MMx

MISSION MONARCH - EXPERT

The Canadian Monarch Monitoring Protocol & Sampling Grid

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INTRODUCTION

Following the dramatic decline of both Eastern and Western North American migratory monarch populations over the past 20 years, the monitoring and protection of the butterfly's breeding, nectaring, and staging habitat throughout the Canadian range was identified as a conservation priority in Canada. In this regard, long-term surveys in protected areas (such as the counts of migrating and roosting monarchs that took place on the Long Point peninsula, Pelee Island and Point Pelee National Park within the Lake Erie-Lake Ontario Ecoregion) and the contribution of community science programs (such as Mission Monarch) have provided useful, yet spatially biased data.

To optimize monarch research and conservation efforts, the Government of Canada (through the Federal Department of Environment and Climate Change), mandated Montréal Space for Life (through the Mission Monarch program), to design a monitoring protocol and sampling grid largely inspired by the Integrated Monarch Monitoring Program (IMMP) but adapted to the Canadian context. Therefore, thanks to the new Expert component of the Mission Monarch program (Mission Monarch - Expert), Canada now has a standardized monitoring program that can be used by conservation professionals and skilled community scientists to collect **geographically and ecologically representative data** that integrates **monarch habitat** and **monarch use** data into a unified dataset compatible with the IMMP (see ***Appendix A*** for more information on this initiative).

What is Mission Monarch - Expert?

Mission Monarch - Expert (hereinafter referred to as "MMx") is the new **Expert** component of the Mission Monarch program. The goal of this component is to characterize potential monarch habitats and monitor monarch presence and activities while following standard procedures, for the purposes of implementing efficient conservation actions in Canada and allowing continental-scale analyses (compatible with other monarch-related protocols in North America).

To accomplish this, MMx aims to:

- Provide geographically and ecologically representative information to update population and habitat models in Canada;
- Contribute to the trinational (which includes Canada, Mexico and the United States) conservation effort by promoting a national protocol and dataset that is compatible with the IMMP currently used in the United States and offers the possibility to conduct wide-scale analyses;
- Fill the knowledge gaps that cannot be addressed by community science programs alone;
- Track long-term changes in the distribution and abundance of monarchs and their habitats;
- Acquire and share information about how habitat conservation actions affect monarchs and their habitat.

To cover the vast distribution range of this migratory species in Canada, MMx engages a broad network of community members, biologists, resource managers, students, landowners, and other professional or skilled community conservationists who collect data that are incorporated into an open-access dataset available to all for analyses. Data collected through MMx is also shared on the ***Trinational Monarch Knowledge Network (TMKN)***, a trinational database that assembles observations of monarch butterflies and of milkweeds (*Asclepias* spp.) throughout the range of monarchs in North America.

Monitoring Activities

The MMx monitoring protocol consists of different monitoring activities (see *Table 1*) that participants can choose from to characterize potential monarch habitats depending on their interests, skills, and needs. While the rest of the document details the best practices for achieving the long-term goals listed above, **any data shared is valuable** for broader-scale analyses.

Monitoring Sites

This monitoring protocol supports two (2) types of monitoring sites: **A. Random** and **B. Non-random.**

A. Random sites can be selected from randomly generated sampling points provided by the MMx Sampling Grid.

These sites are distributed randomly across Canada and among land-use types that may host potential monarch habitat (see *Appendix B*). Monitoring of these sites ensures an unbiased characterization of potential monarch habitat and use of habitat by monarchs across site types and throughout their potential breeding range.

B. Non-random sites are sites of interest (not listed in the MMx Sampling Grid) that landowners, managers, or organizations wish to monitor for conservation, research, or long-term assessment purposes.

These sites of interest can be sites with management plans that promote pollinator conservation, protected or restored areas for monarch conservation, or any potential monarch habitat that is to be monitored by managers, organizations, or individuals following a standard protocol.

Application of MMx

MMx will improve our knowledge of monarch biology and ecology across site types throughout Canada. Unlike other monitoring programs with more simple protocols (adapted for the general public), the more comprehensive approach of the monitoring activities within MMx will make it easier to assess the interactions between monarch life stages and habitat characteristics, both spatially and temporally. **This will help address some priority questions regarding:**

- The understanding of milkweed distribution and abundance to allow for targeted conservation efforts;
- The characteristics that constitute a quality habitat (milkweed and nectar resources) for monarchs;
- The monarch nectaring and breeding hotspots;
- The impact of pesticides and herbicides on monarchs (including herbicide-resistant crops and neonicotinoids);
- The assessment of the monarch breeding population size during the summer;
- The effects of habitat loss and degradation.

