closed habitats ($P < 0.01$, Fig 2.16), with higher species richness observed in open environments than closed in all monitoring years. The variation between monitoring years are similar in both habitat types (Figure 2.15). Fluctuation in species richness between years is not confined to one habitat type.

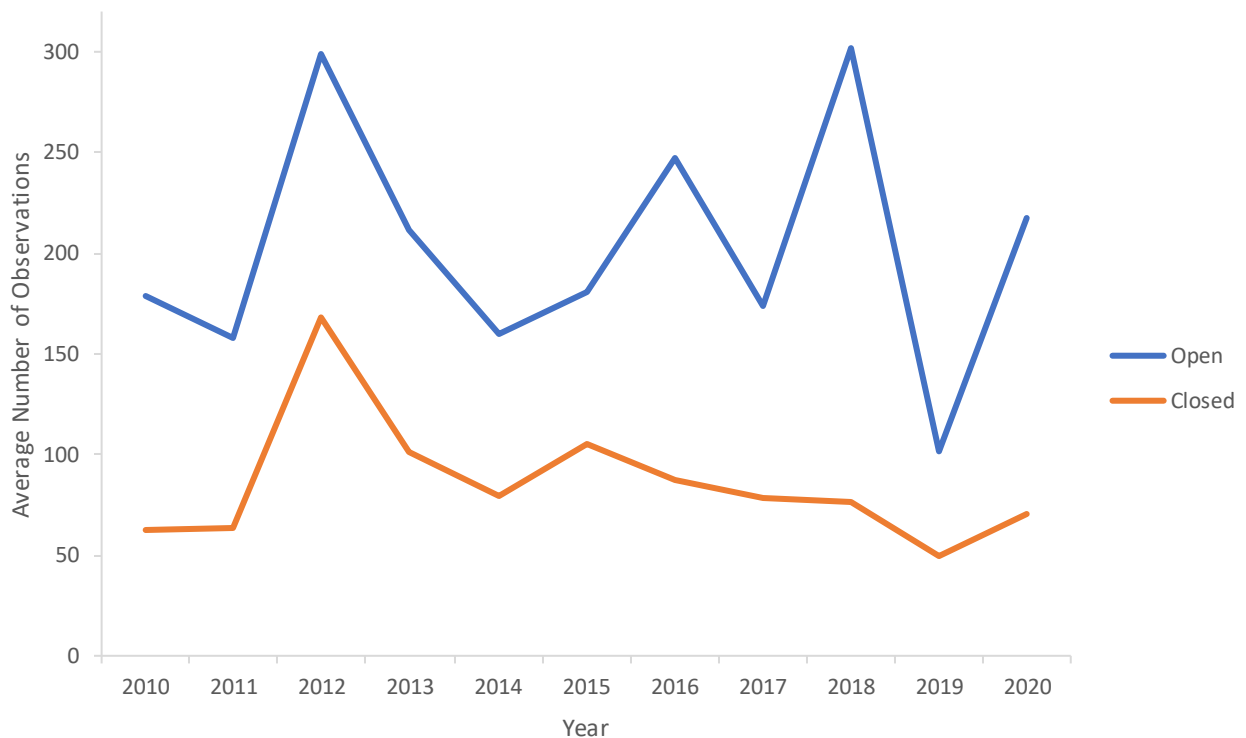


Figure 2.15: A comparison of the average number of butterfly observations made in open and closed environments along the four butterfly transects from 2010 to 2020.

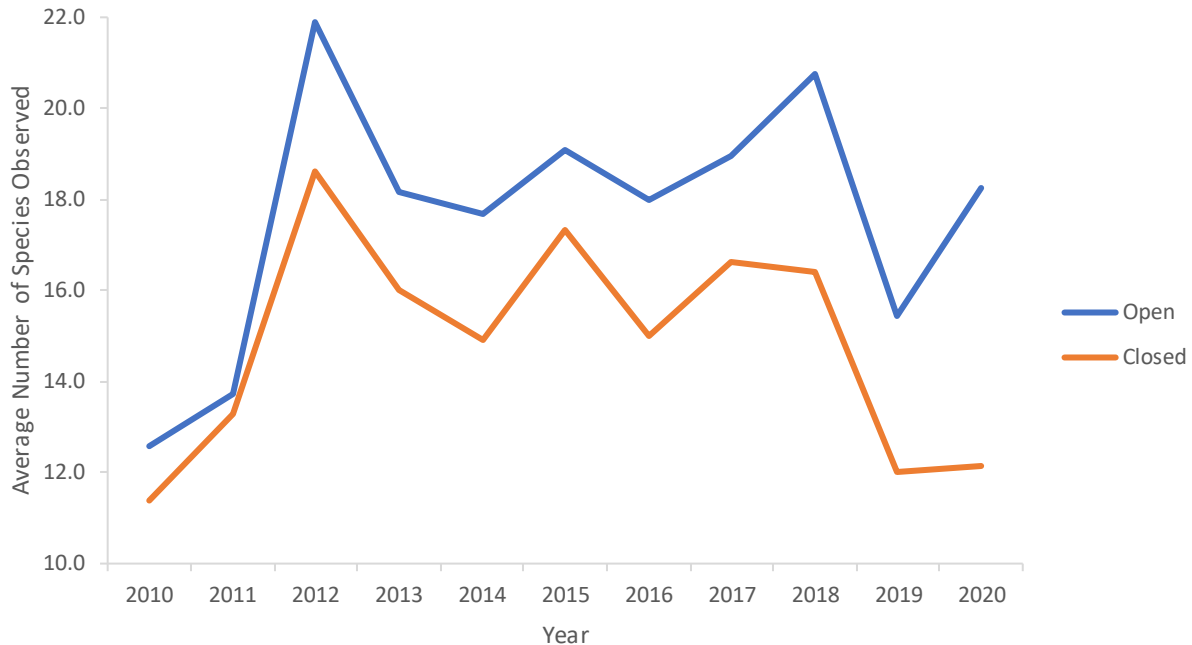


Figure 2.16: A comparison of the average for species richness observed in open and closed environments along the four butterfly transects from 2010 to 2020.

2.3.9 2020 Butterfly Count

The 15th Annual Butterfly Count was held at **rare Charitable Research Reserve** on July 26th, 2020. In total, 384 individuals were observed from 29 butterfly species. The 2020 Butterfly Count had four observers with total party hours of 10.5. Participation was limited to staff and key invited volunteers as the COVID-19 pandemic restricted public participation. Results have been submitted to the North American Butterfly Association and can also be seen below.

Observations: Black Swallowtail 9, E. Tiger Sw. 2, Cabbage White 153, Clouded Sulphur 46, Orange Su. 3, Banded Hairstreak 1, E. Tailed-Blue 1, Gr. Spangled Fritillary 16, Pearl Crescent 12, E. Comma 1, Gray Comma 2, Mourning Cloak 3, Red Admiral 2, Red-spotted Purple 5, Viceroy 1, Tawny Emperor 7, N. Pearly-eye 4, Little Wood-Satyr 12, Com. Wood-Nymph 53, Monarch 26, Silver-spotted Skipper 7, Wild Indigo Duskywing 3, European Sk. 1, N. Broken-Dash 1, Delaware Sk. 4, Hobomok Sk. 1, Black Da. 1, Dun Sk. 6. Unidentified: Azure sp. 1. **Total** 29 species, 384 individuals

2.4.0 Discussion

2.4.1 Overall Abundance and Diversity

The number of butterflies observed during monitoring has fluctuated over the last eleven years with variations between years as great as 5,000 individuals. Despite annual changes in abundances, there has been no upward or downward trend in global abundances of butterflies from 2010 to 2020. Factors such as population cycling and annual weather variations influence

annual fluctuations in abundance (Harrison et al. 2015) and likely contribute to the annual variations observed at **rare**. The absence of a significant trend over monitoring years to date may indicate that there has been no significant response to changes in appropriate habitat and climatic conditions. These changes can occur gradually and may not yet be detectable without continued long term monitoring (Parmesan and Yohe 2003; Thackeray et al. 2016).

Species richness in 2020 was above average with 52 species, but was the lowest species richness recorded since 2016. Twenty of the known species at **rare** that were not observed in 2020 are considered rare or uncommon in Waterloo Region (Linton 2012), and few individuals (often only one for each species) have been observed in previous monitoring years (e.g. Mulberry Wing, Columbine Duskywing, Compton's Tortoiseshell, Silvery Checkerspot, Harvester). These rare species may have simply eluded observation during monitoring this year. Species presence at **rare** can be cross checked with observations posted to eButterfly and observations at the Annual Butterfly Count to provide more insight on presence or absence. When counting incidental observations in addition to monitoring observations, 58 species were observed at **rare** in 2020. Due to the rarity of species and different observers each year, it is also likely that observation bias plays a role in the number of species observed each year. Although protocol and training aim to limit bias to the extent possible, monitoring protocols based on count data are subjected to a certain degree of observational bias (Dennis et al. 2006).

Species evenness and the Shannon diversity index were low in 2020 (Table 2.1). This drop is influenced by the top two most abundant species of 2020, Cabbage White and Clouded Sulphur which made up 56 percent of all butterflies observed (Figure 2.3). Since two species of the 52 observed dominated total observation, evenness of the population was low, and therefore the Shannon's index, which considers evenness when calculated, also decreased. This is not necessarily a cause of concern, as these two species have accounted for 25 to 65 percent of observations each year of monitoring (see section 2.4.3 for more on these species).

To identify if changes in the butterfly population at **rare** occurred within certain habitat types, the abundance and species richness were compared between two habitat categories: open (meadows, alvars, wetlands) and closed (forested areas). These varying environments can have differing sub-populations due to specific habitat requirements of some butterfly species, for instance Northern Pearly-Eye can be found in deciduous and mixed wood habitats whereas Bronze Copper butterflies are found in marsh edges and wet meadows (Carmichael and Vance 2003). Open habitats had significantly higher abundance and richness than closed habitats (Figure 2.15 & 2.16). Habitat requirement for butterflies is determined through availability of larval hostplant, conditions for caterpillar survival, nectar source for adult butterflies, and overwintering sites (Dennis 2010). Open habitats support these requirements for a larger population and wider variety of butterflies. Although there are more open habitats present along monitoring transects (18 sections vs 14 sections), meaning more time was spent observing at designated stops in open areas (approximately 50 minutes more per week), closed sections are often larger sections that cover more distance. Therefore, closed sections would require more time to complete and this minimizes the discrepancy in time spent monitoring these two habitats.

Throughout the 14-week monitoring period, spikes in abundance were observed at two different times during monitoring, early July and mid August (Figure 2.13). These spikes are more prominent in years where overall abundance were highest (e.g. 2012 and 2018). Species with one generation or a minimum of three generations appear to be driving these documented

fluctuations, whereas species with two generations are relatively consistent throughout the monitoring season (Figure 2.14). While changes in number of generations per year has been a documented response to climate change in some insects (Teder 2020), in locations where both single and multi generational species exist, the number of generations in butterfly species appear to be dictated not by the environmental constraints, but species-specific traits such as size, development time, host plant selection, and genetic variation for photoperiodic plasticity (Hasegawa et al. 2019; Teder 2020). Future studies should identify if global fluctuation in abundance coincides with the fluctuation of these two specific groups of butterflies to gain a better understanding of the butterfly populations and what factors are producing the changes seen between years.

2.4.2 Transect Comparison

Butterfly abundance within each transect has fluctuated over the last eleven years of monitoring, and this is particularly apparent at Transect Two. Transect Two has differed by more than 2,000 butterfly observations between monitoring seasons, and two species appear to be driving this variation. Cabbage White and Clouded Sulphur dominate the agricultural fields that possess many host plants of these two butterflies from the pea family (Fabaceae) or mustard family (Brassicaceae), including alfalfa (*Medicago sativa*), white clover (*Trifolium repens*), and wild radish (*Raphanus raphanistrum*) (Carmicheal and Vance 2003; BAMONA 2020b). Cabbage White and Clouded Sulphur observations were high in years with high overall abundance and low in years with low total abundance (Figure 2.6). When these species are excluded from comparison, the disparity between years is reduced. This suggests the significant variation in Transect Two between monitoring years can be explained by the variation in these two abundant species. As these species are habitat generalists with multiple host plants it is unlikely the difference in abundance between years is driven by host availability. Despite observed fluctuations, no transects have shown a directional trend over monitoring years, suggesting that abundances over time have remained relatively stable, or possibly longer-term data must be collected to see a significant change.

Abundance between transects in 2020 aligned with the length of each transect, with the greatest number of observations on the longest transect (Transect One) and the lowest number of observations on the shortest transect (Transect 4) (Table 2.6). Species richness between transects was similar, with the exception of Transect Three having more species than Transect Two (Table 2.4 & 2.5). Transect One and Three have consistently had the highest species richness, which is not surprising considering these two transects have the greatest diversity of habitat meeting the needs of a variety of butterfly species. Whereas Transect Two, and in particular Transect Four, have less diverse habitats and are able to support fewer species of butterflies and their host plants.

Similar to what was observed with the global abundance in 2020, Species evenness and the Shannon diversity index in Transect One were low compared to recent years. This may be due to higher counts of a few common species, as the top three species in 2020 accounted for 68 percent of all observations. This is similar with 2011 where the three most abundant species that year accounted for 73 percent. It is not understood why there would be a large spike in certain abundant species after eight years of monitoring in Transect One, but understanding if this

occurrence in numbers persists or increases in the future is important for better understanding of the local butterfly populations

In Transect Three, species evenness and Shannon's diversity index remained relatively constant across monitoring years, with 2020 holding the third highest record. This indicates some stability in butterfly populations over the last eleven years. The stability of butterfly populations may be influenced by the stability of the surrounding environment, as no major landscape changes have occurred on Transect Three throughout the monitoring program.

Near-average species evenness and Shannon's index in Transect Two and Transect Four in 2020 may also be due to higher counts of a few of common species. For example, the three most abundant species accounted for 76 percent and 59 percent, respectively, of observations in 2020. Compared to 51 percent and 44 percent in 2019 where higher species evenness and diversity were recorded. Furthermore, in Transect Two and Four more variability was observed in species evenness and Shannon's diversity index over the past eleven years of monitoring. This indicates less stability in the butterfly population likely due to the restoration work that has taken place in both transects.

Restoration has occurred in part or in whole along Transect Two and Four since the beginning of monitoring. Transect Four was retired from agricultural production and planted as a tallgrass prairie in 2010 and at least 20 hectares along Transect Two have been naturalized, with continued and gradual changes occurring in that area, including tree planting. Although it will take many years for these areas to convert into a forested environment or a healthy tallgrass prairie, it will be interesting to see how these changes to the habitat in Transect Two will impact the butterfly population.

A prescribed burn occurred at Transect Four in 2014 in an effort to further restore the field to a tallgrass prairie by simulating a wild fire. Controlled burns have been increasingly used as a restoration tool in prairie habitats as it can have a positive effect on the development, productivity, and reproduction of plants and indirect positive impacts on butterfly populations (Vogel et al. 2010). Although there have been multiple studies with prescribed burns that have found positive impacts on butterfly populations (Huntzinger 2003; Vogel et al. 2010), there has also been studies that have found no impact or negative effects on butterflies (Fleishman 2000; Vogel et al. 2007), suggesting the response to fire can be species-specific (Panzer 2002; Vogel et al. 2007; Vogel et al. 2010). With this understood, some research suggests the positive impact of prescribed burns are not exhibited on the butterfly population in the current year, but for approximately four years following these restoration efforts (Vogel et al. 2010). This was reflected in our data as four years after the prescribed burn in 2014 there was an increase in the butterfly population (Figure 2.8). A prescribed burn is scheduled in Blair Flats for spring 2021 and it will be important to understand how the numbers change in the subsequent years, and if it will suggest the same positive impact as the results following the 2014 controlled burn did.

2.4.3 Species of Special Interest

Cabbage White butterflies are invasive, habitat generalists that have spread rapidly throughout Canada (Layberry et al. 1998) and have consistently been one of the most abundant butterfly species observed at *rare* across all monitoring years. This butterfly's foodplants belong to the Mustard Family, including another non-native species, Garlic Mustard (*Alliaria petiolata*). This plant can be found throughout *rare* property, and both laboratory and field studies have

demonstrated successful reproduction of Cabbage White butterflies on this host (Davis and Cipollini 2016; Heinen et al 2016). The number of Cabbage White observed at **rare** reflects observations of the Waterloo Region, as data from the Butterfly Atlas shows Cabbage Whites in 2019 accounted for 16% of observations, similar to the 19% observed at **rare**. Data from the Butterfly Atlas for 2020 was unavailable for comparison at time of writing, but should be considered in future reporting.

The European Skipper is another invasive species that has been present in Canada since its original introduction near London, Ontario around 1910 for livestock feed (Layberry et al. 1998). Since that time, the European Skipper has been an abundant population throughout Ontario, aided by their use of many common invasive plants as hosts, such as Timothy Grass (*Phleum pratense*), Orchard Grass (*Dactylis glomerata*), and Quackgrass (*Elymus repens*) (Layberry et al. 1998). European Skipper butterflies (*Thymelicus lineola*) have been observed at **rare** as one of the top three abundant species in multiple monitoring years, but in 2020 European Skipper was only the fifth most abundant species. Although this invasive butterfly has not shown a significant decline in abundance over the last eleven years, there has been a decline since its spike in 2015. This may be a result of natural population cycling, nonetheless if the population continues to remain low or decrease in future monitoring years this could suggest this invasive species is becoming less abundant in the Waterloo Region. Understanding the trend in abundance of European Skippers at **rare** should continue in the future to better understand if this invasive species is truly declining and if other native species will therefore increase.

There are two types of Emperor butterflies found in the Waterloo Region; Tawny Emperor and Hackberry Emperor. These species are classified as vulnerable (S3) and are woodland species often found near their larval foodplant Hackberry trees (*Celtis spp.*) (AAFC 2014). Although some studies have found Tawny Emperor to be less common than Hackberry Emperor (Cech and Tudor 2005), this is not what was observed at **rare** or within the Waterloo Region (Macnaughton et al. 2020). These two closely related species can occupy the same habitat without competing as the larvae of the Tawny Emperor is reported to feed on mature hackberry leaves whereas the Hackberry Emperor larvae prefers new foliage (Cech and Tudor 2005). Adult Emperors can be found feeding from tree sap, rotting fruit, dung, and carrion (BAMONA 2020). Emperor butterflies face pressure from many generalist species of predators and multiple types of stink bugs which predate on Emperor eggs (Friedlander 1984). Additionally, there are parasites that attack Emperors including *Hyposter fugitivus* a larval parasite, and scelionid egg parasites (Friedlander 1984). These pressures have been documented in more southern areas of North America and the level at which these parasites and predators affect Emperors in Ontario is unknown.

The combined abundance of these Emperor species has significantly increased in last eleven years of monitoring (Figure 2.12). The highest combined recorded observations occurred in 2020 with 20 individuals. This increased abundance at **rare** coincides with observations in the Waterloo Region and all of Ontario. Tawny Emperor observations in Waterloo are documented on the Ontario Butterfly Atlas dating back to 1981, and the number of observations has increased in the last decade. A similar increase in Ontario has been documented for both Tawny Emperor and Hackberry Emperor butterflies over the last decade, with the highest number of sightings for both species recorded in 2020 (122 Tawny Emperor, and 147 Hackberry Emperors) (Agrin et al. 2020). Further close monitoring of these species should continue into the future to see if this upward trend continues.

Within Waterloo Region, Monarch butterflies are considered very common, although the observations at *rare* may not reflect this regional status. At *rare* there has not been a significant trend in Monarch abundance observed over the last eleven years, although large fluctuations have occurred from less than 20 individuals to peaks of over 400 Monarchs (Figure 2.9). Similar population trends are observed with the overwintering colonies in Mexico (Vidal and Rendon-Salinas 2014; Agrawal and Inamine 2018; WWF 2020). 2013 was the lowest year for Monarch's recorded at *rare*, this coincided with multiple reports of a sharp decline in the overwintering populations (Vidal and Rendon-Salinas 2014; Agrawal and Inamine 2018). The World Wildlife Fund reported the number of overwintering Monarchs in Mexico in 2013 was the lowest on record since 1993. These similar fluctuations between *rare's* data and the overwintering population in Mexico suggest our numbers are similar to documented results across North America.

Monarchs have declined approximately 84 percent since the mid-1990s (Crewe et al. 2019). In Canada, Monarch's are listed as Special Concern but are currently being reevaluated for the status of Endangered under the Species at Risk Act (Crewe et al. 2019; WWF 2020). There are multiple biotic and abiotic threats to this migrating butterfly including the reduction of milkweed. This is especially a concern in the American mid-west where Monarchs seek milkweed along their migration north each spring (Pleasants and Oberhauser 2012). Agricultural intensification, increases in genetically modified crops, and changes to land use in this area especially threatens milkweed and in turn, Monarchs (Vidal and Rendon-Salinas 2014; Vidal et al. 2014; Agrawal and Inamine 2018). At overwintering sites in Mexico, extreme weather is a major threat, as floods, strong winds, droughts, and fires can eliminate important Monarch habitat (Vidal and Rendon-Salinas 2014; Vidal et al. 2014). In 1992, approximately 83 percent of butterflies in the San Mateo Almomoloa colony perished due to extremely cold weather (Culotta, 1992). A well-known pressure for overwintering Monarch populations is the degradation of forests from illegal logging in Mexico. This illegal logging severely impacts the habitats of these colonies and can lead to the disappearance of wintering colonies altogether (Vidal et al. 2014; Crewe et al. 2019). However, logging bans and increased effort from the Mexican authorities to protect these overwintering sites have resulted in a decrease in illegal logging, over 730 hectares of land was affected in 2005-2007, and this dropped to approximately five hectares in 2014 (Vidal et al. 2014). Due to these enforced bans of logging in Mexico forests, many overwintering colonies have been protected in the last decade and will continue to be in the future. With the expected growing human global population and increasingly severe impacts of climate change a better understanding of these threats to the Monarch population across the continent is important for future research.

2.4.4 Noteworthy Species

Of the 52 species observed in 2020 during monitoring, 19 are considered very common, 11 common, 15 uncommon, and seven rare, according to the Waterloo Regional Status assignment (Linton, 2012). Certain species statuses were changed from Linton (2012) report due to observed changes in the local butterfly population, and these changes were confirmed by local experts.

The Eastern Pine Elfin was observed in 2020, after only two previous records during monitoring (2015 and 2018). This species was classified as rare in Waterloo Region and has only been observed 23 times since 1996 with majority of observations occurring after 2014 (Macnaughton et al. 2020). Unsurprisingly, the Eastern Pine Elfin caterpillar feeds on White Pine

(*Pinus strobus*), which is present on **rare** property (Linton 2012). These growing observations of the elfin, suggests this species may become more common in the Waterloo Region and at **rare** in the future. This species can be difficult to observe and identify due to their small size, neutral colouration, and the characteristic of remaining close to White Pine trees. Therefore, low detection ability could be contributing to low number of observations during monitoring.

Baltimore Checkerspot is a rare and very distinctive medium sized butterfly with four individuals observed this year, the most of any monitoring year at **rare**. This species has only been recorded in two other instances during monitoring (2012 and 2018). Overall, Baltimore Checkerspot has been observed in the Waterloo Region approximately 362 time since 1934 (Macnaughton et al. 2020).

The Dion Skipper is regionally classified as rare and was observed during monitoring for the first time in eight years. It has only been observed approximately 118 times since 1949 in the Waterloo Region (Macnaughton et al. 2020). Dion Skippers can be found in swamps, marshes, and bogs; and its caterpillar host plants are various sedge species (BAMONA 2020b).

The Silver-spotted Skipper butterfly is one of the largest and most recognizable skippers found throughout Southern Canada. This skipper is considered regionally uncommon, nonetheless it was observed in all four transects and the highest number of observations were recorded in 2020 with 38 individuals.

Silvery Blue butterflies are considered regionally rare, but have been observed each monitoring season since 2015 ranging from seven to 32 individuals. Silvery Blue butterflies have had a large recent range expansion, and this is likely due to the increasing range of non-native plants the butterfly will use as a host, such as Cow Vetch (*Vicia cracca*) (Layberry et al. 1998). This species status should be updated and considered as uncommon for the Waterloo Region. Silvery Blue butterflies can be mistaken for Spring Azures due to their similarities in size and colouration, therefore monitors should be trained to identify the differences between these species. Flight times can aid in identification as Silvery Blue butterflies have a limited flight season from May to early July (AAFC 2014b).

