Credit Valley Conservation

Terrestrial Monitoring Program Report

2003 - 2008

Monitoring Wetland Integrity within the Credit River Watershed
Chapter 2: Wetland Anurans

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CREDIT VALLEY CONSERVATION

Monitoring Wetland Integrity within the Credit River Watershed

Chapter 2: Wetland Anurans 2003-2008

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ABSTRACT

Anurans of the Credit River Watershed: Summary of Monitoring Results 2003-2008
Temporal Trends: No significant temporal trends in total anuran richness and total occupancy were detected. A significant increase in the proportion of sites with full chorus was detected across the whole of the watershed and in the Middle physiographic zone.
Spatial Trends: Species richness was significantly higher in the Middle physiographic zone when compared to the Lower zone for five of the six survey years. Non-statistical observations revealed consistently lower anuran measures in the Lower zone when compared to the Middle and Upper zones. There were no significant differences in anuran parameters between the Middle and Upper physiographic zones.
Individual Species Trends: Green Frog occupancy increased in the Lower watershed while Leopard Frog occupancy increased in the Upper watershed. Green Frog and Spring Peeper were the most commonly identified species during the surveys. Spring Peeper was consistently absent from sites within the Lower physiographic zone. American Bullfrog was absent from the Lower and Upper zones, while Western Chorus Frog was absent from the Middle zone.
Relationships with Landscape and Vegetation: Measures associated with habitat loss and fragmentation (habitat patch size, percent urban land cover within 2 kilometres, non-native vegetation) appeared to have the greatest correlation with anuran richness, occupancy, and abundance.
Recommendations: Continue to track trends and relationships closely over subsequent survey years in order to determine if intervention is necessary to maintain stable anuran populations in the watershed. Land use and wetland vegetation data should be kept up-to-date. Call-detection methodology should be reviewed due to current limitations in the estimation of abundance counts.

Monitoring Wetland Integrity within the Credit River Watershed.
Chapter 2: Wetland Anurans 2003-2008

Anuran monitoring was conducted as part of the terrestrial component of an integrated watershed monitoring program established at Credit Valley Conservation. Anuran species were surveyed at 26 wetland sites throughout the Credit River Watershed from 2003 to 2008. Surveys were based on call detection and followed protocols of the Marsh Monitoring Program. Collected data were used to examine temporal and spatial trends in anuran species richness, site occupancy, and abundance, as well as the relationship between anuran parameters and both landscape and wetland vegetation measures. Eight frog species and one toad species were detected over the six year study period. Green Frog (*Rana clamitans*) and Spring Peeper (*Pseudacris crucifera*) were the most commonly detected species. At this point in monitoring, the nature of collected data favoured the use of non-parametric equivalents to chosen parametric statistical tests. No significant trends were detected in total species richness or total site occupancy, though Green Frog occupancy was found to be increasing in the Lower watershed and Leopard Frog occupancy was found to be increasing in the Upper watershed. In addition, there were significant increases in the number of sites with full chorus calling at both the watershed level and within the Middle physiographic zone. From a spatial perspective, anuran measures were consistently diminished in the lower physiographic zone when compared to those in the Middle and Upper zones. This pattern is likely a result of extensive urbanization and development present in the Lower watershed. Urban effects were also evident in comparisons with landscape and vegetation measures in that measures associated directly or indirectly with habitat loss and fragmentation (habitat patch size, percent urban land cover within 2 kilometres, non-native vegetation) had the greatest correlation with anuran richness, occupancy, and abundance. Further years of monitoring data and updated landscape information are required to determine if anuran populations in the Credit River watershed are stable.

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1.0 INTRODUCTION

1.1 BACKGROUND

Ontario has undergone dramatic change since the arrival of European settlers. A landscape that was historically dominated by forest cover and wetlands has experienced rapid removal of wildlife habitat and fragmentation of remaining natural areas by agriculture, residential development and roads (Larson et al. 1999). The amount of natural cover in a landscape often dictates what forest or wetland dependent wildlife species can be supported. Although this is especially evident with the disappearance of large mammals such as the gray wolf (*Canis lupus*) and black bear (*Ursus americanus*) from the highly developed landscapes of southern Ontario, this is also true for smaller organisms such as amphibians, birds, and plants. Habitat loss, and therefore reductions in overall natural cover can negatively affect a species in many ways; a) some species are observed to disappear from the landscape, b) some species are observed to decline in abundance, while c) others fail to reproduce (Noss and Cooperrider 1994). These effects are not mutually exclusive, and may feed back on one another to exacerbate negative impacts.

Fragmentation generally results in the reduction of total habitat available, the isolation of remaining patches, decreases in patch size and often an increase in total patch number (Noss and Cooperrider 1994; Fahrig 2002). Plant and wildlife species that are adapted to living in well-connected landscapes now face the challenge of acquiring all resources crucial to survival and reproduction from isolated habitat patches (Beier and Noss 1998). This is especially difficult for species that are poor dispersers or require large home ranges to fulfill their foraging and breeding needs (Andren and Nurnburger 1994). Isolation from other patches can result in reduced ability to secure required resources, reduced gene flow and ultimately extirpation of a species (Fleury and Brown 1997). Habitat loss and fragmentation can therefore have serious implications for populations, biodiversity and ecosystem functioning (Hess and Fischer 2001; Noss and Cooperrider 1994). In the case of amphibians, the richness and site occurrence of individual species were found to be influenced by environmental variables at both local and landscape scales (Hecnar and M'Closkey 1998; Buskirk 2005).

1.2 THE IMPORTANCE OF LONG TERM MONITORING

The baseline classification of natural areas and populations is important for understanding spatial patterns of structure, composition, processes, and functions. However, the mapping and classification of populations usually produce only a "snapshot" of community status and do not capture the dynamics of the system (Gebhardt et al. 2005). A monitoring program must be implemented if one hopes to obtain an understanding of trends in dynamics and processes. Monitoring, or the investigation of the status of something over time (Clarke et al. 2004), is useful in providing managers with information on which long-term plans can be based because trends over time can be used to infer future conditions. A superior monitoring program should address both biotic and abiotic environments, as well as function across multiple scales. From an ecological perspective, a monitoring program can also provide insight into cause and effect relationships between environmental stressors and ecosystem responses (Reeves et al. 2004). However, this is not always the end result of monitoring. Though statistical associations can be established between indicators, Sutter (2001) suggests that monitoring does not necessarily address causation.

1.3 WHY MONITOR ANURANS?

Despite continued debate on the subject (Blaustein 1994), most researchers are convinced that significant amphibian population declines have occurred both locally and globally in recent times (Alford and Richards 1999; Blaustein et al. 1994; Skerratt et al. 2007). As a result, approximately one-third of the world's 6000 amphibian species have now been classified as threatened (Stuart et al. 2004). Biological and behavioural characteristics of these species may be contributing to the perceived decline. Amphibians, for instance, tend to have relatively narrow habitat tolerances that can amplify the effects of habitat loss (Findlay and Houlahan 1997). In addition, a biphasic life cycle requires access to both aquatic environments and suitable terrestrial habitat for post-metamorphic stages (Todd et al. 2009). The effects of habitat loss are also exacerbated by the limited dispersal abilities of most amphibian species (Gibbs 1998; Bowne and Bowers 2004). Beyond these innate biological explanations, several potential external causes of this global decline have been proposed by researchers. Chief among these causes are habitat loss, disease, toxins in the environment, and large-scale climate change (Alford and Richards 1999).

Most threats to amphibian populations are either directly or indirectly related to habitat loss and fragmentation (Cushman 2006). Not only does habitat loss directly remove breeding and overwintering sites (Hecnar and M'Closkey 1996), but the resulting disturbance and loss of habitat connectivity in the surrounding landscape matrix can have a detrimental effect on isolated populations. Connectivity within a landscape is particularly important for anuran survival because populations have been shown to experience frequent local extinction and turnover (Hecnar and M'Closkey 1996). Positive relationships have been found between amphibian population and the area of forest in the surrounding landscape (Knutson et al. 1999) and between species richness and increased forest cover (Gibbs 1998), while negative relationships have been found between amphibian populations and both urban land (Knutson et al. 1999) and landscape barriers such as roads (Fahrig et al. 1995). This interference in the dispersal of individuals is of concern because studies have revealed considerable reductions in dispersal success and juvenile survival in fragmented landscapes (Cushman 2006; Becker et al. 2007).

Anthropogenic toxins in the environment can also have detrimental effects on amphibians. Beyond any immediate lethal consequences, environmental toxins can affect anuran populations by increasing the susceptibility of both young and mature individuals to disease (through immuno-suppression), reducing reproductive potential, retarding the growth and development, and causing physical deformities that could decrease the ability to avoid predation (Carey and Bryant 1995; Gilbertson et al. 2003). Biological pathogens can have similar health-related impacts. Viruses and fungal infections can cause "waves" of disease to move rapidly through amphibian populations (Laurance et al. 1996). They can also linger within a population, remaining present in healthy individuals and becoming active only when sub-lethal stress suppresses immune capabilities (Bishop 1992). The most critical microbiological threat currently facing anuran populations is Chytridiomycosis, a skin infection caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Longcore et al. 1999). This pathogen has been identified as the most significant disease affecting biodiversity in vertebrates (Skerratt et al. 2007) due to its impact on amphibians worldwide. Though Chytridiomycosis is not of critical concern in Ontario as of 2009, the associated fungus has been found to be widespread in anurans of the Northeastern United States as recently as 2007 (Longcore et al. 2007).

There is evidence in the literature that climate change is influencing anuran behavior and phenology. Broad patterns seem to suggest that some temperate-zone anuran populations are now breeding earlier in the year (Blaustein et al. 2001). In a historical study near Ithaca NY, four of six monitored frog species were found to be calling 10 to 13 days earlier in 1999 than they were in 1900 (Gibbs and Breisch 2001). Data suggested that this temporal shift was due to daily temperature increases during 5 of the 8 months key to gametogenesis. A similar climate-influenced pattern of earlier calling in amphibians was detected by Beebee (1995) in Great Britain. In addition to temperature, it has been proposed that large-scale decreases in rainfall or snow could also affect anuran survivorship and reproductive behavior by causing ponds to fill later in the season and persist for a shorter period. This would provide less time for metamorphosis and potentially lead to increased competition and predation as individuals become concentrated at fewer breeding sites (Donnelly and Crump 1998). In modeling experiments, the ability of anurans to maintain global population levels in the face of climate alteration was found to be highly dependant on long-range dispersal abilities of the affected species (Araujo et al. 2006). Warming in cooler northern ranges would create new colonization opportunities for species with a limited habitat range but unlimited dispersal abilities.

1.4 THE LANDSCAPE OF THE CREDIT RIVER WATERSHED

The Credit Valley Conservation Authority is a municipal-level agency mandated with environmental and hydrological monitoring and regulation within the borders of the Credit River watershed. This watershed encompasses approximately 980 square kilometres of land in southern Ontario, Canada (Credit Valley Conservation 2003). The River flows southeast for nearly 100 kilometres from its headwaters in Orangeville to its drainage point at Lake Ontario. There are 21 subwatersheds within the main watershed boundaries, as well as zones which drain directly into Lake Ontario. These subwatersheds contain almost 1500 kilometres of streams and creeks that empty into the Credit River (Credit Valley Conservation 2003). As of 2007 the watershed was composed of 23% natural communities, 10% successional communities, 37% agricultural land use, and 29% urban area (Credit Valley Conservation 2007c). Approximately 6% of the watershed (about 32% of the natural cover) is classified as wetlands, while 12% of the watershed (63% of natural cover) is forested (Credit Valley Conservation 2007c).

Though encompassing many unique landscape formations, the Credit River watershed can be divided into three main physiographic zones based on topography, morphology, geologic origins, and the boundaries of individual subwatersheds. The Lower physiographic zone is highly urbanized, containing over 85% of the human population of the watershed. The soils in the Lower watershed are primarily clay, resulting in poor surface water infiltration (Credit Valley Conservation 2007b). The topography of this area is relatively flat and has, in places, been significantly altered by human development. The amount of both wetland and forest cover in the Lower watershed has been classified as poor when applying proposed government guidelines (Environment Canada, 2004). The Middle zone contains the Niagara Escarpment, a region of steep slopes, rocky outcrops, and thin soil (Credit Valley Conservation 2005). These topographic factors, along with limited urbanization and the implementation of protective legislation, have resulted in the Middle watershed containing the greatest proportion of natural cover in the watershed. The Upper physiographic zone lies above the escarpment and is characterized by hilly moraines, glacial spillways, and permeable loamy soils (Credit Valley Conservation 2005; Credit Valley Conservation 2007b). Agricultural land use dominates portions of the Upper watershed, though it also consists of several large wetland complexes and a protected moraine (Oak Ridge) that is shared with the Middle zone.

2.0 OBJECTIVE: MONITORING QUESTION

The purpose of the anuran component of the terrestrial monitoring program at Credit Valley Conservation is a) to detect long-term trends in a selected set of indicators in order to identify trends in anuran population health at the watershed scale, b) to infer the impact of these trends on overall watershed and aquatic health, and c) to provide meaningful data on which watershed management decisions can be based. The central question being asked is as follows:

ARE POPULATION TRENDS OF ANURAN SPECIES IN THE CREDIT RIVER WATERSHED STABLE?

More specifically, 1) are species richness and anuran abundance changing through time; 2) have anuran species richness and abundance differed spatially in the watershed; 3) has anuran site occupancy changed through time; and 4) has anuran community similarity changed through time. The framework for anuran monitoring at this stage in the program is reviewed in Table 1. The analysis techniques listed in the table are those chosen to fit the quality and scope of data currently available. This analytical framework may change as the monitoring program progresses and data quality improves.

Table 1. Anuran Monitoring Framework.

Monitoring Question	Monitoring Variable(s)	Unit of Measurement	Analysis Method
Part 1: Anuran Species Richness			
Is anuran species richness changing over time in the Credit River watershed?	Total number of species detected in the watershed	Count	N/A
	Number of species detected per monitoring site	Count	- Temporal Analysis: Friedman Test - Power Analysis: Linear Regression
Are there spatial differences in anuran species richness in the Credit River watershed?	Total number of species detected in each physiographic zone	Count	- Chi-Square test for independence
	Mean number of species detected per site in each physiographic zone.	Count	- Spatial Analysis: Kruskal-Wallis
Is Anuran richness correlated with landscape parameters in the Credit River watershed?	Mean number of species detected per site compared to three landscape metrics	Count	- Correlation: Spearman Rank
Is anuran richness correlated with vegetation parameters in the Credit River watershed?	Mean number of species detected per site compared to several wetland vegetation parameters	Count	- Correlation: Spearman Rank
Part 2: Anuran Site Occupancy			
Is the proportion of sites occupied by anurans changing over time in the Credit River watershed?	Proportion of total sites occupied	Proportion (%)	-Trend Analysis: Cochrane-Armitage - Power Analysis: Cochrane-Armitage
Are there spatial differences in anuran site occupancy in the Credit River watershed?	Number of sites occupied per physiographic zone	Count	- Chi-Square test for independence
Is there a relationship between anuran site occupancy and landscape metrics in the Credit River watershed?	Site occupancy by anurans tested against three landscape metrics	Presence/Absence	- Trend Analysis: Logistic Regression - Power Analysis: Logistic Regression
Is there a relationship between anuran site occupancy and vegetation parameters in the Credit River watershed?	Site occupancy by anuran species tested against several wetland vegetation parameters	Presence/Absence	- Trend Analysis: Logistic Regression - Power Analysis: Logistic Regression

Monitoring Question	Monitoring Variable(s)	Unit of Measurement	Analysis Method
Part 3: Anuran Site Occupancy			
Is anuran abundance changing over time in the Credit River watershed?	Proportion of sites at which full chorus was detected	Proportion (%)	- Trend Analysis: Cochrane-Armitage - Power Analysis: Cochrane-Armitage
Are there spatial differences in anuran abundance in the Credit River watershed?	Mean full chorus detections per site per physiographic zone	Count	Spatial Analysis: Kruskal-Wallis
Is there a relationship between anuran abundance and landscape metrics in the Credit river watershed?	Mean full chorus detections per site tested against three landscape metrics	Count	- Trend Analysis: Logistic Regression - Power Analysis: Logistic Regression
Is there a relationship between anuran abundance and vegetation parameters in the Credit River watershed?	Mean full chorus detections per site tested against several wetland vegetation parameters	Count	- Trend Analysis: Logistic Regression - Power Analysis: Logistic Regression
Part 4: Anuran Community Similarity			
Is anuran community similarity changing over time in the Credit River watershed?	Jaccard’s Similarity Index	Index	- Temporal Analysis: N/A - Power Analysis: Linear Regression

3.0 METHODS

3.1 ANURAN SURVEYS

Data were collected for six years (2003 to 2008) at 26 wetland monitoring sites distributed throughout the Credit River watershed (Figure 1). The 26 anuran monitoring sites are listed in Appendix 1. Eight of these sites were located in the Lower physiographic zone, eight were located in the Middle physiographic zone, and ten were located in the Upper zone. Data collection followed protocols of the Marsh Monitoring Program (Konze and McLaren 1997) and involved three yearly visits to each site spaced a minimum of 15 days apart. Surveys were conducted within the last 15 days of April, of May, and of June in order to capture both early and late breeding species. Monitoring activities during each 15 day period were split into four specific groups of stations, often referred to as travel routes. Each "route" was surveyed on a single night and involved a specific sequence of sites. These surveys began no less than half an hour after sundown and were completed by midnight. Monitoring was restricted to nights with winds measuring less than "3" on the Beaufort scale (less than 12 km/hr) and to nights consistent with a set temperature established for each survey period.

The monitoring station at each wetland site consisted of an area in front of the observer that was semi-circular in shape and roughly 100 m in radius (Figure 2). Protocols specified a three minute survey period at each station during which an estimate of anuran numbers could be established through auditory detection of calls. Calls estimated to be originating beyond the 100m radius were still counted, but noted in the data as being "distant". The estimate of anuran abundance was based on a 4-level ordinal calling code recommended by the marsh monitoring program. A calling code of "0" noted that no frog or toad was heard. A code of "1" (C1) was assigned to a species when calls were not overlapping and, as a result, a specific count of individuals could be achieved. A code of "2" (C2) was an estimate count of a species because some calls were overlapping and not all individuals could be distinguished. A code of "3" (C3) indicated a full chorus, in which calls were continuous and overlapping. It was still possible to distinguish between anuran species when full chorus was present, but it was not possible to estimate the number of individuals. Additional collected information, recorded on-site with a portable weather station (Kestral 3000), included air temperature, relative humidity, maximum wind speed, average wind speed, and wind direction. Water temperature was measured using a digital thermometer (Fisher Scientific).