Additionally, the MMx standard monitoring activities can be used by individuals or organizations to meet their own information needs or research objectives. For instance, these activities can help track how conservation practices, habitat management, and agricultural practices affect monarchs and their habitats.

Finally, MMx engages stakeholders of all kinds involved in the conservation of monarchs through improved monitoring of monarch habitat and use. The MMx protocol guarantees the development of datasets that will benefit not only the surveyors who invest time and effort in these activities, but also monarch conservation at the national and continental level. Broad and diverse participation is necessary to achieve the desired breadth and depth of sampling and to ensure the program's long-term sustainability. Ultimately, monarch conservation relies on the cooperation of all stakeholders not only in protecting and restoring, but also in understanding and evaluating this species and the habitats on which it relies.

IMPLEMENTATION

The following sections will focus on the different steps leading to the implementation of this protocol.

SAMPLING TIMING AND FREQUENCY

Since the presence of monarchs is not mandatory for conducting any of the surveys described in the sections below (Activity 1, 2 and 3), surveys can start as soon as milkweed (*Asclepias* spp.) and/or blooming nectar plants are present in the field. However, it is possible to only start surveying when monarchs arrive at the specific location. Surveys can then be conducted throughout the summer until most of the monarchs fly back to central Mexico. This means the sampling period may vary greatly between and within provinces across Canada. We therefore encourage you to consult your local experts to find out when milkweed sprouts in your region, when nectar plants start blooming, and when monarchs arrive during spring migration.

Sampling frequency depends on the surveys that you wish to conduct. Best practice would be to conduct all surveys at a sampling site throughout the summer while following the recommended sampling frequency detailed in Table 1. However, if you don't think you can survey a sampling site as often as recommended, we invite you to carry out as many surveys as possible during the ***International Monarch Monitoring Blitz (IMMB)***.

NOTE

The IMMB is an annual event devoted to the research for monarch conservation. To learn more about the IMMB, or this year's event dates, go to the the *CEC's International Monarch Monitoring Blitz webpage* or subscribe to the *Mission Monarch newsletter*.

Table 1 Recommended sampling frequencies for each monitoring activity included in this protocol and estimated duration (details are included in the dedicated sections below.)

Monitoring activity	Sampling frequency	Duration
Plot Description	**Each visit***	**15-30 min**
Activity 1 Milkweed and Blooming Plant Survey	**Monthly**	**1-4 hours**
Activity 2 Immature Monarch Survey	**Weekly**	**1 hour**
Activity 3 Adult Monarch Survey	**Bi-weekly**	**30 minutes**

* Site description must be completed before any other monitoring activity

SITE SELECTION

Overview

Site selection is the first step in MMx. There are two (2) options: **A. Select a random site from randomly generated sampling points** provided by the MMx Sampling Grid or **B. Select a non-random site of your choice** (not provided by the MMx Sampling Grid). The sampling site is where the monitoring plot will be established to conduct the different surveys.

Although both options are valid, this section will elaborate on the most suitable context for each option, based on the surveyor's goals and objectives. It will also describe the steps to follow and how to obtain permission to monitor a given site (if necessary).

Option 1 Select a random site from the MMx Sampling Grid

Context

Selecting a site from randomly generated sampling points ensures the unbiased characterization of potential monarch habitat and use of habitat by monarchs across site types and throughout their potential breeding range in Canada.

This is the preferred option for collecting ecologically representative data via a randomized sampling design. This option requires using the MMx Sampling Grid (see ***Appendix B***).

Instructions

Consult the MMx Sampling Grid and locate an accessible Sampling Block (10 x 10 km square). Then, choose a sampling point within that block. Click on the sampling point to access the site's status, type, and coordinates.

Make sure the site's status is "available," which means that it has not yet been monitored by another surveyor in the current year.

The site's type indicates the expected land use of this point (see Site Type categories in ***Table 2***). If you expect to monitor more than one site within the same sampling block in the current year, we invite you to choose different site types in order to diversify the type of land use monitored.