Juvenal's Duskywing are considered regionally rare but continue to be observed in high numbers at **rare** considering their status. However, observations decreased from their recorded high of 81 observations in 2017 to only 16 in 2020. Similarly, Wild Indigo Duskywing were considered regionally rare but have been observed with increasing frequency with the highest number of observations recorded in 2018 at 48 individuals. The Wild Indigo Duskywing should have a status change to very common based on local expert opinions as they have been observed in a minimum of 50 different locations in the Waterloo Region in the past decade (Macnaughton et al. 2020).

One butterfly species observed in 2020 for the first-time during monitoring at **rare** was the Acadian Hairstreak. This species had only been observed previously at **rare** during the 2013 Annual Butterfly Count. Due to the similarity in Hairstreak butterflies and given that butterflies are often identified quickly while they are perching, it is possible these butterflies have been misidentified during monitoring in the past.

2.4.5 Weather Impact

Butterflies are very sensitive to local weather conditions and patterns (Wikström et al. 2009). Generally, butterflies respond positively to warm and dry weather by increasing

development rates during egg and larval stages, and through reproductive success (Roy et al. 2001). Despite annual changes in abundances, there has been no upward or downward trend in global abundances of butterflies throughout monitoring. Weather variations can be an important factor that influences fluctuations in butterfly abundance (Wikstrom et al. 2009; Harrison et al. 2015; Kuussaari et al. 2016), and likely contribute to the annual variations in abundance that have been observed.

At **rare**, weather conditions (temperature, wind speed, and sun availability) during monitoring were compared to the number of butterflies observed and species richness. Temperature had a significant affect on butterfly activity with higher temperatures leading to increased activity and therefore observations in both butterfly measurements. Past studies have shown higher temperatures having a positive effect on butterflies and increasing their rate of activity (Cormont et al. 2011; WallisDeVries et al. 2011; Altermat 2012). Similarly, when there was a lower percent of cloud cover and more sunlight available, both butterfly abundance and richness significantly increased. Conditions of intermediate to high cloudiness have been shown to reduce the amount of flying in certain butterfly species (Cormont et al. 2011; Delattre et al. 2013).

Furthermore, wind speed had a significant effect on both abundance and richness at **rare**; as wind speed decreases both measurement increase. Studies have shown butterfly counts to be low when windspeeds are above three on the Beaufort scale (Dover et al. 1997) and that the duration of flight for butterflies is negatively affected by intermediate wind speed (Cormont et al. 2011). However, other studies have found wind speed to have no effect on butterfly abundance until extreme winds above five on the Beaufort scale are reached (Pivnick and McNeil 1987; Swengel and Swengel 2000; Wikstrom et al. 2009). Due to the monitoring protocol used at **rare** observations are rarely recorded when wind speeds are above four on the Beaufort scale, nonetheless our data shows lower wind speed can have a positive effect on butterfly abundance.

The absence of a global trend over monitoring years may indicate that there has been no significant response (either positive or negative) to changes in appropriate habitat and changing climatic conditions. However, it is also possible that abundance trends cannot be captured within such a short time frame, especially due to annual fluctuations and dependence of butterfly abundances on seasonal weather conditions. As climate change has been altering the abundance of species worldwide and is predicted to place more pressure on many important species in the future (Altermatt 2012; Martay et al. 2017) increasing our understanding of the relationship between weather and butterflies is extremely important. Areas worth investigating in the future to understand the potential affects of climate change in our region include investigating if seasonal appearance of butterflies are advancing in time due to warming as recorded in other areas of the world and with other insects (Walther et al. 2002; Musolin 2007); and have certain species ranges shifted due to climatic change affecting habitat suitability.

2.5.0 Conclusions and Recommendations

In 2020 nearly five thousand individuals from 52 butterfly species were observed. This included species with different regional status, habitat requirements, and species that have not been observed in multiple years or were observed for the first time in 2020. Despite annual fluctuations, no significant directional trend in butterfly abundances occurred over the last eleven monitoring years. This is a promising indication of stable abundances, however continued

monitoring is necessary to capture the gradual changes associated with large scale restoration and climatic change. Analysis of an abundance trend across years should be continued to determine whether abundances are experiencing a directional trend over time.

Abundances and indices of diversity have been heavily influenced by abundant species across monitoring years. To eliminate skewed abundances in analyses due to more prevalent species (e.g. Cabbage Whites and Clouded Sulphur), future analysis should consider independent analysis of each species (see Wepprich et al. 2019).

It cannot be concluded whether restoration efforts in Transects Two and Four have had positive effects on butterfly populations to date. Annual fluctuations in abundance and species richness make it difficult to determine a trend in the short term, and further years of data will be necessary to shed light on the efficacy of management efforts. Future monitoring should closely observe the effects of past restoration work at **rare** and understand how recent tree planting programs and future prescribed burns are affecting the butterfly population, and if these restoration initiatives are successful.

Across North America, Monarch butterflies travel and inhabit landscape dominated by humans. This exposes them to many threats from habitat degradation and fragmentation, to climate change and natural enemies. Therefore, comparison of **rare's** Monarch abundance to the overwintering population in Mexico should continue as a method to understand if fluctuations in **rare's** Monarch abundance coincides with the whole North America population.

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

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3.0 Plethodontid Salamander Monitoring

3.1.0 Introduction

3.1.1 Salamander Taxonomy

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

The woodland or lungless salamanders (Plethodontidae) are the most frequently observed salamander family at **rare**. Primarily observed are Eastern Red-backed salamanders, with occasional sightings of Four-toed salamanders. Plethodontids are the largest family of salamanders worldwide representing 27 genera and nearly 400 recognized species (Larson et al. 2006; Berkovitz and Shellis 2017). They are generally long and slender and are lungless, breathing through their thin, moist skin (Peterman and Semlitsch, 2013). This reliance on cutaneous respiration across moist body surfaces means plethodontid salamanders are sensitive to environmental changes in their micro-habitat (Zorn et al. 2004; Homyack and Kroll 2014). Gas exchange requires their skin to be moist (Welsh and Droege 2001) resulting in high absorption rates potentially exposing the salamander to contaminants in the soil or water.

3.1.2 Global Amphibian Decline

It is estimated that 41 percent of all known amphibian species are in decline (IUCN, 2020). In 2020 alone, 146 amphibian species were added to the International Union for Conservation of Nature's (IUCN) threatened list, totalling over 2,200 amphibians threatened worldwide (IUCN 2020). Most amphibians experience both aquatic and terrestrial stressors, and therefore are uniquely valuable as indicators of environmental stress. As such, there is significant concern over the noted amphibian declines world-wide; however, the causes of such declines are both variable and context dependent (Blaustein and Kiesecker 2002; Caruso and Lips 2012). Alford and Richards (1999) suggest the decline of amphibian populations is a global problem with complex local causes. Habitat destruction and alteration, infectious disease, contaminants, and invasive species are all examples of such causes that have likely contributed to this global decline (Hof et al. 2011; Bruhl et al. 2013; Arntzen et al. 2017; Carter et al. 2019). Multiple studies have found climate change as a contributing factor to this decline (Bombi and D'amen 2009; Griffiths et al. 2010). As salamander vulnerability to extreme temperatures and dependence on moisture causes these species to require specific ecological conditions, that if altered can have large consequences on salamander populations (Grover 2000; Peterman and Semlitsch 2013). Canada has shown an increase in average temperatures with more frequent and intense extremes, that are predicted to continue to increase in the future (Bush et al. 2019). Precipitation in the summer is also projected to decrease in Southern areas of Canada (Bush et al. 2019).

Eastern Red-backed salamanders will be particularly susceptible to these projected trends as they perform cutaneous respiration and require moist environments (Wells 2007). These environmental changes can affect physiological and morphological traits, and alter the survival

and fitness of these salamanders (Gardner et al. 2011). Warmer environmental temperatures limit salamander activity, such as mating and foraging due to the risk of desiccation (Petranka 1998; Sears 2005). Moist and cool environments required for survival may become limited in the future due to changes in the forest resulting in dryer litter layer and upper soil (Caruso et al. 2015). Although, there are examples of species worldwide adapting to climate change (Parmesan 2006), the ability of Eastern Red-backed salamanders to adapt to changing climate conditions is not well understood (Caruso et al. 2015).

3.1.3 Plethodontid Salamanders as Indicator Species

The Eastern Red-backed salamander is the most abundant plethodontid in Eastern Canada (Ontario Nature 2020) and at **rare**. They are completely terrestrial and therefore do not require ponds or vernal pools for development. All life stages of these salamanders occur in the leaf litter and in moist soil under downed woody debris in mature forests (Conant and Collins 1998; Robert et al. 2016). During times of dry or freezing conditions they move underground through the crevices in the soil for protection (Robert et al. 2016). There are two main colour phases of the Eastern Red-backed salamander: a red-backed morph that has dark grey sides and a rough-edged red stripe down the back, and a lead-backed morph that lacks the red stripe and is entirely grey. Both colour morphs can be found across southeastern Canada but proportion of these two phases can vary between areas (Moore and Ouellet 2014).

Woodland plethodontids are useful indicator species for forested ecosystems due to their life history traits, sensitivities to environmental changes and anthropogenic stresses, and relatively stable population demographics (Zorn et al. 2004; Welsh and Hodgson 2013; Homyack and Kroll 2014). Also, their high abundance, and the ease with which they can be sampled make them good indicator species for long-term monitoring (Welsh and Droege 2001).

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 (13m² for males and juveniles and 24m² for females) (Kleeberger and Werner 1982), and display site fidelity, with some species exhibiting occasional territorial behaviours (Maerz and Madison 2000; Peterson et al. 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 (Burton and Likens 1975). These high densities provide an ample food source for predators such as snakes, rodents, and birds. Therefore, their role in transferring energy up trophic levels is invaluable (Zorn et al. 2004; Walton 2013). As predators of invertebrate species that have substantial impact on decomposition and nutrient cycling on the forest floor, plethodontid salamanders help in managing these important ecosystem functions (Walton 2013).

Being lungless, plethodontid respiration is strongly affected by body moisture and the contact between their skin and contaminants (Droege et al. 1997). This sensitivity makes woodland plethodontids useful indicators of ecological stresses, as they are influenced by their micro-climate and water and air quality. Potential stresses include human activities,

(development, pollution, etc.) natural disturbances (storms, fires, etc.) or any event that may alter soil moisture, quality, litter requirements, or sun exposure (Zorn et al. 2004; Mathewson 2007).

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

3.1.4 EMAN Plethodontid Salamander Monitoring at *rare*

In 2004, the Ecological Monitoring and Assessment Network (EMAN) and Parks Canada published a joint National Monitoring Protocol for plethodontid salamanders. First and foremost, this protocol provides a standardized methodology for plethodontid monitoring across Canada with 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). 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 (Zorn et al. 2004).

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

Salamanders successfully began using the ACOs within weeks of establishment and continue to use them despite resultant disturbances from the monitoring process. Baseline population estimates have been established from initial years of monitoring which continued data collection 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 (Twery and Gottschalk 1996; Styers et al. 2010)

3.2.0 Methods

3.2.1 Monitoring Locations

Indian Woods (IW) is an old-growth Sugar Maple-American Beech dominated forest located on the western side of the *rare* property, south of Blair Road and north of Whistle Bare Road. The forest covers approximately 20 acres and contains trees 250+ years old. The Indian Woods salamander monitoring plot is located on the east side of an ephemeral pond near the south edge of the forest (Appendix A, Figure A.3). The plot is accessed by parking at the South Gate on Whistle Bare Road, and walking north along the Grand Allée trail until a second path merges from the west (left) side. This second trail is marked by a blue square sign with a white arrow. From the point of the trail junction, walk east (right) off-trail into the forest towards a large ephemeral pond (approximately 100m). The 32 ACOs are distributed in a large square made up of four lines of eight ACOs each. Boards five, six, and seven were missing prior to 2009.

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

3.2.2 Monitoring Protocol

Approximately three weeks prior to the start of monitoring, all ACOs in both Indian Woods and the Hogsback were visited to ensure proper positioning and clear labelling. If necessary, boards were repositioned 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 beginning of September to the end of October. At the beginning of each monitoring week, water was collected into a squeeze bottle from Lamb's Inn (a well water source) where no additional chemicals have been added. This water was used to calibrate the soil moisture meter (Lincoln Irrigation Corporation, Lincoln, Nebraska, USA) by adjusting the meter with a screw driver to read a

moisture rating of “10: saturated” when the probe was completely immersed in the water. The start time for the entire monitoring plot and Beaufort’s wind and sky codes were recorded on the data sheet at the start of monitoring (see Appendix C, Tables C.1 and C.2 and Figures C.2 and C.3). Presence or absence of precipitation in the 24 hours previous to monitoring was recorded rather than recording precipitation values. This change was made to account for the fact that the Kitchener-Waterloo data was not consistent with personal observations of precipitation on *rare* property (i.e. rain would be recorded for Kitchener-Waterloo which did not occur at *rare*). In Indian Woods, the depth of the ephemeral pond was recorded with a ruler when water was present.

Boards were always visited in sequential order starting with one. Soil temperature (°C) and moisture were collected at each ACO by inserting the probes of the soil thermometer (Ashcroft® Thermometers, USA) and soil moisture meter to a depth of 10cm, as marked with tape on the probes, in the soil beside the board. Canopy cover was also recorded at each ACO as complete (>90% cover), incomplete (10-90% cover), or no cover (<10% cover).

Each ACO was gently turned over and any salamander’s underneath were collected by the observers wearing nitrile gloves and placed into a plastic container with a sponge dampened with 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 (Smart Weigh Scale, model # A18-038) in grams. 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 as weather stations and each weather station represents a specific subset of ACOs (Table 3.1 and 3.2). At each weather station, weather variables including average wind speed (taken as the average after ten seconds), air temperature (°C) and percent relative humidity were collected using the Kestrel 3000 (Nielson-Kellerman, Boothwyn, PA, USA). Additionally, soil pH testing was collected using a Kelway soil tester at both Indian Woods and the Hogsback at each weather station on the last day of monitoring. A complete list of required equipment is available in Appendix B, List B.2.

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

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

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

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

3.2.3 Data Analysis

Data analysis was conducted using R, version 4.0.2 (R Development Core Team 2020). Prior to analysis, assumptions of parametric testing were examined. When transformation was required, the most appropriate transformations were performed, and assumptions were retested with each model. Each salamander monitoring plot (Indian Woods and the Hogsback) was interpreted as representing a unique population, and each ACO within that plot was interpreted as representing a sample of that population. Analyses excluded years 2006 and 2008 as these monitoring seasons had either missing ACOs or did not monitor for the full period as established in 2009.

Monitoring ran for nine successive weeks each year as recommended by EMAN protocols. Due to this monitoring protocol, temporal pseudoreplication is present in our ecological data as our weekly observations are not considered statistically independent. We acknowledge the potential for confounding effects and the statistical limitation this presents, and have cautiously interpreted results with this in mind.

To gain a better understanding how the monitoring week affects observations, we examined weekly changes independently. A univariate ANOVA split by plot was used to investigate weekly differences in Eastern Red-backed salamander abundance, with week as an independent variable. As abundance was the dependent variable, plots were considered separately to account for uneven sample numbers (ACOs) at each plot. This was followed by Tukey post-hoc testing to determine where the differences occurred.

Each monitoring plot had a differing number of ACOs and data had to be standardized to allow for comparisons of count data. Abundance was therefore transformed into catch per unit

effort (CPUE), 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. The CPUE calculation included only Eastern Red-backed salamanders due to low numbers of other species. A two-way ANOVA with plot and year as independent variables and CPUE as the dependent variable was performed to determine differences in CPUE between years and plots, and the interaction between year and plot variables. Where interactions occurred, data were split or combined appropriately for subsequent testing. This was followed by Tukey post-hoc testing to determine where the differences between the levels occurred.

Differences in species composition was examined within each plot to identify which species are more common at *rare*, and to determine changes in abundance within each salamander species. To better understand the occurrence of both colour phases of the Eastern Red-backed salamander, the ratio of lead-backed to red-backed was calculated. The colour phase ratio was determined for each monitoring year to identify directional change within the Eastern Red-backed salamander colour morphs.

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

To better understand fluctuation in salamanders within each life stage, a size class comparison was completed. Only Eastern Red-backed salamanders (both colour phases) were considered. Individuals were classified as adult, intermediate, or juvenile based on their snout-vent length as outlined in Zorn et al. (2004). Age classes were based on snout-vent lengths and 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. Where interactions occurred, data were split appropriately for subsequent testing. Tukey 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. Variables were considered for models based on their inherent relationship with salamanders (i.e. since salamanders live in the soil, soil factors were likely important). A correlation matrix was created to identify multicollinearity between parameters. In this analysis, multicollinearity is considered to be present when correlation is greater than 0.7 and less than -0.7. R^2 values of each correlated pair were compared, and the variable with the larger value was included in the model. Multiple linear regressions were used for each plot to determine which environmental factors (soil temperature, soil moisture, soil pH, pond depth, precipitation, sky and wind codes, wind speed, relative humidity, air temperature, canopy cover, and disturbance) affected total salamander abundance. Hierarchical multiple regressions followed with total abundance as the dependent variable and related parameters as the independent variables. How well each model predicted the dependent variable (the goodness of fit of each model) was tested using the Akaike's Information Criterion (AIC) model selection technique.

3.3.0 Results

3.3.1 Total Abundance

Between September 1st and October 28th, a total of 166 salamanders were observed at **rare Charitable Research Reserve** in 2020. This is the second lowest total observation (after 2016) of all monitoring years with equal sampling effort. Observations were split relatively evenly between sites, with 84 salamanders observed in Indian Woods and 82 observed in Hogsback. This is the second lowest abundance in Hogsback after 2016, and fourth lowest observation in Indian Woods. One species of salamander was observed in Hogsback (Eastern Red-backed) and three species were observed in Indian Woods (Eastern Red-backed, Yellow-spotted, and Blue-spotted).

3.3.2 Eastern Red-backed Salamander Abundance

The CPUE (catch per unit effort) was significantly affected by plot ($p < 0.001$), so Indian Woods and Hogsback were each considered independently of one another. In Indian Woods, CPUE significantly differed between only the highest (2009) and lowest (2020) years on record ($p < 0.001$). In Hogsback, significant differences occurred between calculated CPUE in 2016 and all other years excluding 2018 and 2020 ($p < 0.001$). CPUE in 2020 was significantly different from 2013 and 2014; CPUE in 2013 was significantly different from 2011 and 2018. In Hogsback, the highest recorded CPUE occurred in 2013 and the lowest CPUE was in 2016. Hogsback CPUE was higher compared to Indian Woods in all years except 2016 (Figure 3.1).

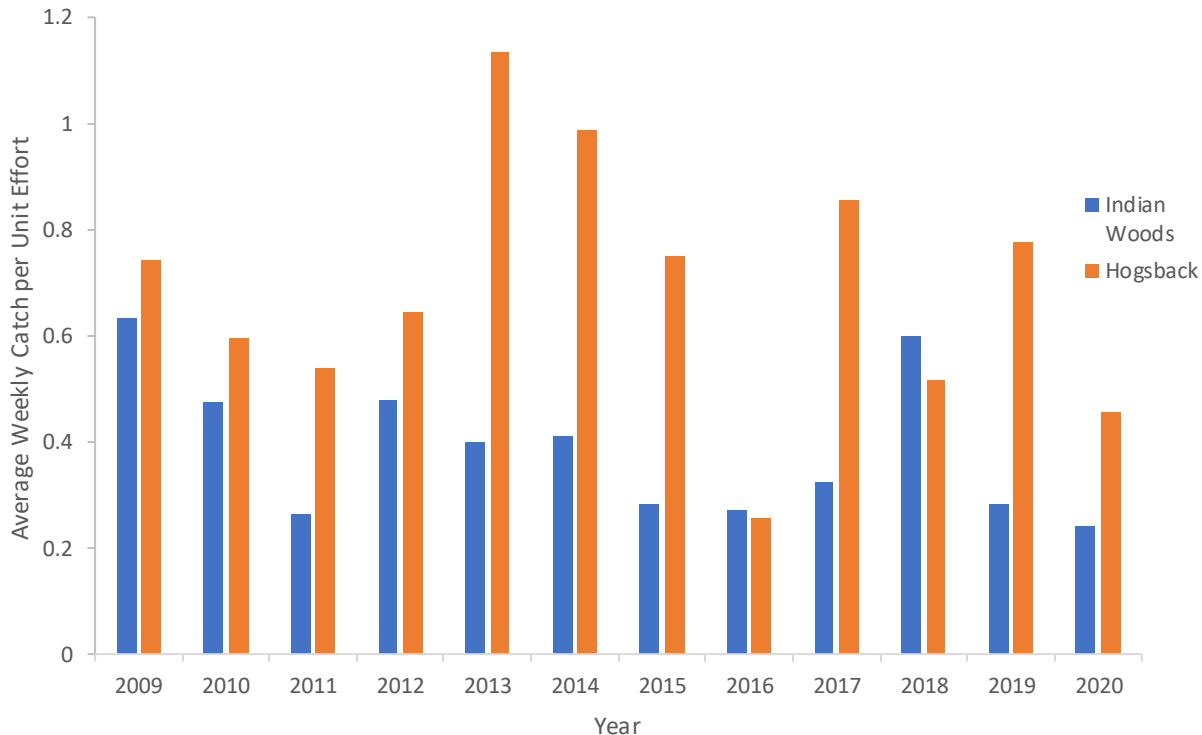
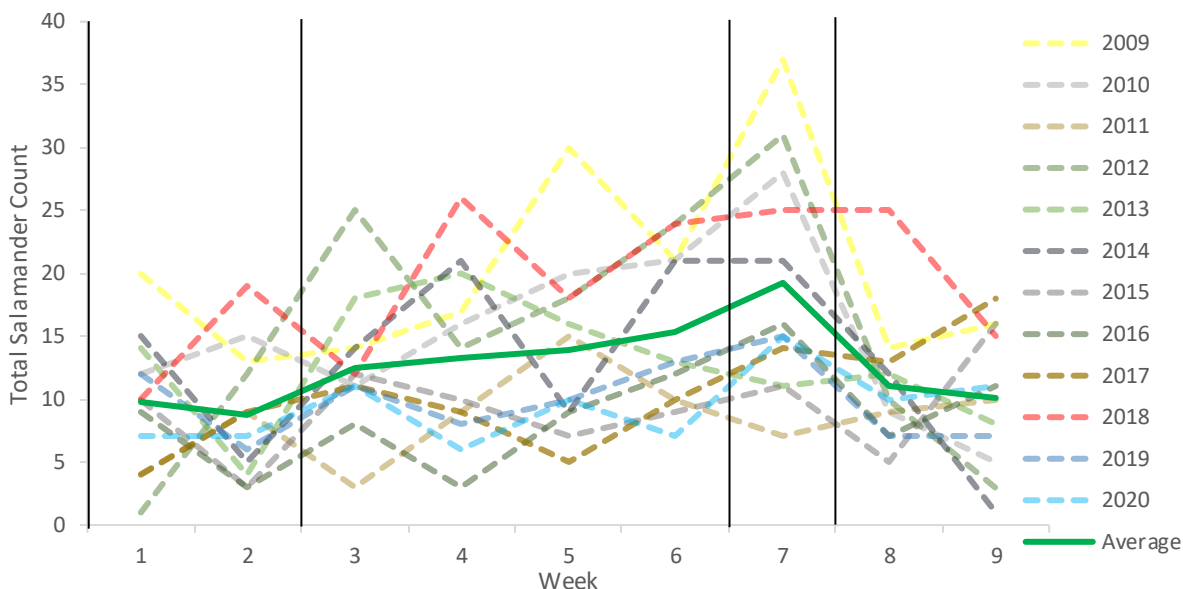


Figure 3.1: Average weekly salamander observation per artificial cover object (ACO) (Catch per Unit Effort) for both Indian Woods and Hogsback from 2009 to 2020.

Salamander abundance was examined across weeks and due to the number of ACOs differing in each plot, Indian Woods and Hogsback were examined independently. No significant difference occurred between weeks in Hogsback ($p>0.05$). However, a significant difference occurred between weeks in Indian Woods ($p<0.01$), with significantly higher abundances documented in week seven than week one and two. Figure 3.2a illustrates abundance in Indian Woods is typically lowest in week two and commonly peaks in week seven.

a.



b.