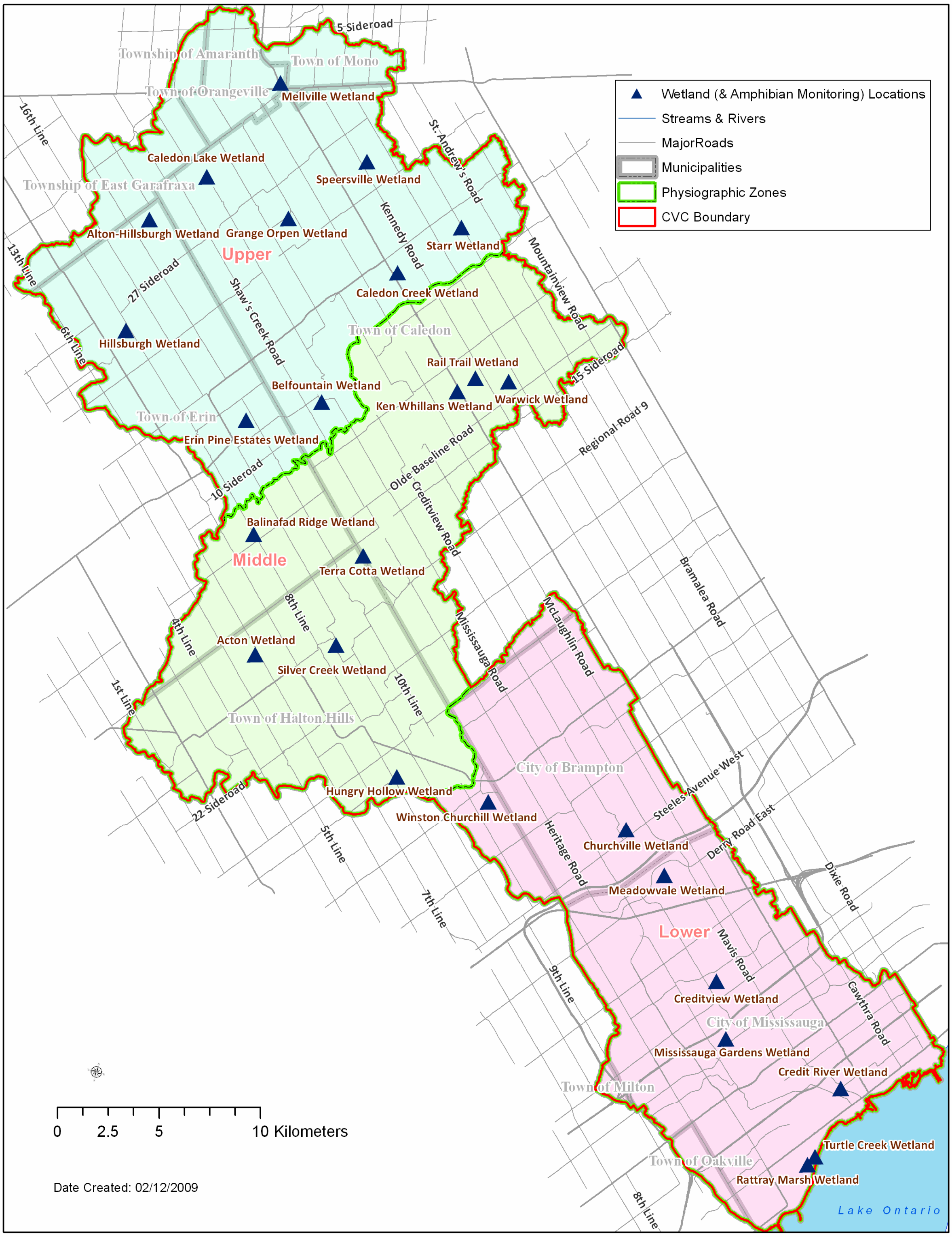

Figure 1. A map of the Credit River Watershed and the jurisdiction of the Credit Valley Conservation authority showing the location of 26 anuran monitoring stations. Physiographic zones have been separated using colour.

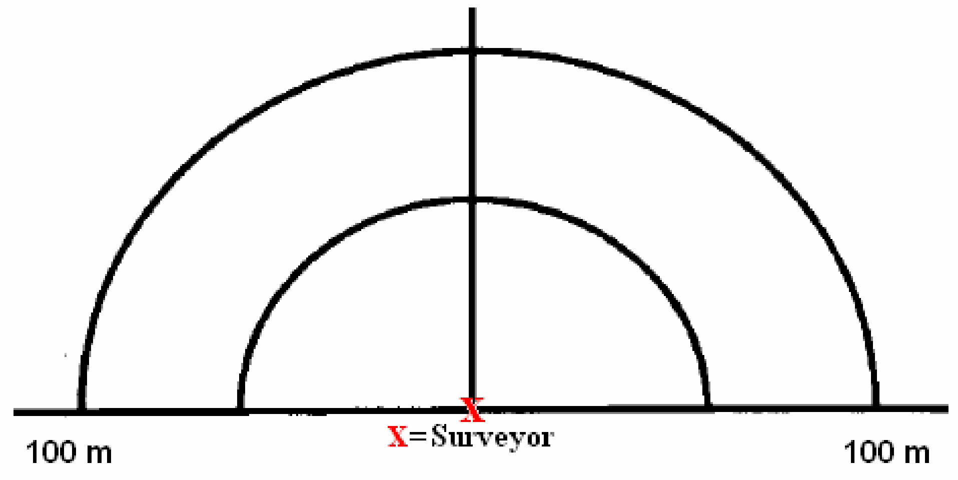

Figure 2. An anuran monitoring station as defined by the Marsh Monitoring Program. The curved lines show the semi-circular boundaries of the station.

3.2 LANDSCAPE PARAMETERS

The three landscape parameters referenced within this report are habitat patch size, the distance from monitoring plots to the nearest road, and the percent urban land use within 2 kilometres of the plot. They are based on orthophotography collected between 2005 and 2007 over the Credit River watershed. Aerial photographs were interpreted and analysed using ArcGIS, a software package capable of generating information on patterns, structure, and change within a landscape (Credit Valley Conservation Authority 2007a). The measure of "habitat patch size" refers to the total size of the contiguous area of natural habitat within which an anuran monitoring station was nested. The Ecological Land Classification (ELC) community series included in the definition of natural habitat are listed in Appendix 2. Habitat patch size was selected as a landscape parameter because several studies have found negative relationships between anuran population trends and both habitat loss and landscape fragmentation (Cushman 2006). The "distance to the nearest road" measure was chosen because the presence of roads can often limit the movement of species (Gibbs 1998), lead to increased anuran mortality rates (Fahrig et al. 1995; Hels and Buchwald 2001; Mazerolle 2004), or result in altered reproductive behaviour (Eigenbrod et al. 2009; Parris et al. 2009). The percent urban land use within 2 kilometres was measured from the outside of a 30 metre buffer surrounding the approximate centroid of each wetland site. The ELC land use types included in the definition of urban land use are listed in Appendix 2. This matrix measurement was chosen because studies have found negative associations between urban expansion and both anuran abundance (Knutson et al. 1999) and the presence of suitable anuran habitat (Baldwin and DeMaynadier 2009). In addition, Findlay and Houlahan (1997) found that taxa richness increased with an associated increase in the amount of forest cover within 2 kilometres of wetland sites in southeastern Ontario.

3.3 VEGETATION PARAMETERS

Measures of vegetation used in this report are based on data collected through the wetland component of the Terrestrial Monitoring program at Credit Valley Conservation. Data were collected from ground vegetation and regeneration subplots arranged along a 50 metre hydrological gradient at selected wetland monitoring plots. Most anuran monitoring stations had been intentionally paired with a wetland vegetation monitoring plot so that direct comparisons could be made between fauna and vegetation. Vegetation parameters were separated into three general groups (Table 2). Parameters in the ground vegetation group included all herbaceous vegetation regardless of height, as well as all woody vegetation less than 16 cm in height. Parameters in the Regeneration group contained woody vegetation greater than 16 cm in height and less than 10 cm DBH, excluding woody vines. The parameter in the combined vegetation group included all ground vegetation as well as all regenerating woody vegetation greater than 16 cm in height.

Table 2. Wetland vegetation parameters used in comparisons with anuran monitoring data.

Data Group	Parameters
Wetland Ground Vegetation	- Total species per site - Total native species per site - Total non-native species per site - Proportion of non-native species per site
Wetland Woody Regeneration	- Total species per site - Total native species per site - Total non-native species per site - Proportion of non-native species per site
Wetland Combined Vegetation	- Mean coefficient of conservatism score per site

It has been shown that vegetation density and structure can influence amphibian survival (Purrenhage and Boone 2009). Ground vegetation was compared to anuran parameters because the associated plants in this category are the dominant living component of most wetland communities and are likely to have an influence on ground dwelling fauna. Regenerating woody vegetation was compared to anuran parameters because climbing frog species may show a preference for wetlands with a greater variety of vertical structure. The mean Coefficient of Conservatism (mCC), the only combined vegetation parameter, was chosen to examine the influence of vegetation quality on anurans. mCC is a measure based on species-specific scores listed in the Floristic Quality Assessment System for Southern Ontario (Oldham et al. 1995). These Coefficient of Conservatism scores, ranging from zero to ten and assigned only to native species, are estimates of sensitivity and habitat fidelity. The higher the mean Coefficient of Conservatism of a given site, the greater the habitat sensitivity and specificity of the constituent native plants. The ubiquitous Canada Goldenrod (*Solidago canadensis*), for instance, has been assigned a score of 1, while the rare alvar species Dwarf Lake Iris (*Iris lacustris*) has been assigned a score of 10.

3.4 DATA PREPARATION

All frog observations not confirmed to species (including those labelled as "unknown") were removed from the data set before analysis. Observations of Pickerel Frog (*Rana palustris*) were included in species richness and abundance analyses but excluded from all other statistical testing because the associated sample size (only three observations over six years) was too small.

A species presence at a given monitoring station on a given year was based on any call detection of that species, regardless of calling code or the number of visits (out of three) at which it was recorded. The maximum calling code recorded for each species during the three yearly site visits was used when analysing abundance data.

The mean species richness over six years of monitoring was calculated for each site so that correlations could be made between species richness and landscape parameters. This was necessary because landscape data were not available in a year-by-year form but rather as a single "snapshot" representation of land parameters during the general period of monitoring. For logistic regression tests between site occupancy (presence/absence) over the six-year survey period and landscape parameters, a site was considered occupied if the species or group in question was detected in at least two of the six survey years. This was done to avoid the inclusion of temporally isolated occurrences.

Comparisons between anuran data and wetland vegetation parameters could only be made for the years 2005 through 2008 because vegetation community information prior to these years is considered unreliable due to staff turnover and sampling inconsistencies. In addition, comparisons with vegetation data could only be made using 18 of the 26 anuran monitoring stations because this is the number of sites at which both anurans and vegetation were surveyed for the years 2005 through 2008. For logistic regression analyses between site occupancy (species presence/absence) and vegetation parameters, a site was considered occupied if the anuran species was detected in at least two of the four survey years. For similar analyses using full chorus calls, positive detection was defined as full chorus occurring at a site during at least two of four survey years. Vegetation parameters at each site were averaged over the four years in order to be comparable to these anuran occupancy and abundance data.

The Jaccard's Similarity Index was calculated for total species richness between each set of two consecutive survey years (five calculations in total) for both the whole of the watershed and within physiographic zones. It was also calculated between the first and last survey years (2003 and 2008) for both the whole of the watershed and within physiographic zones. Finally, the index was calculated between each two-zone combination (Lower with Middle, etc.) for each separate survey year. The Similarity Index is a calculated value used to compare the species similarity between two samples. A high index value is indicative of low species turnover between tested groups. The Jaccard's Index equation is as follows:

$$Sj = \frac{a}{a + b + c}$$

Where S_j = Jaccard's Similarity coefficient
a = The number of species in group 1 and group 2 (joint occurrences)
b = The number of species in group 1 but not in group 2
c = The number of species in group 2 but not in group 1

3.5 STATISTICAL ANALYSIS

Linear regression and repeated measures ANOVA, the parametric statistical tests chosen in the original study framework to analyse anuran richness and abundance data, could not be performed at the current stage of monitoring. The small number of species detected throughout the survey, combined with a data set replete with zero or near-zero values, made it difficult to meet parametric assumptions. Data were tested for normality and homoscedacticity with Shapiro-Wilks and Levene's test for homogeneity of variance, respectively. The variance of anuran parameters was often consistent between years, but normal distributions could not be obtained even after data transformations. In addition, linear trend analyses were not performed on any variable consisting of less than ten data points because outliers in such a case would have too great an influence on test results. Parametric and non-parametric tests used to analyse anuran data at the current stage of monitoring are described below. All analysis was completed using STATISTICA version 7 (Statsoft Inc.) unless otherwise noted, with results being considered significant at $p < 0.05$.

3.5.1 Trend Analysis

Trend analysis was preformed on anuran occupancy data to examine possible sequential patterns across the six study years. Cochrane-Armitage trend analysis was employed for these tests because the anuran occurrence data was in the form of proportions (such as the percent of total sites occupied per year). The Cochrane-Armitage test is a non-parametric analysis of trend designed to examine proportion instead of count data, and is the closest proportion-based equivalent of parametric linear regression. Tests were conducted using *XL*STAT, a third party add-on to Microsoft Excel.

3.5.2 Temporal Analysis

Temporal analysis was performed in order to examine patterns in anuran richness data among the six survey years. Per-site data were analysed using the Friedman test (Zar 1974), a non-parametric equivalent of a repeated measures ANOVA which tests the null hypothesis that observations in different groups share the same median values. The Friedman test is preferable over other non-parametric options when dealing with trend data because it takes into account that values between groups (years in this case) are dependant. This is not a true trend analysis, however, because it is not testing for a sequential change in the dependant variable. It is instead testing whether there are significant differences in the median values of at least two of the groups. In lieu of post-hoc analysis, which is not available for the Freidman test, a trend must be inferred after visually examining the size and directions of any differences between individual groups.

3.5.3 Spatial Analysis

Spatial analysis was performed to examine differences in anuran data among the three physiographic zones of the Credit River watershed. The Chi-square test for independence was used to look for interaction between year and zone effects. This non-parametric test for independence is based on a probability distribution of observed and expected values between groups (year) and conditions (zone). Chi-square comparisons were completed using free software provided by Preacher (2001). If independence was shown between year and zone, physiographic zones were then compared using a Kruskal-Wallis test. This analysis of variance by ranks (Zar 1974) is a non-parametric version of a One-Way ANOVA in which treatment levels of a predictor variable (zones in this case) are independent of one another. Zone effects had to be analysed on a year by year basis because Kruskal-Wallis cannot account for year effect when all survey years are used in the same analysis. An associated post hoc test was then run to determine which zone relationships were significant.

3.5.4 Comparisons with Landscape and Vegetation Parameters

Relationships between continuous anuran variables (such as "mean species per site") and continuous landscape or vegetation parameters were examined using Spearman rank correlations. The binary nature of anuran occupancy and full chorus detection data, however, made comparisons using rank-based correlation inappropriate. Instead, binary occupancy and abundance data was tested against landscape and vegetation parameters using simple logistic regression with one continuous predictor. Though still considered a parametric option, logistic regression is a non-linear estimation method in which the binary response variable is assumed to follow a binomial distribution (Quinn and Keough 2002). The assumptions of normality and homoscedacticity do not apply as with linear regression.

3.5.5 Statistical Process Control

Statistical Process Control (SPC) is an application which uses ecological time series to develop monitoring thresholds. Though similar to regression, SPC is not based on hypothesis testing. Instead, this application seeks to identify instances when a time series exhibits non-random behaviour. A series demonstrating this non-random behaviour is considered "out of control" and, as such, unsuitable for developing a monitoring baseline. If the time series exhibits natural random variability around a reference point (usually the mean), the series is considered "in control". Data that are in control can be treated as a baseline from which monitoring thresholds can be generated. A minimum of five years of "in control" data are required to set monitoring thresholds.

SPC control charts are divided into six zones based on the mean value of a time series and its standard deviation adjusted to sample size (Figure 3). The Upper and Lower critical limits, defined as +/- 3 standard deviations from the mean, encompass the "in control" range of the time series. Control charts for stable systems will not contain any points outside the +/- 3 SD "in control" range. In such a case, the series represents appropriate reference conditions from which the +/- 3 SD critical limit thresholds can be adopted. Series with points outside the "in control" range may represent an ecosystem under stress or moving slowly toward some alternate state. A data set in this type of flux would not provide appropriate reference conditions on which thresholds could be based.

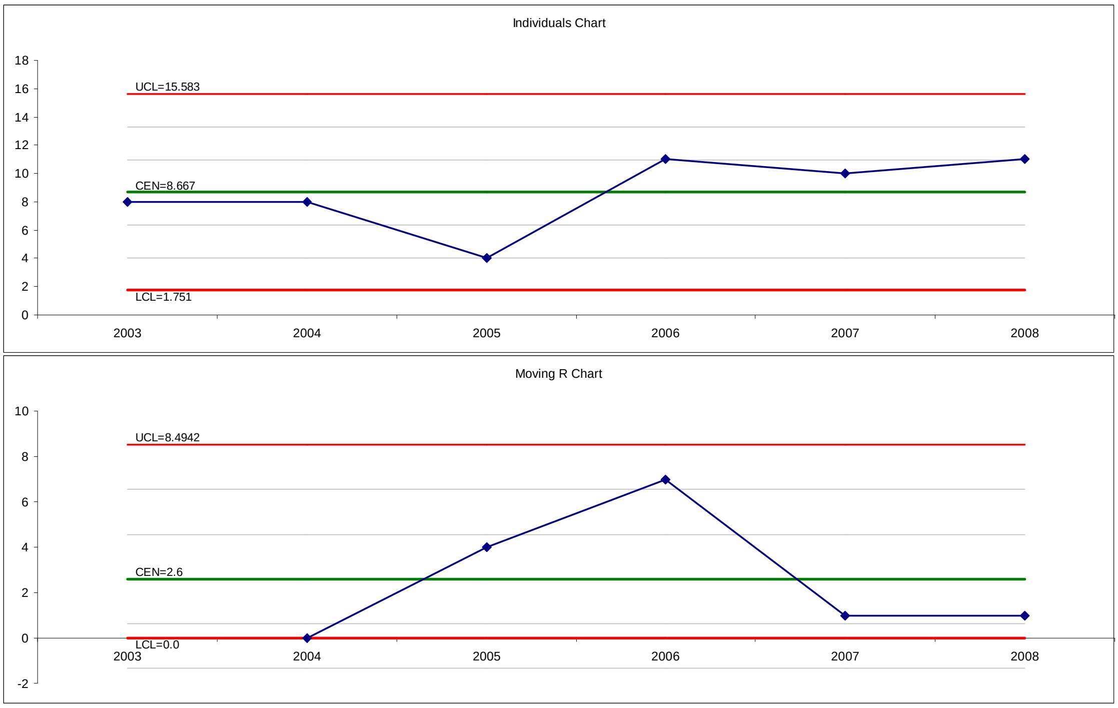

Figure 3. Statistical Process Control charts for Leopard Frog site occupancy. Both the individual moving average chart (showing the parameter values for each year) and the Moving Range chart (showing the amount of change in the parameter between years) are "in control" from 2003 to 2008. The green line indicates the time series mean and the red lines show the upper and lower critical limits (+/- 3standard deviations). Since the data set is in control, the mean from 2003-2008 can be used as a baseline for future monitoring.

