









Wetland Amphibian Monitoring Protocol

Terrestrial Long-term Fixed Plot Monitoring Program

Regional Watershed Monitoring and Reporting

January 2016







Report prepared by: Paul Prior, Fauna Biologist

Sue Hayes, Project Manager, Terrestrial Field

Inventories

Lyndsay Cartwright, Data Analyst

Reviewed by: Scott Jarvie, Associate Director,

Environmental Monitoring and Data

Management

This report may be referenced as:

Toronto and Region Conservation Authority (TRCA). 2016. Wetland Amphibian Monitoring Protocol - Terrestrial Long-term Fixed Plot Monitoring Program – Regional Watershed Monitoring and



Table of Contents

page		
4	INTRODUCTION	1.0
4	STUDY DESIGN	2.0
5	EQUIPMENT & MATERIALS	3.0
6	PLOT SET-UP METHODOLOGY	4.0
7	DATA COLLECTION METHODOLOGY	5.0
10	DATA MANAGEMENT AND ANALYSIS	6.0
12	REFERENCES	7.0



List of Tables

Table 1: Frog and toad species present within the TRCA jurisdiction
Table 2: Sample size required to detect a 50% decline based on 90% power for each species 5
Table 3: List of required equipment and materials for plot set-up and seasonal monitoring 6
Table 4: Beaufort wind codes9
Table 5: Call level codes for amphibians
Table 6: Background noise codes
List of Figures
Figure 1: Peek calling periods for frog and toad species in the TRCA jurisdiction
List of Appendices
Appendix A: Marsh monitoring program habitat description form
Appendix B: Marsh monitoring program amphibian data collection form14
Appendix C: Marsh monitoring program amphibian route summary form15



1.0 INTRODUCTION

Wetlands are recognized as being highly diverse, productive and dynamic ecosystems that serve a vital environmental role. Acting as a water filtration system, they prevent sedimentation, slow surface run-off and improve water quality. They also provide valuable breeding and spawning habitat for numerous bird, fish, mammal, and amphibian species. Throughout Ontario, there are five types of freshwater wetlands, namely, marshes, bogs, fens, swamps and shallow open water.

The wetland amphibian protocol used by the Toronto and Region Conservation Authority (TRCA) has been adopted from the Marsh Monitoring Program (MMP) and focuses on amphibian species in marsh habitats. A marsh is defined as "a vegetated, wet area, periodically or regularly inundated up to a depth of 2 metres with standing or slowly moving water" (Bird Studies Canada, 2009). Marshes are home to several amphibian species that are extremely sensitive to environmental change. The long-term monitoring of the richness and distribution of these species throughout the TRCA jurisdiction can aid in the assessment of the overall health of the region.

2.0 STUDY DESIGN

Ensuring a sample size that is appropriate to detect region wide trends is the primary objective of the monitoring program. However, with additional funds and resources in the future it will also be desirable to increase the sample size in order to have the ability to look at differences between three land-use zones (urban, urbanizing and rural).

Program Objective:

 To assess the overall trend of species richness in frog and toad populations in the TRCA jurisdiction

Amphibian surveys are limited to frog and toad species found throughout the TRCA jurisdiction. The unique and distinctive territorial and/or mating calls sung by male frogs and toads, in the spring and summer, are used as the main means of species identification. Within the TRCA jurisdiction there are currently nine frog species and one toad species that can be found (Table 1).



Table 1. Frog and toad species present within the TRCA jurisdiction.

Common Name	Scientific Name
American toad	Anaxyrus americanus
Gray treefrog	Hyla versicolor
Spring peeper	Pseudacris crucifer crucifer
Chorus frog	Pseudacris triseriata
Wood frog	Lithobates sylvatica
Northern leopard frog	Lithobates pipiens
Pickerel frog	Lithobates palustris
Green frog	Lithobates clamitans
Mink frog	Lithobates septentrionalis
Bullfrog	Lithobates catesbeiana

An *a priori* power analysis was conducted in 2008 (Zorn 2008) to determine the appropriate number of monitoring plots needed to achieve sufficient power. In 2015, a further power analysis (retrospective) was conducted to ensure the appropriate number of plots are monitored for assessing spatial and temporal trends in frog species richness, the number of L1-L3 frog species and the occurrence of specific frog species (American toad, green frog, northern leopard frog, spring peeper, tetraploid grey treefrog and wood frog). The sample sizes used in this power analysis were based on sample sizes used in TRCA (2015a).