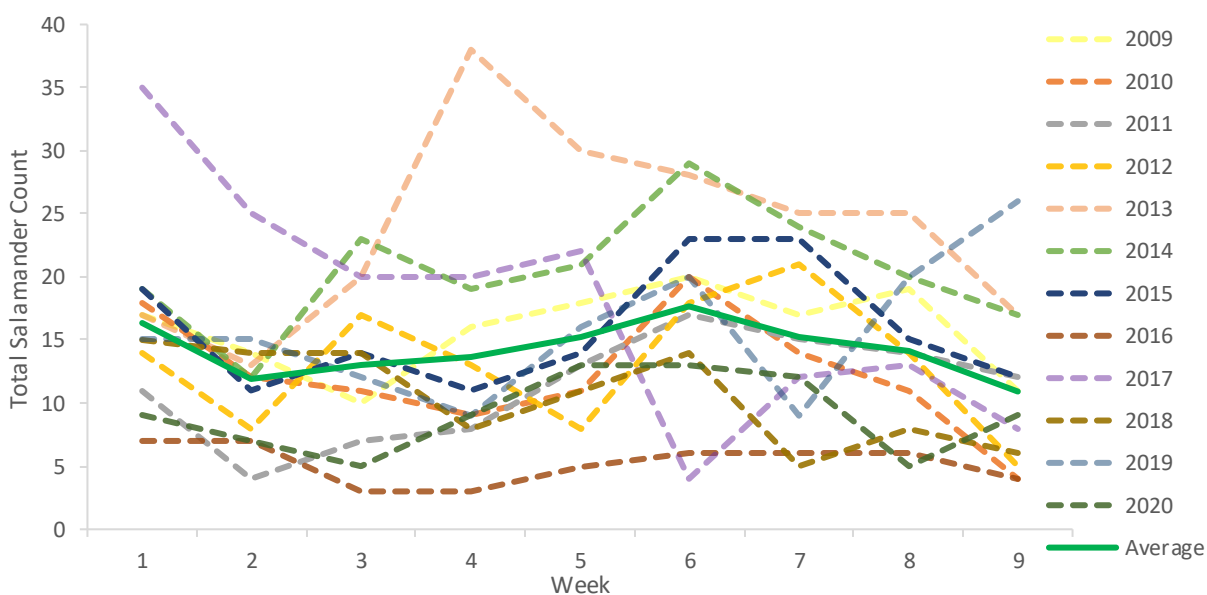


Figure 3.2: Total weekly salamander counts in Indian Woods (a.) and the Hogsback (b.) from 2009-2016. Data from 2006 and 2008 is excluded due to unequal sampling effort.

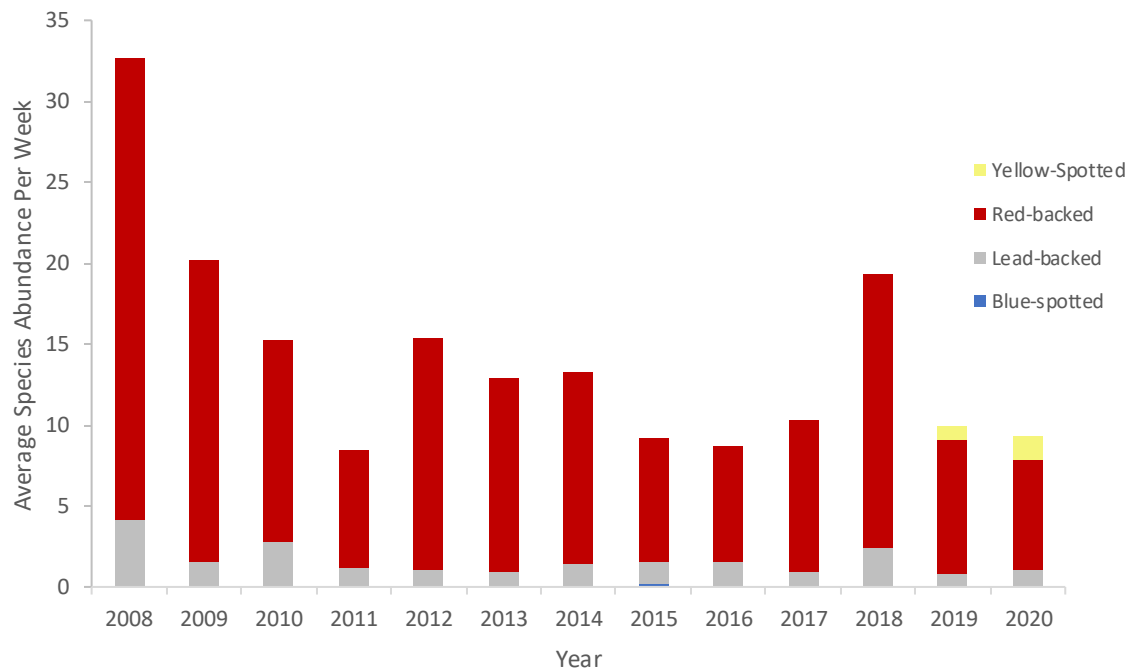
3.3.3 Salamander Species Composition

Eastern Red-backed salamanders have been the dominant species at both sites every year since monitoring began (Figure 3.3). Within Indian Woods, two other species have been observed in low quantities, Yellow-spotted and Blue-Spotted salamanders. Yellow-spotted salamanders had not been observed in Indian Woods until 2019, when seven observations were made, followed by thirteen observations in 2020. Blue-spotted Salamanders have been observed only six times since 2009 in this plot.

In Hogsback, sporadic observations of Yellow-spotted and Blue-spotted salamanders have been made since 2009, typically not more than four individuals a year. The Four-Toed salamander has only ever been observed in Hogsback at *rare*, and only fifteen individuals have been observed since the beginning of monitoring. In 2020, only Eastern Red-backed Salamanders were observed in Hogsback.

The ratio of lead-back to red-backed morphs for the Eastern Red-backed salamanders was examined across monitoring years within each monitoring site (Figure 3.4). The ratio between colour morphs in Indian Woods show no directional trend over time. In Hogsback the ratio observed over the last 12 monitoring years shows a slight decrease in lead-backed morph compared to red-backed.

a.



b.

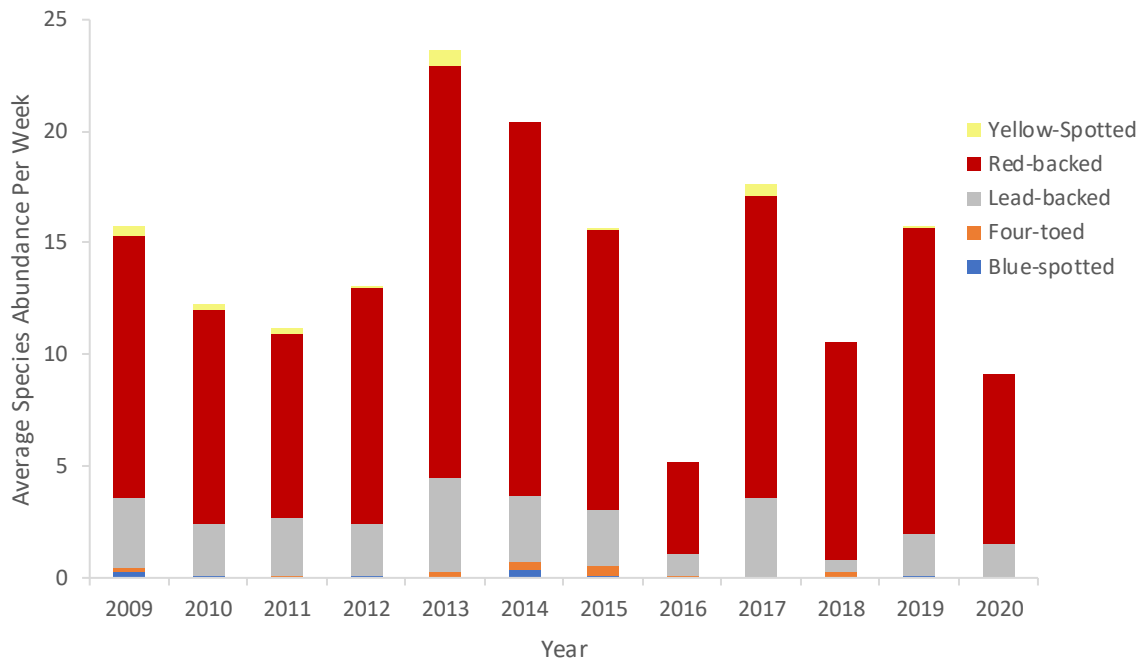


Figure 3.3: Mean salamander abundance by species for each monitoring year in Indian Woods (a.) and the Hogsback (b.). Red-backed and Lead-backed are two colour morphs of the same species, the Eastern Red-backed Salamander.



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

3.3.4 Eastern Red-backed Salamander Size Class Distribution

Size class did not interact with year or plot; therefore, Indian Woods and Hogsback were considered simultaneously in a univariate ANOVA with abundance as the dependent variable and size class as the independent. A significant difference did occur between class sizes ($p < 0.001$), with significantly more adult salamanders observed than intermediate and juveniles, and significantly more intermediate than juvenile salamanders observed. Differences in size class are represented in Figure 3.5.

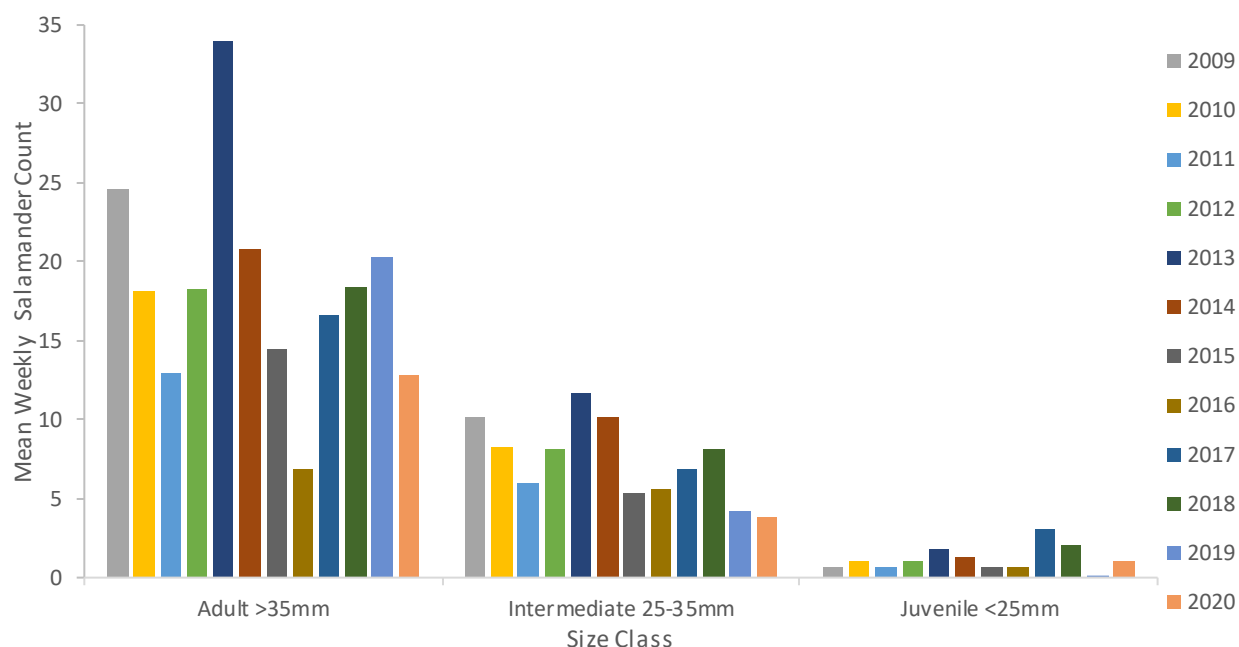


Figure 3.5: Mean size distribution of salamanders observed weekly during monitoring in Indian Woods and the Hogsback combined from 2009-2016.

3.3.5 Environmental Parameters

Correlation analysis between environmental parameters in Indian Woods identified a positive correlation between soil temperature and air temperature ($r^2 = 0.766$) and pH and disturbance ($r^2 = -0.713$). Soil temperature and disturbance were excluded from the model as they had lower R^2 values. The best model predicting salamander abundance in the Indian Woods included soil moisture, air temperature, sky code, and precipitation ($p < 0.001$). Air temperature and Beaufort sky code had significant, negative relationships with salamander abundance in Indian Woods ($p < 0.05$).

Correlation analysis between environmental parameters in the Hogsback identified a correlation between soil pH and disturbance ($r^2 = -0.732$). Soil pH was excluded from the model because it had a lower R^2 value than disturbance ($r^2 = 0.0338$ and 0.0423 respectively). The best model for predicting salamander abundance in Hogsback included soil moisture, wind code, sky code, and disturbance ($p < 0.001$). Soil moisture had a positive, significant relationship with salamander abundance ($p < 0.001$), while Beaufort sky code and disturbance had significant negative relationships with abundance ($p < 0.005$).

Average monthly temperature during monitoring was mid range in September and in October it was second lowest on record (Figure 3.6). Total precipitation was mid-range for September and October in 2020 (Figure 3.7).

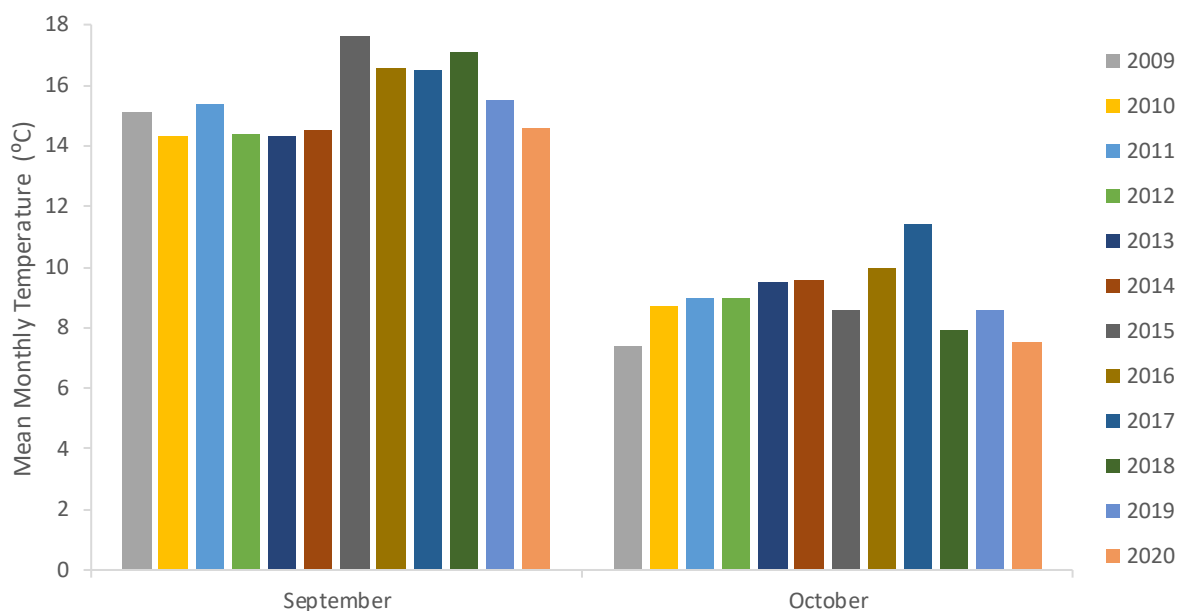


Figure 3.6: Mean monthly temperatures for Waterloo Region during the salamander monitoring season in 2009 from Environment Canada- data from Waterloo International Airport Weather Station, and 2010-2020 data from Kitchener-Waterloo Weather Station.

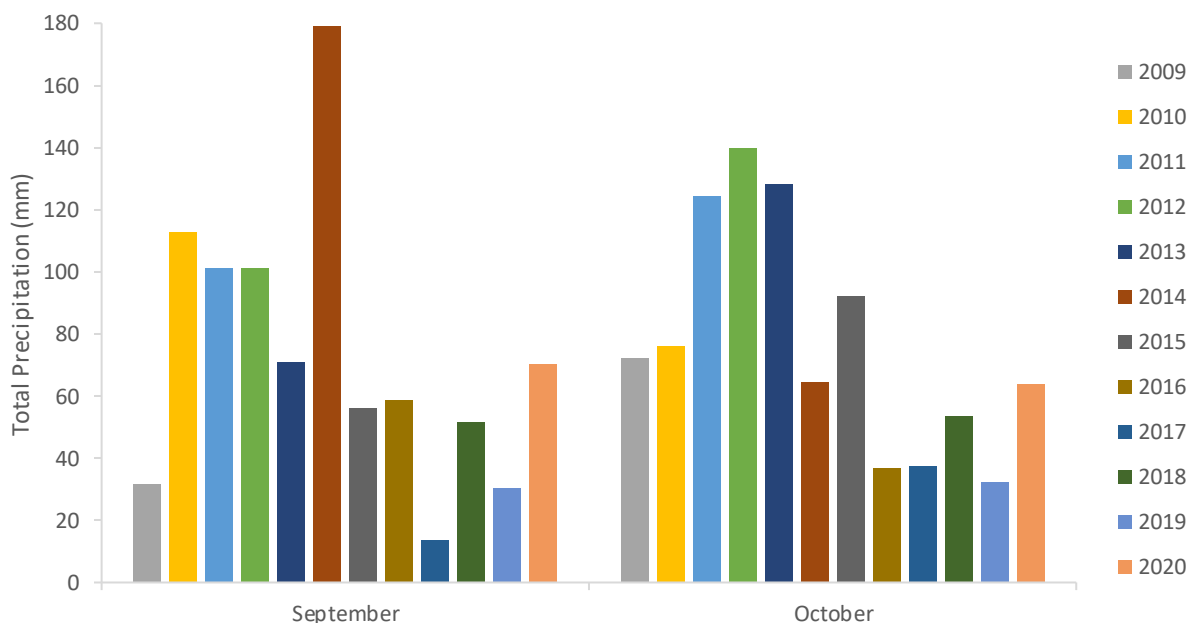


Figure 3.7: Total monthly rainfall for Waterloo Region during the salamander monitoring season in 2009 from Environment Canada- data from Waterloo International Airport Weather Station, and 2010-2016 data from Kitchener-Waterloo Weather Station.

3.4.0 Discussion

3.4.1. *Eastern Red-Backed Salamander Abundance*

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

The 2020 monitoring season is the second lowest year after 2016 for total salamander observations in both plots, and abundances were not within the threshold ranges for either plot. Since establishing the threshold, three monitoring years were below Hogsback threshold and five below Indian Woods. The number of years outside the monitoring threshold at each plot is concerning at *rare*. Similarly, when assessing populations by catch per unit effort, CPUE was the lowest of all monitoring years in Indian Woods, and the second lowest in Hogsback (Figure 3.1). There has been a slight decline in salamander abundance in Indian Woods for the majority of monitoring years since initial observations, and while there is no significant downward trend yet detected, it is certainly a concern that 2020 numbers were significantly lower than 2009 and the lowest recorded to date. While Hogsback shows more variation between monitoring years with no clear directional trend in observations. There are several possible environmental explanations for these low and concerning observations.

Due to salamander requirement for cool and moist habitats, temperature plays an important part in salamander fitness. High temperatures cause salamander skin to dry out more quickly and, as a consequence, limit their surface activity (Feder and Pough 1975). Salamanders have been found active with temperatures as low as 1°C and over 20°C, with thermal optimum for performance at approximately 15°C (Catenazzi 2016; Fontaine et al. 2018). Air temperature was found to have a significant, negative relationship with salamander abundance in Indian Woods, and was correlated with soil temperature. This is most apparent in 2016 as it had the lowest recorded abundance (125 salamanders) occurring with the highest mean air temperature (19.6°C). However, air temperature does not explain the low abundance observed in Indian Woods in 2020 as it had the second lowest mean air temperature at 14.5°C of all monitoring years. While air temperature is certainly a factor contributing to number of observations, it is not the only important consideration.

Beaufort sky codes are a measurement of the amount of sunlight, cloud cover, and rain, and are therefore representative of the temperature and precipitation on a given monitoring day (see Appendix C, Table C.2). Higher Beaufort sky codes indicate more precipitation and less sunlight. A significant negative relationship exists between Beaufort Sky codes and salamander abundance in both plots, likely due to salamanders not being limited to suitable refuge under ACO during damp and cooler days. The average sky code of 1.2 in 2020 for Indian Woods was below

the total average of 1.6 (meaning monitoring occurred on more clear, dry days than average) and likely was not a contributing factor. However, the average sky code of 2.4 in Hogsback for 2020 was the second highest observed during monitoring and is likely a contributing factor for lower observed abundance. Monitoring in each plot occurs at the same time of day on subsequent weekdays, so weather conditions during monitoring can vary by plot.

Low survivorship during the previous winter to monitoring seasons may be another explanation for the low number of observations in 2020. The dominant overwintering strategy of Eastern Red-backed salamanders is avoidance of sub-zero temperatures by retreating into the soil column (Storey and Storey 1986), and these salamanders have been observed as deep as one meter in the soil (Grizzell 1949; Hoff 1977). With little or no snow cover, harsh winters may be particularly difficult to survive as snow pack acts as an insulator against ambient air temperatures protecting animals beneath the snow (Aitchinson 2001). Therefore, little snowfall or high snowmelt from multiple days above freezing may expose salamanders to harsher temperatures or cause them to expend additional energy to retreat further below a frost line. There is currently not a lot known about winter activity level and how far salamanders are able to retreat underground

The amount of precipitation and the number of days with an average air temperature above zero were examined for winter 2019/2020 (Table 3.6). An above average amount of rainfall and days above freezing temperatures were documented in winter preceding monitoring, with an average amount of snowfall. It is possible these sporadic warmer temperatures disrupted the protective snow required for insulation this past winter. However, other monitoring season with similar overwintering conditions did not show a decrease in salamander abundance as seen in 2020. Therefore, overwintering conditions alone can not explain variation in abundance, but is likely a contributing factor. Furthermore, given that weather measurements are not taken in monitoring plots, rather gathered from nearby weather stations, it is difficult to draw site-specific conclusions. In-situ measurement of snow accumulation during the winter months would be useful for determining the effects of weather on salamander populations, and should be considered in the future as part of the monitoring program at *rare*. Site specific snow measurement equipment can range from a few hundred to thousands of dollars depending on the sophistication and accuracy of the equipment. Snowmetrics is an avalanche forecasting and snow research business offering snow depth probes (approximately \$100 each) that can be installed in desirable locations to read snow depth up to one metre (with extensions available). One probe can be installed at each monitoring location and potentially each weather station to increase accuracy. This technique for snow depth measurement is relatively inexpensive for equipment, however human resource cost from repeatedly visiting the sites each winter will accumulate. Alternatively, there are remote ultrasonic snow depth sensors that provide precise, reliable, and maintenance free measurements. This sophisticated equipment will record both continuous snow depth and temperature measurements onto a datalogger. The only potential expense related to human resources for this technique would be for the battery replacement that is required (every month or monitoring season depending on the specific ultrasonic sensor). However, the cost of this type of sensor depends on the accuracy, brand, frequency of readings, and battery life; and ranges within the thousands. The USH-9 snow depth sensor manufactured by the Hydrological Services America is one potential brand that could be utilized at *rare* in the future if resources allow.

Table 3.3: The total amount of precipitation and days above freezing from December to January from 2011-2020, from Environment Canada-data the Kitchener-Waterloo Weather Station. Precipitation on days above 0 °C was considered rain and below was considered snow, as data did not specify precipitation type.

Year	Days Above Freezing	Rainfall (mm)	Snowfall (mm)
2011	7	19.2	46.2
2012	27	102.6	46.3
2013	19	121.4	68.2
2014	11	45.7	58.4
2015	13	17.3	29.3
2016	35	55.7	64.8
2017	31	150.7	44
2018	19	69.5	56.2
2019	19	88.3	38
2020	23	94.72	50.88
Average	20.5	76.5	50.22

The vernal pool in Indian Woods was particularly dry this monitoring season as water was only present for two weeks, compared to years with high abundance (ex. 2009) where water was present each week of monitoring. Although precipitation levels were not significantly lower during monitoring compared to other years (Figure 3.7), other factors could be causing these observed differences in the pond water level. It is possible anthropogenic disturbances such as nearby aggregate mining operations have had a part in lowering the water table. Regardless, if the pond fails to fill with water in the future, this area may cease to be a productive breeding site for salamanders with aquatic juvenile phases. Although Red-backed salamanders live a completely terrestrial life not requiring this pool for breeding, a reduced water table may mean they must burrow deeper into the soil to find moisture in periods of drought, potentially reducing their visibility during salamander monitoring, and limiting the amount of time they can spend on the surface foraging before finding moisture refuge. Regardless of the cause, loss of moisture in the plots likely play a heavy role in observed salamander abundance considering the positive relationship soil moisture had with Eastern Red-backed salamander abundance in Hogsback. This is apparent in 2020, as the average moisture level (5.7) and salamander abundance (82) were both the lowest of the past four monitoring years.