Use the site's coordinates to locate the sampling site in the field.

NOTE

When choosing a sampling site, the best practice is to repeatedly monitor the same site throughout the summer based on the recommended sampling frequencies in *Table 1*.

Visiting multiple sites only once during the summer is not recommended. Visiting the same site multiple times while following the recommended sampling frequencies is the best way to evaluate the quality of the site.

Option 2 Select a non-random site of your choice

Context

Selecting a non-randomly generated site does not ensure the unbiased characterization of potential monarch habitat and use of habitat by monarchs across site types. However, if there is interest in characterizing potential monarch habitat and/or monitoring monarch use of habitat outside of the MMx Sampling Grid, we recommend using the MMx monitoring activities to facilitate analysis of continental-scale data.

This is the preferred option for collecting standardized data on sites of interest (e.g., sites with management plans that promote pollinators, protected or restored areas for monarch conservation, etc.) that are not included in the MMx Sampling grid. This applies to individuals or organizations interested in carrying out monarch-related surveys in specific locations.

Instructions

Visit the site of interest and make sure it can support the MMx activities detailed in the sections below. Here are the criteria that apply to non-random sites:

1. Surveyors need to have permission to access the site (see the ***Obtaining Permission to Monitor*** section to confirm that you have the necessary permission);
2. The non-random site must consist mainly of accepted site types targeted by this protocol (see ***Table 2*** for accepted site types within the MMx).

NOTE

If the intention is to use this protocol within a research project, make sure that the needs of the project and this program are compatible. If they are not, this protocol can be used as inspiration, but note that data collected through a modified protocol may not be compatible with the MMx database and, as such, may not be integrated.

Table 2 MMx site types. Accepted sites to be monitored with MMx are in white. The unaccepted site types (no monitoring) are in grey. Site types are based on the *2015 Land Cover of North America at 30 metres* produced by the North American Land Change Monitoring System (NALCMS).

Site Types	Code	Description
Agriculture	**AGC**	Areas associated with intensively managed crops, including annual crops, perennial grasses for grazing, and woody crops.
Developed	**DEV**	Areas that contain urban constructed materials for human activities, such as cities, towns, transportation, etc.
Grassland	**UGS**	Areas dominated by graminoid or herbaceous vegetation, generally accounting for > 80 percent of total vegetation cover.
Protected land	**PAG** **PGS** **PWL**	Protected agricultural land Protected grassland Protected wetland
Rights-of-way	**ROW**	Vegetation habitat ≥ 4 metres wide next to roads, railways, and transmission/power lines.
Wetland	**UWL**	Areas dominated by perennial herbaceous and woody wetland vegetation which is influenced by the water table at or near the surface over extensive periods of time (either coastal or inland). Includes marshes, swamps, bogs, etc.
Forest	**FOE** **FOD** **FOM**	Needleleaf evergreen forest generally taller than three metres. Broadleaf deciduous forest generally taller than three metres. Mixed forest generally taller than three metres (neither needleleaf nor broadleaf tree species occupy > 75 percent of total tree cover).
Open water	**OWR**	Areas of open water, generally with < 25 percent cover of non-water cover types.
Barren land	**BLD**	Areas characterized by bare rock, gravel, sand, silt, clay, or other earthen material, with little (< 10 percent) or no "green" vegetation present.

Obtaining Permission to Monitor

Unless the person or organization is the owner of the property on which the sampling site is located, the landowners or land managers must be informed of your presence and of the activities carried out on their property. See ***Appendix C*** for tips on how to get in touch with landowners or land managers and obtain permission to monitor. You will be asked to upload a copy of the form when you register a site in the online MMx user portal. After granting permission to access and monitor their land, they must sign the ***Landowner Waiver & Release of Liability Form*** before any activities can begin. This agreement contains important details on site access, data sharing permissions, and contact information.

MONITORING PLOT SETUP

Overview

Surveys typically occur within a 1 hectare monitoring plot at a sampling site. The shape of the plots may differ slightly based on the type and/or shape of the site being monitored. Defining the plot boundaries is important to ensure that surveys occur consistently in the same location at the site, that similar sized areas (plots) are assessed across sites, and that the plot size and location are properly tracked within MMx.

The following section presents the steps to properly set up the monitoring plot. This includes exploring the site, selecting the best arrangement possible for the monitoring plot, identifying the plot boundaries, and properly recording all the necessary information for the database.