Values that exceed the critical limits are associated with significant increasing or decreasing trends and should always be investigated thoroughly. Often in monitoring, however, early detection of changing trends or significant decline in parameters is desired in order to recognise problems before the effects become irreversible. To address these early detection concerns, the upper and lower warning limits on control charts are represented by +/- 2 standard deviations from the mean. Trends of series that exceed the warning limits but not the critical limits should be tracked closely for further changes. A time series with values falling within the warning limits is exhibiting natural variability and is considered to be stable and not of concern based on SPC alone. All statistical control charts for anuran data were generated using SPC XL software package, a third-party add-on application to Microsoft Excel.

3.5.6 Power Analysis

Power analysis uses baseline monitoring data – such as the "in control" time series detected using SPC – to determine if additional sampling is required to detect significant trends or desired effect sizes. In ecological monitoring, additional sampling can be interpreted as an increase in the number of monitoring sites at which data is collected. Effect size refers to the detectable change in the time series over a given interval, and is often related to a specific monitoring threshold. Power analysis is most frequently used to calculate the sample size required to detect a significant change, or to calculate a specific effect size given the variance of the time series being considered. It can also be used to determine the maximum effect size detectable in a time series given the scope of sampling. All power analysis presented in this report were conducted using PASS (NCSS Inc.). The form of Power Analysis applied to a given parameter was dependant on the specific statistical test being used to examine the associated data. Tests were run using a set power level (1-beta) of 90% and a significance level (alpha) of 20%, the analysis design recommended by an external statistician (P. Zorn, personal communication). Effect sizes were designed to be detected over a five year monitoring period.

Power analysis of the anuran data was conducted for the linear trend analyses stipulated in the original monitoring framework and for the Cochrane-Armitage and logistic regression analyses actually applied in this report. Though linear regression was not applied to monitoring data at this time, linear trend analysis was still examined for power because monitoring thresholds (1 SD, 2 SD, and 3 SD) were calculated for the planned linear analyses using SPC. Power analysis specific to both linear regression and Cochrane-Armitage allowed for the calculation of required site numbers for detecting SPC thresholds as well as the calculation of minimum effect sizes. Power analysis specific to logistic regression requires continuous covariates (the predictor variables) to be normally distributed, so landscape and vegetation parameters (the covariates) were - when required - normalized by transformation before power analysis proceeded. It should be noted that the actual logistic regressions reported in the results section were performed with raw (pre-normalized) covariates. Though not necessarily identical in results, the normalized and non-normalized results should be comparable because covariate transformations did not change the significance status of relationships with binary anuran data. PASS presents the effect size for logistic regressions as an odds ratio, a descriptive relationship with limited interpretive value. As a result, power analysis was only used to calculate the minimum number of monitoring sites required to detect a significant logistic relationship between the binary anuran parameter and chosen landscape or vegetation covariate.

4.0 RESULTS AND DISCUSSION

4.1 DESCRIPTIVE RESULTS

4.1.1 Identified Species

Nine anuran species were detected at monitoring stations during the study period (Table 3). The associated species of conservation concern (SCC) tier rankings listed in Table 3 are based on rarity and status of concern within the boundaries of the Credit River watershed (Credit Valley Conservation 2009), while the S ranking of species is a provincial signifier assigned by the Ministry of Natural Resources. Tier 3 species American Toad (*Bufo americanus*), Gray Tree Frog (*Hyla versicolor*), Green Frog (*Lithobates clamitans*), Leopard Frog (*Rana pipiens*), and Spring Peeper (*Pseudacris crucifera*) are Species of Urban Interest that are thought to be relatively secure in more natural environments. Tier 2 species Bullfrog (*Rana catesbeiana*), Pickerel Frog (*Rana palustris*), and Wood Frog (*Lithobates sylvaticus*) are known to be locally rare or are exhibiting signs of decline. Western Chorus Frog is ranked as Tier 1 because it is formally considered a species of conservation concern. This denotes that it has been designated as threatened or endangered in Canada, or has been designated rare at a provincial level. The only anuran previously detected in the watershed but not appearing in the survey records was Mink Frog (*Rana septentrionalis*), a Tier 2 species listed as "secure" provincially.

Table 3. Anuran species detected at anuran monitoring stations in the Credit River watershed from 2003 to 2008.

Common Name	Latin Name	S Ranking (Provincial)	SCC Tier (Local)
American Bullfrog	*Rana catesbeiana*	Uncommon	2
American Toad	*Bufo americanus*	Secure	3
Gray Tree Frog	*Hyla versicolor*	Secure	3
Green Frog	*Lithobates clamitans*	Secure	3
Northern Leopard Frog	*Rana pipiens*	Secure	3
Pickerel Frog	*Rana palustris*	Uncommon	2
Spring Peeper	*Pseudacris crucifera*	Secure	3
Western Chorus Frog	*Pseudacris triseriata*	Uncommon	1
Wood Frog	*Lithobates sylvatica*	Secure	2

Gray Tree Frog, Spring Peeper, and Western Chorus Frog are all classified as tree frogs, or Hylidae. They can be distinguished by the presence of discs on the tips of their toes, a morphological adaptation that assists in vertical movement (Harding 1997). Despite this climbing ability, tree frogs such as Western Chorus Frog may never leave near-ground terrestrial habitats for arboreal ones (MacCulloch 2002). American Bullfrog, Green Frog, Northern Leopard Frog, Pickerel Frog, and Wood Frog are known as true frog species, or Ranidae. These frogs are usually associated with aquatic habitats, though some species (such as Wood Frog) may favour wooded terrestrial habitats during non-breeding periods (MacCulloch 2002). As a member of the Bufonidae family, American Toad is distinguishable from the eight recorded frog species by its short legs, stocky body shape, and thickened skin that protects against water loss (Harding 1997).

4.1.2 Anuran Site Occupancy

It is not surprising that Green Frog and Spring Peeper were detected at the greatest number of monitoring sites throughout the watershed (Figure 4), as these are considered two of the most abundant frog species in the Great Lakes region (Harding 1997). American Bullfrog, Western Chorus Frog, and Wood Frog – the three recorded species assigned an SCC Tier ranking of 2 (locally rare) - occupied fewer total sites than those species listed as Tier 3. Pickerel Frog was only recorded on multiple years at a single site (Silver Creek). It should be noted that "detection", in this case, has been defined as presence at a site in at least two of the six survey years. Based on a non-statistical observation of trends in occupancy patterns of individual species (Appendix 3), Green Frog and Spring Peeper were also the only anurans to exhibit relatively stable levels of site occupancy over the whole of the watershed. Year-to-year occupancy numbers for the other seven species were notably more unstable. American Toad had the most erratic between-year occupancy of any detected species, both at the whole of the watershed and within physiographic zones. This may be a product of low site fidelity in the reproductive behaviour of this species because Petranka and Holbrook (2006) found that American Toads rarely use the same breeding sites from year to year. It should also be noted that Mink Frog, though not detected in the surveys, was frequently heard by other monitoring crews in the upper portions of the watershed.

The above conclusions assume that the detection probability for all species at all sites was 1. In reality, detectability may vary between species and will usually be less than absolute. MacKenzie et al. (2002) recommend a likelihood-based method for estimating anuran occupancy rates when detection probability is less than 1. As an example, they determined that estimated toad occupancy using the likelihood model was 44% higher than the proportion of sites at which the species was actually identified. Evidence of competition between species for site occupancy was not examined at this point in monitoring, though patterns of intra-species competition may not be significant when other site-level characteristics are considered. In a Swiss study, Buskirk (2005) found that occurrence of amphibian species was positively correlated with the densities of other species and concluded that competition was a less important predictor than variation in site quality.

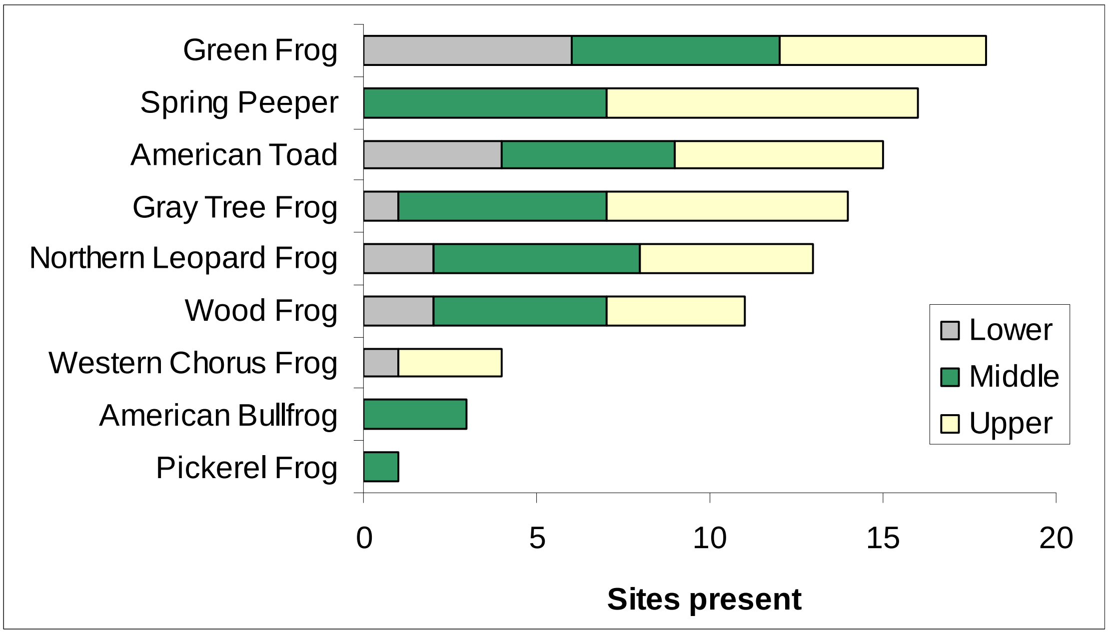

Figure 4. The total number of monitoring sites each anuran species occupied over the course of the study period, separated by physiographic zone. Occupancy has been defined as presence at a site in at least two of the six survey years.

4.1.3 Anuran Abundance

The call-based method of estimating anuran abundance places strict limitations on the type of population data that can be reported and analysed. Foremost, the semi-ordinal nature of the call numbering system makes the call values non-proportional. This prohibits any combination of call values for analytical purposes, including the calculation of such parameters as “Mean calling level per species”. It is relatively easy to obtain an accurate count when individuals are spread out evenly across the sampling area but difficult if the same numbers of individuals are clustered. In addition, call counts provide no information on age structure or caller health (Van Wieren and Zorn 2005). Clearly, surveys were dominated by the detection of call code one, or individual calls (Figure 5). This level was nonetheless excluded from analysis because its detection, though providing the desired individual counts, tells only part of the population story. The other part is hidden within the records of call codes two and three. Accurate individual counts are obscured by the overlapping calling associated with these two higher levels, so the three call codes are not relatable in terms of establishing complete anuran abundance numbers. However, full chorus detection can be used to identify wetland sites with large meta-populations of certain species or to identify those species which occur at the highest numbers in the watershed. These points form our justification for including only call code three in all abundance analyses.

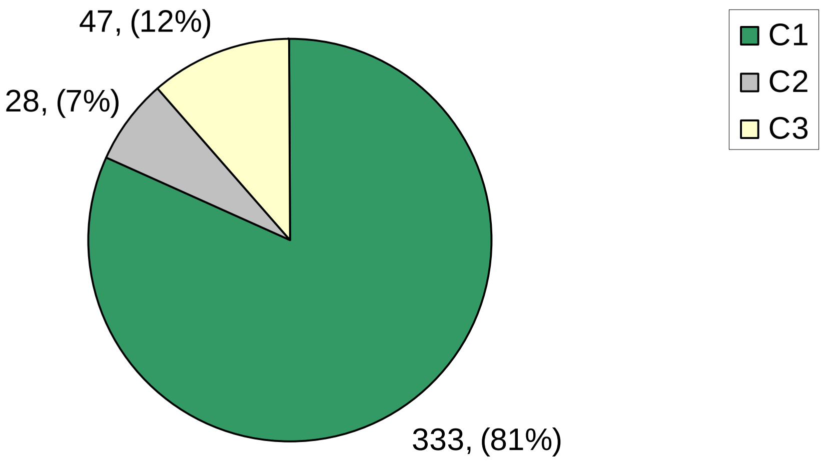

Figure 5. The total number and proportion (%) of calls detected in each of three calling levels, based on the maximum level detected per site per year.

Relative rankings of anuran species based on call detections (Figure 6) are similar to the rankings by site occupancy (Figure 4) save that Spring Peeper outranks Green Frog in terms of total detections. American Toad had a relatively low total detections count given the species' high occupancy ranking, suggesting that individuals are well dispersed throughout the watershed but not locally abundant. The breakdown of total full chorus detections (n = 47) by anuran species (Figure 7) shows that Spring Peeper alone was responsible for 68% of all full chorus detections while tree frog species together were responsible for over 90% of full chorus detections.

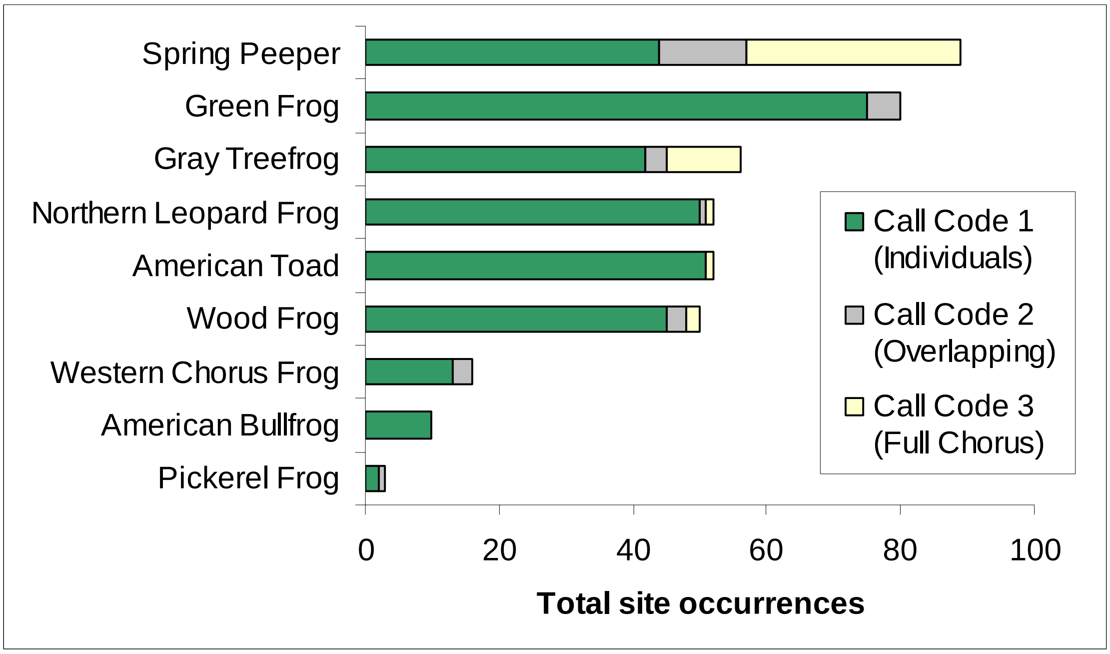

Figure 6. The distributions of C1, C2, and C3 call detections as separated by anuran species. Data is presented for all detections from 2003 to 2008 (408 in total), based on the maximum level detected per site per year.

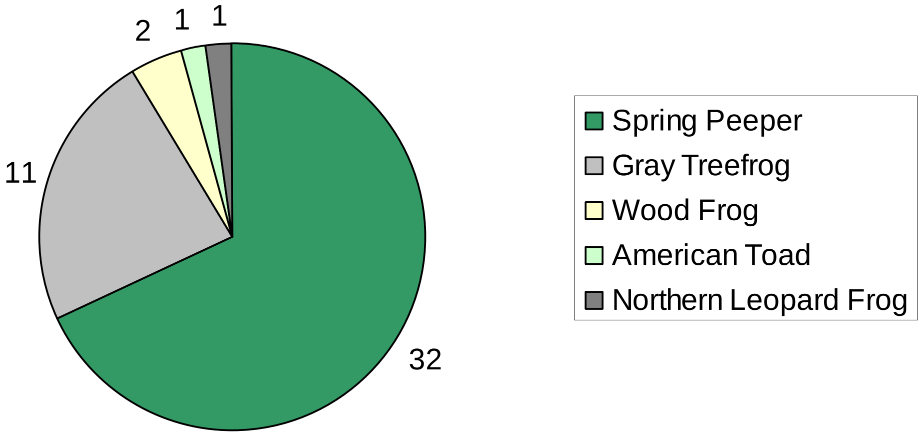

Figure 7. The distributions of C1, C2, and C3 call detections as separated by anuran species. Data is presented for all detections from 2003 to 2008 (408 in total), based on the maximum level detected per site per year.