Disturbance under and around each ACO is recorded each visit and these disturbances include ants, snakes, frogs, mold growth under ACOs, rodent activity, and displaced boards. There was a significant negative relationship between salamander abundance and disturbance in Hogsback. For example, in 2020 disturbance was the third highest on record (average of nine ACOs disturbed weekly) and abundance was relatively low (82). Contrary to this, 2019 monitoring season had high rates of disturbance (over fourteen ACOs affected weekly) as well as high salamander observations (142 individuals). Again, disturbance appears to be one of many potentially contributing factors impacting total observations.

One environmental factor that could be contributing to the observed changes in abundance is the availability of refuge for salamander. Downed woody debris provides habitat and refuge for multiple species, including salamanders, as it is a buffer against changes in

temperature and moisture (Garcia et al. 2020). More available woody debris near monitoring plots due to extreme weather events or tree death may cause a decrease in observed salamanders as there would be less reliance on ACO for survival. ACOs are used for sampling as they attract salamanders because they mimic natural cover and provided adequate microclimate and protection from predators (Zorn et al. 2004; MacNeil and Williams 2013). Recently, multiple large trees within the forest health monitoring plot adjacent to Indian Woods salamander ACOs have fallen, and this downed woody debris would increase available refuge and potentially lower then number of observations under monitoring ACOs. To better understand the relationship between downed woody debris and salamander observations at *rare*, monitoring should include recording substantial changes in downed woody debris within and adjacent to plots. It may be prudent to conduct a wide spread count of observed Eastern Red-backed Salamanders under nearby natural downed woody debris during week 7 of monitoring each year to understand if ACO results are representative of the natural population.

In all likelihood, there is no one direct cause of the abundances seen in any year and factors including temperature, moisture, available cover, and disturbances influencing the environment all have an impact (Heatwole 1962; Feder and Pough 1975; Jaeger 1979, 1980; Feder 1983; Feder and Londos 1984; Herbeck and Larsen 1999). Non-climatic factors may also contribute to the observed differences in abundance at *rare* and is described in detail in section 3.4.2.

Weekly patterns in abundance in 2020 generally mirror those of previous years, with Indian Woods abundances lowest in the second week and highest in the seventh week (Figure 3.2 (a)). Significant differences occurred between week seven and weeks one and two. Weekly patterns in the Hogsback are less clear, with the number of salamanders observed staying relatively consistent between weeks. Figure 3.2 (b) clearly shows that 2020 abundances in the Hogsback were consistently lower than the average from the past 12 years of monitoring.

The slight decreases observed in both plots in 2020 is a concern for salamander populations at *rare* if this pattern persists or worsen in the future. It is recommended that monitoring continue to help attribute cause and severity of changes to Eastern Red-backed salamander populations.

3.4.2 Potential Impacts on Abundance

3.4.2.1 Invasive Species

Exotic species invasions are a significant global problem that has the potential to threaten salamanders at *rare*. In Canada, there are no native earthworms, meaning the nineteen confirmed species in Ontario are all non-native. Of these confirmed species, seventeen originate from Europe/Asia and two from the United States (ISAP 2020). Invasive earthworms impact local salamander populations by altering the physical and chemical properties of the soil, exposing the soil through the consumption of leaf litter, and causing understory species decline (ISAP 2020). The more recent introduction of invasive Asian earthworms (*Amyntas* sp.) are causing increasing concerns for salamander populations. *Amyntas* earthworms began colonizing forests in northeastern U.S. as early as the 1900's and have reached Canada in more recent decades, particularly along the Ontario border (Gates 1958; Ziemba et al. 2015; Moore et al. 2018). Although there has been only one report confirming the presence of this earthworm in Ontario (Reynolds 2014), they are expected to spread further north due to climate and soil properties

being conducive of a larger expansion (Moore et al. 2018). As of now, limited research is available on the extent of these Asian earthworm invasion in Canada.

The presence and microhabitat changes from *Amyntas* has been documented to increase the rate of activity of Eastern Red-backed salamander, causing more movement between cover objects, the leaf litter, and soil (Ziemba et al. 2015). This displacement is likely due to irritation to salamanders' sensitive skin from the mucous-like secretion *Amyntas* produce (Gorsuch and Owen 2014). The increase in activity in lungless salamanders especially during sub-optimal thermal and moisture windows can have negative energetic consequences, and increase the rate of predation (Holmes and Robinson 1988; Ziemba et al. 2015). Furthermore, close proximity of *Amyntas* is believed to alter other behaviours of Eastern Red-back salamanders including territorial defense, mating, and egg brooding (Ziemba et al. 2015).

Overall, *Amyntas* earthworms have the potential to negatively affect salamander populations and due to the recent introduction of this invasive species there is no known control, and preventing the spread is the current best protection. Typically, invasive European earthworms can move up to 30 feet per year in the soil, whereas *Amyntas* have been documented traveling up to seventeen acres in one year (Laushman et al. 2018), causing increasing concern of the impact of these invasive earthworms. Although it is unclear the extent of the invasive Asian Earthworm in Ontario, this species has the potential to threaten salamanders at **rare** in the future, if not currently. If available expertise is found, **rare** should consider conducting comprehensive earthworm surveys in both forests.

3.4.2.2 Emerging Infectious Disease

There are many significant diseases that have negatively impacted global amphibian populations. Fortunately, there has been no evidence at **rare** of any disease to date affecting the salamander population. However, it is important to be aware and informed of significant or newly emerging diseases and their potential future impacts at **rare**. A common fungal pathogen is *Batrachochytrium dendrobatidis* (Bd) and is found in moist and aquatic environments (Densmore and Green, 2007). This disease has been associated with Eastern Red-backed salamanders and has caused population declines of amphibian species around the world (Skerratt et al. 2007; Becker and Harris 2010). Besides an increase in mortality, common symptoms to identify this disease during monitoring is a decrease in body mass, skin ulcers, and skin thickening (Becker and Harris 2010; UTIA 2020).

A more recent and concerning salamander fungus discovery is *Batrachochytrium salamandrivorans* (Bsal), another fatal skin disease (Smith et al. 2018; Waddle et al. 2020). This similar fungal pathogen is causing massive salamander death in Europe and could cause significant morbidity and mortality if introduced to the Canadian salamander population (Smith et al. 2018). The risk of introducing Bsal is high due to the large number of Asian salamanders traded between and Europe and North America (Yap et al. 2015). Therefore, a new Act addressing international trade of amphibian species affected by this fungus has put a stop of all trade as a method to prevent the spread into Canada (Yap et al. 2015). Common symptoms to identify Bsal include necrotic skin lesions, shedding of skin, decrease in body mass, and erythema (redness of skin) (UTIA 2020). As Bd and Bsal are both fungal skin diseases with similar symptoms, visual observations may not be adequate to distinguish between them, and collecting skin swabs for analysis of DNA through qPCR can identify the causation of observed symptoms (Blooi et al.

2013). Monitors at **rare** should be on high alert for symptoms or evidence of amphibian diseases at monitoring sites and across the property during future monitoring.

3.4.3 Salamander Species Composition

While the monitoring program at **rare** is primarily designed for plethodontid salamanders (Zorn et al. 2004), other species have been observed on the property. Since 2008 two mole salamander species were observed in Indian Woods. The Blue-spotted salamander was observed in low numbers in six of the past thirteen years, and the Yellow-spotted salamander was observed only in the last two monitoring years (with seven and thirteen observations, respectively). Mole salamanders are more easily found in spring during their breeding season (Skidds and Golet 2007), and therefore low numbers in the fall are likely not unusual. These salamander species have an aquatic stage for breeding and require nearby vernal pools or other suitable water source.

Vernal ponds (or ephemeral pools) are seasonal wetlands that do not have a permanent connection to a water source, they are typically filled from snowmelt and rainfall in the spring and dry out with time. Vernal ponds offer salamanders a safe and fishless breeding habitat and are important for maintaining salamander populations (Millikin et al. 2019). Multiple characteristics of vernal ponds influence species composition through impacts on egg development and hatching success (Skidds et al. 2007; Milliken et al. 2019). Larger pools with longer hydroperiods (time when water is present) have a positive affect on abundance (Snodgrass et al. 2000; Skidds et al. 2007). Water pH is important as low levels (<5.5) can cause a decrease in hatching success, larval development, and survival (Brodman 1993). Furthermore, the vegetation structure and tree canopy cover around the vernal pond positively affect abundance as they provide refuge for larvae and maintain water temperature (Kern et al. 2013; Scheffers et al. 2013). Therefore, the changes seen in mole species, specifically the Yellow-spotted salamander in recent years may be caused by changes in their aquatic habitat. Currently only pond depth is recorded during monitoring, however more characteristics should be documented in the future to better understand if the changes occurring correlate with shifts in species composition.

Historically species diversity has been higher in Hogsback than Indian Woods (Figure 3.3), as Four-toed salamanders, another member of the plethodontid family, have been observed in low numbers (one to four individuals) in seven monitoring years since 2009. These salamanders are typically found in sphagnum moss or boggy woodlands (Conant and Collins 1998), the later of which is found in the Hogsback forest stand. However, in 2020 only Eastern Red-backed salamanders were observed in Hogsback, and dry conditions may have contributed to this low diversity as breeding grounds were dry compared to previous years. Similar to Indian Woods, data collection in the spring would be beneficial to gain more accurate population data, particularly for mole salamanders which breed in the spring.

Eastern Red-backed salamanders have been dominant in both plots across monitoring years (Figure 3.3), with the proportion of red-backed phase individuals being consistently higher than the proportion of lead-backed individuals (Figure 3.4). This is unsurprising, as the lead-backed phase salamanders are known to experience preferential predation pressure (Kutcha 2005). Studies have found lead-backed salamanders have a higher percentage of autotomized tails (Moreno 1989; Venesky and Anthony 2007), possibly indicating a higher attack rate on the darker colour morph. Additionally, documented differences in these colour morphs response to predators can contribute to the disproportion seen at **rare**. Lead-backed individuals show higher

levels of mobility when exposed to predators, whereas red-backed individuals show immobility behaviour, likely due to its red colouring acting as a warning signal to predators (Venesky and Anthony 2007). Red-backed morphs have also been found more successful at camouflaging in majority of backgrounds than lead-backed morphs (Hantak and Kuchta 2018). Therefore, the vulnerability of lead-backed morphs to predators could contribute to the disproportionate ratio observed at each plot.

Studies of spatial variation suggest lead-backed morphs are more closely associated with warmer and drier climates than the red-backed morphs (Gibbs and Karraker 2006; Anthony et al. 2008). Evidence of temperature related selection colour morphs in Eastern Red-backed individuals include frequency of lead-backed phase mortality increasing in colder areas, lead-backed morphs retreating into the soil earlier in the fall, and lead-backed morphs having lower standard metabolic rates allowing for increased mobility in warmer environments (Lotter and Scott 1977; Moreno 1989). Lead-backed morphs have been observed in lower percentages after week six of monitoring at *rare* and this could be attributed to retreating earlier into the soil. Due to temperature preference between colour morphs it is believed increased global temperatures will result in a shift from red-backed to lead-backed dominance in temperate areas (Gibbs and Karraker 2006), and the ratio of colour morphs should continue to be examined for this shift in Eastern Red-backed salamanders in the future. However, there are doubts in the scientific community about the influence of climate on colour morphs and their validity as an indicator for climate change (Moore and Ouellet 2014).

3.4.4 Size Class Distribution

Consistent with previous monitoring years, the greatest proportion of Eastern Red-backed salamanders in 2020 fell within the snout-vent length range of 35mm-45mm. Based on size class categories outlined in Zorn et al. (2004), significantly more adults were found in both plots than intermediates and juveniles, and further there are significantly more intermediates observed than juveniles (Figure 3.5). However, within each size class there has been no significant change in abundance across monitoring years despite the fluctuation observed. The proportion of salamanders in each size class appears to remain relatively consistent, despite changes in the total number of salamanders observed each year. For example, 2013 had a large number of total salamanders and this increase can be seen across all size class. Similarly, when there was a decrease in total observed salamanders in 2011, this was reflected in each category (Figure 3.5). Therefore, the factors driving these fluctuations in abundance is not restricted to a size class and factors impacting number of salamanders are more or less equally impacting each life stage.

It should be noted that juvenile populations may be underrepresented by ACO sampling. Larger and dominant salamanders may be exhibiting territorial behaviours that outcompete juveniles for space (Marsh and Goicochea 2003), and over time larger salamanders will displace smaller individuals and skew results. Low observations of juveniles in tandem with intermediates or adults at *rare* indicates that territorial behaviour may be occurring. Although Red-backed salamanders have also been shown to exhibit kin selection, allowing related juveniles into their territories in stressful conditions (Horne and Jaeger 1988; Jaeger et al. 1995; Simons et al. 1997) this seems to be occurring minimally during fall at *rare* as the occurrence of multiple individuals under one ACO is limited.

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

Size class distribution should continue to be monitored closely at **rare** to understand the local impact of climate change, as changes in climate are reported to affect amphibian body size (Gardener et al. 2011; Secord et al. 2012). As Eastern Red-backed salamanders are particularly sensitive to warm and dry environments, when temperatures increase as projected this will limit activity such as foraging, and reduce body size (Sears 2005; Caruso et al. 2015). An increase in abundance of smaller salamanders may be a precursor to overall abundance changes, as body size is an integrative trait linked to fitness and survivorship (Blankenhorn 2000; Reading 2007).

3.5.0 Conclusions and Recommendations

After twelve years of consistent and consecutive monitoring, this program has established baseline data of expected salamander populations in both Indian Woods and Hogsback and will continue to compare future years to these baselines. Observed salamander abundances in both plots have fallen below threshold levels in multiple non-consecutive years, and species composition was low relative to previous years. Although, they have not been consecutive, five of the past seven years of monitoring in Indian Woods fell below thresholds and should be monitored closely in future years. Soil moisture, air temperature, disturbance, and Beaufort sky code significantly impact salamander abundance and may have contributed to low observations in 2020, however the cause of declines observed in **rare** forests are unknown. As this program acts as a warning sign for environmental change, falling numbers coupled with ongoing human pressures from agriculture, development projects, and the potential for cumulative effects from aggregate extraction highlight the need for continued monitoring at **rare**. Only by continuing long-term monitoring, can **rare** best assess the impact of land management decisions both on and adjacent to the property.

Including a spring monitoring season in addition to fall monitoring would be helpful to capture abundances of mole salamanders. However, should resources only allow monitoring in one season it is recommended to prioritize the continuation of fall monitoring. Additionally, collecting more thorough data on environmental conditions from the vernal pond in Indian Woods, in-situ snow accumulation measurements, and of the quantity of surrounding downed woody debris may provide more insight on changes to species composition in the future. Therefore, it is recommended that a full nine week fall monitoring program continue at both forest sites.

3.6.0 Literature Cited

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4.0 Forest Canopy and Tree Biodiversity Monitoring

4.1 Introduction

4.1.1 Forest Health Monitoring

Forests are critical to environmental health and stability (Environment Canada and Canadian Forest Service 2004). Covering over 30 percent of all the land on our planet, forests house a significant amount of the world's biodiversity and contribute to the livelihoods of over a fifth of the global population (FAO and UNEP 2020). Forests have an important role in supporting life on earth, from providing food, shelter, and energy to being a safeguard for communities via soil conservation, water cycling, and air quality mediation (Nowak et al. 2013; Larson 2014; FAO and UNEP 2020). Established global policies and recommendations related to the safeguarding of terrestrial environments is an important and necessary step to protect these valuable habitats. Global agreements to restore and promote sustainable ecosystems include conserving and enhancing greenhouse gas sinks and reservoirs, reaching long term temperature goals, controlling and eradicating priority invasive species, integrating ecosystem and biodiversity values into future development processes, and taking action to prevent the extinction of threatened species are critical to promote a healthy environment (UN 2015; UNFCCC 2016).

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; Suffling et al. 2003; Butt et al. 2012). Due to rapid development and land use changes, forest species have been removed and land cover has been significantly altered. What remains are highly fragmented forests which are much smaller in size than they were historically (Waldron 2003). Forests are also under pressure from many other biotic and abiotic factors. Widespread invasive species have caused drastic changes to forest stand composition and forest nutrient cycles, threatening to alter the ecology of forest systems profoundly (Jones and McDermott 2017; Ichii et al. 2019). Impacts to forests from climate change are thought to be equally far-reaching and may exacerbate the spread of invasive species (Thorne et al. 2018; Shin et al. 2019). Natural disturbances to forests from insects and disease will become more severe with warmer climates (Weed et al. 2013). Forests will also have to adapt to more instances of extreme weather such as heat stress, flooding, and drought (Howard 2019). These factors demonstrate the number of pressures impacting our forests and highlight the need to monitor the health of our remaining forest stands.

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

4.1.2 EMAN Forest Monitoring at *rare*

With the rapid development of southern Ontario, there are very few undisturbed remnant old-growth forests remaining (Ontario Ministry Natural Resources 1999). At the ***rare* Charitable Research Reserve**, one such remnant old growth exists: a Sugar Maple-American Beech (*Acer saccharum* – *Fagus grandifolia*) dominated forest named Indian Woods, which has trees more than 250 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 facing current developmental pressure along its boundaries. All of these forest ecosystems contribute immensely to the region by sequestering carbon dioxide and improving air and water quality (Butt 2011), as well as providing increasingly uncommon habitat to numerous plants and animals that require mature forest interior to thrive (Ontario Ministry of Natural Resources 1999).

Forests face diverse challenges in the landscape of Waterloo Region. The ***rare*** reserve is bordered by conventional farm fields, aggregate mining operations, subdivisions, and busy roads. Neighbouring lands to the southeast are currently facing drastic change as a new housing development is built. By acquiring baseline records of the conditions of the ***rare*** forests and continuing long term monitoring, changes in the forest stands may be detected early, allowing for the development and implementation of an effective management plan to protect ***rare*** forest ecosystems.

The research questions being addressed with long term forest canopy tree biodiversity monitoring were identified at the establishment of the program (McCarter 2009):

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)?

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 within the plot, and diameter at breast height (DBH) were collected in this first year. In 2010, three plots were established in the Hogsback forest so that all three major forested areas on the ***rare*** property would be represented in the monitoring program. An Ecological Monitoring and Assessment Network (EMAN) Tree Health Protocol was added to the monitoring program in 2010, and all nine forest plots were monitored annually up to and including 2015, when it was decided to conduct monitoring every five years moving forward. This altered protocol is still consistent with the EMAN protocol as the minimum monitoring protocol is every five years, and multiple organizations follow this protocol such as the Credit Valley Conservation, Toronto and Region Conservation Authority, and Conservation Halton. A shrub and small tree monitoring program was

piloted in 2013-2014 but was ultimately replaced with the widespread Vegetation Sampling Protocol implemented across the property.

4.2 Methods

4.2.1 Forest Plot Locations

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

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

Indian Woods: A remnant old-growth forest, rare for southwestern Ontario (OMNR 1999), is located south of Blair Road and north of Whistle Bare Road, on the west side of the property. The three forest plots in Indian Woods are oriented in a north-south line in the centre of the forest, approximately 100m east of the Grand Allée. The third plot can be accessed by turning east into the forest off the Grand Allée towards the salamander monitoring plot and continuing to the top of the hill overlooking the pond. The first and second plots can be found by heading north from the third plot (Appendix A). The plots are approximately 30m apart and the flagging tape on the corners of each plot should be visible from the adjacent plot.

Hogsback: Located at the south-west corner of the property, the Hogsback is bisected by Cruickston Creek and bordered by the Newman Drive subdivision to the west. 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 wet years. The second forest plot overlaps with the Hogsback salamander monitoring plot. The first plot is found approximately 30m north of the second plot on the same elevated ridge, and the third plot is located 30m southwest of the second plot, separated by a small boggy area (Appendix A). This site can be accessed from Springbank Gardens by passing the pavilion and travelling south along the small hedgerow and fence line toward the forest, then east along the forest perimeter. The forest can be entered at part of fence lowered with a fallen log, at the southern edge of Hogsback Field.

4.2.2 Plot Establishment

Following the EMAN Forest Canopy Tree Biodiversity Monitoring Protocol (Environment Canada and Canadian Forest Service 2004), the plots established in 2009 and 2010 at **rare** are permanent 20m x 20m plots located in the forest interior. According to EMAN, plots should not be

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

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

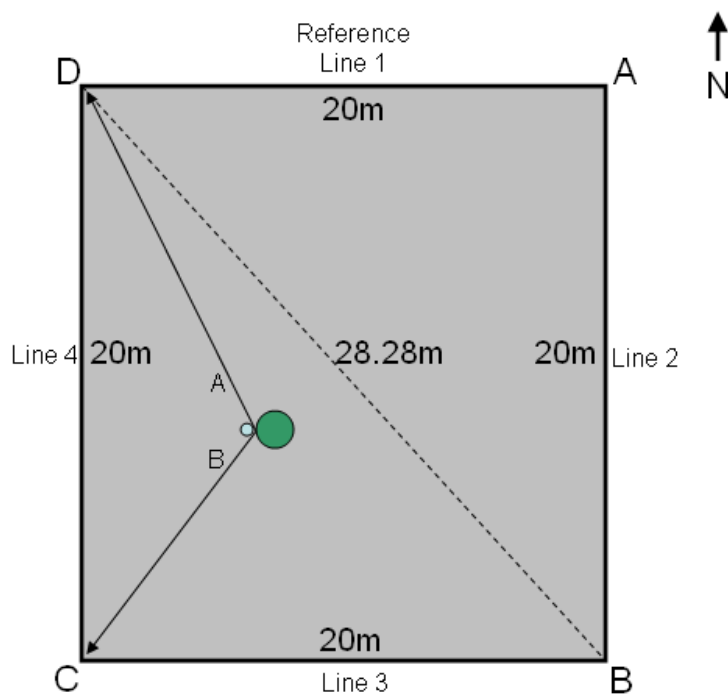


Figure 4.1: Diagram of an EMAN forest canopy tree biodiversity plots from McCarter 2009. The A and B distances are used to map the position of the tree within the plot. The A distance is measured from the tree to the corner to the right of the observer standing facing the reference line. The B distance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

4.2.3 Summer Monitoring Procedure: Canopy-Tree Monitoring

Each plot is visited once in the summer while the leaves are still present for ease of identification and canopy assessments. At each plot, the following variables were recorded for each tree in the monitoring plots: diameter at breast height (Woven Fibre Glass 5m Diameter Tape, Richter Measuring Tools), and tree condition based on Environment Canada and Canada Forestry Services EMAN codes (Table 4.1). Tree health was monitored by recording stem defects, crown class, crown rating (Table 4.2), and any other health notes, again based on Environment Canada's EMAN protocol. Marginal trees in each plot were checked to see if they had graduated into the 10cm DBH size class (minimum for inclusion). Several trees were large enough to be added to the plots in 2020.

4.2.4 Fall Monitoring Procedure: Tree Height Measurements

Each plot is visited once in the fall after all the leaves have fallen to allow for more accurate height measurements. At each plot, tree heights were measured using the Hagl f Electronic Clinometer and the Mastercraft  Fibre glass measuring tape. An updated protocol based on MNRF protocol aimed at improving tree height accuracy is included below. Due to time constraints, measurements were taken multiple times by one observer in 2020, but would ideally be taken by two trained observers to achieve the most accurate results.

Surveying Method:

1. Measurements will be taken with the clinometer by two observers.
 - a. Make sure that the batteries in the instrument are fully charged (many electronic clinometers will continue functioning with poor batteries, but will not provide accurate measurements).
2. Total height is considered the tree base to highest live part of the crown (Figure 4.2)
3. Walk around the tree to determine the highest live part of crown, and evaluate lean.
4. Choose one sighting position that allows a view of the highest live part of the crown.
 - a. If the tree is leaning (the high point of the crown is offset from the base of the crown by more than 1 m) an offset sighting position should be used (see figure 4.2).
5. Each observer will follow the clinometer manual instructions to take height measurements.
6. Record measurements for each observer at the sighting position.
 - a. Each observer takes multiple measurements until measurements of the two observers are within 50 centimeters or as close as possible, and two measurements are averaged.