Power was sufficient (>81%) for the analysis of comparing the rural and urban zones (10 rural, 10 urban) and for analyzing temporal trends regionally (14 monitoring plots). Power was also sufficient (>95%) for analyzing temporal trends in the rural zone alone for frog species richness and the number of L1-L3 frog species. Power was low for analyzing temporal trends in the urban zone alone for frog species richness and the number of L1-L3 frog species; however, it was decided that it is more important to examine potential losses in the rural zone given the small number of frog species currently in the urban zone. Temporal trends for the rural and urban zones alone for specific frog species were not analyzed. Full details of the 2015 power analysis can be found in TRCA (2015b).

3.0 EQUIPMENT & MATERIALS

Different materials and equipment are needed depending on whether the plot is being set-up for the very first time or if visited for seasonal monitoring (Table 3).



Table 3. List of required equipment and materials for plot set-up and seasonal monitoring.

Set-up Equipment	Seasonal Monitoring and Maintenance Equipment
• Compass	Compass
Aerial photo of general station area	 Map showing plot locations
Hand held GPS unit	Amphibian data form
• ½ to ¾ metre posts of iron rebar	Habitat Description form
Spray paint	• Thermometer
Flagging tape	 Flashlight (spare batteries)
Pens and pencils	Bug repellent
Clipboard	Watch/ stopwatch
Habitat description forms	Clipboard and pencils
·	Flagging tape

4.0 PLOT SET-UP METHODOLOGY

In order to reduce staff travel time during the field season, amphibian stations and wetland bird stations were placed at the same locations or were placed in close proximity to one another. Site access was also a consideration in order to reduce the amount of travel time between stations.

Monitoring stations are set-up at the edge of wetlands that are generally large enough to accommodate at least one station (needing to be 100 m radius semi-circle in size). As larger wetlands are not evenly distributed across the jurisdiction, some monitoring sites are smaller than the 100 m radius semi-circle. By allowing smaller sized wetlands to be included, a more even distribution of stations is created across the jurisdiction and provides a gradient of wetland types and quality to be represented in the TRCA's monitoring program.

A survey route can consist of one to eight monitoring stations. The number of stations on a given survey route depends on the size of wetland. Smaller wetlands can accommodate fewer stations than a larger wetland. Stations must be at least 500 m apart to ensure that observers cannot hear the calling activity at a previously monitored station (prevent double counting). Regardless of the wetland size, a potential station site is only suitable if the 100 m radius semi-circle is dominated by at least 50% marsh habitat. The main characteristic of a marsh habitat is that they are wet areas where the predominant vegetation is non-woody species (trees and shrubs) such as cattails, grasses, sedges and rushes. These areas are inundated with water (maximum depth of 2 m) for a portion or all of the year (MMP, 2009).

Stations are set-up and oriented to maximize the area of wetland being sampled by sight and ear. Choosing an elevated focal point is useful for this reason. Sites that are partially blocked by trees and shrubs do not pose a problem as long as the station can still be monitored by ear.

Selected monitoring stations are marked by a ½ to ¾ metre iron rebar post hammered down,



enabling the top section to remain visible. As amphibian surveys are performed at night, the stakes are marked with reflective flagging tape allowing them to be relocated annually (when flashlight is passed over it), even when vegetation has grown taller than stakes. The northing and easting is taken using the hand held GPS unit and recorded onto the data sheet. To help define the outer limits of the 100 m semi-circle, interval distances of 25, 50, 75 m can be measured out to the front and to either side of the station and marked with flagging tape.