4.1.4 Anuran Health at Individual Monitoring Sites

The watershed health perspectives adopted by the CVC integrated monitoring program require that results presented in Parts 1 through 4, along with the statistical process control and power analysis sections, focus primarily on temporal and spatial trends at the level of either the entire watershed or within and between the three physiographic zones. However, it seems pertinent to highlight some individual stations (Table 4) that may in the future function as reference sites. The qualitative "Healthy" and "Unhealthy" rankings are based on high or low values of mean per-site species richness, mean per-site full chorus detection, and the number of survey years in which anurans occupied the sites. Appendix 4 summarizes anuran parameters for each individual monitoring station in the study.

Table 4. The monitoring sites with the healthiest and unhealthiest anuran communities. [b]

Zone	Monitoring Site	Mean Species Richness [a]	Years Occupied	Mean Full Chorus Detections [a]
	Healthiest Anuran Communities			
Lower	Winston Churchill Wetland	4	6	0
Middle	Balinafad Ridge	5	6	1
Both Upper and Whole of the Watershed	Speersville Wetland	5.17	6	1.33
	Unhealthiest Anuran Communities			
Both Lower and Whole of the Watershed	Mississauga Gardens	0.33	2	0
Middle	Hungry Hollow Wetland	0.83	4	0
Upper	Hillsburgh Wetland	0.83	3	0
	Grange Orpen Wetland	0.67	3	0

[a] Per year, over six years
[b] Rankings are qualitative but based on high or low values of mean per-site species richness, mean per-site full chorus detection, and the number of survey years in which anuran occupied the sites.

The healthiest monitoring site in the whole of the watershed was Speersville wetland, a marsh located in the Upper physiographic zone. An isolated location, in combination with the permanent presence of water, may be influencing the ranking of this site. The healthiest site in the Lower physiographic zone (Winston Churchill Wetland) is also relatively isolated, contains permanent water, and is located in a portion of that zone with the least amount of urban influence. Conversely, the unhealthiest site in the Lower zone (Mississauga Gardens) is located close to a highway and in the Middle of a major urban centre. Hungry Hollow, the unhealthiest site in the Middle physiographic zone, is in a moist regenerating lowland that has little permanent water present and is at times dominated by red ants. These ants produce a stinging bite and have the potential to ward off other fauna species (Todd et al. 2008). Balinifad ridge may be the healthiest site in the Middle watershed due to the presence of ample permanent water and a variety of vegetation, while Grange Orpen wetland may be one of the unhealthiest sites in the Upper watershed because conditions there were often quite dry. Exogenous or site-level factors influencing the ranking of Hillsburgh Wetland remain unclear.

Identifying populations of species of concern in the Lower watershed is important from a conservation perspective due to both the scarcity of natural area in that zone and the threats to sensitive species presented by continued urbanization. Western Chorus

Frog, a locally-ranked species of concern (Credit Valley Conservation 2009), was detected each survey year at Creditview Wetland in the urbanized Lower zone. This is unexpected given that Creditview is a small patch of natural habitat isolated within a densely populated urban community. The site is likely supporting a remnant population left over from habitation that occurred before development. The persistence of Chorus Frog at this site could be due to restoration efforts and an absence of direct disturbance, as the wetland is fenced-off and public access is restricted. Lower levels of direct disturbance, along with proximity to the Middle watershed, may also be responsible for the fact that Winston Churchill Wetland was the only site in the Lower physiographic zone at which both Gray Treefrog and Spring Peeper were detected over the six survey years.

4.2 TEMPORAL ANALYSIS

4.2.1 Anuran Species Richness

Monitoring Question: Is anuran species richness changing over time in the Credit River Watershed?

- Trend analysis could not be applied to total and mean richness at this time.
- There was a significant difference in mean species richness between at least two of the survey years.

Trend analysis could not be performed on total species counts at either the watershed level or within zones due to a deficit of data points (only six over the survey period). There were no observable trends in the richness data over the six year study period (Figure 8). The slight increase in total species observed in 2004, 2005, and 2008 was due to the detection of Pickerel Frog during those three years. Aside from this anomaly, however, each of the other eight anuran species found in the watershed were detected during every year of the study. These results are consistent with a study of anuran richness in southwestern Ontario ponds by Hecnar and M'Closkey (1998), which found no significant change in regional species richness over time. Figure 8 also shows the total number of anuran species detected in each physiographic zone by year. The lower richness in the Upper zone in 2005 is due to the absence of American Toad and Northern Leopard Frog that year.

There appeared to be no strong trend in mean species richness over the study period either watershed-wide or within physiographic zones (Figure 9). The Friedman test did show, however, that there was a significant difference in mean per-site species richness (watershed-wide) between at least two of the six survey years (X^2 = 13.514, p = 0.019). This difference was likely between the lower means in 2004/2005 and the higher mean in 2008. Friedman tests on individual physiographic zones also found that there was a significant difference in per-site species richness in the Upper watershed between at least two of the six survey years (X^2 = 15.608, p = 0.008), likely a result of the decreased richness in 2005.

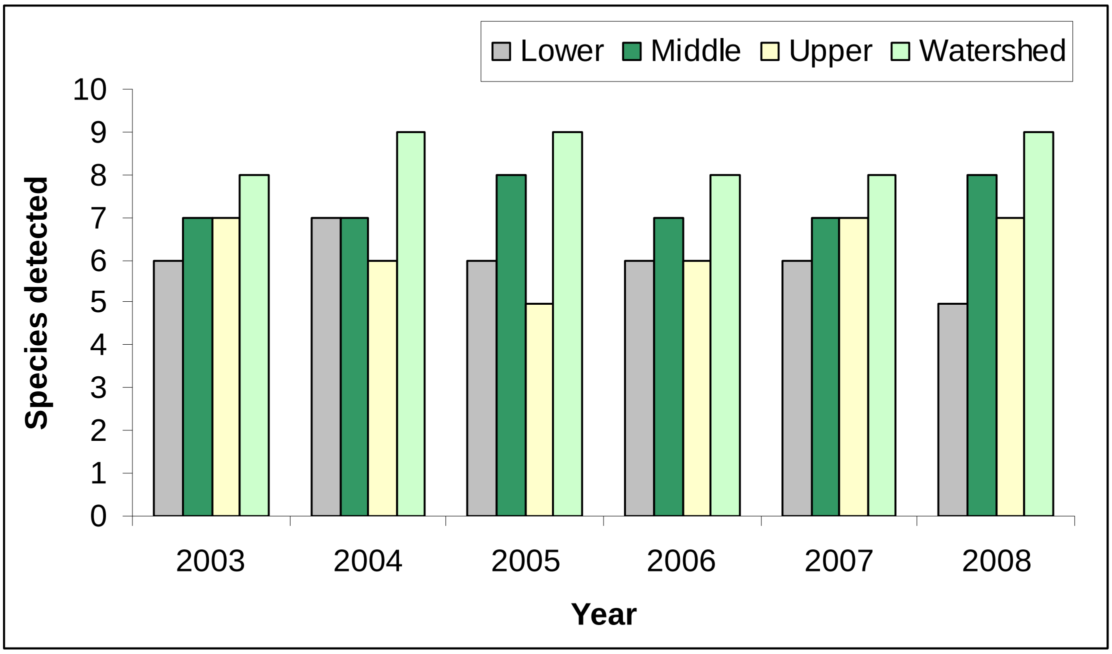

Figure 8. The total number of anuran species detected in the Credit River watershed as a whole and separated by physiographic zone for the years 2003 through 2008.

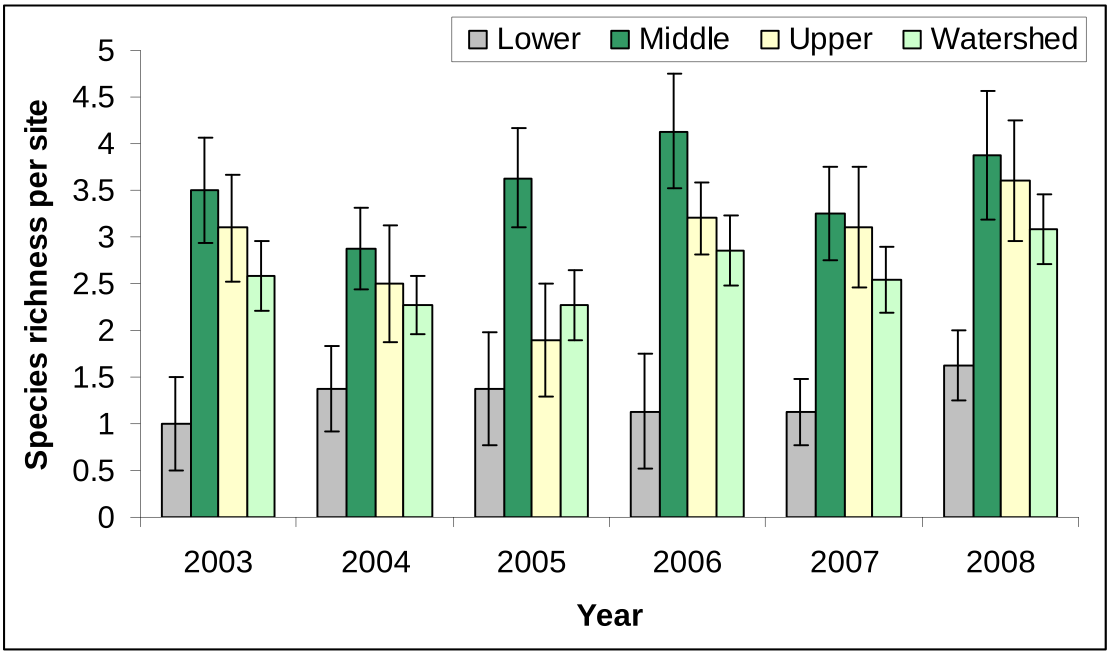

Figure 9. The mean per-site richness of anuran species detected in the Credit River watershed as a whole and separated by physiographic zone for the years 2003 through 2008, +/- standard error.

4.2.2 Anuran Site Occupancy

***Monitoring Question: Is the total number of sites occupied by anurans changing over time in the Credit River watershed*?**

- There was no significant trend in total site occupancy (all species combined) over the monitoring period.
- Proportional site occupancy of Green Frog increased over time in the Lower physiographic zone while proportional occupancy of Leopard Frog increased over time in the Upper zone.
- Green Frog occupied the greatest number of monitoring sites over the course of the study.
- Spring Peeper occupied the greatest proportion of monitoring sites in the Middle and Upper physiographic zones over the course of the monitoring period.

Proportion of monitoring sites occupied by at least one anuran species, by year, was chosen for this analysis due to discrepancies between the numbers of sites included in each zone. Cochrane-Armitage trend analysis found no significant trend in the proportion of sites occupied at either the watershed level (z = 1.132, p = 0.258, Figure 9) or within physiographic zones (Lower: z = 1.255, p = 0.209; Middle: z = 0.423, p = 0.672; Upper: z = 0.667, p = 0.505, Figure 10). This is in agreement with results of the Ontario Backyard Frog Survey, which found no consistent trends in site occupancy between 1995 and 2001 (De Solla et al. 2006). However, site occupancy was noticeably lower in the Upper physiographic zone in 2005. As with anuran richness in that zone in 2005, this low proportion could have been a result of the absence of both American Toad and Northern Leopard Frog.

There were only two significant trends in the proportional site occupancy of individual species at either the whole of the watershed or within individual physiographic zones. The proportion of sites occupied by Green Frog increased over time in the Lower watershed and the proportion of sites occupied by Leopard Frog increased over time in the Upper watershed (z = 2.051, p = 0.040 and z = 2.151, p = 0.031, respectively, Figure 11). The proportional site occupancy of all species both watershed-wide and within zones is presented as figures in Appendix 3. It is unclear whether the two detected trends are harbingers of long-term population changes or simply products of the relatively short study duration. The significant results for Leopard Frog, for instance, were probably a result of that species' absence from the Upper zone in 2005. Additional years of data may be required to determine if the patterns are unidirectional, cyclic, or merely part of random fluctuations.

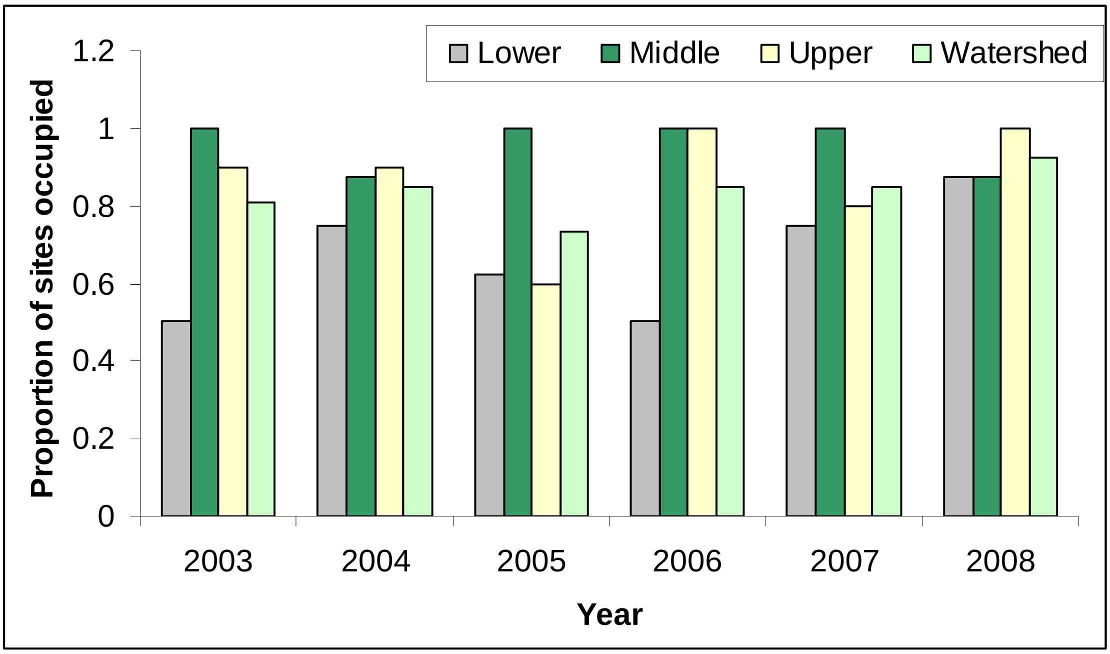

Figure 10. The proportion of monitoring sites occupied by at least one anuran species by year, presented for the whole of the Credit River watershed and by individual physiographic zones. No significant trends in total occupancy were identified.

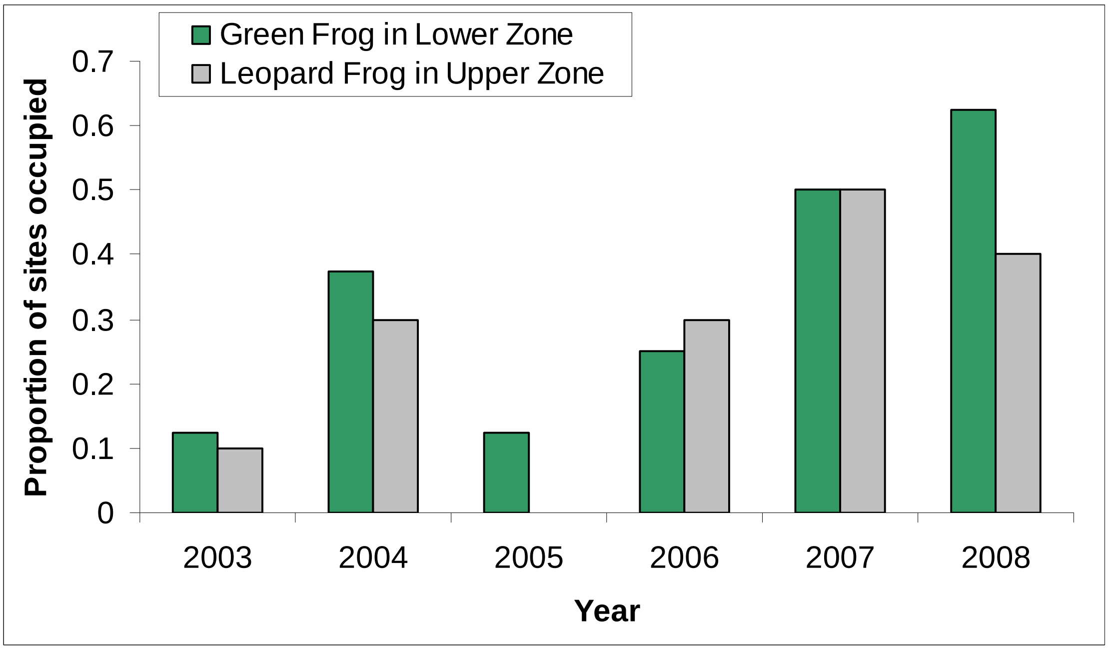

Figure 11. The proportion of monitoring sites occupied by Green Frog in the Lower physiographic zone and the proportion of sites occupied by Leopard Frog in the Upper zone from 2003 to 2008. Both data sets show a significantly increasing trend over time.

4.2.3 Anuran Abundance

Monitoring Question: Is anuran abundance changing over time in the Credit River watershed?

- There was a significant increase in the proportion of sites at which full chorus was detected in both the whole of the watershed and in the Middle physiographic zone.

Cochrane-Armitage analysis revealed a significant increase in the proportion of sites with full chorus at both the watershed level (Z = 2.48, p = 0.013) and within the Middle physiographic zone (Z = 2.16, p = 0.031). Full chorus was never detected in the Lower physiographic zone and the increasing trend apparent in the Upper zone was not significant (Z = 1.65, p = 0.099) (Figure 12). It is difficult to explain these significant trends, partially because similar patterns were not visible in richness and occupancy data. For example, we would perhaps expect to see an increase in Spring Peeper site occupancy associated with an increase in the proportion of sites with full chorus because Spring Peeper was the species most likely to be detected calling at full chorus (Figure 11). It is still possible that the proportional increase in full chorus is due to rapid growth of localized populations. It is possible, however, that the increase is instead due to physical factors such as humidity and springtime temperatures or some unknown sampling or non-sampling error.