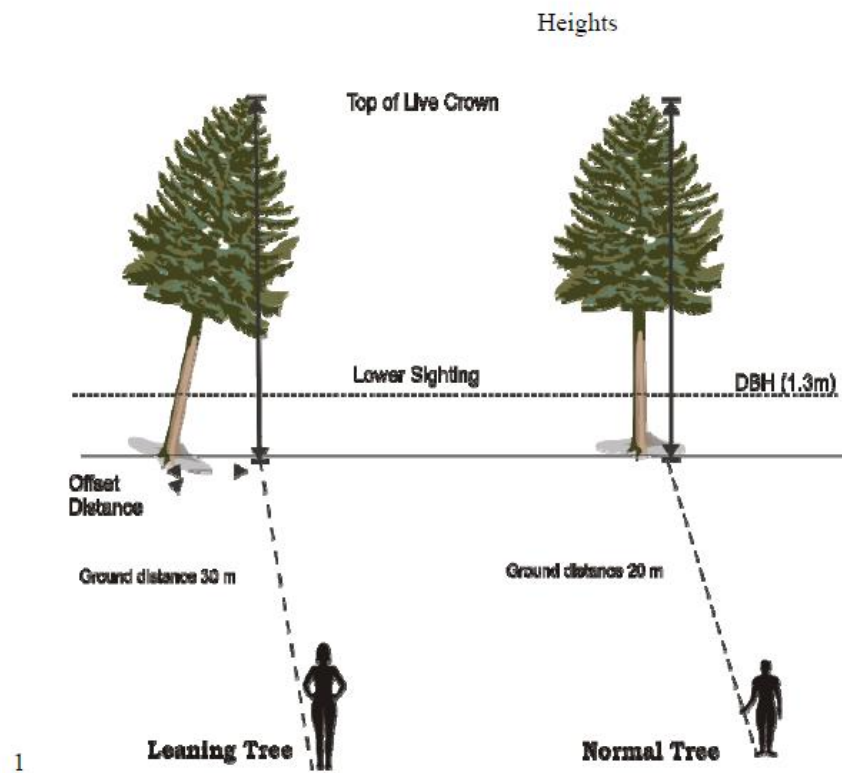


Figure 4.2: Sighting positions for leaning and normal trees (MNRF Procedural Manual, 2016).

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

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

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

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

4.2.5 Data Analysis

All data were analyzed using Microsoft Excel 2020. For each location, summary statistics were calculated by combining the data from the three plots which together represent the forest stand. For each stand, the number of trees present, the mean diameter at breast height (DBH), and the total basal area (sum of cross-sectional area of all trees within a plot, based on DBH measurements) were recorded. These data were used to calculate the Shannon diversity index (H) and species evenness value (E_H) for each forest stand. The relative density, relative frequency, and relative dominance were also calculated, and results were combined to give an importance value (IV) to each species within each stand (Roberts-Pichette & Gillespie, 1999). Only living trees were included in these calculations; formulas used for all calculations are found in Figures 4.3 to 4.6

In addition, differences between size classes at each forest stand was investigated. Trees were assigned one of eight size classes based on their DBH measurements in meters (0.1-0.19,

0.2-0.29, 0.3-0.39, 0.4-0.49, 0.5-0.59, 0.6-0.69, 0.7-0.79, 0.8+; hereon referred to as size class one through eight). Size classes within each forest stands from 2009 to 2020 were compared to identify differences and within each size class over time.

To better understand tree crown health the proportion of trees with healthy/light-moderate decline and severe decline in canopy was determined for dominant and codominant trees. Categories were expanded (i.e. healthy and light/moderate crown decline were all considered healthy trees) to reduce potential errors from an individual's judgement (CVC 2010). Trees considered intermediate or suppressed were excluded due this crown class typically receiving less sunlight and often exhibit severed dieback in healthy forests (M^cAfee 2004). A monitoring threshold of 10% was used to determine the significance of severe decline as surpassing this threshold for two consecutive years is an early warning sign of health decline (M^cAfee 2004).

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

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

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

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

Figure 4.4: 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 4.5: Formula for calculating the relative dominance of tree species in a forest stand, with all three plots per stand combined.

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

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

4.3 Results

4.3.1 Canopy Tree Monitoring: Abundance and Dominance

The Cliffs and Alvars forest plots were comprised of five species, all from different families (Figure 4.3). Sugar Maple and American Beech are the two most dominant species in all three plots. No new mortalities have occurred since monitoring in 2016, and three new tree recruitments reached the minimum 10cm DBH requirements to be added to the canopy monitoring protocol (two Sugar Maples, one American Beech). The Shannon diversity index for this forest is 1.31, with a marginal but steady decline observed over monitoring years. Species evenness is 0.81, and has remained relatively stable over monitoring years (Table 4.3).

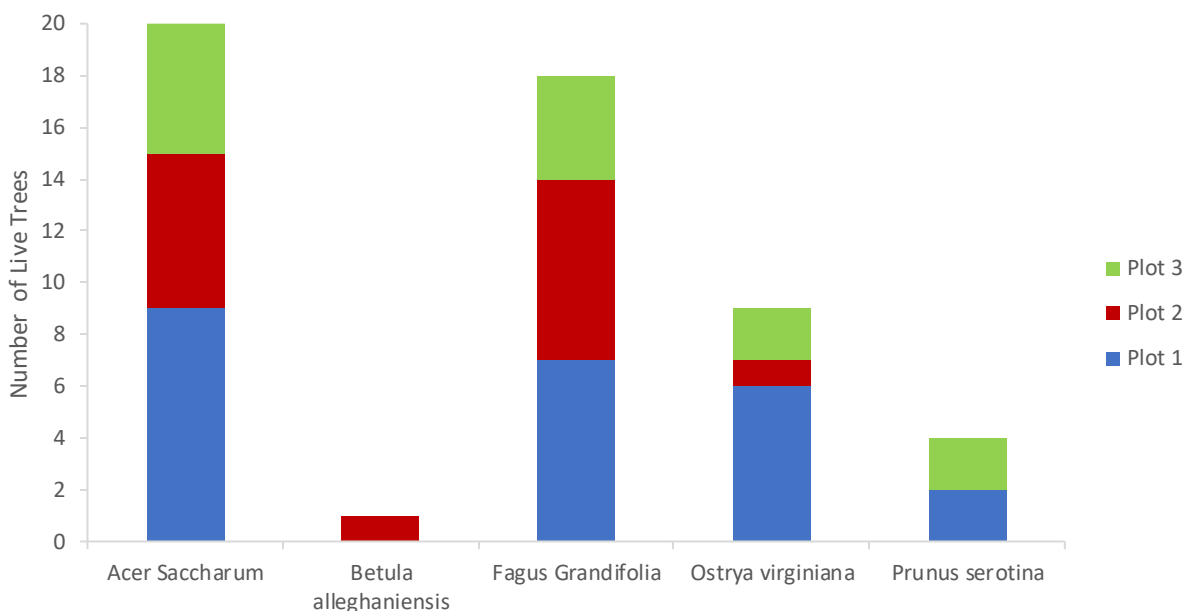


Figure 4.3: Tree species composition and abundance from the three forest plots in Cliffs and Alvars, as recorded in 2020.

The lowest number of observed tree species is in the Indian Woods forest plots with only four species from three different families present (Figure 4.4). Sugar Maple is the most dominant species in plots two and three, whereas American Beech is the most dominant species found in plot one. There have been no mortalities and two new recruitments (two American Beech) in Indian Woods since monitoring in 2016. The Shannon diversity index and species evenness have marginally increased since monitoring began and are the highest recorded to date in Indian Woods at 0.88 and 0.63, respectively (Table 4.3).

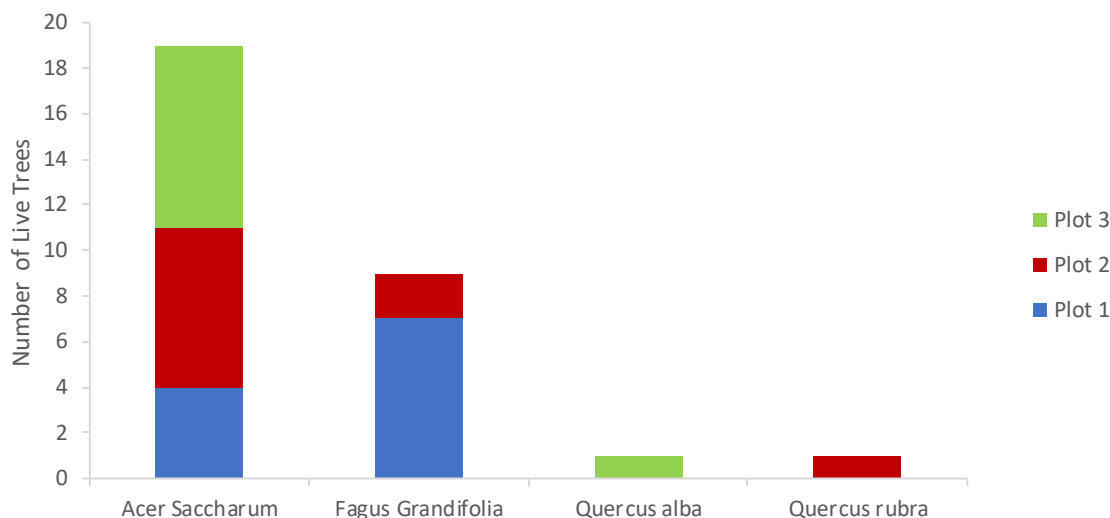


Figure 4.4: Tree species composition and abundance from the three forest plots in Indian Woods, as recorded in 2020.

The Hogsback forest has the highest species abundance across all three forest stands with nine species from six different families (Figure 4.5). Sugar Maple is the dominant species for the forest stand, but American Hop-Hornbeam is the most dominant species in plot three. Six new mortalities occurred (Red Maple, Ash, American Hop-Hornbeam, American Beech), and three new tree recruits were added to the canopy monitoring (two American Beech and one Sugar Maple). Species evenness is 0.86 and the Shannon diversity index is 1.90. Both have marginally but steadily declined over monitoring (Table 4.3).

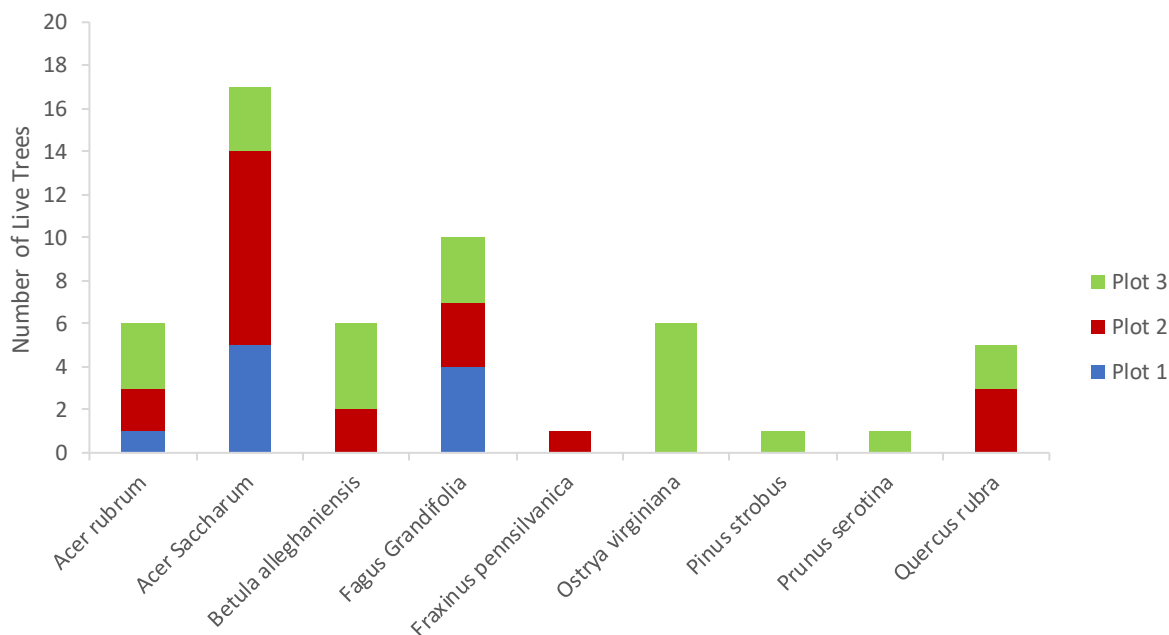


Figure 4.5: Tree species composition and abundance from the three forest plots in Hogsback in 2020.

Table 4.3: Summary of the forest canopy monitoring observations, along with the Shannon diversity index and species evenness for each forest stand across all monitoring years.

		Measures					
		Number of Live Trees	Number of dead trees	Number of Species	Mean Stem DBH (cm)	Shannon-Weiner Diversity Index	Species Evenness Value
Cliffs and Alvars	2009	48	7	7	23.07	1.49	0.83
	2010	50	6	7	23.34	1.56	0.80
	2011	49	8	7	23.30	1.48	0.76
	2012	49	9	6	23.40	1.40	0.78
	2013	49	9	6	23.40	1.40	0.78
	2014	48	10	6	23.90	1.41	0.79
	2015	47	11	5	24.30	1.34	0.83
	2016	47	11	5	24.55	1.32	0.83
	2020	50	11	5	24.53	1.31	0.81
Indian Woods	2009	34	4	5	32.97	0.84	0.52
	2010	32	7	4	32.11	0.75	0.54
	2011	32	7	4	32.30	0.75	0.54
	2012	29	10	4	33.10	0.79	0.57
	2013	31	10	4	32.90	0.76	0.55
	2014	30	11	4	33.30	0.78	0.56
	2015	28	15	4	30.10	0.85	0.61
	2016	28	15	4	31.10	0.85	0.61
	2020	30	15	4	31.04	0.88	0.63
Hogback	2010	54	6	10	24.92	2.08	0.90
	2011	54	6	10	25.10	2.08	0.90
	2012	54	6	10	24.49	2.08	0.90
	2013	56	6	10	25.30	2.05	0.89
	2014	57	6	10	24.70	2.04	0.86
	2015	56	7	10	25.20	2.03	0.88
	2016	54	8	10	25.92	2.01	0.87
	2020	51	14	9	26.10	1.90	0.86

4.3.2 Canopy Tree Monitoring: Stand Characteristics and Size Class Abundance

In Indian Woods and Hogsback forests, Sugar Maple had the highest importance value, whereas in Cliffs and Alvars, American Beech recorded the highest importance value. The importance value, along with the abundance, basal area, relative density, relative frequency, and relative dominance for all species present in each forest stand can be found in Table 4.4.

Small trees (size class one) dominated each forest stand and across all monitoring years (Figure 4.9). Size class distribution within Cliffs and Alvars and Hogsback forests were similar, with a large difference between size class one and the remaining seven classes. The number of trees within the remaining seven classes decreased as size class increased. With the exception of size class three containing more trees than size class four in Cliffs and Alvars, and with Hogsback size class four containing more trees than three.

The abundance of trees in Indian Woods was more evenly distributed between size classes compared to Hogsback and Cliffs and Alvars forests. Multiple larger size classes have a higher abundance than smaller size classes, but the dominant tree size is still below 0.5m DBH.

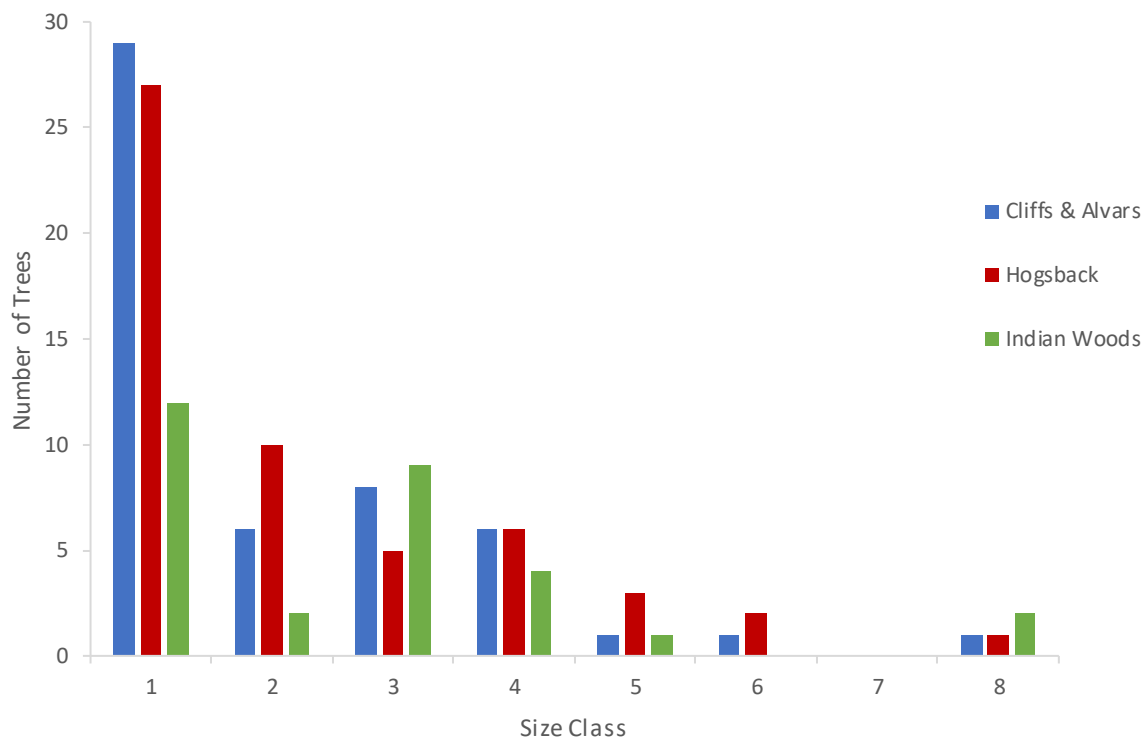


Figure 4.9: Number of trees in each size class across years in all forest stands.

Table 4.4: 2020 tree species composition and summary statistics for the three forest stands monitored at *rare*.

Location	Species name	Abundance	Basal Area (m2)	Relative Density	Relative Frequency	Relative Dominance	Importance Value
Cliffs and Alvars	<i>Acer saccharum</i>	20	0.81	38.00	25.00	22.78	85.78
	<i>Betula alleghaniensis</i>	1	0.16	2.00	8.33	4.39	14.73
	<i>Fagus grandifolia</i>	18	1.48	36.00	25.00	41.42	102.42
	<i>Ostrya virginiana</i>	8	0.88	16.00	25.00	24.53	65.53
	<i>Prunus serotina</i>	4	0.25	8.00	16.67	6.88	31.55
Indian Woods	<i>Acer saccharum</i>	19	1.38	63.33	42.86	65.06	171.25
	<i>Fagus grandifolia</i>	9	1.56	30.00	28.57	7.99	66.56
	<i>Quercus alba</i>	1	0.17	3.33	14.29	21.04	38.66
	<i>Quercus rubra</i>	1	0.10	3.33	14.29	5.90	23.52
Hogsback	<i>Acer rubrum</i>	6	1.17	11.76	33.04	28.62	73.43
	<i>Acer saccharum</i>	16	1.07	31.37	33.04	26.19	90.60
	<i>Betula alleghaniensis</i>	6	0.17	11.76	21.81	4.12	37.69
	<i>Fagus grandifolia</i>	10	1.04	19.61	33.04	25.41	78.06
	<i>Fraxinus pennsylvanica</i>	1	0.08	1.96	10.90	1.88	14.74
	<i>Ostrya virginiana</i>	5	0.10	9.80	10.90	2.53	23.24
	<i>Pinus strobus</i>	1	0.01	1.96	10.90	0.32	13.19
	<i>Prunus serotina</i>	1	0.07	1.96	10.90	1.77	14.63
	<i>Quercus rubra</i>	5	0.38	9.80	21.81	9.15	40.76

4.3.3 Forest Health

Overall, 170 defects were observed from the three forest stands in 2020 (Figure 4.10). The three most abundant defects were cankers (40%), open wounds (20%), and dry seams (18%); these defects combined accounted for 78% of all observed defects. Hogsback forest had the highest amount of stem defects, observed on 88% of all living trees within the stand. In Cliffs and Alvars, defects were observed on 86% of the living trees and in Indian Woods, where the lowest number of defects were recorded, 87% of the living trees within the stand had observed defects.

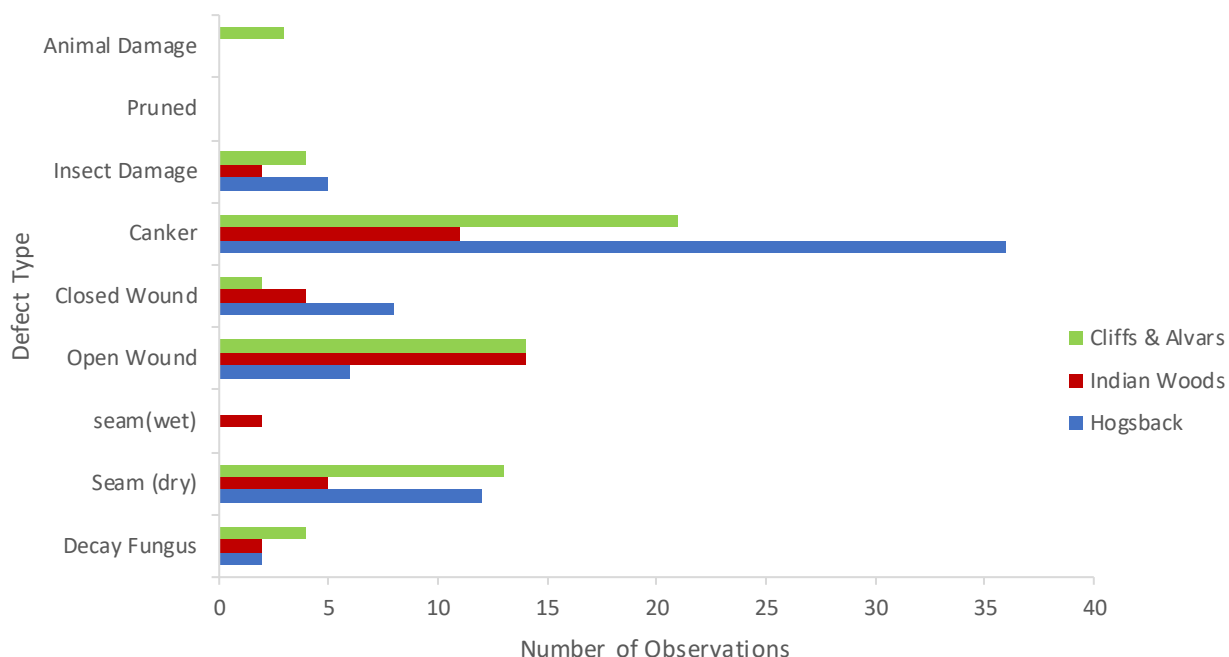


Figure 4.10: Occurrences of stem defects in *rare* forest stands in 2020.

While there have been no major trends in crown health for all forest plots observed since 2010 when crown rating data was first collected, the proportion of trees with severe crown decline is less in 2020 compared to 2010 for two forest plots (Figure 4.11). The proportion of trees with severe decline has rarely exceeded the 10% threshold (M'Affee 2004) and it has never been surpassed for two or more consecutive years. Cliffs and Alvars has exceeded this threshold the most of the three plots, while Hogsback crown health appears the most stable.

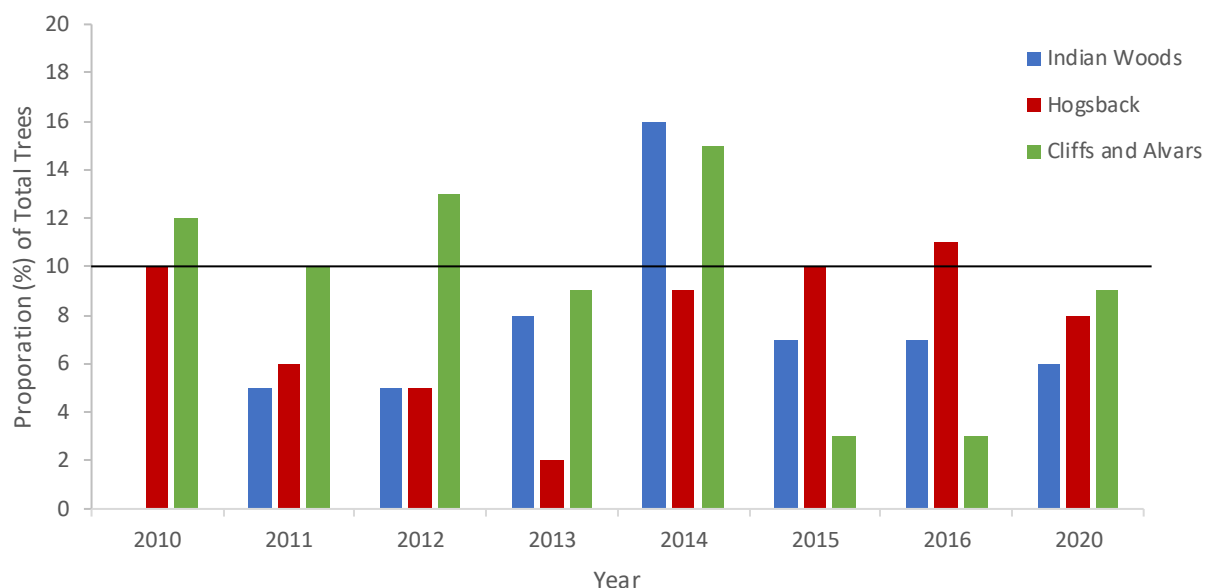


Figure 4.11: Comparison of the total number of dominant and codominant trees for each forest plot with severe decline in canopy crown rating. The black solid line represents the 10% threshold.