Miscellaneous monarch observations may also be recorded.

Attributes Measured or Assessed

- Original (if random site) and verified site type
- Sampling Point ID
- Land ownership type
- Conservation status and management (if applicable)
- Plot setup
  - Shape
  - Coordinates
  - Orientation
  - Dimension

Supplies Needed

- **GPS device**
- **Compass**
- **Long-distance measuring instrument or device (measuring wheel, transect tape, distance tracking device, etc.)**
- **Plot boundary markers (stakes, pin flags, etc.)**
- ***Monitoring Plot Setup Form*, clipboard, and pencils**

STEP 1

Site Exploration to Monitor

On arriving at the sampling site coordinates, explore the surroundings to get a general idea of the site's characteristics. This will help you in the following steps of this activity. Pay attention to the dominant site type present (refer to ***Table 2***), the approximate size of the site (based on the actual site type in the field), the other site types that might be present within the monitoring plot, and the milkweed distribution and density across the site.

Those observations will be useful when completing the Plot Description Form (see ***Plot Description Activity***) and when selecting the milkweed sampling options of the Immature Monarch Survey (see Step 1 of ***Activity 2***).

While exploring, make sure that the dominant site type of the sampling site is one of the accepted site types to be monitored with MMx (***Table 2***).
If it is not an accepted site type, indicate which site type it is in the ***Monitoring Plot Setup Form*** and do not proceed with the subsequent steps since unaccepted site types are not to be monitored (see Step 4 - Verified Site Type). Select another sampling point of the same site type in the MMx Sampling Grid.

If it is an accepted site type, but not the site type indicated in the MMx Sampling Grid, record the actual site type in the ***Monitoring Plot Setup Form*** and continue following the steps.

NOTE

If you plan to conduct an Adult Monarch Survey (see *Activity 3*) on the same day that you are setting up the monitoring plot, it is recommended to wait at least one hour between the plot setup and the Activity 3 survey so as to minimize the effect of your presence on the behaviour of butterflies.

STEP 2

Selecting Plot Orientation, Shape and Size

The best-case scenario would be to have a standard rectangle plot (see ***Figure 1***) with the following characteristics:

- 1 hectare (50 x 200 m)
- oriented northward (0º)
- starting point (P1) located at the sampling point
- less than 10% of unaccepted site type within the plot (e.g., open water, dense tree canopy, etc.)

There is very little chance that all these requirements will be met. More often than not, the **orientation, shape** (***Table 3***), and **size** of the plot (***Table 4***) should be adapted to best fit within the site.

NOTE

A geographic information system (GIS) tool (e.g., Google Earth) can be used in advance to better visualize the area to monitor, since the proposed alterations mentioned below may be difficult to achieve in the field. Keep in mind that the actual site's characteristics may differ from those projected by these GIS tools.

Possible alternatives to the standard rectangle plot

Change Sampling Point Location

Best practice would be to use the sampling point as the starting point (P1) of the plot. However, the starting point can be located elsewhere on the site if that helps to better fit the plot within the sampling site. This also applies when the sampling point of a given site type is located just outside of that site type due to geomatic inaccuracy.

Change Plot Orientation

A northward orientation (0º) of the plot is to be privileged when starting at the sampling point.
If necessary, shift its orientation clockwise until it fits within the site. The shape or size of the plot should be changed ONLY if the plot's orientation does not fit within the site even after shifting.

Change Plot Shape

Rectangle plots with a 1 x 4 ratio (e.g., 50 x 200 m) are to be privileged over any other plot shape. If no rectangle plot of 1 ha fits within the site necessary, try fitting a square plot of equal area.

Change Plot Size

Plots of 1 ha are to be privileged in all cases. If the plot must be downsized, it should not be less than 0.25 ha (*Table 4*). If this is not possible, indicate that the site is too small to be monitored (with estimated size of the site) in the *Monitoring Plot Setup Form* and do not proceed with the subsequent steps.

Tips and Warning

This only applies to sampling points from the sampling grid (random sites). **Sites of your choice (non-random sites) can be smaller than 0.25 ha.**

Special circumstances

If the sampling point of an unprotected or protected Agriculture site type (AGC or PAG) is in active production, shift the sampling point to the nearest field corner, set up a **linear plot** along the field's border, and change the site type code for it to be considered as a border (AGC becomes AGE and PAG becomes PAE).