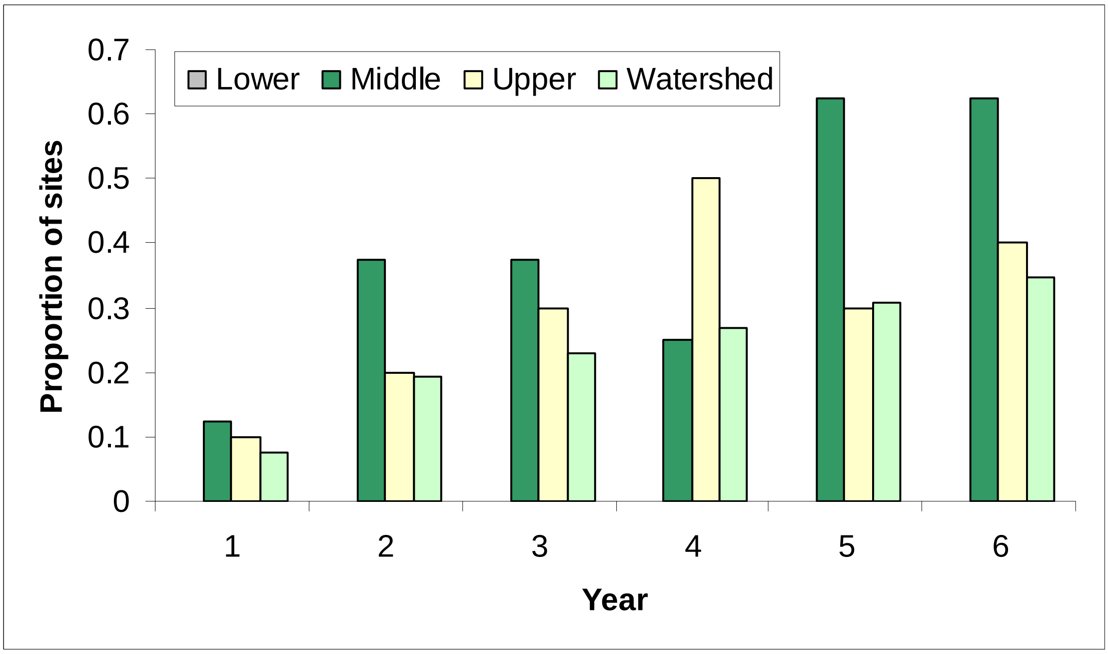

Figure 12. The proportion of monitoring sites at which full chorus (C3) was detected, separated by year and physiographic zone. Significant increases were found across the whole of the watershed and in the Middle zone.

4.2.4 Anuran Community Similarity

Monitoring Question: Is anuran community similarity changing between consecutive years in the Credit River watershed?

- Trend analysis could not be applied to community similarity calculations at this time
- Similarity was lowest in the Lower watershed when considering change between the first and last survey years.

Trend analysis could not be performed on Jaccard's Similarity Index Scores at either the watershed level or within zones due to a deficit of data points (only five over the survey period). There was no obvious trend in community similarity over the six year study period (Figure 13), with the low index score for the Upper physiographic zone in 2006 likely being a product of the absence of American Toad and Northern Leopard Frog in 2005. Values in the first five bar-groupings are based on the anuran species turnover between consecutive years. The index values can be viewed as a measure of species turnover between survey years. Higher index values indicate a greater species similarity between two groups, with a value of 1.0 indicating complete similarity. When comparing similarity index calculations between each combination of two zones for each year of the study, there were no observable trends in any of the zone relationships over the six survey years (Figure 14).

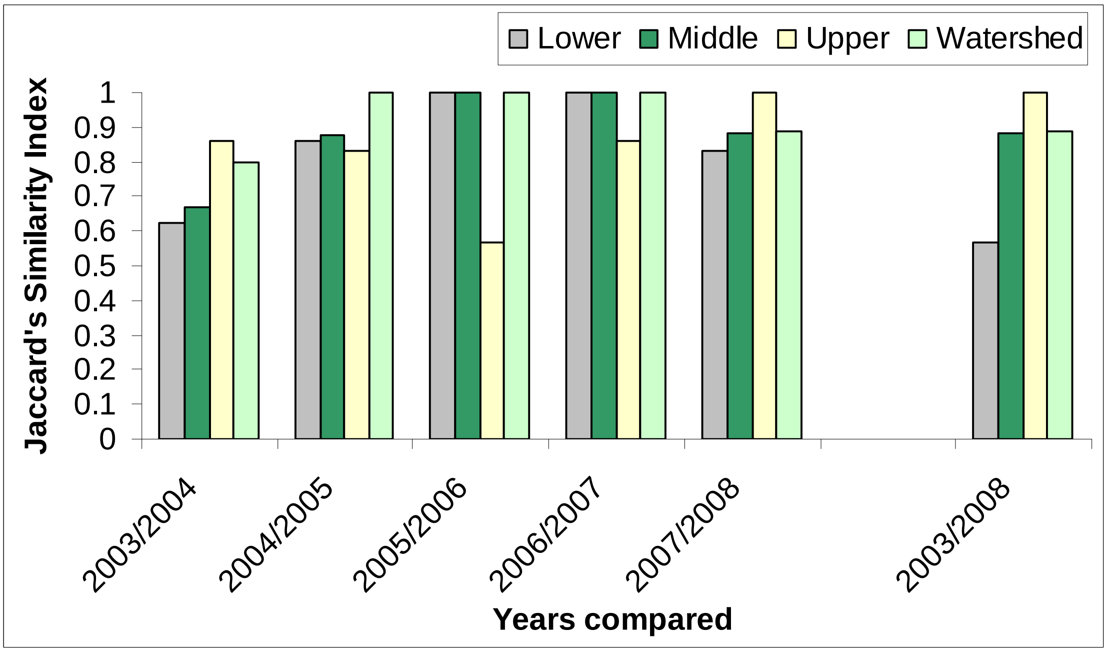

Figure 13. Jaccard's Similarity Index values between consecutive years for the whole of the watershed and within each individual physiographic zone. The set of bars on the right side of the figure compare first and last survey years.

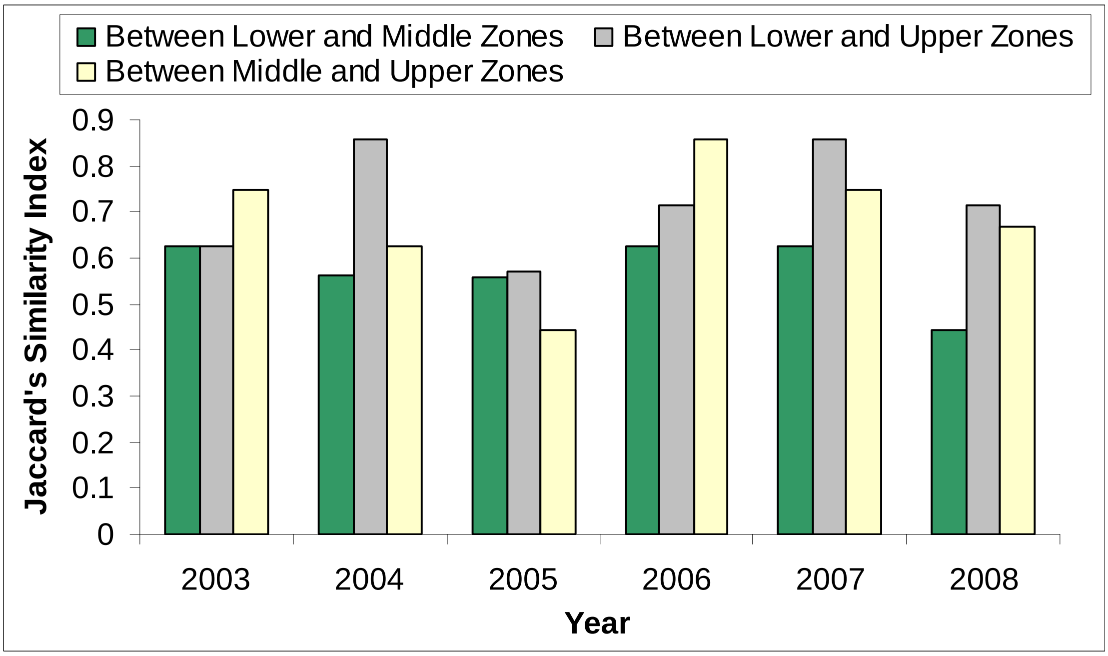

Figure 14. Jaccard's Similarity Index values between each combination of two physiographic zones for each survey year.

Overall species composition did not change in the Upper zone when the first and last survey years were compared (Figure 13, similarity index of 1.0), while compositional change was more pronounced in the Lower watershed over the same period (similarity index of 0.57). American Bullfrog and Gray Treefrog were present in the Lower zone in 2003 but not in 2008, whereas Wood Frog was present in 2008 but not in 2003. It is unclear what factors, if any, were responsible for this change in species over the study period. In general, large changes in similarity index values must be evaluated closely before any conclusions can be made. This is because there are so few choices in the available "pool" of potential anuran species. Outside the introduction to the watershed of a previously undetected invader or rare native such as Fowler's Toad, some combination of the same nine or ten species will be detected each survey year.

4.3 SPATIAL ANALYSIS

4.3.1 Anuran Species Richness

Monitoring Question: Are there spatial differences in anuran species richness in the Credit River watershed?

- Mean per-site species richness was significantly less in the Lower physiographic zone when compared to the Middle physiographic zone for all years except 2004.

The Chi-Square test for independence did not find a significant association between Year and Zone (X^2 = 0.503, p > 0.999). Non-parametric Kruskal-Wallis tests showed significant per-site species richness differences between physiographic zones in all study years except 2004 (Table 5). Post-hoc testing revealed that these results were primarily due to significant differences between values in the Lower and Middle physiographic zones (Figure 9), though the near significant differences ($p < 0.1$) that existed between the Lower and Upper zones in 2003, 2006, and 2007 could be considered biologically significant. These patterns in richness are likely being driven by differences in land use, as the greatest contrasts in both land composition and the associated measures of landscape disturbance within the watershed exist between the predominantly urban areas of the Lower zone and the protected escarpment and moraine areas of the Middle zone. It should be noted that there were no significant relationships detected post-hoc between the Middle and Upper zones. Urbanizing areas do exist in the Upper watershed and in pockets of the Middle watershed, though they are not present to the same extent or intensity as in the Lower zone.

Table 5. Test statistics, p values, and the associated significant (p<0.05) or near-significant post-hoc results for Kruskal-Wallis tests of per-site species richness performed between physiographic zones for each survey year.

Year	**Test Statistic (*H*)**	***p* value**	**Zone differences determining results (post-hoc)** [a]
2003	8.367	0.015	**Lower-Middle (*p* = 0.028)** **Lower-Upper (*p* = 0.052)**
2004	4.086	0.130	None
2005	6.088	0.048	**Lower-Middle (*p* = 0.069)**
2006	9.426	0.009	**Lower-Middle (*p* = 0.010)** Lower-Upper (*p* = 0.099)
2007	7.260	0.027	**Lower-Middle (*p* = 0.053)** Lower-Upper (*p* = 0.072)
2008	6.622	0.037	**Lower-Middle (*p* = 0.056)**

[a] Significant and near-significant post-hoc results are presented in bold.

4.3.2 Anuran Site Occupancy

Monitoring Question: Are there spatial differences in anuran site occupancy in the Credit River watershed?

- There are insufficient data to make any conclusions regarding spatial relationships in overall site occupancy.
- Spring Peeper was consistently absent from sites in the Lower physiographic zone.

The binary nature of anuran occupancy data precludes statistical spatial analysis. However, patterns in total sites occupied (Figure 10) seem to suggest that fewer sites were occupied by anurans in the Lower physiographic zone when compared to the Middle and Upper physiographic zones. In 2003 and 2006, for instance, anurans were detected in only 50% of sites in the Lower physiographic zone while all sites in the Middle zone were occupied. This may have been due to lower than normal occupancy rates of the common species Green Frog in the lower zone during those two years

(Appendix 3), or the absence of Wood Frog from that zone in 2003. The pattern seemed to have diminished by 2008, a year in which proportional site occupancy was similar between the three physiographic zones and Green Frog occupancy was at its highest level in the Lower zone. The Chi-Square test for independence using “percent of sites occupied” data found no significant association between Year and Zone ($X^2 = 16.339$, $p = 0.090$).

Spring Peeper was consistently absent from the Lower physiographic zone despite being the most common species in the Middle and Upper zones. This spatial discrepancy may be due to the fact that Spring Peeper appears especially sensitive to habitat patch size (Table **7**). The dominance of urbanization in the Lower zone has resulted in decreased patch sizes and connectivity of natural areas and fewer significant wooded wetlands. Alternatively, the lack of detection in the Lower zone may be a product of inequalities in the sampling design. Spring Peeper requires wooded habitats for at least part of its life cycle (MacCulloch 2002), yet a majority of monitoring stations in the Lower watershed are located at open marsh sites. Though American Bullfrog and Western Chorus Frog were also consistently missing from individual physiographic zones (the Lower/Upper and Middle, respectively), their low occurrence numbers make it difficult to speculate on the root causes of observed spatial distributions.

4.3.3 Anuran Abundance

Monitoring Question: Are there spatial differences in anuran abundance in the Credit River watershed?

- Data are insufficient to make any spatial comparisons of full chorus detection between the three physiographic zones.
- Full chorus has not been detected in the Lower watershed

The Kruskal-Wallis test could not be performed on per-site C3 calling data between physiographic zones for each year because full chorus was never detected in the Lower zone from 2003 to 2008 (Kruskal-Wallis requires a minimum of three groups for comparisons). Results of Mann-Whitney U tests for each separate survey year found no significant differences in full chorus detection between the Middle and Upper physiographic zones (p values were all greater than 0.6). It is difficult to conclude whether the absence of full chorus detection in the Lower watershed is due to exogenous factors affecting all species or simply the lack of Spring Peeper at sites in the Lower physiographic zone.

4.4 RELATIONSHIPS WITH LANDSCAPE PARAMETERS

4.4.1 Anuran Species Richness

Monitoring Question: Is anuran species richness correlated with landscape parameters in the Credit River watershed?

- Mean per-site anuran species richness was positively correlated with habitat patch size and negatively correlated with the percent urban land cover within 2 kilometres of the monitoring site.

The Spearman Rank tests using mean species richness data revealed a moderately strong positive correlation between mean per-site anuran species richness and habitat patch size ($\rho = 0.523$, $p = 0.006$) and a strong negative correlation between per-site richness and percent urban land use within 2 kilometres ($\rho = -0.745$, $p < 0.001$). No significant correlation was detected between mean species richness and the distance from monitoring sites to the nearest road ($\rho = 0.029$, $p = 0.885$. The significant results here are consistent with Parris (2006), who found an increase in species richness with pond size within urban areas of Melbourne, Australia. In general, a larger size will be more habitat-diverse and, hence, increase the likelihood that a patch will encompass the specific habitat requirements of a greater number of species. Inversely, a small habitat patch may be homogenous in structure and encompass the specific habitat requirements of only a few species. The negative correlation with percent urban cover is not surprising, as bulk habitat loss and reduction of patch connectivity are associated with increased urban development. There may be no significant correlation with distance to the nearest road because the nearest road may not bisect an anuran corridor. Further study of such landscape configuration issues is required.

4.4.2 Anuran Site Occupancy

Monitoring Question: Is there a relationship between anuran site occupancy and landscape parameters in the Credit River watershed?

- There was a significant positive relationship between habitat patch size and site occupancy of both Gray Tree Frog and Spring Peeper.
- There were no significant relationships between site occupancy and the distance to the nearest road for any anuran species.

There was a significant positive relationship between habitat patch size and site occupancy of both Gray Tree Frog ($X^2 = 11.756$, $p = 0.006$) and Spring Peeper ($X^2 = 14.433$, $p < 0.001$), and a nearly significant positive relationship between patch size and Wood Frog ($X^2 = 3.694$, $p = 0.055$) (Table 6). These three species favour wooded habitats during non-breeding periods and require temporary or permanent ponds in woodlands for breeding purposes (MacCulloch 2002). An increase in patch size would likely result in an associated increase in suitable wooded and permanent water habitat, a relationship supported by the fact that Gray Tree Frog and Spring Peeper were detected at every station located within a habitat patch greater than 170 hectares (8 stations) and 115 hectares (12 stations) respectively. A nearly significant positive relationship with patch size was also found for Bullfrog (X^2 = 3.58, p = 0.058), but there were too few observations of this species to have any amount of confidence in apparent trends.

Though roads have been shown to have a significant influence on anuran population dynamics (Mazerolle 2004; Fahrig et al 2005; Eigenbrod et al. 2009), there were no significant relationships found between site occupancy of individual species and the distance of monitoring sites from the nearest road (Table 7). This may be partially due to the fact that "distance to the nearest road" is a weak surrogate for road disturbance in the surrounding landscape, missing the complexity of landscape configuration and failing to address the often restricted nature of frog dispersal routes. It ignores the influence of multiple roads surrounding a monitoring site, and it fails to consider differences in road size, type, and frequency of usage. As might be expected, the road parameter is not significantly correlated with habitat patch size (P = 0.106, p = 0.607). In addition, roads represent a mortality risk (Fahrig et al. 1995; Hels and Buchwald 2001; Mazerolle 2004) so they may have a greater influence on anuran abundance than on occupancy (results section 3). The density of roads surrounding monitoring sites - a measure appearing more frequently in the literature - would likely be a more useful surrogate for landscape disturbance.

Table 6. Summarised results for logistic regressions between habitat patch size and site occupancy of eight anuran species.

Anuran Species	**Test Statistic (X^2)**	***p* value**	**Significant Relationship**
American Toad	0.223	0.637	None
Bullfrog	3.58	0.058	None
Gray Tree Frog	11.756	**0.006**	**Positive**
Green Frog	1.007	0.316	None
Leopard Frog	0.035	0.852	None
Spring Peeper	14.433	**< 0.001**	**Positive**
Western Chorus Frog	0.243	0.622	None
Wood Frog	3.694	0.055	None

Table 7. Summarised results for logistic regressions between distance to the nearest road and site occupancy of eight anuran species.

Anuran Species	**Test Statistic (X^2)**	***p* value**	**Significant Relationship**
American Toad	3.409	0.065	None
Bullfrog	0.041	0.839	None
Gray Tree Frog	2.473	0.116	None
Green Frog	1.692	0.193	None
Leopard Frog	0.272	0.602	None
Spring Peeper	0.381	0.537	None
Western Chorus Frog	1.168	0.280	None
Wood Frog	1.403	0.236	None

There were significant negative relationships between percent urban cover within 2 kilometres and site occupancy of Gray Tree Frog, Leopard Frog, Spring Peeper, and Wood Frog (Table 8). In general, it has been shown that landscape characteristics are important in determining the spatial pattern of frog occurrence at ponds (Mazerolle et al. 2005). This is particularly true in urbanizing areas, where development has been shown to negatively influence the amount of suitable anuran habitat in the landscape (Baldwin and DeMaynadier 2009). As is the case with decreasing patch size or the presence of roads, widespread urbanization can lead to isolated metapopulations. This is turn can increase the risk of local extinctions because highly mobile juveniles are unable to disperse between local population centres (Cushman 2006, Becker et al. 2007).