4.3.4. Tree Mortality

Mortalities for co-dominant and dominant trees for each forest area over the last 12-year monitoring period on average is below 5% (Table 4.5). Indian Woods had the highest rate of decline within a single monitoring year, however decline was most recently documented in Hogsback. Cliffs and Alvars had the most stable and lowest rate of decline of all three forest stands.

Table 4.5: Change in number of living dominant and co-dominant trees between each year of monitoring.

	Indian Woods			Cliffs and Alvars			Hogsback		
	Dominant /Co-Dominant Trees	Newly Dead	% Change	Dominant /Co-Dominant Trees	Newly Dead	% Change	Dominant /Co-Dominant Trees	Newly Dead	% Change
2016-2020	18	0	0	41	0	0	36	3	1.92
2015-2016	17	0	0	33	0	0	36	1	2.70
2014-2015	16	3	15.75	29	1	3.33	31	1	3.13
2013-2014	19	0	0	30	1	3.23	32	0	0
2012-2013	19	0	0	31	0	0	32	0	0
2011-2012	19	1	5.0	31	1	3.13	32	0	0
2010-2011	20	0	0	32	1	3.03	32	0	0
2009-2010	20	3	13.04	33	0	0	32	0	0
	Average Change		4.22	Average Change		1.59	Average Change		0.96

At **rare**, the species with the highest number of mortalities was Sugar Maple with eight deaths since monitoring began. This species also had the highest abundance with 55 individuals as of 2020. The mortality rate for Sugar Maple for the combined plots was 12.6%. American Beech had the second highest number of deaths at five individuals and is the second most abundant species (42 trees), and had a mortality rate of 11.9%. However, ash species (white, green, and black) had the highest mortality rate at 100% (with eight combined deaths) as of 2020. The total amount of tree death and new recruits for each plot can be found in Table 4.6.

Table 4.6: The total number of mortalities and new tree recruits for each monitoring plot from 2009 to 2020 at **rare**.

	Indian Woods		Cliffs and Alvars		Hogsback	
	New Recruits	Mortalities	New Recruits	Mortalities	New Recruits	Mortalities
2009-2010	1	3	1	0	No data	No data
2010-2011	0	0	0	1	0	0
2011-2012	0	3	1	1	0	0
2012-2013	2	0	0	0	2	0
2013-2014	0	0	0	1	1	0
2014-2015	2	4	0	1	0	1
2015-2016	0	0	0	0	0	2
2016-2020	2	0	3	0	3	6
Totals	7	10	5	4	6	9

4.4 Discussion

4.4.1 Canopy Tree Monitoring: Abundance and Dominance

American Beech and Sugar Maple dominate **rare** forests. This is not surprising, as Sugar Maple and American Beech are very commonly seen together as co-dominant species in northeastern North American late-successional or 'climax' forests (Takahashi & Lechowicz 2008), and can comprise up to 80% of the canopy (Menard et al. 2014). In these Maple-Beech forest stands, the relative dominance of each species varies with regional climate conditions across their range (Poulson and Platt 1996; Gravel et al. 2011; Menard et al. 2014). There is evidence that American Beech has been recruiting more new canopy trees over the past 40 years than Sugar Maple has in Maple-Beech forests throughout North America (Gravel et al. 2011). This trend is weakly observed in forest plots at **rare** with ten new American Beech recruits and seven new Sugar Maple recruits since 2009. However, there is a high incidence of Beech Bark Disease on the American Beech within the plots making it unlikely beech will come to dominant the forest stands at **rare** in the near future. Beech Bark Disease and its impact on American Beech trees at **rare** is discussed in-depth in section 4.4.5.

The Cliffs and Alvars forest is a mature stand co-dominated by Sugar Maple and American Beech, which combined account for 76% of trees and 75% of canopy trees in all three monitoring plots. These dominant species as well as another observed species in this forest, American Hop-hornbeam, prefer well drained, upland habitats and are shade tolerant (Laird Farrar 1995). Black Cherry and Yellow Birch, also found in Cliffs and Alvars plots in low densities, prefer moist soil

and are less tolerant to shade, so are typically found in canopy gaps. They are commonly documented in mixed forests dominated by American Beech and Sugar Maple (Laird Farrar 1995). Species evenness and diversity indices have had a small but steady opposing change since monitoring began in this forest, with a slight increase in evenness and decrease in diversity. This can largely be attributed to the loss of White Ash and Butternut, both species are vulnerable to species specific pest/pathogens that have decimated populations in all plots (in-depth discussion in sections 4.4.6 and 4.4.7), and the increase in new recruits primarily from the two-dominant and shade-tolerant species (Sugar Maple and American Beech). As a forest approaches a Maple-Beech climax we would expect tree diversity to drop, as increased stability in the community from limited disturbance allows dominance of late successional trees to exclude other species (Lienard et al. 2015). It is important to note that this diversity index is not a measure of the overall diversity of the system. Mature forests support a high diversity of wildlife including numerous birds, fungi, lichen, insects, salamanders, and mammals.

Indian Woods is a remnant old-growth forest dominated by Sugar Maple, which makes up 63% of trees and 93% of canopy trees in this forest stand. Due to this single-species dominance, Indian Woods has the lowest tree species diversity and evenness of all three *rare* forest stands (Table 4.3). Old-growth forests are often considered to have reached a climax community stage, with the habitat continuing in a relatively steady state subject to environmental conditions (Krebs 2011).

Hogsback forest is a forest-wetland complex with a greater diversity of habitat and therefore species than the other two forest stands monitored at *rare*. Similar to Cliffs and Alvars, Hogsback diversity index has slightly but steadily declined as the total number of tree species present in the plots has declined since monitoring began. The wetland areas are a source of increased diversity that can support species that thrive in wet soils, like Yellow Birch and Red Maple (Sibley 2009). While Sugar Maple and American Beech co-dominated Hogsback (51% of trees and 53% of canopy trees), they tend to thrive in areas with drier soils (Laird Farrar 1995) and is likely the reason they do not dominate to the extent they occur in Cliffs and Alvars and Indian Woods. This increased diversity of Hogsback will allow this forested area to be more resilient to disease, disturbance, invasive species, and species loss than the other forested area (Montagnini et al. 1995; Balvanera et al. 2006; Garcia-Gonzalo et al. 2012; Hantsch et al. 2014). Mixed forests increase the chance of some trees having resistance to these hazards, allowing specific species to continue to maintain forest cover, sustain ecosystem functions, and are available as replacement for damaged species (Jactel et al. 2017). For instance, the affect of Emerald Ash Borers on Hogsback forest health would have been greater if this system was dominated by ash species.

4.4.2 Canopy Tree Monitoring: Stand Characteristics and Size Class

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

As Sugar Maple and American Beech dominated the majority of plots, it is unsurprising to see these species also have the two highest IVs across all stands. This aligns with information gleaned from the diversity and evenness indices, demonstrating once again that forests at *rare* are in or approaching a climax maple-beech community. It is interesting to note the value of some canopy associate species like Red Maple and oaks. In Indian Woods, only one Red Oak and White Oak were present within plots, each considerably large relative to other trees in the area. Large oak trees in ecosystems are important even when found in low abundance, as their foliage in the canopy and acorn production are important inputs for terrestrial ecosystems and support a diversity of organisms (Dey 2014). In Hogsback, Red Maple have an importance value closely ranked with that of American Beech. Although they are few in number, the Red Maples present are large and thus exert a strong influence on the forest community as a whole. 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 a climax status, and Red Maple may be able to remain a dominant presence with a relatively high IV (Walters and Yawney 1990).

The DBH size class 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's structure (Burns and Honkala 1990). The size class distribution is useful baseline data for future comparisons examining recruitment and replacement patterns of each stand (Forrester & Runkle 2000; Parker 2003). Both the Cliffs and Alvars and the Hogsback forests had a greater abundance of trees in size class one. Therefore, although the forests stands are late-successional, many younger individuals, particularly shade tolerant species such as Sugar Maple and American Beech, are successfully becoming part of the larger canopy. Size class distribution in both of these forests appears to be right-skewed, typical of a stand comprised mostly of young trees with fewer in the larger size classes. An exception exists in the Cliffs and Alvars, where size class three had higher abundance than size class two. Historically, this forest stand was grazed by cattle in the early twentieth century, and this could account for this increased number of trees in size class three. These trees may have been large enough at the time of the grazing to not be stripped completely by the cattle. Smaller trees and likely the trees now in class two, were more likely to have been grazed, potentially resulting in fewer individuals today.

Indian Woods, a remnant old growth forest, had different distribution than the other two forest stands. Tree abundances was more evenly dispersed among all of the size classes, particularly the first four. This indicates that, although regeneration is occurring in this forest stand, it is settling as a climax community forest where Sugar Maple and Beech trees are stable in the understory for many years using a series of gaps to reach the canopy (Forrester & Runkle 2000).

4.4.3. Mortality Rate

Crown dieback in trees can be used as an early indicator of many of the stresses a tree faces and, as a consequence, as a measure of forest health (Schomaker et al. 2007). The rate of tree mortality can affect ecosystem services, forest structure, composition, and the amount of organic material on the forest floor (Mantgem et al. 2009). Sajan (2006) outlines thresholds for forest stand health using EMAN crown rating codes. Annual mortality rate of dominant and co-dominant trees should not surpass 5% in two consecutive monitoring years for a forest stand to be considered healthy (Sajan 2006; CVC 2010). Mortality rates in *rare* forests largely did not exceed the 5% mortality threshold (Table 4.5), with the lowest average annual mortality rate

observed in Hogsback. However, Hogsback forest is the only monitored stand where co-dominate or dominate tree mortality has occurred since 2015 and an average mortality rate of 2.5% has been maintained in that time. Of the five tree deaths since 2015 in Hogsback, three have occurred in ash species, one American Beech, and one Sugar Maple. American Beech and ash trees are both impacted by separate invasive insects that have been observed at **rare** and can cause high occurrences of tree death (refer to section 4.3.6 and 4.3.7). Therefore, mortality at Hogsback does not appear to be from weather events or a natural death, but from the stress of these pests. There are no remaining ash species within Hogsback plots as of 2020. Close monitoring of Hogsback should continue to monitor if mortality rates continue to increase.

High annual mortality rates that reach or exceed threshold levels have been documented three times in Indian Woods since monitoring began. Indian Woods has far fewer dominant and co-dominant trees than the other monitored stands, meaning when tree loss does occur, it has a higher impact on the calculated mortality rate. Although more tree loss had occurred in Indian Woods dominant and co-dominant trees than in the other two forest stands, the cause of death for these trees appears to be mostly weather related. In winter 2014-2015, a significant ice storm caused a dominant tree to fall, knocking over two other co-dominant trees in the same area. Trees in Indian Woods are comparatively larger and older and are thus likely more susceptible to extreme weather events than the other forest stands which have smaller, younger trees. This is a normal characteristic of old-growth forests, where natural disturbances cause occasional death of the oldest and largest trees, which are then replaced by understory trees (Krebs 2011). Additionally, Indian Woods small stand size may lower its resilience and contribute to its susceptibility to environmental hazards (MacArthur and Wilson 1967; Godefroid and Koedam 2003). Indian Woods was the only forest stand at **rare** where establishing three plots 90m from the forest edge as recommended by the EMAN protocol was not possible. Long term restoration plans are in place at **rare** to increase Indian Woods size through tree planting, and ultimately connect the stand to Hogsback to increase resilience in both forested areas.

As a consequence of having the most tree deaths, Indian Woods also had the largest number of new recruits over the monitoring period (Table 4.6). New recruits are able to capitalize on canopy gaps left by large fallen trees, and eventually take their spot in the canopy. The number of new recruits indicates this is an area of active growth, however so far there has been a disproportionate number of deaths to recruits. In all likelihood an influx of new recruits will be seen over the next years as several large canopy gaps have opened. While crossing the 5% threshold can be indicative of an unhealthy forest, examining the underlying causes of mortality in this case results in a better understanding. With no present trend indicating a continued and sustained mortality loss, Indian Woods appears to be a healthy system, albeit more susceptible to environmental hazards, and monitoring should continue to alert **rare** to additional stressors. To increase the resilience of Indian Woods minimizing disturbance of healthy trees by removing hazard trees will prevent damage from unpredicted events like the ice storm of 2014/2015. Restoration planting in canopy gaps with trees favoured by the predicted warming climate such as oak species, and documenting and removing trees affected by Beech Bark Disease reduces the threat to remaining American Beech (the second most dominant species), and will aid in the resilience of Indian Woods.

4.4.4 Forest Health

Canopy dynamics (i.e. gaps and closures) are known to influence the regeneration and growth rates of forests, as well as species composition (Weiskittel and Hix 2003). Forest canopies are in constant flux. Openings are created by disturbance and subsequently filled by individuals in the understory. Of the three forest stands, Indian Woods had the highest amount of tree death, this coincided with having the largest proportion of intermediate and suppressed trees in 2020. This demonstrates how a shift in resource availability can change canopy dynamics. It would be concerning if these slight changes in tree death and canopy decline did not follow with new individuals to fill this space in the canopy. As Cliffs and Alvars and Hogsback have less tree mortality and crown decline throughout monitoring, there is less availability of resources such as sunlight and space, and this can contribute to the lower proportion of intermediate and suppressed trees in each stand. However, the proportion of canopy trees with severe decline rarely exceeded the 10% threshold in any plots and does not indicate any early signs of forest health decline at **rare**.

As succession progresses in forests and the canopy closes, the composition of canopy trees shifts toward more shade tolerant species like Sugar Maple and American Beech in eastern deciduous forests (Fox 1977), and this has been observed at **rare**. In 2020, five new American Beech recruits and four new Sugar Maples were added to the forest plots and all new recruits since 2009 have been one of these dominant species. These species are able to grow suppressed in the understory and exploit canopy gaps when they occur, outcompeting other shade-intolerant species (Weiskittel and Hix 2003).

Overall, the forest stands monitored at **rare** display normal characteristics of mature forest stands with majority of trees on **rare** property appearing to be healthy. Given the information collected from monitoring, no specific management practices are recommended for most species. However, three problem groups have been identified: American Beech, ash species, and Butternut. Each of these species or species groups have a serious pest or disease that has caused significant tree mortality and has the potential to persist into the future. These groups are each given their own separate section to discuss the extent of the problem and identify potential management practices.

4.4.5 American Beech Pests

American Beech trees have thin bark that increases their vulnerability to scale insect infestation (Calic et al. 2017). A major concern for American Beech trees across Central/Eastern Canada and at **rare** is Beech Bark Disease (BBD). BBD is caused by an infestation of one or more species of a fungus called *Neonectria* (Calic et al. 2017). The fungus typically enters a tree that has been stressed due to feeding from a non-native scale insect called *Cryptococcus fagisuga* (Calic et al. 2017). The fungal infestation causes a range of health problems from reduced growth to crown dieback, and potentially death. Mortality from individuals infected by the fungus can be up to 50% (Kasson and Livingston 2011), and only 1% of beech trees are resistant to this scale insect (Houston and Houston 2000). Potential instances of BBD have been identified via forest health monitoring on **rare** property since 2010. The non-native scale insect has been known to be present in southern Ontario since 2003 (Morin et al. 2007) and has been officially documented in Cambridge as of 2012, likely spread from firewood (McLaughlin and Greifenhagen 2012). American Beech is an important food source for many species and BBD reduces nut

production on which many wildlife species rely (Rosemier and Storer 2010; Cale et al. 2015). American Beech constitutes a considerable portion of the over-story in the Cliffs and Alvars and the Hogsback forests, and unfortunately, a widespread BBD infection at **rare** could potentially cause significant losses in these forest areas.

Signs of BBD were present in all forest areas at **rare** during 2020 monitoring. Of the 35 living American Beech within the forest plots, nine trees (25.7%) were identified as having infestations. Note that the *Neonectria* fungi associated with BBD are most visible in the fall when they begin to fruit and are bright red in colour (McLaughlin and Greifenhagen 2012). This means some infected trees may have been overlooked, as monitoring is primarily completed in late summer, therefore BBD surveying should be added to the fall monitoring protocols when tree heights are determined. However, a University of Waterloo research project is currently taking place at **rare** with the use of drones to increase identification of trees affected by BBD, in hopes to better understand the spread of this disease.

BBD infestations proceed in three phases; (1) the advancing front where feeding from the scale insect begins, (2) a killing phase, which can last between 3-20 years (typically 3- 5) where *Neonectria* infects trees causing death, followed by (3) the 'aftermath' forest in which the fungus is still present, but is no longer actively causing rapid tree mortality (Morin et al. 2007; Cale et al. 2017). Four beech deaths have occurred since 2009 suggesting we are likely in the killing phase of BBD at **rare**. Control measures such as pesticide application have not been fully effective in reducing scale insect number due to their waxy protective covering (McCullough et al. 2001), and the physical removal of infested trees can have financial and feasibility constraints (Wiggins et al. 2004). A less costly measure with greater potential mortality is to allow BBD to progress and gather seed from resistant trees for reseedling (McLaughlin and Greifenhagen 2012).

A recent emerging threat to beech trees is Beech Leaf Disease (BLD), which affects both native and ornamental beech species. This disease is associated with nematodes, of the subspecies *Litylenchus crenatae mccannii* (Marra and LaMondia 2020). Symptoms of BLD were first recognized in 2012 in Ohio near Lake Erie, and have been observed in different regions of Ontario since 2017 (Martin et al. 2019). Symptomatic trees with this disease possess dark interveinal bands on their leaves with varying coverage, irregular and thickened leaves with curled margins, aborted buds, and branch and crown dieback (Volk et al. 2019; Carta et al. 2020). Mortality from BLD has been observed to occur within seven years from detection (Carta et al. 2020). The spread of BLD is not well understood but other nematodes are known to be spread through water, mites, and animals. To minimize the spread of BLD, leaves from potentially affected forest floors should not be transported and shoes should be cleaned after exposure. Due to recent discovery of this disease there are currently no known method to control or manage BLD, but there are experimental chemical treatments currently being tested (Volk et al. 2019). Although BLD has not yet been recognized in **rare** forest stands educating staff and members of the public, and monitoring for these symptoms in the future is important for protecting American Beech trees on **rare** property and understanding the health and stability of these forests.

In past monitoring years several American Beech trees were observed with Beech Blight Aphids (*Grylloprociphilus imbricator*). Beech Blight Aphids are an insect pest that feeds exclusively on beech and can form dense colonies on branches and create fungal masses of sooty mold (*Scorias spongiosa*) from its secreted honeydew (Cook-Patton et al. 2014). While this insect on its own is unlikely to kill or severely harm mature host tree, in combination with other

stresses (i.e. drought, BBD) it may result in tree damage or death, and direct effects on beech seedlings are unknown (Childs 2011; Ramage and Mangana 2017). Beech Blight Aphids were not observed within the plots in 2020 however further monitoring is required to identify if this threat re-emerges at **rare**.

4.4.6 Emerald Ash Borer and the Ash Tree Decline

Emerald Ash Borer (EAB; *Agrilus planepennis*) is a major pest for ash trees in North America and it is capable of infesting and killing even the healthiest of ash trees (OMNR 2020). Native to Asia and Eastern Russia, EAB was first detected in Canada in 2002 and has spread through much of eastern Canada (Herms and McCullough 2014; OMNR 2020), including southern Ontario. EAB was first documented in Waterloo Region in 2010 at Highway 401 and Homer Waterson Boulevard in Kitchener (Region of Waterloo 2010); a location only a few kilometers away from the **rare** property. EAB has killed millions of ash trees and is considered one of the most destructive invasive insects in North America (Lovett et al. 2016; Morin et al. 2017). Additionally, EABs are a threat to ash-dependent invertebrates, as taxonomic experts state approximately 93 insect and six mite species could become extirpated in North America by the loss of the ash population (USDA 2020).

Evidence of EAB within the forests at **rare** has been noted, although no individual adults have actually been observed. Evidence includes small D-shaped exit holes left by adults that emerge from under the bark, as well as dieback of ash tree crowns. All eleven ash trees located within the forest monitoring plots at **rare** are dead. While they do not make up a large portion of the canopy, the majority of ash will die off in these forested areas ultimately changing the canopy composition in these forests. As it is unlikely young ash tree recruits will survive to replace the dying ash trees in the canopy, Sugar Maples are likely to fill the canopy due to their high abundance and ability to grow rapidly in the presence of limited light availability (NRCS 2016). This occurrence will cause forest diversity to decline and composition will shift towards dominant species like Sugar Maple, as is typical in later-successional old growth forests. Overall, low numbers of ash trees and high occurrences of EAB indicators mirror the declining trends in ash trees across North American forests.

Given that a population of EAB can travel up to 20km over a single year (Prasad et al. 2010), it is likely that populations made their way to **rare** around the time of the first observation in Kitchener in 2010. Early detection of EAB is difficult, as the insects first attack the canopy of all but the smallest ash trees and by the time signs and symptoms are detected trees are usually already heavily infested (Cappaert et al. 2005; Polland et al. 2011). Because of this, visual surveys rarely detect infestations until the populations have increased and multiple generations have dispersed (Cappaert et al. 2005). Unlike many other insect infestations, EAB will not only attack stressed or damaged trees, but will also attack the healthiest of trees, resulting in death only three to four years after infestation; saplings and small trees may succumb to the insect's damage in as little as one year (Herms and McCullough 2014).

Management of EAB infestations is difficult and, in many cases, removal of infested/dead trees is required. Where invasion is detected early on, and/or trees are very significant to the landscape, insecticide can be applied, but should take a site-specific approach (Herms et al. 2014; Herms and McCullough 2014). Insecticides are costly and reapplication is often necessary in subsequent years (Herms et al. 2009). However, insecticides can often still be less costly than

tree removal in urban settings (McCullough and Mercader 2012). Many environmental and practical concerns limit the use of insecticides in a forest environment, however select trees could be saved in this manner, and they may provide re-seeding potential once the EAB epidemic has passed. Selective cutting of infested trees is similarly difficult and costly and is probably not a feasible method of managing trees on a property wide scale. Seed collection from any individual ash trees that appear to resist EAB could be used for reintroducing ash back into the forests at **rare**.

In order to limit the further spread of EAB, best management practices include not transporting ash wood, branches, or logs, including firewood (buy local, burn local), dropping off infested trees at the appropriate drop off sites, and reporting any signs of EAB to the Canadian Food Inspection Agency (Region of Waterloo 2010). A more detailed report and management plan for EAB and ash is available on the **rare** server.

In 2019, **rare** partnered with University of Waterloo to bring onboard a postdoctoral fellow to evaluate EAB and forest resiliency at **rare**. This research explored the link between plant and parasitoid populations to provide a strong basis on which to create and improve ecological restoration and rehabilitation programs. The overall objective of understanding the abundance and diversity of parasitic insects could provide conservation authorities and environmental groups with a cost-efficient measure to maintain biodiverse and resilient forest through limiting invasive species populations with parasitoids. This research could identify natural enemies of EAB and potential aid in the resistance of future disturbance at **rare**.

4.4.7 Butternut Death

One of twelve Butternut trees on **rare** property falls within the monitoring plots in the Cliffs and Alvares stand. This solitary Butternut tree in monitoring plots was observed to have severe crown dieback and extensive wounds before it was eventually found dead. 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 was a relatively recent introduction to North America (Broders & Boland 2010). Symptoms of the disease include elongated and sunken cankers, which commonly originate at leaf scars, buds, or wounds; and black substance can emanate from the cracks (MNRF 2020). 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 (Broders et al. 2015; Tanguay et al. 2018; MNRF 2020). As no remaining living Butternut are located in any monitoring plots, continued observation of other 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. In 2020, forty Butternut seedlings with forty companion trees/shrubs were planted on **rare** property near the riparian area of Newman Creek. As part of an offset planting program for a nearby development project where two butternut trees were lost. This was an important step to improve the population of this endangered species and will need continued monitoring in the future.