5.0 DATA COLLECTION METHODOLOGY

This protocol is based on the following:

Marsh Monitoring Program Participant's Handbook for Surveying Amphibians. 2009 Edition. 13 pages. Published by Bird Studies Canada in cooperation with Environment Canada and the U.S. Environmental Protection Agency. February 2009.

Once stations are set-up at appropriate locations a habitat description form is filled in to describe the marsh habitat (Appendix A - same form used for wetland birds). This form is completed once annually around late May to mid-June (facilitates identification of vegetation). The date, assigned route number, assigned observer number and name, along with an indication of survey type (i.e. bird and or amphibian) and station letter (each station on a survey route is alphabetized i.e., the first station would have a station letter of "A" the second station would be "B" etc.) are filled in on the data form. There are an additional seven sections to the form. Part A asks for estimated percentages of the total sample area that is covered by emergent vegetation, open water (including floating plants), exposed mud/sand/rock, trees and shrubs (values should add up to 100%). In part B the density of floating plant cover in open water zones (none, slight, moderate, dense) is indicated. Part C is an indication of wetland permanency based on 3 categories of permanent (almost never dries up, water is usually quite deep), semi-permanent (dries up in some years of low precipitation; water fairly shallow) or seasonal (usually flooded in spring and early summer, but tends to dry up in late summer or in dry years). Part D is an indication of the overall marsh size based on the following categories of tiny (between 1.5 and 2.5 ha), small (between 2.5 and 5 ha), medium (between 5 and 25 ha), large (between 25 and 50 ha) and huge (greater than 50 ha). In part E the type of habitat behind the station (determines whether plot is located in marsh edge or interior) is indicated based on five categories; marsh, field, forest, urban, and other. In part F you are asked to indicate if there are human influences affecting the sample area (either positive or negative). The last section asks for the percentage coverage of dominant emergent vegetation (record of the four most dominantly occurring emergent vegetation species) (Note: These species do not need to add up to 100%). In addition, a sketch map of key habitat features showing the position of vegetation within the monitoring station is included. Also, an indication of the compass bearing for the marsh orientation of the survey is documented.

Monitoring for frog and toad species are done three times each year during the peak breeding times for the individual species. There are generally three peak calling times throughout the field season to capture the various species (Figure 1).



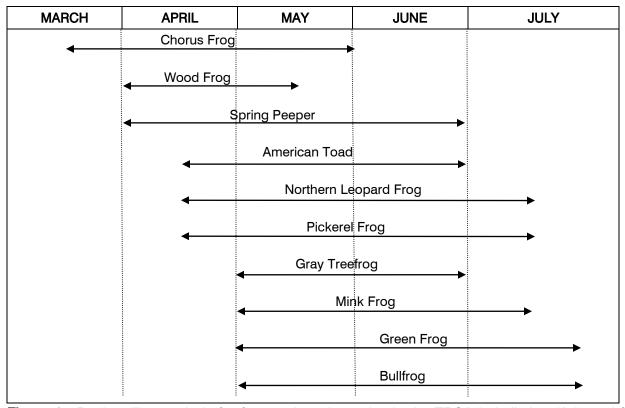


Figure 1: Peek calling periods for frog and toad species in the TRCA jurisdiction (Adapted from Marsh Monitoring Program Participant's Handbook for Surveying Amphibians, 2009)

The early breeders (chorus frog, wood frog and spring peeper) are captured during April visits, the mid-breeders (American toad, northern leopard frog and pickerel frog) during May visits and the late breeders (gray treefrog, mink frog and bullfrog) during June visits. Even though all ten species listed in Figure 1 are not used in the data analysis due to insufficient sample size, there is enough overlap of the three survey times that if the species were present it could be theoretically detected. Each visit is separated by at least 15 days.