Table 8. Summarised results for logistic regressions between the percent urban cover within 2 kilometres of the monitoring site and site occupancy of eight anuran species.

Anuran Species	**Test Statistic (X^2)**	***p* value**	**Significant Relationship**
American Toad	0.566	0.452	None
Bullfrog	3.294	0.069	None
Gray Tree Frog	15.759	**<0.001**	**Negative**
Green Frog	0.350	0.554	None
Leopard Frog	9.297	**0.002**	**Negative**
Spring Peeper	14.975	**<0.001**	**Negative**
Western Chorus Frog	0.150	0.700	None
Wood Frog	6.640	**0.010**	**Negative**

4.4.3 Anuran Abundance

Monitoring Question: Is there a relationship between anuran abundance and landscape parameters in the Credit River Watershed?

- There were significant positive relationship between full chorus detection and both habitat patch size and the distance to the nearest road.
- There was a significant negative relationship between full chorus detection and the percent urban cover within 2 kilometres.

Logistic comparisons between the detection of full chorus and the three chosen landscape parameters produced several significant results. There was a significant positive relationship between detection of full chorus and both habitat patch size (X^2 = 12.790, p < 0.001) and the distance to the nearest road (X^2 = 3.856, p = 0.049), while a significant negative relationship existed between full chorus and the percent urban cover within 2 kilometres (X^2 = 15.970, p < 0.001). The strong relationships with both habitat patch size and the percent urban cover in the landscape could be the result of an associated increase in the amount of suitable wetland and forest habitat supporting larger anuran populations. The relationship with patch size could also be driven by the fact that site occupancy of Spring Peeper, the species most commonly detected at full chorus, had a strong positive relationship with habitat patch size (Table 6). The positive relationship between full chorus detection and distance to the nearest road could be a result of anuran population sensitivities in that the habitat fragmentation and mortality risk suggested by the roads parameter could be leading to smaller populations and, hence, decreased probability of detecting full chorus. Noise from adjacent roadways could also be making it more difficult to detect anuran calls. These detection problems were noted at the Hutttonville and Ken Whillans sites, while low flying jets occasionally delayed the start of surveys at the Meadowvale site. Bee and Swanson (2007), for instance, found that realistic levels of traffic noise limited the range of anuran acoustic signals in Minnesota.

4.5 RELATIONSHIPS WITH VEGETATION PARAMETERS

4.5.1 Anuran Species Richness

Monitoring Question: Is anuran species richness correlated with wetland vegetation parameters in the Credit River watershed?

- The only significant finding was a negative correlation between anuran species richness and non-native species richness in the wetland ground vegetation layer.

Each selected vegetation parameter was converted to mean per-site values across all years so they could be compared to mean per-site anuran richness values. The only significant relationship was a negative correlation between anuran species richness and non-native species richness in the wetland ground vegetation layer (ρ = -0.542, p = 0.020, Table 9). Though there is the temptation to look for a direct relationship between non-native vegetation on anuran richness, this and any other significant correlations must be considered cautiously and no causal relationships should be inferred. The non-native vegetation richness parameter may not be directly influencing anuran richness but simply be acting as an indirect surrogate of landscape disturbance and urbanization, elements that are themselves expressed more directly by landscape measures. Non-native vegetation is likely to be more prevalent at stations located in habitat patches with greater edge length and closer proximity to anthropogenic dispersal routes.

Table 9. Spearman Rank correlations between anuran richness and wetland vegetation parameters, ranked by the absolute size of the correlation.

Vegetation Parameters	Correlation Coefficient (ρ)	*p* Value
Mean non-native richness (ground vegetation)	**-0.542**	**0.020**
Mean proportion non-native species (ground vegetation)	-0.429	0.076
Mean native richness (regeneration)	0.426	0.078
Mean coefficient of conservatism (combined vegetation)	0.412	0.090
Mean total species richness (regeneration)	0.373	0.127
Mean non-native richness (regeneration)	-0.284	0.253
Mean proportion non-native species (regeneration)	-0.257	0.302
Mean total species richness (ground vegetation)	-0.115	0.648
Mean native richness (ground vegetation)	0.065	0.797

4.5.2 Anuran Site Occupancy

Monitoring Question: Is there a relationship between anuran site occupancy and vegetation parameters in the Credit River watershed?

- There were several significant negative relationships between occupancy of individual species and measures of non-native vegetation.
-There were significant positive relationships between occupancy of individual species and both total and native regeneration richness.

Several significant logistic relationships were found between site occupancy of individual anuran species and the chosen wetland vegetation parameters (Table 10). As with the rank correlations between anuran richness and the same vegetation parameters, the significant negative results here were associated with non-native plant richness covariates. Again, these measures of non-native vegetation may be acting as surrogates for habitat loss and landscape fragmentation. Many of the significant positive relationships between vegetation and occupancy involved tree frogs (Gray Treefrog, Spring Peeper) and richness measures of regenerating woody plant species. The increased structural diversity at the monitoring stations that is implicit in an increase in regeneration diversity may be encouraging tree frog species to remain in the immediate vicinity of the surveyed wetlands even after breeding has been completed. Open marsh sites with little woody plant diversity would not provide the stratified habitat required by these species during non-breeding periods. The positive relationship between Spring Peeper occupancy and mean coefficient of conservatism is tougher to explain, as a) the literature provides no evidence that the quality of native plant species has any effect on anuran populations and b) mCC could be expressing any number of environmental variables. Vegetation with high habitat fidelity may be more prevalent within the interior communities associated with increasing habitat patch size, which is itself a landscape parameter significantly related to Spring Peeper occupancy.

Table 10. The significant and near significant results of logistic regression tests between site occupancy of individual anuran species and the chosen wetland vegetation parameters.

Species	Vegetation Parameter	Test Statistic (X^2)	*p* Value	Significant Trend
Gray Tree Frog	Ground vegetation non-native richness	3.649	0.056	Negative
	Ground vegetation proportion non-natives	3.747	0.053	Negative
	Regeneration total species richness	3.928	0.048	Positive
	Regeneration native richness	4.299	0.038	Positive
Leopard Frog	Ground vegetation non-native richness	4.225	0.040	Negative
Spring Peeper	Ground vegetation non-native richness	8.434	0.004	Negative
	Ground vegetation proportion natives	11.576	0.001	Positive
	Regeneration non-native richness	3.880	0.049	Negative
	Regeneration proportion non-natives	4.764	0.029	Negative
	Combined vegetation Mean Coefficient of Conservatism	8.815	0.003	Positive
Wood Frog	Regeneration total species richness	6.674	0.010	Positive
	Regeneration native richness	6.173	0.013	Positive

4.5.3 Anuran Abundance

Monitoring Question: Is there a relationship between anuran abundance and wetland vegetation parameters in the Credit River watershed?

- There were significant negative relationships between full chorus detection and measures of non-native vegetation.
- There were significant positive relationships between full chorus detection and both total and native regeneration richness.

There were several significant logistic relationships found between full chorus detection and selected wetland vegetation parameters (Table 11). As with the relationships between vegetation and richness or occupancy, the significant negative results are mostly associated with measures of non-native vegetation. This could again be related to the kind of landscape alteration that promotes the growth and transport of invasive plant species. The negative trends in abundance could also be due to properties of the vegetation itself. For example, it has been shown that larval amphibian development can be slowed by secondary compounds leached from invasive plants such as Purple Loosestrife (*Lythrum salicaria*) (Maerz et al. 2005). The significant positive results were associated with regeneration richness (both native and total) and mean Coefficient of Conservatism. Please see the occupancy results section (page 33) for a discussion of the potential connections between anuran parameters and both regeneration richness and mean coefficient of conservatism values.

Table 11. Significant results of logistic regressions between full chorus detection and selected wetland vegetation parameters, listed by confidence level.

Vegetation Parameter	Test Statistic (X^2)	*p* value	Significant Relationship
Mean coefficient of conservatism	7.313	0.007	Positive
Native richness (regeneration)	6.550	0.010	Positive
Proportion non-natives (ground vegetation)	6.481	0.011	Negative
Total species richness (regeneration)	5.671	0.017	Positive
Non-native richness (ground vegetation)	5.426	0.020	Negative
Proportion non-natives (regeneration)	4.743	0.029	Negative
Non-native richness (regeneration)	4.490	0.034	Negative

4.6 STATISTICAL PROCESS CONTROL

The upper and lower critical and warning limits calculated for each anuran trend parameter with at least five years of available data (Table 12) will be adopted as monitoring thresholds to which future anuran monitoring data can be compared. Future values that exceed the upper or lower warning limits (2 standard deviations) will signify emergent increasing or declining population trends, while values that exceed the upper and lower critical limits (3 standard deviations) will signify a significant deviation from natural variability. Such deviations would warrant immediate management action before trends became irreversible. No monitoring thresholds can be applied to those parameters deemed to be out of control or those displaying no variance over the monitoring period.

In general, the spread in values between the upper and lower critical limits of individual parameters seem larger in the Upper physiographic zone when compared to similar parameters in the Lower zone. For instance, the spread of “mean species per site” values between upper and lower limits in the Lower zone was 1.2 species units while the same spread in the Upper zone was 3.2 units. This pattern is likely due to increased variability in the yearly values of parameters associated with the Upper watershed. Among measures of site occupancy by individual species, American Toad had the largest spread in values between the upper and lower critical limits while Green Frog and Western Chorus Frog had the smallest spreads. It should be noted that critical and warning thresholds presented in the table do not exceed the constraints of realistic values, such as the maximum proportion of monitoring sites occupied (100%) or the maximum Jaccard’s score (1.0), even if calculated values exceeded these constraints. Values that exceed these constraints cannot be realistically detected and, therefore, cannot be adopted as thresholds.

Table 12. Monitoring thresholds for all anuran trend parameters extracted from Statistical Process Control individual (moving average) IMR charts for data collected from 2003 through 2008.

Parameter	Thresholds [a]			
	Upper Critical Threshold	**Upper Warning Threshold**	**Lower Warning Threshold**	**Lower Critical Threshold**
Species Richness				
Total species	9.6	9.21	7.79	7.4
Total species (Lower zone)	7.2	6.9	5.5	5.1
Total species (Middle zone)	Not "in control"			
Total species (Upper zone)	8.5	7.8	4.9	4.2
Species per site	3.5	3.2	2.0	1.7
Species per site (Lower zone)	1.9	1.7	0.9	0.7
Species per site (Middle zone)	5.3	4.7	2.4	1.7
Species per site (Upper zone)	4.5	4.0	1.8	1.3
Proportional Site Occupancy (%)				
Sites Occupied	>100	95.6	71.1	64.9
Sites Occupied (Lower)	>100	97.7	35.6	20.1
Sites Occupied (Middle)	>100	>100	82.5	75.9
Sites Occupied (Upper)	>100	>100	47.7	28.2
American Bullfrog	Not "in control"			
American Bullfrog (Lower)	Not "in control"			
American Bullfrog (Middle)	32.1	27.6	9.9	5.5
American Bullfrog (Upper)	No Records			
American Toad	>100	87.9	<0	<0
American Toad (Lower)	75.7	60.2	<0	<0
American Toad (Middle)	>100	>100	<0	<0
American Toad (Upper)	>100	>100	<0	<0
Gray Tree Frog	70.7	59.1	12.7	1.1
Gray Tree Frog (Lower)	Not "in control"			
Gray Tree Frog (Middle)	>100	86.0	<0	<0
Gray Tree Frog (Upper)	>100	94.2	9.1	<0
Green Frog	60.9	57.5	43.8	40.4
Green Frog (Lower)	86.5	68.8	<0	<0
Green Frog (Middle)	88.7	82.1	55.5	48.8
Green Frog (Upper)	92.6	78.4	21.6	7.4
Leopard Frog	59.9	51.1	15.6	6.7
Leopard Frog (Lower)	Not "in control"			
Leopard Frog (Middle)	>100	94.1	14.3	<0
Leopard Frog (Upper)	85.2	65.7	<0	<0
Spring Peeper	73.4	69.3	46.4	40.7
Spring Peeper (Lower)	15.4	10.9	<0	<0
Spring Peeper (Middle)	>100	96.6	70.0	63.4
Spring Peeper (Upper)	>100	>100	55.2	42.8
Chorus Frog	20.5	17.1	3.4	0
Chorus Frog (Lower)	No Variance			
Chorus Frog (Middle)	No Records			
Chorus Frog (Upper)	43.3	34.4	<0	<0
Wood Frog	54.6	47.1	17.0	9.5
Wood Frog (Lower)	34.5	27.9	1.3	<0
Wood Frog (Middle)	83.3	72.2	27.8	16.8
Wood Frog (Upper)	68.9	56.5	6.8	<0

Parameter	Thresholds [a]			
	Upper Critical Threshold	Upper Warning Threshold	Lower Warning Threshold	Lower Critical Threshold
Abundance				
Total C3 call records	13.7	11.7	3.9	2.0
Total C3 call records (Lower)	No Records			
Total C3 call records (Middle)	6.7	5.6	1.4	0.4
Total C3 call records (Upper)	9.1	7.7	1.9	0.5
Community Similarity				
Jaccard's index	1.00	1.00	0.78	0.71
Jaccard's index (Lower zone)	1.00	1.00	0.62	0.50
Jaccard's index (Middle zone)	1.00	1.00	0.66	0.56
Jaccard's index (Upper zone)	1.00	1.00	0.50	0.35

[a] Limits are presented in the specific units used to measure each given parameter.

4.7 POWER ANALYSIS

The Calculated numbers of monitoring sites required to detect linear trends that exceed each threshold identified through Statistical Process Control (1 SD, 2 SD or 3 SD) were found to be identical between all anuran trend parameters (Table 13). This odd pattern is a product of the Power Analysis procedure specific to linear regression, which itself uses standard deviation as a key variable in calculations. Though results show that a one standard deviation change in values cannot be detected in any parameter by the current program (approximately 32 sites are required), values that remain within this "green" zone are not considered deviant from a management perspective. Of greater importance is the fact that there are currently enough monitoring plots in the program to detect warning thresholds (7.2 plots needed) and critical thresholds (less than 3 plots needed) across the whole of the watershed and within the Lower, Middle, and Upper physiographic zones.

The smallest minimum detectable effect size based on the current complement of monitoring sites (26 in the watershed, 8 in the Lower and Middle zones, 10 in the Upper zone) was 6.6% for the total species trend in the whole of the watershed (Table 13). This means that the monitoring program has sufficient power to detect a 6.6% change from the baseline mean (about 1 species) in the mean number of anuran species in the watershed over a five year period. High variability in some parameters, along with the small number of sites in each physiographic zone, resulted in proportionally higher minimum detectable effect sizes. For example, it is only possible to detect a minimum change of 102.7% from the baseline mean (or about 5 detections) in the number of full chorus records in the Upper physiographic zone.

The current program of 26 monitoring sites only has the power to detect the two standard deviation warning threshold for Toad occupancy across the whole of the watershed, while the three standard deviation critical threshold can only be detected for Toad occupancy across the whole of the watershed and within the Upper physiographic zone (Table 14). The minimum detectable effect sizes were greater than three standard deviations for every other proportion parameter. For instance, a minimum change of 7.9 standard deviations can be detected in Spring Peeper occupancy at the watershed level given the current complement of monitoring sites (Table 14). Two conclusions can be drawn from these results: either a) there are not enough anuran monitoring sites in the program to detect the desired changes in proportional occupancy, or b) the 1, 2, and 3 standard deviation thresholds used for linear regression are not appropriate when dealing with proportional data.

The calculated number of monitoring sites required to determine a significant logistic relationship between the binary anuran variables and the landscape or wetland vegetation covariates differed greatly between the many variable and covariate combinations (Table 15). All test results with landscape covariates have been presented. However, only statistically significant relationships ($p < 0.05$) with vegetation covariates were tested for power due to the sheer number of possible parameter combinations. The exceptions are that no relationships with the percent urban cover within 2 kilometres, mean coefficient of conservatism values, non-native regeneration richness, and proportion non-native regenerating species are presented because the distribution of these parameters could not be normalised. The calculated N values (required number of sites) were within the current complement of sites (26) for each statistically significant logistic relationship, save for full chorus detection compared to the distance to the nearest road ($p = 0.0496$, n =75). For example, the significant relationship between Leopard Frog occupancy and non-native ground vegetation richness had a significance level of 0.004 and could legitimately be detected with a sample size of 19. Required sample sizes were often quite high for non-significant logistic relationships. For instance, over 30 000 monitoring sites would be required to detect a significant relationship between Spring Peeper occupancy and the distance to the nearest road. Further examination of these results is necessary because the number of sites required to detect a relationship may change as more survey years are added to the data set or as updated landscape information becomes available.

Table 13. Results of linear regression power analysis for anuran parameters that will be eventually be examined for temporal trends.