4.4.8 Difficulties, Limitations, and Recommended Changes to Forest Canopy Tree Monitoring

The forest canopy tree monitoring program at **rare** has the potential to identify changes within **rare's** three major forest stands early, so appropriate action to address emerging issues can occur. However, it should be noted that the EMAN protocol includes qualitative data collection which can result in subjectivity. A monitoring intern is hired to conduct monitoring protocols annually, and while all receive the same training and follow the same protocols, observer bias is expected particularly in reporting of crown ratings, stem defects, and tree heights. In this report, an effort was made to include crown ratings in evaluating forest health, but categories were expanded (i.e. healthy and light/moderate crown decline all considered healthy trees) to reduce potential errors from an individual's judgement (Bavrilic and Bowers 2009; CVC 2010).

Stem defect assessments are limited, not just in their subjectivity, but also in that they do not assess the severity of a defect. A 5cm open wound on a tree's lower stem and an open wound nearly girdling the entirety of the stem are both equally classified as an 'open wound'. Furthermore, many minor stem defects have little impact on the health of a tree and are commonplace on many species (USDA 2015). The United States Department of Agriculture (USDA) Forest Service contains a more in-depth protocol for assessing stem defects (called tree damages) and sets minimum thresholds for recording on certain types of defects such as open wounds. It is recommended these guidelines, or some other threshold be implemented with the stem defect assessments to ensure recorded defects are indicative of a danger to a tree's health.

One measure not included within forest health monitoring at **rare** that is present in the EMAN protocol is the inclusion of estimates of stand age (Roberts-Pichette and Gillespie 1999). Tree age can be estimated with the use of a tree corer and gives insight into stand age and growth patterns across time. EMAN protocol recommends coring five trees from each species found in each forest plot, but from trees outside of established plots to avoid damage to trees within plots (Roberts-Pichette and Gillespie 1999). Based on this protocol, a one-time tree coring effort could be made to discover average stand age and historical growth with replications in coring effort. Roberts and Gillespie (1999) also recommend taking cores from newly dead trees. This could be compared to average stand age to help determine if the death was directly caused by an external stressor and could easily be added to yearly canopy assessments. Tree coring has occurred in some areas at **rare** in the past and continuing this assessment may enhance our understanding of these environments and the cause of recent tree deaths in **rare's** forest stands.

4.5 Conclusions and a Summary of Recommendations

Over the past 12-year monitoring period at **rare**, there have been few changes in the forest stands in terms of diversity, size class, dominance, and canopy composition. The most appreciable difference over the monitoring period has been the decline of ash trees as a result of Emerald Ash Borer. American Beech are also showing signs of decline and developing management and/or monitoring programs targeting species of special concern is of the utmost importance. The three following types of trees should be targeted:

1. Ash species: Extend monitoring efforts on **rare** property as low sample size in the 2016 EAB survey may limit our understanding of the true impact of EAB. Create a recovery program for

ash on the property by collecting seed from ash trees, especially those that appear unaffected by EAB.

2. American Beech: Create a targeted survey to examine the extent of BBD on the property, which should take place in the fall. At the very least, examine Beech trees within the plots for signs of BBD. Collect seed for future Beech recovery program prioritizing trees that appear unaffected by BBD. Consider options of selective cutting and removal of heavily infested trees.

3. Butternut: Develop a specific monitoring protocol for Butternut canker to efficiently survey conditions for the eleven Butternut located outside of the forest plots and the newly planted trees near Newman Creek. Collect seed for future Butternut recovery programs from individuals with no sign of Butternut canker.

Long term data collection and analysis is required in order to fully understand if the integrity of the three forest stands are in fact being maintained or improved through management strategies. With constant changes in the surrounding land use, continued monitoring is important for early detection of changes in the health of *rare* forests. However, there are some changes that need to be made to Forest Health Monitoring should the program and data be viable into the future. The following modifications to data collection and forest canopy monitoring report are recommended:

1. Modify the current stem defect system to incorporate minimum damage thresholds and take more detailed notes and/or pictures about the extent of observed damage.

2. Incorporate tree-aging into the monitoring program with core assessment from newly dead trees within plots, and from trees outside of plots to calculate average stand age in areas where historical data does not exist. If possible *rare* should consider new or ongoing research partnership as a method to complete this work.

3. Establish and implement a plan to increase forest resilience in vulnerable areas through combined approaches, including the removal of hazard or pest/pathogen infected trees and restoration planting.

These modifications are not drastic changes to the forest monitoring protocols, but will allow more useful data to be collected and summarized on a more efficient timescale.

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5.0 Soil Humus Decay Rate Monitoring

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, that is a significant global producer of carbon dioxide, as well as methane and nitrogen gases (Berg and McClaugherty 2008; Zhen et al. 2018). Soil humus, the stable organic material remaining after initial decomposition, acts as the reservoir for 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 and McClaugherty 2008; Makkonen et al. 2012; Berg 2014). The rate at which decomposition occurs is dependent on many factors, including litter quality, decomposed biomass, the species composition and abundance of the decomposer organisms in the soil, and a suite of ecological conditions including soil temperature, moisture, pH, and aeration (Parks Canada 2006; Bradford et al. 2016; Wang et al. 2021; Zhai et al. 2021).

Although decomposition may appear undesirable it is a vital ecological process that critically influences the health of terrestrial ecosystems (Gregorich et al. 2017). Through decomposition approximately 90% of carbon-rich plant litter returns to the soil and decays into mineral components, including soluble nutrients and carbon dioxide (Gessner et al. 2010). Changes to the soil decay rates can lower soil health and alter many ecosystem functions, such as air and water quality, soil resilience, nutrient retention, and conserving biodiversity (Steffen et al. 2015; Turmel et al. 2015; Baer and Birge 2018). Furthermore, decomposition rates can have pronounced effects on atmospheric carbon dioxide and consequently global climate (Gregorich et al. 2017).

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 amounts of carbon are stored in trees, soils, and decaying plant litter (Luyssaert et al. 2008). Any change in the balance between carbon uptake (through photosynthesis) and carbon release (through decay) could have an impact on levels of atmospheric carbon dioxide, an important greenhouse gas linked to global climate change (Bardgett et al. 2013). 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 (Bardgett et al. 2013).

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 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 in 2009 in the Cliffs and Alvars forest. The success of the first monitoring year resulted in an expansion of the study in 2010 by the establishment of monitoring plots in both Indian Woods and Hogsback forest stands. Monitoring has occurred at all three forest sites in each subsequent year.

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 gained 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 sticks 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 microorganisms responsible for decomposition. A significant change in decay rates over time would reflect a change in the physical or biological soil environments.

As decay rates can be influenced by a suite of factors including climate, moisture, nutrient availability, litter quality, and soil microfauna (EMAN 2004; Parks Canada 2006; Bradford et al. 2016; Wang et al. 2021; Zhai et al. 2021), measurement of decay rate can be used as indicators of these factors and of soil health. The following questions have been identified from previous monitoring reports and the EMAN protocol:

1. Is the ecosystem integrity of the forest being maintained or improved under the management of *rare*?
2. 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, aggregate extraction)?
3. What are the long term trends in forest litter decomposition at *rare* and how do the three forest stands compare to one another?

5.2 Methods

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 or during 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 (to 0.001g) on a Sartorius 1265MP balance. A sample datasheet to record stick weight pre and post decay can be found in the Appendix (Figure C.5). After recording their mass, decay sticks were tagged with pre-labelled aluminum tags attached with approximately 10cm of extra-strong fishing line. With the exception of the initial

year of monitoring, decay sticks were placed in 100% vinyl mesh bags (dimension: 17cm x 4cm with an approximate pocket size of 16cm x 3cm; hole size: 3mmx 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 (Gallardo et al. 1995; Albers et al. 2004; Moore et al. 2005). A complete list of equipment required for installation can be found in List B.4 of the Appendix.

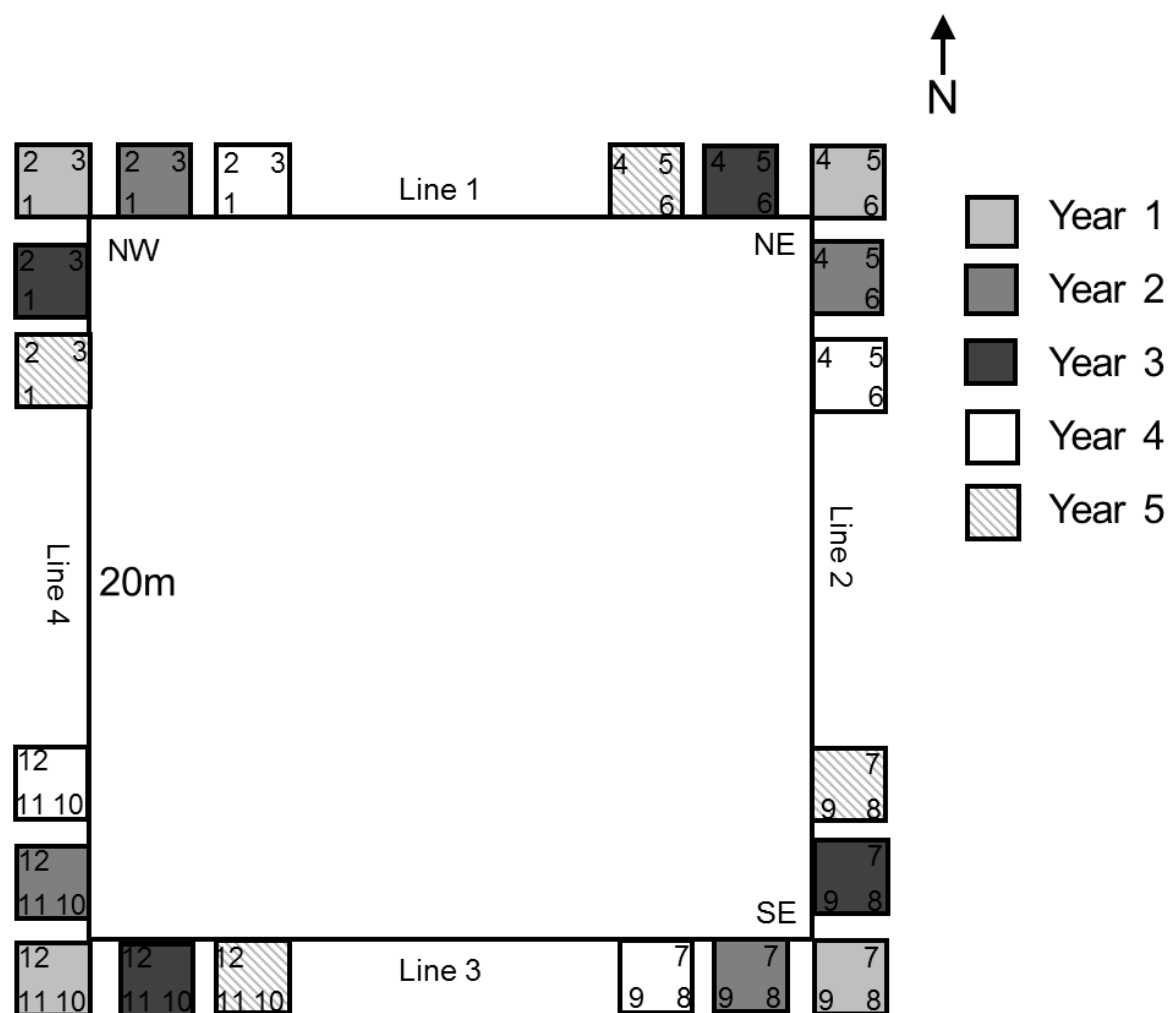


Figure 5.1: Distribution of annual soil humus decay rate (ADR) plots (numbered 1-12) around a forest canopy tree biodiversity plot. Twelve ADR plots are arranged around the corners of each plot; three located in the originally recommended location of the corner and moved counter-clockwise and clock-wise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

A 1m² 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 2020, decay sticks were installed on November 2nd in the Cliffs and Alvars, November 4th in the Indian Woods, and November 6th in the Hogsback.

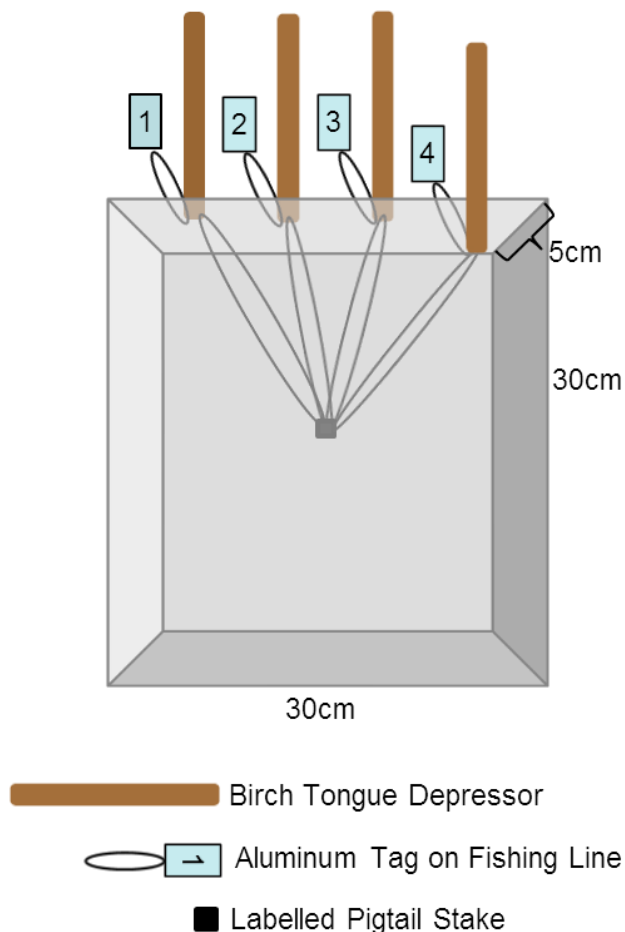


Figure 5.2: Diagram of annual soil humus decay rate (ADR) monitoring plot set-up as viewed from above. Decay sticks 1-3 are installed parallel to the soil surface at a depth of 5cm, separated 10cm from each other. Stick 4 is placed on the soil surface, and all decay sticks are tied to the central pigtail 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 was slowly removed where decay sticks were suspected to be. 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 the Appendix (List B.4).

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 (to 0.001g). Weights were recorded on a datasheet found in the Appendix (Figure C.5).

5.2.4 Data Analysis

Data were analysed using Microsoft Excel 14.0.6 (Microsoft 2010) and R version 4.0.2 (R Development Core Team 2020). Prior to analysis, parametric assumptions were examined. When transformation was required, a square root transformation was applied followed by cautious interpretation of the results (Ahrens et al. 1990).

Percent dry weight loss for each decay stick was calculated, as changes in dry weight can be examined as a proxy for soil decomposition (NRC 2007). Weight loss was compared across years, sites, and stick position using a multivariate analysis of variance (ANOVA). Where interactions occurred, data were split or combined appropriately for subsequent testing, and was followed by Tukey post-hoc testing to determine where differences occurred. To determine how weather could be impacting decay rates a generalized linear model was run. Temperature and precipitation were the independent variables and percent of mass loss was the dependent variable. The interaction of the two independent variables was investigated and the significance was tested with an iterative F-test (Crawley 2007).

5.3 Results

5.3.1 Annual Decay Rates

In 2020, 143 of a possible 144 sticks were recovered from the annual decay rate plots. One surface stick was lost during this sampling year from the Cliffs and Alvares forest stand. Decay sticks positioned below ground lost significantly more mass than those positioned on the soil surface ($p < 0.001$), regardless of year or site (Table 5.1). Significant differences were observed between all three sites, with the lowest rates recorded in Hogsback and the highest observed in Indian Woods ($p < 0.005$).

Table 5.1: Decay rates measured as percent mass loss of decay sticks from Cliffs and Alvars, Indian Woods, and Hogsback forest stands from 2011 to 2020. (SD= Standard Deviation).

	Cliffs & Alvars		Indian Woods		Hogsback	
	Average (%)	SD (-/+)	Average (%)	SD (-/+)	Average (%)	SD (-/+)
All Sticks	33.2	17	37.1	19	29.8	17
Below Ground Sticks	39.5	11	42.6	14	33	17
Above Ground Sticks	12.5	9	19.7	14	20.1	15

Decay rates, as represented by percent mass lost, varied with a combination of site and year ($p < 0.001$), so data were split by site and reanalysed separately with one-way ANOVAS, followed by Tukey's post hoc test to determine where the differences occurred. In Cliffs and Alvars, the highest observed decay rate was recorded in 2012, which was significantly higher than most other years (2011, 2015, and 2017 to 2020). 2014 had the second highest rate which was significantly higher than 2015 and 2018 to 2020. The lowest observed decay rate was recorded in 2019, which was significantly lower than 2012-2014 and 2016 ($p < 0.001$, Fig. 5.3). In Hogsback, decay rates in 2011 were the lowest recorded and significantly different than most years (2012 to 2014, 2016, and 2018). The highest recorded rates were observed in 2018 and were significantly higher than 2011, 2015, 2019, and 2020. Decay rates in 2014 were second highest, and significantly different than just the lowest two year (2011 and 2019) ($p < 0.001$, Fig. 5.3). In Indian Woods, the highest decay rates were observed in 2012 and 2013 respectively, and both were significantly different from the years with the lowest recorded rates, 2011 and 2020. 2012 was additionally significantly higher than 2019 ($p < 0.001$, Fig. 5.3).



Figure 5.3: Average decay rate comparison over monitoring years for each forest stand.

Average annual temperature and precipitation were compared to the decay rates observed. These weather variables had no significant effect on decay rates at *rare*. The annual average temperature and precipitation compared to decay rates are found in Figure 5.4.

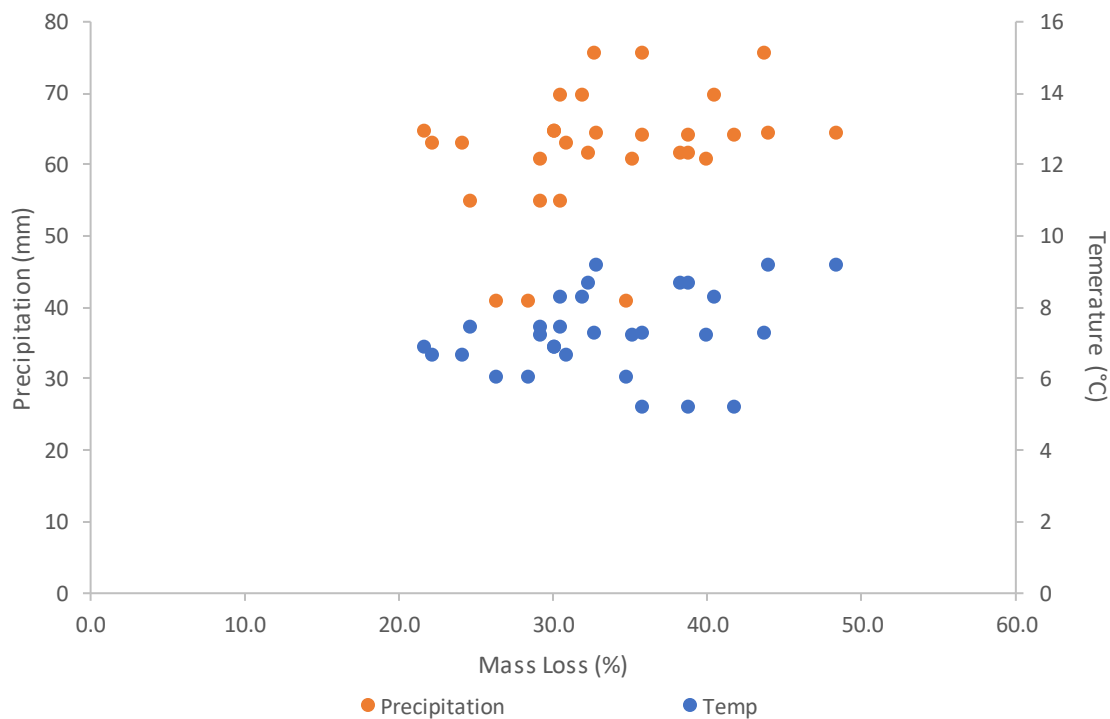


Figure 5.4: A comparison of average temperature and precipitation for Waterloo Region compared to decay rates (percent mass loss) for all three forest stands (Environment Canada- data from Kitchener-Waterloo Weather Station).

5.3.2 Comparison with Baseline Data

EMAN protocol suggests the first five years of monitoring data be used to create a baseline for monitoring programs in order to accurately identify trends. Data from 2010 to 2015 were used to identify averages and standard deviations for decay rates for each plot (Table 5.2).

Using these data, we can compare recent monitoring results to the averages for each location to determine if a monitoring season fell within or outside of these averages. Values that are outside of the given ranges may be indicative of environmental change impacting *rare's* forest ecosystems, particularly if observed repeatedly in consecutive years. In 2018, decay rates observed in Cliffs and Alvars and Hogsback forests were respectively below and above the established baseline range. In 2019 and 2020, all monitoring plots were below the baseline range (Table 5.2).

Table 5.2: Average decay rate (%) for each monitoring plot for monitoring seasons 2016 to 2020. Baseline range was determined from 2010 to 2015 data, numbers below baseline range are bolded and above baseline range are italic.

Plot	Baseline	Standard Deviation	2016	2017	2018	2019	2020
Cliffs & Alvars	35.9	+/- 6	38.3	31.9	29.1	24.1	29.1
Indian Woods	39.1	+/- 7	38.7	40.5	35.1	30.8	30.4
Hogsback	29.8	+/- 5	32.2	30.5	<i>40</i>	22.1	24.6

5.4 Discussion

To better understand soil health at *rare*, the percent of mass loss of decay sticks was determined for both buried and above ground placements. As expected, decay sticks placed below ground had significantly higher rates of decay than those placed at ground level (Table 5.1) Rates of decay can be strongly influenced from a variety of factors including the activity of invertebrates and microfauna in the soil (Lin et al. 2019; Song et al. 2020). Organisms that help facilitate decomposition in soil include saprotrophic bacteria and fungi, nematodes, protozoans, mites, earthworms, springtails, and rotifers (Adl 2003; Stirling et al. 2016). Decay sticks placed below ground were more accessible to these soil organisms and moisture, thus higher decay rates are expected as was observed.

Decomposition differed in all three monitoring locations (Table 5.1). Hogsback forest had significantly lower decay rates than the other two forest stands. 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. Sticks in this corner have had consistently lower rates of decay (average 25% mass lost) than the other three corners in this plot (average 32% mass lost). This difference is likely from anaerobic conditions. If decay sticks are continuously exposed to extremely high moisture levels or are completely submerged in water, decay rates can be slowed by lack of oxygen to support microbial activity (Schlentner and Van Cleve 1985; USDA 2007). Because a quarter of the sticks in the Hogsback are anticipated to be

exposed to these anaerobic conditions, decay rates in the Hogsback are expected to remain lower than the other forest stands in future years.

Furthermore, Cliffs and Alvars had significantly lower decay rates than Indian Woods (Table 5.1). Soil moisture, soil mineral composition, and the diversity and abundance of organisms in the soil likely all contribute to these observed differences. Soil mineral composition is the inorganic components of soil consisting of clay, silt, sand, and gravel (Adl 2003). The proportion in which these four minerals are present in soils alters its physical and chemical properties and directly impacts decomposition (Adl 2003). Different mineral composition alters the amount of space in the soil for water, oxygen, and microfauna which in turn alters the rate of decay (Swift et al, 1979; Tibbett et al. 2004; Tumer et al. 2013). Size distribution of soil minerals can affect the diversity and abundance of organisms, as a larger proportion of microfauna prefer soil with a higher silt/sand composition than soil with clay or gravel (Adl 2003). Sequentially, this will affect decay rates (Wagg et al. 2014; Mustonen et al. 2020). Data from a University of Waterloo soil research project at **rare** provides some supports for this theory (Seuradge 2015), as Hogsback soil composition had the lowest ratio of sand and silt of the three forest. However, Cliffs and Alvars had a larger quantity of sand and silt in its soil than Indian Woods. Cliffs and Alvars is a much more heterogenous forest than the other two forest stands, so plot-specific soil samples may be valuable to further understand these differences. Regardless, differences in soil composition and diversity of decomposer organisms are likely contributing to the variances observed between sites to some extent.