If the sampling point selected is a Rights-of-way site type (ROW) that is less than 25 m wide (within which no rectangle plot can fit), set up a **linear plot** starting from the sampling point, or measure the width of the site and shift the starting point to the mid-width.

If the sampling point selected is a Developed site type (DEV) located on or near a roadside with a less than 25 m wide habitat edge (within which no rectangle plot can fit), set up a **linear plot** starting from the sampling point, or measure the width of the roadside and shift the starting point to the mid-width.

Table 3 Accepted plot shape and corresponding site types

Shape	Corresponding Site Type Codes
Rectangle	AGC, DEV, UGS, WET, PAG, PGS, PLW
Square	AGC, DEV, UGS, WET, PAG, PGS, PLW
Linear	DEV, ROW, AGE*, PAE*

* New site type if the sampling point is shifted to the nearest field corner.
AGE = Agriculture edge and PAE = Protected agricultural land edge.

Table 4 Ideal and minimum plot dimensions based on plot shape

	Rectangle		Square	Linear	
	Length (m)	**Width (m)**	**Length (m)**	**Length (m)**	**Area (ha)**
Ideal	200	50	100	500	1
Minimum*	100	25	50	250	0.25

* Only applies to random sites from the MMx Sampling Grid.
Non-random sites can be smaller than 0.25 ha.

STEP 3

Plot Setup

Plot setup instructions (presented between brackets [ ]) are for best-case scenarios of standard rectangle, square, and linear plots. More often than not, the coordinates, direction, and distances between boundaries will differ from what is suggested. Record the correct information in the ***Monitoring Plot Setup Form*** while completing STEP 4.

Standard Rectangle Plot (see Figure 1)

R1. Navigate to the [sampling point coordinates] and mark the spot as P1. If the sampling point is not the plot's starting point (P1), record these coordinates.

R2. With a compass or a GPS device, align yourself at [0] degrees (North) to find the direction of P2.

R3. With a long-distance measuring instrument or device, walk [200 m] in the direction previously determined.

R4. Mark this as P2 and record the coordinates.

R5. [Add] 90 degrees to the original orientation, record that direction, and walk [50 m] in this new direction.

R6. Mark this as P3 and record the coordinates.

R7. Repeat step R5 but walk [200 m] in this new direction.

R8. Mark this as P4 and record the coordinates.

The monitoring plot is now mapped.

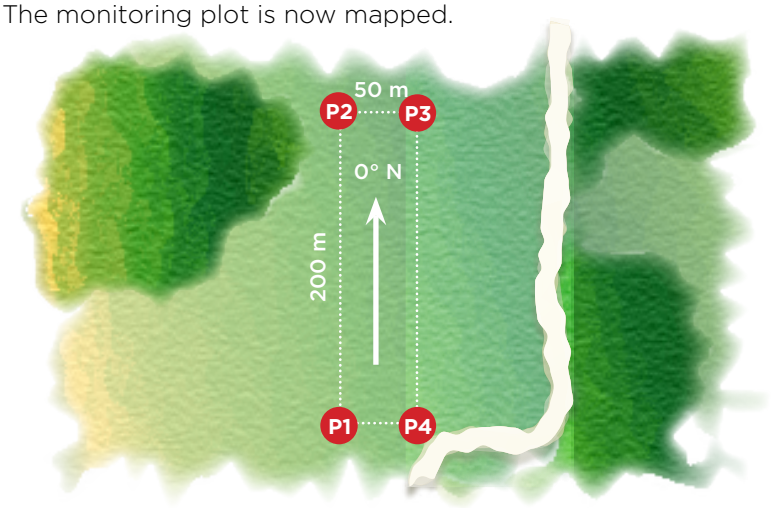

Figure 1 Standard rectangle plot setup

NOTE

Plot markers can be left in the field to facilitate recurring activities. However, to avoid injuries or damage to equipment, make sure to inform the landowners and/or land managers that these materials have been left on the site. Also, avoid objects that may be hazardous and become hidden when the vegetation grows, and remove markers at the end of the survey season.

Standard Square Plot (see Figure 2 for a non-standard square plot)

S1. Navigate to the [sampling point coordinates] and mark the spot as P1. If the sampling point is not the plot's starting point (P2), record these coordinates.