Anuran Parameter [b]	Sites Required to Detect SPC Thresholds			Minimum Detectable Effect Size	
	1 SD	2SD (Warning)	3SD (Critical)	% Change	Units Change [a]
Anuran Species Richness					
Mean species per site (Watershed)	31.8	7.2	2.6	7.0	0.35
Mean species per site (Lower zone)	31.8	7.2	2.6	35.2	0.4
Mean species per site (Middle zone)	31.8	7.2	2.6	24	0.85
Mean species per site (Upper zone)	31.8	7.2	2.6	36.2	1.05
Total species (Watershed)	31.8	7.2	2.6	7.0	1 species
Total species (Lower zone)	31.8	7.2	2.6	20	2 species
Total species (Middle zone)	31.8	7.2	2.6	13.6	1 species
Total species (Upper zone)	31.8	7.2	2.6	22.1	2 species
Anuran Abundance					
Total full chorus detections (Watershed)	31.8	7.2	2.6	54.3	5 detections
Total full chorus detections (Middle zone)	31.8	7.2	2.6	81.4	3 detections
Total full chorus detections (Upper zone)	31.8	7.2	2.6	102.7	5 detections
Anuran Community Similarity					
Jaccard's Similarity Index (Watershed)	31.8	7.2	2.6	10.9	0.1
Jaccard's Similarity Index (Lower zone)	31.8	7.2	2.6	29.0	0.25
Jaccard's Similarity Index (Middle zone)	31.8	7.2	2.6	29.1	0.25
Jaccard's Similarity Index (Upper zone)	31.8	7.2	2.6	30.3	0.25

[a] Rounded up to the nearest whole number for parameters that cannot be m
[b] Listed are the numbers of monitoring sites required to detect SPC thresholds, as well as the minimum detectable effect size given the current study design

Table 14. Power analysis results for trends in proportional site occupancy.[a]

Occupancy Category	Spatial Level	Sites required to detect SPC thresholds			Minimum detectable effect size (SD)
		1 SD	2 SD	3SD	
Total Occupancy	Watershed	154	76	50	5.66
	Lower zone	62	31	20	7.43
	Middle zone	150	73	49	17.4
	Upper zone	63	31	20	6.03
Bullfrog	Watershed	503	247	163	18
	Lower zone	191	94	62	22
	Middle zone	141	69	46	16.4
	Upper zone	-	-	-	-
American Toad	Watershed	46	**23**	**15**	1.74
	Lower zone	74	36	24	8.7
	Middle zone	37	18	12	4.49
	Upper zone	27	13	**9**	2.62
Gray Tree Frog	Watershed	93	46	30	3.48
	Lower zone	191	94	62	22
	Middle zone	50	25	16	5.97
	Upper zone	48	24	16	4.65
Green Frog	Watershed	171	84	56	6.28
	Lower zone	46	23	13	5.5
	Middle zone	141	69	46	16.4
	Upper zone	67	33	22	6.38
Leopard Frog	Watershed	93	46	30	3.48
	Lower zone	94	46	30	11
	Middle zone	50	25	16	5.97
	Upper zone	51	25	16	4.85
Spring Peeper	Watershed	217	106	70	7.9
	Lower zone	191	94	62	22
	Middle zone	150	73	49	17.4
	Upper zone	87	43	28	8.24
Chorus Frog	Watershed	247	121	80	8.96
	Lower zone	-	-	-	-
	Middle zone	-	-	-	-
	Upper zone	92	45	30	8.74
Wood Frog	Watershed	135	66	44	4.97
	Lower zone	102	50	33	11.9
	Middle zone	85	42	28	10.04
	Upper zone	56	27	18	5.34
Full Chorus Calling	Watershed	100	49	32	3.72
	Lower zone	-	-	-	-
	Middle zone	47	23	15	5.6
	Upper zone	67	33	22	6.38

[a] Presented are the calculated number of monitoring sites required to detect the SPC thresholds and the minimum detectable effect size expressed in standard deviations.

Table 15. The calculated number of monitoring sites (N) required to detect a significant logistic relationship between the binary anuran variables and landscape or wetland vegetation covariates.

Binary Dependant Variable	Continuous Covariate	Logistic Regression *p* Value	Sites Required (N)
Bullfrog Occupancy	Patch Size (Sqrt)	0.058	67
	Distance to Road (Sqrt)	0.839	14769
Toad Occupancy	Patch Size (Sqrt)	0.637	2666
	Distance to Road (Sqrt)	0.065	95
Gray Tree Frog Occupancy	Patch Size (Sqrt)	0.006	6
	Distance to Road (Sqrt)	0.116	215
	Ground Vegetation Non-Native Richness	0.056	22
	Ground Vegetation Proportion Non-Natives	0.053	21
	Regeneration Total Species Richness	0.048	21
	Regeneration Native Richness	0.038	20
Green Frog Occupancy	Patch Size (Sqrt)	0.316	206
	Distance to Road (Sqrt)	0.193	248
Leopard Frog Occupancy	Patch Size (Sqrt)	0.852	287
	Distance to Road (Sqrt)	0.602	3388
	Ground Vegetation Non-Native Richness	0.04	19
Spring Peeper Occupancy	Patch Size (Sqrt)	< 0.001	5
	Distance to Road (Sqrt)	0.537	31425
	Ground Vegetation Non-Native Richness	0.004	7
	Ground Vegetation Proportion Non-Natives	0.001	3
Chorus Frog Occupancy	Patch Size (Sqrt)	0.622	3642
	Distance to Road (Sqrt)	0.280	187
Wood Frog Occupancy	Patch Size (Sqrt)	0.055	45
	Distance to Road (Sqrt)	0.236	415
	Regeneration Total Species Richness	0.010	9
	Regeneration Native Richness	0.013	12
Full Chorus Detection	Patch Size (Sqrt)	< 0.001	6
	Distance to Road (Sqrt)	0.0496	75
	Ground Vegetation Proportion Non-Natives	0.011	15
	Ground Vegetation Non-Native Richness	0.020	9
	Regeneration Total Species Richness	0.017	13
	Regeneration Native Richness	0.010	7

5.0 CONCLUSIONS

No critical signs of decline were apparent in anuran populations over the study period. Parameters appeared stable (or at least oscillated randomly) over the six years of monitoring. At times, the detection of temporal and spatial trends was hindered both by a deficit of sampling points and by an inability to apply more robust statistical techniques to the collected data, namely linear trend analysis and Repeated Measures ANOVA. These problems may be alleviated as additional years of survey data are incorporated into analyses or as patterns in anuran richness, occupancy, and abundance change through time. If, however, the analytical problems persist, the study framework or monitoring protocol may have to be altered to better address monitoring questions. For example, the usefulness of the ordinal call classification system in accurately estimating anuran abundance numbers may have to be reviewed. Unfortunately, there appears to be few alternatives available that fit within the resource constraints of the current monitoring program.

Four significant temporal trends were found to exist between 2003 and 2008. Proportional site occupancy of Green Frog increased in the Lower physiographic zone while proportional occupancy of Leopard Frog increased in the Upper zone. An unexplainable significant increase in the proportion of sites with full chorus calling was also observed, both watershed-wide and within the Middle physiographic zone. Species richness and total site occupancy did experience yearly drops and surges in both the whole of the watershed and in individual physiographic zones, but there was no overall movement of values towards improvement or decline. As such, few predictions can be made concerning future conditions of anuran populations in the watershed. Additional years of monitoring may be required to recognise any temporal patterns outside unstable population dynamics (De Solla et al. 2006) or the cycle of extinction and re-colonization often associated with metapopulations (Marsh and Trenham 2000; Smith and Green 2005) that have been reported in the literature.

Spatial trends in anuran parameters were more pronounced over the study period. Though the only statistically significant spatial pattern was detected in species richness between the Lower and Middle zones for five of six survey years, anuran parameter values were consistently diminished in the Lower physiographic zone when compared to those in the Upper and Middle zones. Fewer monitoring sites were occupied by any species in the Lower zone, and no full chorus calls were detected there over the six year study. The absence of full chorus in the Lower zone could be an early indicator of declining populations and is likely capturing the influence of pervasive urban development and associated habitat fragmentation in the lower portion of the watershed, as well as a sampling inequality that is biased towards marsh sites in urban areas. Despite dissimilarities in topography and land use, spatial distinctions between the Middle and Upper zones were rarely substantial.

Green Frog and Spring Peeper were the most commonly detected anuran species in the watershed. Based on a non-statistical observation of trends, these two species were also the only ones to exhibit relatively stable levels of site occupancy over the whole of the watershed (Appendix 3). Year-to-year occupancy numbers for the other seven species were notably more unstable, with American Toad occupancy being the most erratic. The regional distribution of some species appeared to favour or exclude certain landscape divisions. Spring Peeper was mostly absent from the Lower physiographic zone, whereas American Bullfrog was not recorded in either the Lower or Upper zones. Western Chorus Frog was absent from the Middle zone while exhibiting seemingly stable occupancy at a few sites in the Lower and Upper zones. Unfortunately, a relative shortage of detections excludes us from making any conclusions regarding population trends in this species of concern.

Direct or indirect measures of habitat loss and fragmentation (such as habitat patch size, the percent urban land cover within 2 kilometres of monitoring sites, and the richness of non-native vegetation) had the strongest correlation with anuran richness, occupancy, and abundance. These relationships between anuran parameters and potential environmental predictors should be subject to more comprehensive multiple-variable modeling as analysis continues, and be tracked intently as updated landscape and vegetation community data becomes available. In reference to individual monitoring sites, two locations appeared to represent the best and worst of anuran communities in the watershed. Speersville Wetland, a marsh in the Upper physiographic zone, was rated the healthiest anuran community because it had the highest mean species richness, the second highest mean full chorus detection, and was occupied by anurans in each of the six survey years. The Mississauga Gardens site, a marsh located within a major urban centre in the Lower physiographic zone, was rated the least healthy anuran community because it had the lowest mean species richness, no detections of full chorus calling, and was occupied by anurans for only two of the six survey years.

Additional years of data may be required to confirm current trends, so it is unnecessary to pursue a change in the number of monitoring sites at this time. It is recommended, however, that trends and spatial relationships in anuran richness, occupancy, and abundance continue to be tracked closely over subsequent survey years in order to determine if intervention is necessary to maintain stable populations in the watershed. Land-use and composition information, as well as wetland vegetation data, should be kept up-to-date so that analysis can be based on the most accurate data available. In addition, call detection methodology should be reviewed due to current limitations in the estimation of abundance counts. Through this integration and continued focus on program improvement, a more complete and holistic understanding of anuran population health in the Credit River watershed can be gained.

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APPENDIX A: ANURAN MONITORING SITES IN THE CREDIT RIVER WATERSHED 2003 TO 2008.

Site Name	Site Number	Upper Tier Municipality	Lower Tier Municipality	Physiographic Zone	Habitat Patch Size (Ha)
Rattray Marsh	W-01	Peel Region	Mississauga	Lower	92.3
Credit River Marsh	W-02	Peel Region	Mississauga	Lower	0.3
Creditview Wetland	W-03	Peel Region	Mississauga	Lower	5.4
Meadowvale ESA Wetland	W-04	Peel Region	Mississauga	Lower	42.3
Winston Churchill Wetland	W-12	Halton Hills	Halton Hills	Lower	24.7
Turtle Creek	WNV-01	Peel Region	Mississauga	Lower	92.3
Mississauga Gardens	WNV-02	Peel Region	Mississauga	Lower	52.4
Churchville	WNV-03	Peel Region	Brampton	Lower	16.8
Hungry Hollow Wetland	W-06	Halton Hills	Halton Hills	Middle	114.1
Acton Swamp	W-07	Halton Hills	Halton Hills	Middle	127.9
Terra Cotta Wetland	W-08	Peel Region	Town of Caledon	Middle	753.3
Ken Whillans RMA	W-09	Peel Region	Town of Caledon	Middle	165.9
Warwick Wetland	W-10	Peel Region	Town of Caledon	Middle	191.2
Rail Trail	A-01	Peel Region	Town of Caledon	Middle	158.9
Balinafad Ridge	A-02	Wellington	Town of Erin	Middle	223.6
Silver Creek	A-03	Halton Hills	Halton Hills	Middle	370.1
Erin Pine Estates Wetland	W-11	Wellington	Town of Erin	Upper	93.1
Hillsburgh Wetland	W-13	Wellington	Town of Erin	Upper	14.4
Grange Orpen Wetland	W-15	Peel Region	Town of Caledon	Upper	137.6
Starr Wetland	W-16	Peel Region	Town of Caledon	Upper	291.3
Speersville Wetland	W-17	Peel Region	Town of Caledon	Upper	218.9
Caledon Lake Bog	W0-18	Peel Region	Town of Caledon	Upper	554.8
Mellville Marsh	W-19	Amaranth	Orangeville	Upper	5.5
Belfountain Wetland	W-20	Peel Region	Town of Caledon	Upper	31.2
Caledon Creek	WNV-4	Peel Region	Town of Caledon	Upper	78.3
Alton-Hillsburgh	A-04	Wellington	Town of Erin	Upper	321.3

APPENDIX B: ECOLOGICAL LAND CLASSIFICATION (ELC) COMMUNITY SERIES AND LAND USE TYPES INCORPORATED INTO THE DEFINITIONS OF NATURAL, AGRICULTURAL, AND URBAN LAND COVER.

ELC Community Series	ELC Code	Land use Cover (aggregated)
Coniferous forest	FOC	
Coniferous plantation	CUP3	
Coniferous swamp	SWC	
Cultural meadow	CUM	
Cultural savannah	CUS	
Cultural thicket	CUT	
Cultural woodland	CUW	
Deciduous forest	FOD	
Deciduous plantation	CUP1	
Deciduous swamp	SWD	
Marsh	MA	**Natural**
Mixed forest	FOM	
Mixed plantation	CUP2	
Mixed swamp	SWM	
Open Aquatic	OAO	
Open Beach/Bar	BBO	
Open Bluff	BLO	
Shrub bluff	BLS	
Shrub bog	BOS	
Thicket swamp	SWT	
Treed beach/Bar	BBT	
Treed bog	BOT	

Land Use Type	ELC Code	Land use Cover (aggregated)
Commercial/Industrial Open Space	MOC	
Educational/Institutional Open Space	MOI	
Inactive Aggregate	AI	
Intensive Agriculture	AGI	
Manicured Open Space	MOS	
Non-intensive Agriculture	AGN	**Agriculture**
Other Open Space	MOO	
Private Open Space	MOP	
Recreational Open Space	MOR	
Wet Meadow	WET	
Active Aggregate	AA	
Airport	TPA	
Collector	TPC	
Commercial/Industrial	CIC	
Construction [a]	CON	
Educational/Institutional	CII	
High Density Residential	URH	
High Rise Residential	URR	**Urban**
Highway	TPH	
Landfill	LF	
Low Density Residential	URL	
Medium Density Residential	URM	
Railway	TPX	
Regional Road	TPR	
Residential Estate	URE	
Rural Development	RD	
Urban	URB	
Mixed Residential	URX	

[a] New addition Nov 2009

APPENDIX C: ANURAN PROPORTIONAL OCCUPANCY

Table 1. Proportion of monitoring sites occupied by American Bullfrog.

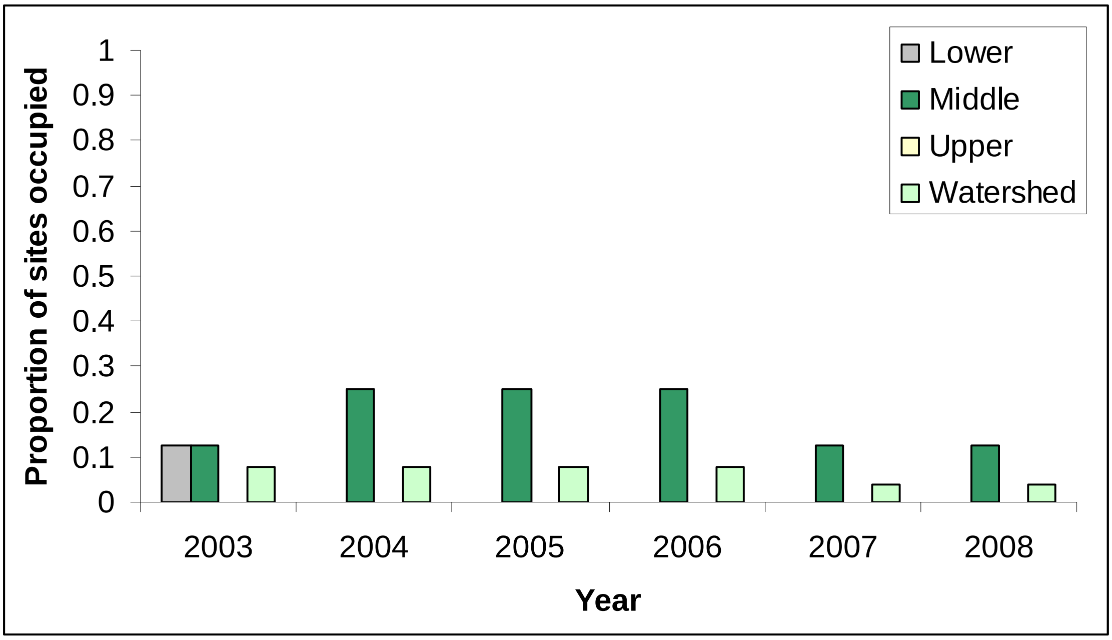
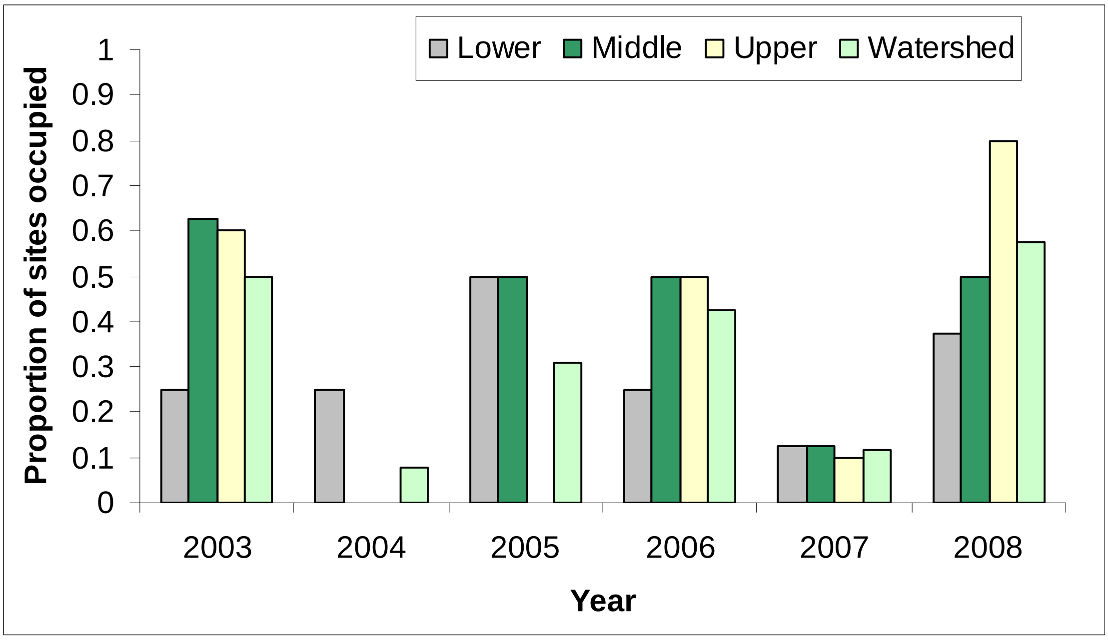


Table 2. Proportion of monitoring sites occupied by American Toad.



APPENDIX C: ANURAN PROPORTIONAL OCCUPANCY (Cont'd)

Table 3. Proportion of monitoring sites occupied by Grey Tree Frog.