Temperature guidelines change with each visit. For the first visit night temperatures are above 5°C, at least 10°C for the second visit and at least 17°C for the third and final visit. Calling activity is at its highest when environmental conditions are most favourable (i.e. warm, damp nights with little to no wind). Wind conditions cannot be above a three on the Beaufort scale (Table 4) and no heavy or continuous rain. Not only will strong winds dry out the skin of the amphibian causing them to remain under water and hinder calling activity but it will also impair the ability of the observer to effectively listen to any calling/singing. For this reason, close attention must be paid to weather and temperature conditions in order to select an appropriate night to monitor and to ensure that survey conditions are standardized. If conditions change for the worse while conducting the survey the survey is terminated until conditions are appropriate. Monitoring is done in teams consisting of two people.



Table 4. Beaufort wind codes (taken from Marsh Monitoring Program Participant's Handbook for Surveying Amphibians, 2009)

Beaufort Scale	Wind Speed (km/h)	Description	Visual Cues
0	0 - 2	Calm	Smoke rises vertically
1	3 - 5	Light	Smoke drifts
2	6 - 11	Slight breeze	Leaves rustle
3	12 - 19	Gentle breeze	Lighter branches sway
4	20 - 30	Moderate breeze	Dust rises, branches move
5	31 - 39	Fresh breeze	Small trees sway

Surveys start one half hour after sunset and end before midnight. Surveys follow a point count method, and as such, at each station observers will determine the intensity (call code 1, 2 or 3) of calling activity and provide an abundance count for individuals (Table 5). To begin a survey, observers first navigate to a station and stand facing the wetland in the direction of the pre-set station compass bearing. Most calling would have temporarily ceased with the arrival of the observers at the station. For this reason observers stand quietly for 1 minute at the designated focal point to allow the amphibians to settle-down. Following this time, the observer listens to the calling activity for a total of 3 minutes using a stop watch or timer. The calling activity for each species is categorized according to its intensity into one of three call code levels. Using the appropriate species codes the count of all the species that are heard calling from within the 100 m semi-circle and from outside the 100 m semi-circle is recorded (Appendix B). A small sketch depicting this information and including the direction from which the marsh was sampled (i.e. 32° NNE) is also created using the data collection form. For each visit the assigned route number, route name, assigned observer number, observer name, visit number (1, 2 or 3), date, survey start and finish time of the route using 24 hour clock, background noise code (1 to 4) (Table 6), Beaufort wind scale number, cloud cover and air temperature estimates are recorded. Proper units are always recorded to prevent confusion in data entry. All comments are noted. Before leaving a station, forms are checked to make sure that they are completely filled out. "N/A" or a dash is placed in all blank boxes.

Table 5. Call level codes for amphibians (taken from Marsh Monitoring Program Participant's Handbook for Surveying Amphibians, 2009)

Call Code	Description		
1	Calls not simultaneous, number of individual can be accurately counted.		
2	Some calls simultaneous, number of individuals can be reliably estimated		
3	Full chorus, calls continuous and overlapping, number of individuals cannot be reliably estimated		



Table 6. Background noise codes (taken from Marsh Monitoring Program Participant's Handbook for Surveying Amphibians, 2009)

Index	Description
0	No appreciable effect (e.g., owl calling)
1	Slightly affecting sampling (e.g., distant traffic, dog barking, car passing)
2	Moderately affecting sampling (e.g., distant traffic, 2-5 cars passing)
3	Seriously affecting sampling (e.g., continuous traffic nearby, 6-10 cars passing)
4	Profoundly affecting sampling (e.g., continuous traffic passing, construction noise)

6.0 DATA MANAGEMENT AND ANALYSIS

Data Management

At the end of the field season, once all three monitoring surveys have been completed, the Amphibian Route Summary Form (Appendix C) is filled in and sent to Bird Studies Canada. In addition, all data are entered into a corporate TRCA access database and all field collection forms are stored in a corporate filing system.

Data Analysis for the 2015 Terrestrial Long-term Monitoring Program Report (TRCA 2015a)

Frog data were retrieved from the TRCA Natural Heritage Monitoring database using the query function selected for all species, at all sites (excluding special projects) for all years (2009-2013). Before calculations were completed, the initial data set was examined to ensure equal sampling effort by checking what sites did not have 3 site visits over the season. The following sites and years were excluded because sampling effort was inconsistent (less than 3 visits in one year or different numbers of stations surveyed among years): Duffins (WF-13) only 1 station was surveyed in 2009, none 2010-2012 and 2 stations in 2013; South Queen St East of Main (WF-21) only had visits 2 and 3 in 2010.