S2. With a compass or a GPS device, align yourself at [0] degrees (North) to find the direction of P2.

S3. With a long-distance measuring instrument or device, walk [100 m] in the direction previously determined.

S4. Mark this as P2 and record the coordinates.

S5. [Add] 90 degrees to the original orientation, record that direction, and walk [100 m] in this new direction.

S6. Mark this as P3 and record the coordinates.

S7. Repeat S5.

S8. Mark this as P4 and record the coordinates.

The monitoring plot is now mapped.

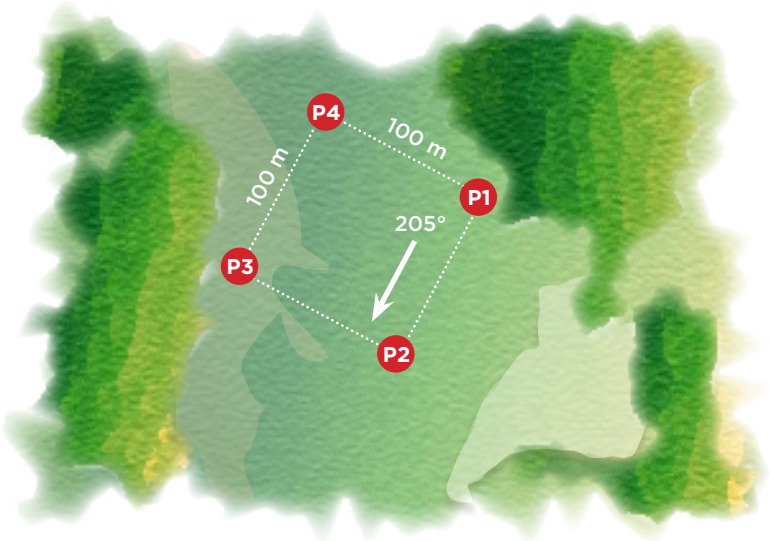

Figure 2 Non-standard square plot. Orientation from P1 to P2 is 205° raher than 0°.

Standard Linear Plot (see Figure 3)

L1. Navigate to the [sampling point coordinates] and mark the spot as P1. If the sampling point is not the plot's starting point (P1), record these coordinates. If located near a road, do not enter or walk across the roadway.

L2. With a compass or a GPS device, select a direction of travel towards P2.

   a. On roadsides, travel in the direction of oncoming traffic and record the true direction followed.

   b. In agricultural fields, travel in the direction where the entire plot will fit in and record the direction followed. If the linear plot fits in both directions, flip a coin to randomly determine the direction and record the direction followed.

L3. With a long-distance measuring instrument or device, walk [500 m] in the direction previously determined.

L4. Mark this as P2 and record the coordinates.

The monitoring plot is now mapped.