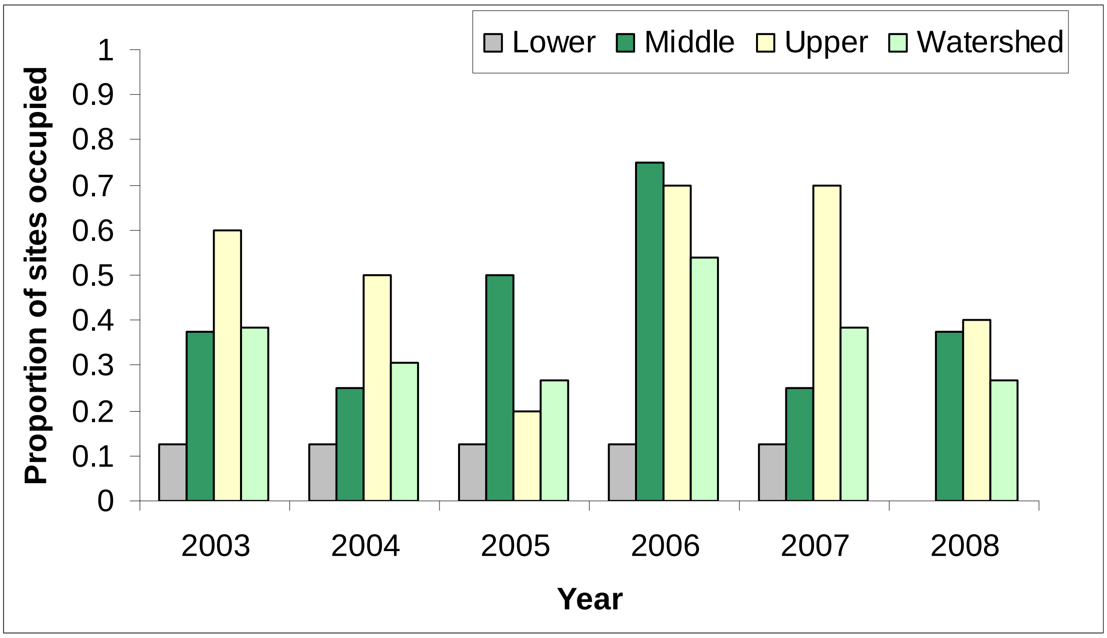
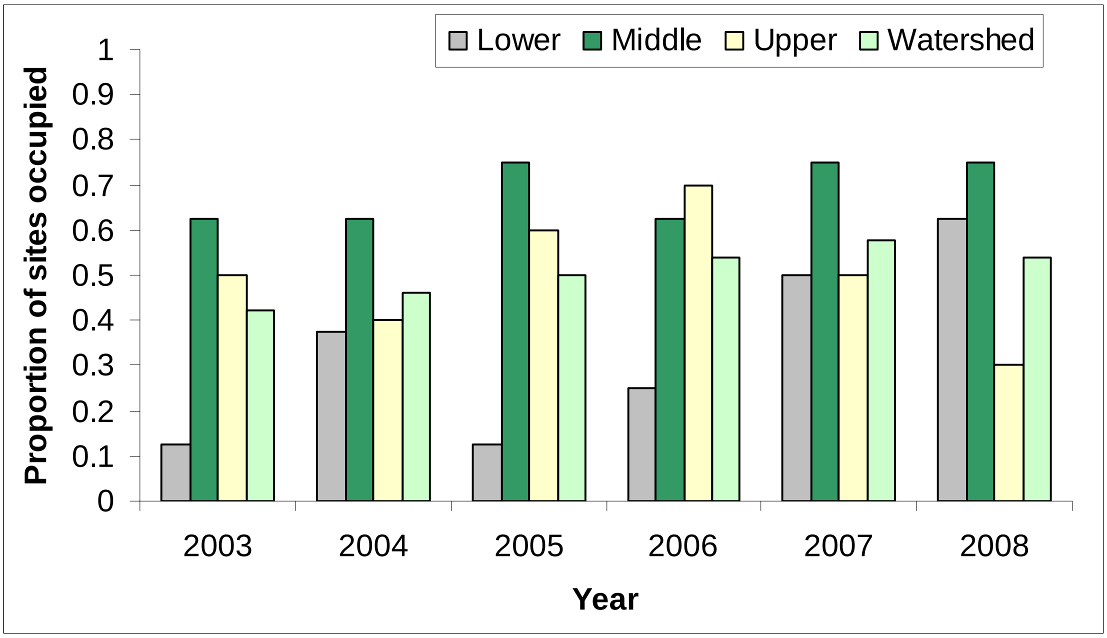


Table 4. Proportion of monitoring sites occupied by Green Frop.



APPENDIX C: ANURAN PROPORTIONAL OCCUPANCY (Cont'd)

Table 5. Proportion of monitoring sites occupied by Northern Leopard Frog.

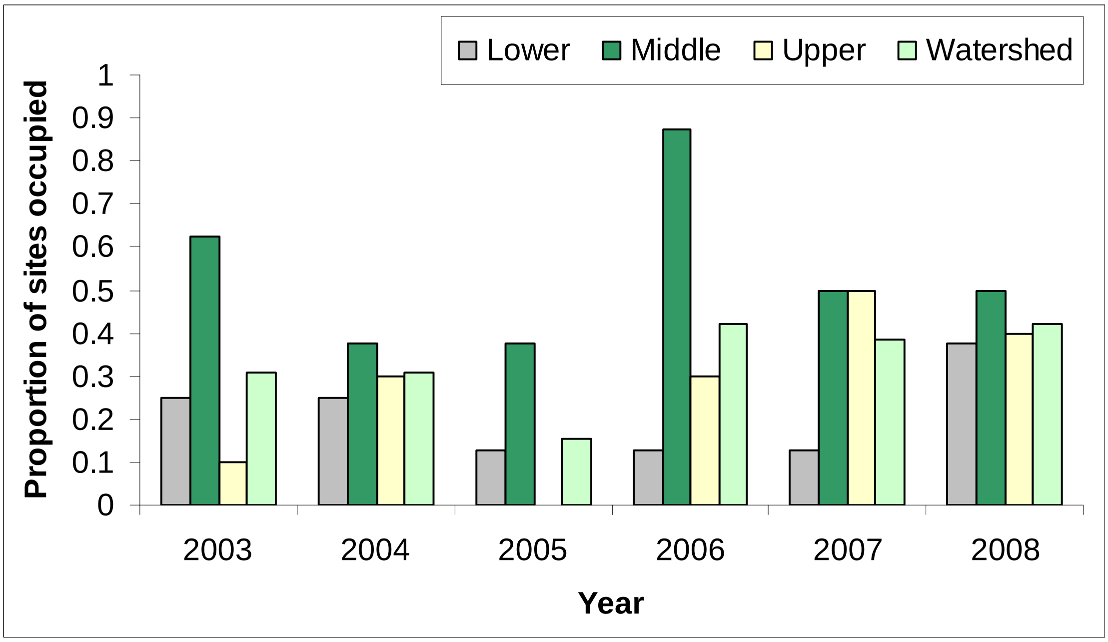
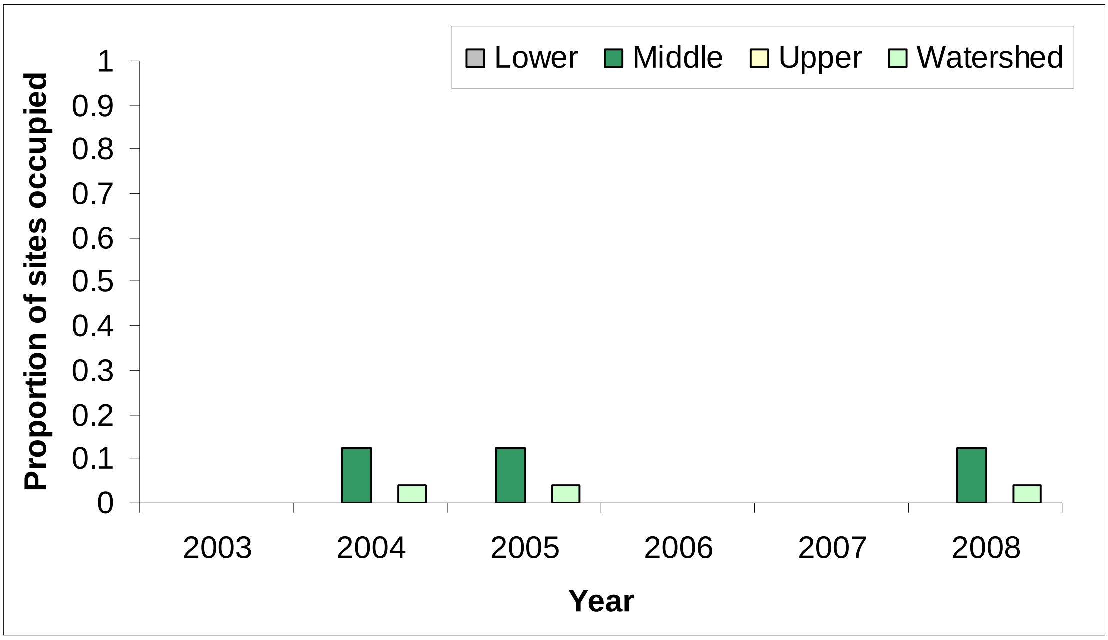


Table 6. Proportion of monitoring sites occupied by Pickerel Frog.



APPENDIX C: ANURAN PROPORTIONAL OCCUPANCY (Cont'd)

Table 7. Proportion of monitoring sites occupied by Spring Peeper.

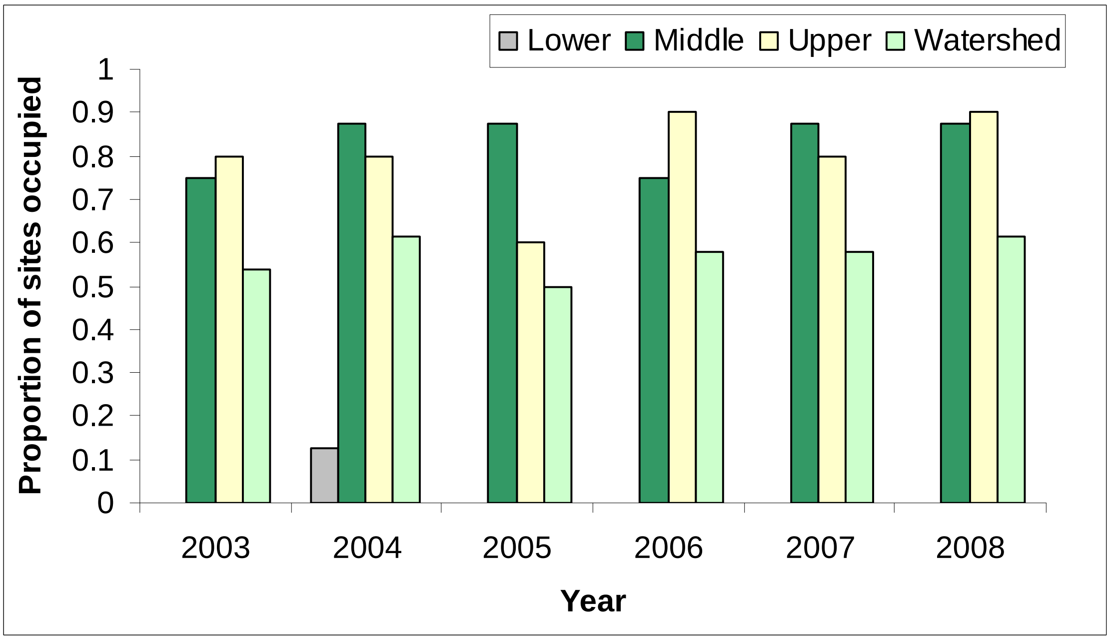
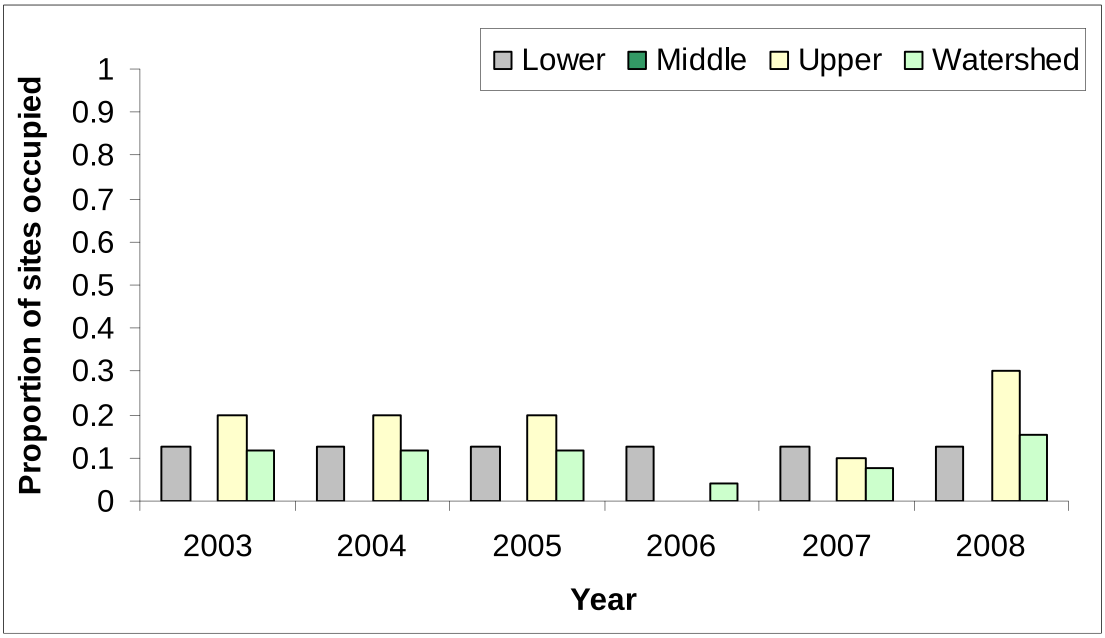
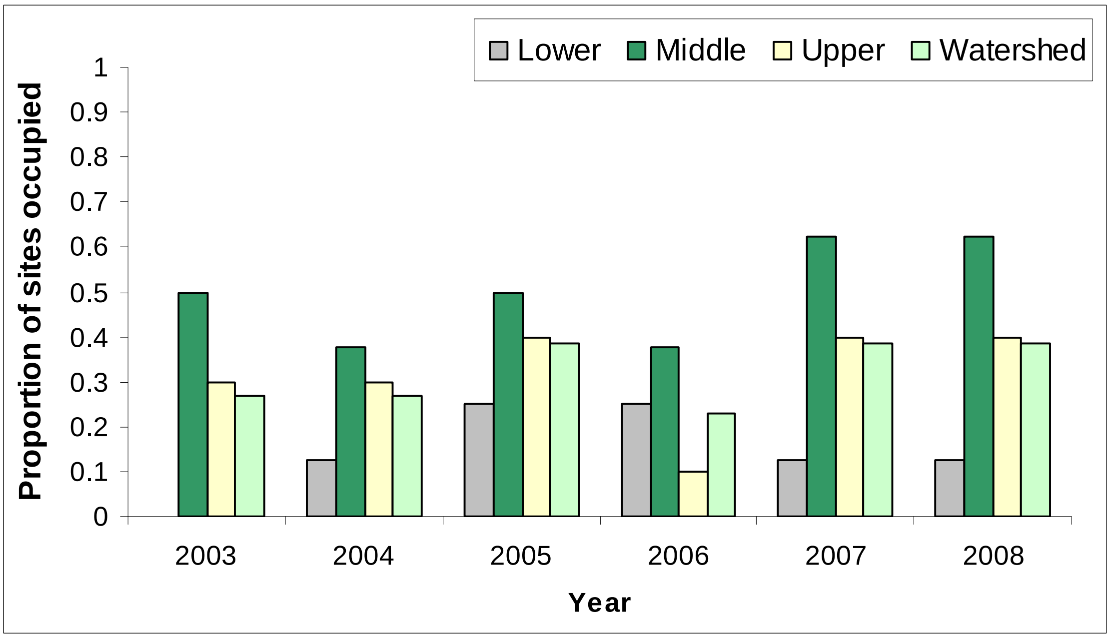


Table 8. Proportion of monitoring sites occupied by Western Chorus Frog.



APPENDIX C: ANURAN PROPORTIONAL OCCUPANCY (Cont'd)

Table 9. Proportion of monitoring sites occupied by Wood Frog.



APPENDIX D: PARAMETER VALUES ASSOCIATED WITH EACH INDIVIDUAL ANURAN MONITORING STATION IN THE CREDIT RIVER WATERSHED.

Site Name	Site Number	Physiographic Zone	Mean Species Richness [a, b]	Years Occupied [a, b]	Mean Full Chorus Detections [a,b]
Rattray Marsh	W-01	Lower	1.17	5	0
Credit River Marsh	W-02	Lower	0.5	4	0
Creditview Wetland	W-03	Lower	1.83	6	0
Meadowvale ESA Wetland	W-04	Lower	1	3	0
Hungry Hollow Wetland	W-06	Middle	0.83	4	0
Acton Swamp	W-07	Middle	3.67	6	0.5
Terra Cotta Wetland	W-08	Middle	4	6	0.83
Ken Whillans RMA	W-09	Middle	2.5	6	0
Warwick Wetland	W-10	Middle	4.17	6	0.33
Erin Pine Estates Wetland	W-11	Upper	1.83	5	0
Winston Churchill Wetland	W-12	Lower	4	6	0
Hillsburgh Wetland	W-13	Upper	0.83	3	0
Grange Orpen Wetland	W-15	Upper	0.67	3	0
Starr Wetland	W-16	Upper	2	5	0.17
Speersville Wetland	W-17	Upper	5.17	6	1.33
Caledon Lake Bog	W-18	Upper	4.17	6	0.33
Mellville Marsh	W-19	Upper	3.17	6	0
Belfountain Wetland	W-20	Upper	4.67	6	1
Rail Trail	A-01	Middle	4.67	6	0
Balinafad Ridge	A-02	Middle	5	6	1
Silver Creek	A-03	Middle	3.5	6	0.83
Alton-Hillsburgh	A-04	Upper	4.33	6	1.5
Turtle Creek	WNV-01	Lower	0.5	3	0
Mississauga Gardens	WNV-02	Lower	0.33	2	0
Churchville	WNV-03	Lower	0.83	4	0
Caledon Creek	WNV-04	Upper	2.17	6	0

[a] Per year, over six survey year
[b] The highest values within each parameter are highlighted in green, while the lowest values are highlighted in red.