Data were sorted in Excel first by plot ID then year then station then date. Data entries were deleted if the species was detected outside the 100 m radius. For the calculation of species richness, repeated entries of the same species at the same site and station were deleted because a species was often detected at more than one visit. Average species richness per site per year was calculated by determining the total number of species found at a particular station in a particular site then averaging those values based on the number of stations at a site. Most sites only had one station. To determine the percent of sites occupied, species presence/absence was noted at each site in each year. Temporal and spatial trends were determined for each species independently.

For both temporal and spatial analysis, summary tables with site as row and year as column were used. For temporal trends, data analysis attempted to maximize the number of years with the same list of sites consistently surveyed each year. This often resulted in limiting the number of sites included because new sites were added in more recent years. Keeping the same group of



sites studied in each year allows for valid comparisons among years. The list of sites and years included for the temporal analysis can be found in the appendix of TRCA (2015a). The current baseline year for the temporal data is 2009 but in future years a later baseline year may be used in order to increase the number of sites included in the analysis.

Temporal trends were statistically analyzed using Mann-Kendall tests in an established Microsoft Excel™ spreadsheet provided by the Ministry of Natural Resources and Forestry. The Mann-Kendall test is a non-parametric test for identifying monotonic trends in time series data. This test was chosen over traditional regression analyses because the data did not meet the assumption of independent samples required for regression analyses. When analyzing time-series data, data collected at the same site from one year to the next are not independent. This made the Mann-Kendall test the best option. The Mann-Kendall test uses the S statistic to determine an associated p-value. If the value of S is zero, there is no trend in the data. If a data value from a later time period is higher than a data value from an earlier time period, S is incremented by one. On the other hand, if a data value from a later time period is lower than a data value sampled earlier, S is decremented by one. The net result of all such increments and decrements yields the final value of S (TRCA 2011). For example, a very high positive value of S is an indicator of an increasing trend, and a very low negative value indicates a decreasing trend (TRCA 2011). A p-value of less than 0.05 denotes a significant trend (increasing or decreasing) and a p-value of greater than 0.05 indicates that there is no increase or decrease over time and that the variable of interest is stable.

For spatial analysis, data analysis attempted to maximize the number of sites. This often resulted in using more recent years of data because new sites were added in more recent years. Often the most recent 2-4 years of data were used because they contained a consistent set of sites in each year. Spatial analysis only included 2013-2014 data to maximize the number of sites (10 rural and 10 urban). An average value across the selected years was calculated for each site and this single value per site was used for analysis of species richness and # L1-L3 species. For the spatial analysis of the percent of sites occupied, 2013 and 2014 were pooled and species presence/absence was examined across the two year period. For example, if a species was absent at a site in 2013 but present at the same site in 2014, this species would be recorded as overall present at that site. The list of sites and years included for the spatial analysis can be found in the appendix of TRCA (2015a).

Spatial trend analysis for average species richness and # L1-L3 species was conducted using SAS JMP statistical software (SAS Institute Inc. 2008). Differences between urban and rural land use zones were analyzed using independent t-tests. An independent t-test is a parametric test that compares the mean value between two groups (e.g. urban and rural land use zones). This test is reported using the test statistic, t, and an associated p-value where a p-value of less than 0.05 indicates a difference between groups. A p-value of greater than 0.05 indicates that there is no difference between groups. Before performing t-tests, all data were checked for normality and homoscedasticity because these are two assumptions of using parametric statistics. If these assumptions were not met, data transformations were attempted to improve normality or heteroscedasticity. If data transformations were not effective, a Wilcoxon test was conducted (Z-statistic). This is the non-parametric version of an independent t-test and is the appropriate test to



proceed with if the data do not meet assumptions. For TRCA (2015a), an independent t-test was used but this may not be the appropriate test to use in the future if the data violate the assumptions of using parametric statistics listed previously. When comparing the proportion of sites occupied between the urban and rural zone a separate chi-square test was used for each species as per Zar (1999).