Examining differences in decay rate across years is the most important measure in understanding and tracking changes in soil health at **rare**. The past two monitoring years significantly differed from several previous monitoring seasons in all three forest stands and observed rates were outside of established baseline levels (Figure 5.3; Table 5.2). Additionally, in 2018 two locations fell outside the baseline range (Cliffs and Alvars was below average and Hogsback was above average). While recent change should be monitored carefully, it is less alarming that these trends are in the direction of slowing decomposition and that decay rates at **rare** are not increasing with time. This means the amount of carbon released from **rare's** soil has not risen, or accelerated the rise in atmospheric carbon dioxide, a greenhouse gas linked to climate change (Bardgett et al. 2013).

However, decreases in decay rates are concerning as decomposition releases chemicals critical for life, and ramification on ecosystem functions could occur. A decrease in decomposition can alter the amount of carbon, nutrients (such as nitrogen, phosphorous, sulphur), and energy in the soil; altering growth and reproduction of the ecosystems (Swift et al. 1979). This will lead to a decrease in diversity and abundance of not only microfauna in the soil, but of plants and organisms of higher trophic levels (Swift et al. 1979; Gessner et al. 2010). As changes in soil composition have a slow turnover, decreases in decay rates have only been observed recently at **rare**. Of these recent variations three of the seven occurrences were less than 1% different from the baseline range (Table 5.2), raising doubts on how critical the observed changes in decomposition are, and if mitigation measures will be necessary. Therefore, we do not entirely understand the severity of these changes in decomposition, and continued monitoring is essential to identify if they persist or further decline.

Decomposition is widely known to be driven by climate with moisture and temperature as the principle factors that impact rate of decay (Bradford et al. 2016; Amani et al. 2019). Higher

temperatures increase microbial activity which can in turn increase decay rates (Singh and Gupta 1977; Wall et al. 2008; Zhang et al. 2015). The same trend is seen in soil moisture, as increased soil moisture permits increased microbial and plant activity (to a point) resulting in higher decay rates (Wall et al. 2008; Riutta et al. 2016). Therefore, annual temperature and precipitation rates were compared to decay rates at *rare*. Neither temperature nor precipitation had a significant effect on decay rates (Figure 5.4). To improve this analysis for future monitoring years, site specific variables, including soil moisture and temperature, could be measured with the use of data logging equipment rather than relying on weather stations located off property approximately 12km away. With in-situ data, we would be able to more precisely compare decay rates with climate data to better understand what factors are driving decomposition. Equipment required to collect site specific data include a 5TM Moisture and Temperature Sensor and a Standard Em50 Data Logger at each monitoring location (Cliffs and Alvars, Hogsback, Indian Woods) and would cost approximately \$3000.

In addition to weather, there are multiple factors that could be altering soil decay rates at *rare*. Pesticides impacting non-target organisms from local farming or metal contamination from aggregate mining could be reducing microbial decomposer in *rare* forests (Gessner et al. 2010). Excess nitrogen can decrease the diversity of fungal decomposer, and potentially affect soil insects (Allison and Martiny 2008; Gessner et al. 2010). Additionally, acidification of soil can alter fungal and detritivore community composition and reduce diversity in forests (Gessner et al. 2010). Data collected in Indian Woods and Hogsback during salamander monitoring suggests pH has slightly decreased in both stands in the past three years and potentially contributing to the decrease in decomposition (no data available for Cliffs and Alvars). However, this provides only a general impression of the chemical composition in these forest stands, as these samples were not collected at the ADR plots and exact microhabitat may vary. A collection of soil samples for analysis should be completed to better understand soil properties and factors affecting decay rates. Samples collected can be sent to the University of Guelph Soil and Nutrient Laboratory to gain a better understanding of pH, nutrient concentrations, and heavy metal concentrations at all sites. This would be an expense of approximately \$250 per sample and should be completed every five or ten years depending on available resources. Sampling chemical properties in each forest stand will help *rare* understand how land use is influencing soil properties, which can be related to other monitoring programs. It also has the potential to help develop management practices, such as increasing buffers around forest stands in response to unfavourable results.

5.5 Conclusion

Changing decay rates can be indicative of global climate change or local environmental pressures. Monitoring at *rare* does not show an increase in decay rates and therefore mitigation measures are not required to prevent an increase in atmospheric carbon dioxide. However, there was a decrease in decomposition in the last two monitoring years, that if persists or worsens, can limit the amount of resources and energy available within these terrestrial ecosystems. As these processes occur over long timescales, it is pertinent to continue monitoring to be able to separate changes in climate from changes caused by other external pressures.

Furthermore, improvements can be made to the soil monitoring program by monitoring soil moisture, temperature, and/or other climate variables in plots each month to allow for a closer

comparison of these variables to decay rates. The collection of soil samples for analysis of chemical composition will also improve our understanding of soil properties. These monitoring improvements would require additional field equipment and expenses.

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Appendix A: Maps and Coordinates

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

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

Section one (N 43° 22.980' W 80° 21.541')

- Riparian grassland (milkweed, goldenrod, grasses)
- Stop past the sedge wetland, toward the river at the solitary shrub

Section two (N 43° 23.025' W 80° 21.426')

- Riparian meadow with trees and shrubs on south side (starts when pass single tree)
- Generally, walk towards wooden post
- Stop at old fallen tree in middle of field, within direct view of the osprey tower, 100m

Section three (N 43° 23.058' W 80° 21.222')

- Riparian area with trees on south side (grasses, sedges, small shrubs, goldenrod) (starts when cross stream on trail)
- Stop in open grass area with small hill on right hand side just after trail turns away from river, before continuing into forest

Section four (N 43° 23.120' W 80° 21.017')

- Mainly coniferous forest trail with open canopy areas, on cliffs
- Stop when path forks to small lookout over the river to the left, break in cedar dominance

Section five (N 43° 22.986' W 80° 20.625')

- Deciduous forest trail (after stake in ground)
- Stop at large fallen tree over trail, trail has moved around log; cliffs on south side and open meadow (milkweed, raspberry, goldenrod, one Oak) on north side

Section six (N 43° 22.761' W 80° 20.617')

- Open shrub land (at the turn after the hill)
- Stop at alvar on the left hand side of trail right after the old car on the right hand side, large red pine on trail edge and large white pine further back near alvar

Section seven (N 43° 22.767' W 80° 20.697')

- Deciduous forest trail (turn to go into alvar after woods stop and open up again)
- Stop at large alvar, ~10m after tall Oak tree

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

- Open shrub land (after big left turn)
- Stop on second boardwalk

Section nine (N 43° 22.793' W 80° 20.901')

- Grand Trunk Trail, deciduous forest
- Stop at culvert in wetland

Section ten (N 43° 22.901' W 80° 21.250')

- Grand Trunk Trail, dense shrub growth on both sides of trail

- Stop at entrance to Osprey Tower path to the north, and path to Slit Barn to the south

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Section one (N 43° 22.584' W 080° 22.569')

- Coniferous forest (Ash trees, Cedar trees, shrubs)
- Stop at swampy meadow just past culvert (goldenrod, cattails, milkweed)

Section two (N 43° 22.601' W 080° 22.469')

- Meadow (milkweed, goldenrod, grasses, sedges)
- Stop at junction of trails

Section three (N 43° 22.541' W 080° 22.454')

- Black Locust plantation and meadow
- Stop halfway through plantation area, where tree has grown around top wire of fence on east side

Section four (N 43° 22.482' W 080° 22.430')

- Meadow (milkweed, goldenrod, grasses, sedges) on west side of transect, Spruce tree forest on east side
- Stop at third large Spruce tree on east side, about halfway down the straight portion of the trail

Section five (N 43° 22.424' W 080° 22.301')

- Spruce and deciduous forest
- Stop where wet area ends (will change from year to year), small clearing to the north, several small trees leaning across path

Section six (N 43° 22.476' W 080° 22.064)

- Meadow (grasses, sedges) and Walnut tree plantation
- Stop halfway down straight section of walnut trees, dead and fallen White Pine on north side with young maples around it

Section seven (N 43° 22.568' W 080° 22.158')

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

Section eight (N 43° 22.635' W 080° 22.273')

- Maple Lane Trail (deciduous forest of Sugar Maple and shrubs)
- Stop near small pile of logs on south side of trail

Section nine (N 43° 22.606' W 080° 22.437')

- Meadow (vetch, goldenrod, grasses, sedges, scattered trees and shrubs)
- Stop halfway before the junction of trails, between two stumps on north side of trail

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

***Park before first driveway**

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

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

Section two (N 43° 23.131' W 080° 22.523')

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

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

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

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

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

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

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

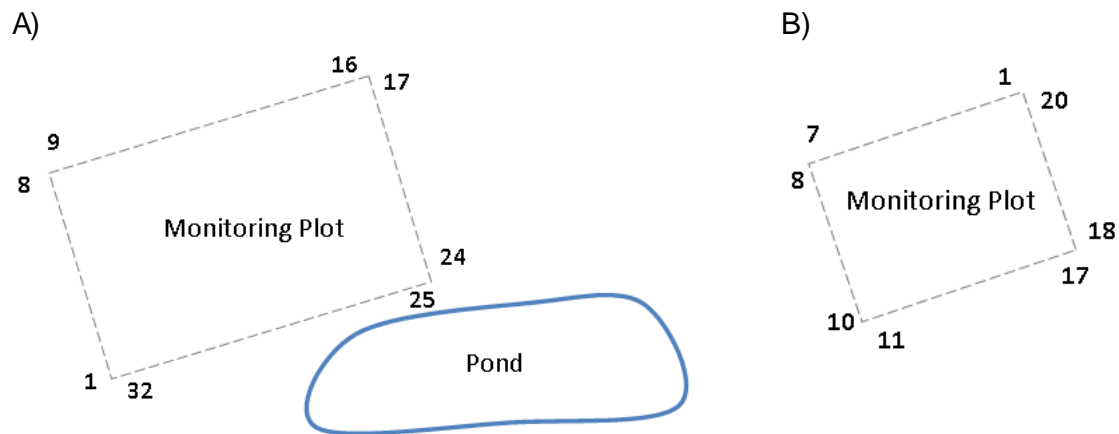


Figure A.3: Layout of artificial cover objects (ACOs) on salamander monitoring plots in A) Indian Woods and B) Hogsback.

Table A.2: GPS coordinates of plots used for forest canopy and tree biodiversity monitoring in Indian Woods, Cliffs and Alvars, and the Hogsback.

Plot	Latitude (DMS)	Longitude (DMS)	Latitude (DD)	Longitude (DD)
IW1	43°22'27.27"	80°21'51.45"	43.37424	80.36651
IW2	43°22'26.12"	80°21'56.08"	43.37392	80.36558
IW3	43°22'23.62"	80°21'54.78"	43.37323	80.36522
CA1	43°22'46.30"	80°21'1.34"	43.37953	80.35037
CA2	43°22'44.64"	80°21'0.21"	43.37907	80.35006
CA3	43°22'43.72"	80°20'57.91"	43.37881	80.34942
HB1	43°22'24.18"	80°21'11.10"	43.37338	80.35308
HB2	43°22'23.28"	80°21'12.66"	43.37313	80.35352
HB3	43°22'22.08"	80°21'14.46"	43.3728	80.35402

APPENDIX B: Field Equipment

List B.1: Suggested butterfly monitoring field equipment

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

List B.2: Salamander monitoring equipment list

- Field data sheets A and B on waterproof paper
- Clipboard
- Pencils
- Nitrile gloves
- 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 Lamb's Inn well water
- Flagging tape
- Aluminum tags
- Digital camera
- Luster Leaf Rapitest Digital Soil pH Meter

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

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

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

List B.4: 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: Field Codes and Data Sheets

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

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

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

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

Date:				Start:						Temp_START:					
Transect:				Finish:						TEMP_END:					
Observer:	A			B			C			D			E		
	Sun:			Sun:			Sun:			Sun:			Sun:		
Recorder:	Wind:			Wind:			Wind:			Wind:			Wind:		
	W1	S	W2	W1	S	W2	W1	S	W2	W1	S	W2	W1	S	W2
Cabbage White															
Clouded Sulphur															
Monarch															
Red Admiral															
Notes:															

Figure C.1: Sample of butterfly monitoring field sheet (available on *rare* server).

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

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

[illegible]

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

APPENDIX D: Species Lists

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

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

†Denotes observation only seen during annual butterfly count

*Denotes incidental observation outside of monitoring or annual count

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

Table D.4: 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 (1/4).

Species	Earliest Record By Year											Annual Butterfly Count	Earliest Record at rare
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020		
Aphrodite Fritillary												July 17 (2016)	July 17 (2016)
Acadian Hairstreak											Jul-14	July 13 (2008)	July 13 (2008)
American Lady	May-20		May-15	May-22	Jul-17	May-20	May-17	Jul-28	May-28	May-31	Jul-17	July 10 (2010)	May 15 (2012)
American Snout			Jul-11					Jul 21	Jul 30			July 10 (2010)	July 10 (2010)
Appalachian Brown		Jul-06	Jun-18	Jul-02	Jul-03			Jul-11	Jun-21	Jun-24	Jun-30	July 2 (2011)	June 18 (2012)
Arctic Skipper	Jun-03			Jun-04	Jun-03		Jun-10		Jun-07			July 10 (2010)	June 3 (2010)
Baltimore Checkerspot			Jun-26					Jul-06			Jun-29	July 3 (2011)	June 26 (2012)
Banded Hairstreak		Jul-12	Jun-25	Jul-15	Jul-03	Jun-29	Jul-18	Jul-11	Jul-18	Jul-24	Jul-14	July 2 (2011)	June 25 (2012)
Black Dash	Jun-08		Jul-14	Jul-30	Jul-30	Jul-28	Aug-4	Jul-18	Jul-4	Aug-15	Jul-15	July 10 (2010)	June 8 (2010)
Black Swallowtail	May-04	May-30	May-14	May-22	May-23	May-20	May-17	May-17	May-25	Jun-17	May-20	July 10 (2010)	May 4 (2010)
Broad-Winged			Jul-14	Jul-12	Jul-18							July 10 (2010)	July 10 (2010)
Bronze Copper			Jun-06		Jun-20	Jun-24	Jun-14	Aug-14	Jun-21	Jul-1	Jun-25	July 2 (2011)	June 6 (2012)
Cabbage White	May-03	May-19	May-14	May-21	May-21	May-20	My-17	May-15	May-23	May-21	May-20	July 2 (2011)	May 3 (2010)
Clouded Sulphur	May-04	May-31	May-14	May-21	May-24	May-19	May-17	May-15	May-23	Jun-6	May-22	July 10 (2010)	May 4 (2010)
Columbine Duskywing	May-19		May-31			May-19							May 19 (2010)
Common Buckeye		Sep-15	Jun-06					Jul-4					June 6 (2012)
Common Sootywing		Aug-04	Jun-07	May-22	Jun-06	May-26	Jul-11	Jun-7	May-24	Jul-8	Jun-10	July 10 (2010)	May 22 (2013)
Common Wood-Nymph	Jun-25	Jun-14	Jun-18	Jun-13	Jun-19		Jun-29	Jun-14	Jun-28	Jul-10	Jun-16	July 2 (2011)	June 13 (2013)
Compton Tortoiseshell		Jul-12								Aug-12			July 12 (2011)
Coral Hairstreak						Jul-08		Jul-4	Jul-4	Jul-8	Jul-6	July 2 (2011)	July 2 (2011)
Crossline Skipper						Jul-15	Jul-12	Jul-14	Jul-18		Jul-13	July 2 (2011)	July 2 (2011)

Table D.4: The earliest record of observation for each butterfly species historically observed at *the rare Charitable Research Reserve* (2/4).

Species	Earliest Record By Year											Annual Butterfly Count	Earliest Record at rare
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020		
Delaware Skipper	May-24	Jul-11	Jul-09	Jul-04	Jul-10	Jul-06	Jul-07	Jul-04	Jun-25	Jun-18	Jul-06	July 10 (2010)	June 2 (2009)
Dion Skipper			Jul-14								Jul-15	July 13 (2008)	July 13 (2008)
Dun Skipper		Jul-06	Jun-26	Jul-12	Jul-04	Jul-08	Jul-12	Jun-28	Jun-11	Jul-16	Jul-14	July 10 (2010)	June 26 (2012)
Eastern Comma	May-14	Jun-01	May-15	May-27	Jun-19	Jun-16	May-19	May-15	May-23	May-21	Jun-25	July 10 (2010)	May 14 (2010)
Eastern Pine Elfin						May-20			Jun-8		May-27		May 20 (2015)
Eastern Tailed Blue		Jul-27	Jul-14	Jul-15	Aug-06	Jun-26	May-30		Jul-3	Jul-10	May-22	July 11 (2006)	July 3 (2018)
Eastern Tiger Swallowtail	May-19	Jun-01	May-14	May-22	Jun-06	May-21	May-24	May-17	May-25	May-27	Jun-1	July 2 (2011)	May 14 (2012)
European Skipper	May-24	Jun-14	May-15	May-30	Jun-10	May-19	Jun-09	Jun-12	Jun-11	Jul-1	Jun-15	July 2 (2011)	May 15 (2012)
Eyed Brown	Jun-15	Jul-05	Jun-08	Jun-25	Jun-20	Jun-16	Jun-9	Jun-28	Jun-25	Jul-8	Jun-29	July 2 (2011)	June 8 (2012)
Giant Swallowtail		Jun-08	May-15	May-30	Jun-10	May-27	May-24	May-30	May-23	Jun-6	Jun-2	July 11 (2006)	May 15 (2012)
Grey Comma						Jun-22	Aug-2	Jul-17	May-23		May-21	July 18 (2020)	July 17 (2017)
Great Spangled Fritillary	Jun-21	Jul-11	Jun-18	Jul-02	Jun-25	Jun-29	Jun-27	Jul-4	Jun-15	Jul-8	Jul-8	July 10 (2010)	June 15 (2018)
Hackberry Emperor											Aug-19		Aug 19 (2020)
Harvester		Aug-19			Jun-21	Jun-22		Aug-1					Jun 21 (2014)
Hickory Hairstreak												July 11 (2006)	July 11 (2006)
Hobomok Skipper	May-26	Jun-01	May-30	Jun-04	Jun-06	May-27	May-31	May-30	May-30	Jun-11	Jun-8	July 2 (2011)	May 26 (2010)
Inornate Ringlet	May-19	Jun-06	May-14	May-21	Jun-02	May-26	May-24	May-29	May-28	Jun-3	Jun-1	July 2 (2011)	May 14 (2012)
Juvenal's Duskywing	May-26	May-25	May-14	May-21	May-23	May-20	May-17	May-15	May-23	May-31	May-22		May 14 (2012)
Least Skipper		Aug-05	May-28	Jul-30	Jun-19	Jun-23	Jun-14	Jun-14	Jun-7	Jul-8	Jun-30	July 19 (2009)	May 28 (2012)
Leonard's Skipper							Aug-14		Aug-18	Aug-12			Aug 14 (2016)
Little Glassywing		Jul-06	Jul-10	Jul-12	Jun-30	Jul-03	Jul-18	Jul-14	Jul-4			July 2 (2011)	July 2 (2011)

Table D.4: The earliest record of observation for each butterfly species historically observed at *the rare Charitable Research Reserve* (3/4).

Species	Earliest Record By Year											Annual Butterfly Count	Earliest Record at rare
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020		
Little Wood-Satyr	Jun-03	Jun-08	May-30	Jun-04	Jun-10	May-28	Jun-06	Jun-12	Jun-7	Jun-24	Jun-9	July 2 (2011)	May 28 (2015)
Little Yellow												July 11 (2006)	July 11 (2006)
Long Dash		Jun-14	May-28	Jul-04	Jun-27	Jun-17		Jul-17	Jun-19	Jul-24		July 2 (2011)	May 28 (2012)
Meadow Fritillary		Jun-14	May-28	Jul-04	Jun-27	Jun-17				Jun-27		July 10 (2010)	July 10 (2010)
Milbert's Tortoiseshell	Jun-21	Jul-19	Jun-11		Jun-16	May-21	Jun-14		Jun-26		Jun-25		June 11 (2012)
Monarch	Jun-25	May-30	May-14	Jun-19	May-30	Jun-11	Jun-09	May-23	May-24	May-24	Jun-9	July 2 (2011)	May 14 (2012)
Mourning Cloak	May-04	Jun-07	May-14	May-21	May-24	Jun-22	May-17	May-23	May-23	May-31	May-20	July 10 (2010)	May 4 (2010)
Mulberry Wing								Jul-31	Jul-4			July 20 (2013)	July 4 (2018)
Mustard White		Aug-12											Aug 12 (2011)
Northern Broken-Dash			Jun-26		Jul-04	Jul-03	Jul-11	Jul-14	Jul-4	Jul-31	Jul-8	July 10 (2010)	June 26 (2012)
Northern Crescent	Jun-03	Jun-07	Jun-04	Jun-12	Jun-03	Jun-02	Jun-09	Jun-7	May-23	Jul-3	Jun-3	July 10 (2010)	May 21 (2009)
Northern Pearly-Eye	Jun-03	Jun-20	Jun-11	Jun-13	Jun-19	Jun-10	Jul-17	Jun-14	Jun-15	Jul-03	Jun-25	July 10 (2010)	June 3 (2010)
Orange Sulphur	Jun-30	Jul-19	May-14	Jun-04	May-30	Jun-02	Jun-10	Jun-7	May-24	Aug-12	Jun-26	July 10 (2010)	May 14 (2012)
Painted Lady	May-04		May-15	May-21	Jul-07	May-19	May-17	May-24		May-31	Jul-6		May 4 (2010)
Pearl Crescent		May-25	May-14	May-22	May-26	May-19	May-17	May 15	May 24	May-24	May-22	July 2 (2011)	May 14 (2012)
Peck's Skipper		Jul-11	Jun-18	Jul-06	Jul-03	Jun-22	Jul-12	Jun-8	Jul-26			July 2 (2011)	June 8 (2017)
Question Mark	May-19	Jun-07	May-17	Jun-14	Jul-21	May-28	May-19	Jun-7	Jun-21	Jun-7	May-20	July 10 (2010)	May 17 (2012)
Red Admiral	May-03	May-25	May-14	Jun-04	May-21	May-19	May-17	May-15	May-29	May-21	May-20	July 10 (2010)	May 3 (2010)
Red-Spotted Purple	Jun-01	Jun-14	May-25	Jun-04	Jun-19	Jun-02	Jun-14	Jun-12	Jun-7	Jun-12	Jun-17	July 10 (2010)	May 25 (2012)
Silver-Bordered Fritillary				Jun-03		May-27	May-16					July 2 (2011)	May 16 (2016)
Silver-Spotted Skipper	Jun-08	Jun-20	Jun-25	Jun-13	Jun-13	Jun-11	May-31	Jun-26	May-30	Jul-3	Jun-25	July 10 (2010)	June 8 (2010)

Table D.4: The earliest record of observation for each butterfly species historically observed at *the rare* Charitable Research Reserve (4/4).

Species	Earliest Record By Year											Annual Butterfly Count	Earliest Record at rare
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020		
SilveryBlue						Jun-02	May-31	Jun-7	May-24	Jun-6	May-21		June 2 (2015)
Silvery Checkerspot			Jun-20		Jul-18					Jul-3			June 20 (2012)
Spicebush Swallowtail										Jul-23	Aug-21		July 23 (2019)
Spring Azure	May-04	May-20	May-15	May-21	May-21	May-20	May-17	May-15	May-23	May-21	May-20		May 4 (2010)
Striped Hairstreak		Jul-26		Jul-12	Jul-18	Jul-02	Jul-12		Jul-19	Aug-12	Jul-20	July 11 (2006)	July 02 (2015)
Summer Azure	Jun-08	Jul-05	Jun-11	Jun-13	Jun-13	Jun-11	Jun-09	Jun-14	Jun-7	Jun-7	Jun-1	July 2 (2011)	June 1 (2020)
Tawny Emperor		Aug-04	Jul-17	Jul-25	Jul-28	Jul-16	Jul-06		Jul-3	Aug-16	Jul-14	July 10 (2010)	July 3 (2018)
Tawny-edged Skipper		Jul-22		Jul-16	Jul-17	Jun-16	Jul-05	Jul-26	Jun-18		Jun-9	July 2 (2011)	June 16 (2015)
Variegated Fritillary			Jul-05							Aug-21			July 5 (2012)
Viceroy	Jun-08	Jun-20	May-25	Jun-04	May-28	May-26	Jun-09	Jun-8	May-28	Jun-12	Jul-09	July 10 (2010)	May 25 (2012)
White Admiral		Jun-14	Aug-01		Aug-18	Jun-22				Aug-12		July 11 (2006)	June 14 (2011)
Wild Indigo Duskywing	May-17		Jul-11		Jul-28	May-28	Jun-09	Jul-25	May-28	Aug-14	May-27	July 2 (2011)	May 17 (2010)