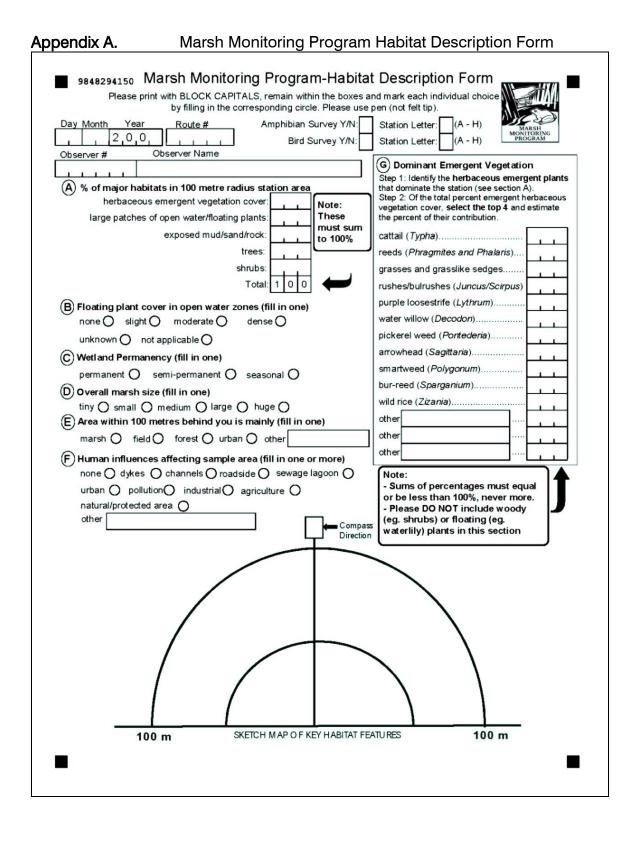
7.0 REFERENCES

- Marsh Monitoring Program Participant's Handbook: Getting Started. 2009 Edition. 13 pages. Published by Bird Studies Canada in cooperation with Environment Canada and the U.S. Environmental Protection Agency. February 2009.
- Marsh Monitoring Program Participant's Handbook for Surveying Amphibians. 2009 Edition. 13 pages. Published by Bird Studies Canada in cooperation with Environment Canada and the U.S. Environmental Protection Agency. February 2009.
- TRCA. 2011. Regional Watershed Monitoring Program: Surface Water Quality Summary 2006-2010. 49 pp + appendices.
- TRCA. 2015a. Terrestrial Long Term Monitoring: Spatial and Temporal Trends 2008-2014.
- TRCA. 2015b. A Retrospective Power Analysis for the Terrestrial Long Term Monitoring Program.
- Zar, J. H. 1999. Biostatistical analysis, 4th edition. New Jersey, USA.
- Zorn, Paul. 2008. A *Priori* Power Analysis for Toronto and Region Conservation Authority's Regional Watershed Monitoring Program. Report prepared for TRCA. Ottawa, ON



APPENDICES

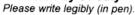






Appendix B. Marsh Monitoring Program Amphibian Data Form

Marsh Monitoring Program - Amphibian Data Form Return by 31 July





VISIT INFORMATION

Route #: ON499 Route Name: Mud Lake Marsh
Observer #: 18649 Observer Name: Kathy Jones
Visit #: Day:
Cloud Cover (10th): Temperature (°C or °F): 15C Beaufort Wind Scale (0-6):
Precipitation (check one): None/Dry Damp/Haze/Fog Drizzle Rain

CALL LEVEL CODES

Code 1: Calls not simultaneous, number of individuals can be accurately counted

Code 2: Some calls simultaneous, number of individuals can be reliably estimated

Code 3: Full chorus, calls continuous and overlapping, number of individuals cannot be reliably estimated

Amphdfrm2008.cdr, rev 02/2008