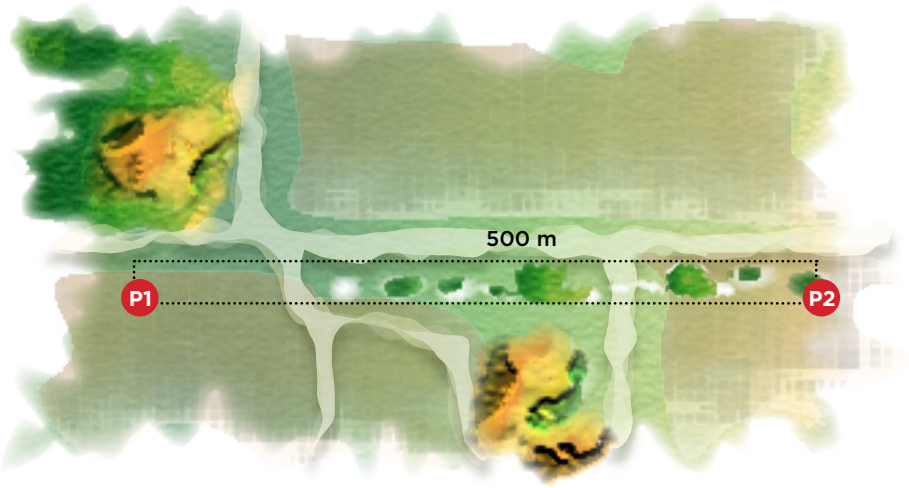

Figure 3 Standard linear plot setup

STEP 4

Fill the Monitoring Plot Setup Form

Record all information in the Monitoring Plot Setup Form

Sampling Point Information

- **Random site:** Record the Point ID from the mapping tool (e.g., CA-2849-30).
- **Non-random site:** Generate a temporary unique Sampling Point ID as follows -> Type the "initials" of the observer (e.g., Jamie Smith -> JS), the date following the YYMMDD format, and the number of the site visited (if more than one in the day), all separated by dashes "-". For example, if it's the first site visited by Jamie Smith on July 1, 2022, the temporary unique Sampling Point ID will be JS-220701-1.

Original Site Type

- Site selected from MMx Sampling Grid: Record the Site Type classification of the sampling point from the mapping tool
- Site of your choice: Record "NA"

Verified Site Type

Choose the Site Type that best describes your site when on the field (*Table 2*). If it is not an accepted site type, do not proceed with the subsequent steps since unaccepted site types are not to be monitored. Instead, submit the information collected and select another sampling point of the same site type in the MMx Sampling Grid (start over from STEP 1 of this section).

Special circumstances

- If the sampling plot is located in the nearest field corner of an Agriculture site type (AGC), the verified site type should be AGE.
- If the sampling plot is located in the nearest field corner of a Protected agricultural land site type (PAG), the verified site type should be PAE.
- If the sampling plot is located on or near a roadside, railway, or transmission/power line, the verified site type should be ROW.

Land Ownership Type: Identify whether the land is privately owned or public. For privately owned land, indicate whether or not the public have access to the land.

Conservation Status: Record whether the plot area is managed for conservation. If yes, fill out the *Conservation Site Project Description Form*.

NOTE

The MMx Sampling Grid occasionally features classification errors. If a site type was misclassified, record the code for the correct type and monitor the plot accordingly.

Conservation sites and other managed habitat for monarchs (or pollinators)

Land managers interested in evaluating conservation/management projects may want to place more than one monitoring plot within the site area, especially in large areas. The manager may also want to place plots in specific areas of the site according to site characteristics (e.g., soil moisture, seeding). The number of plots to establish is at the discretion of the land manager, based on the heterogeneity of the habitat and their information needs.

One monitoring plot for each 5 hectares is recommended, but not required. For sites that accommodate linear plots (e.g., rights-of-way), a 500 m linear plot at each 5 km is recommended, but not required.

Conservation sites may include land set aside for pollinators, areas with management practices that value the presence of pollinators (e.g., selective mowing), etc.

Plot Shape: Record the shape of the plot (see ***Table 3***).

Plot Size: Record the size (in hectares) of the plot.

Plot Coordinates: Record GPS coordinates for the four boundaries (P1 to P4) of the monitoring plot (as explained in STEP 3: Plot Setup).

Starting and Following Directions: Record the starting direction, which is the direction from P1 to P2 (as explained in STEP 3: Plot Setup). Also record the direction from P2 to P3, and from P3 to P4.

Plot Dimension: Record distance (in m) between each boundary (P1-P2, P2-P3, P3-P4, and P4-P1) (as explained in STEP 3: Plot Setup).

STEP 5

Miscellaneous Monarch Observations

If monarchs of any stage are observed outside pertinent monarch-related surveys (Activity 2 and 3), record them as Miscellaneous Monarch Observations at the end of the ***Monitoring Plot Setup Form***. Record dead monarchs as well, but note them separately in parentheses "( )".

For immature stages (eggs, caterpillars, chrysalis), tally the stages observed in the plot and record the associated plant species.

For adult monarchs, record their behaviour and the associated plant species if they are observed nectaring, ovipositing, or roosting.

PLOT DESCRIPTION

Overview

Plot Description can be conducted during or after the Monitoring Plot Setup. Afterwards, a subset of the data fields will need to be updated before surveys are conducted within the plot (see ***Activity 1***, ***Activity 2*** and ***Activity 3***). It is **mandatory** that these fields are updated since the information will be used in combination with the observations recorded in the survey activities presented in the following sections **(i.e., Plot Description must be completed or updated on every visit to the site)**.

The following section identifies the adjacent site type and the ecology, disturbances, and other features of the habitat within the monitoring plot.

Miscellaneous monarch observations may also be recorded.

Attributes Measured or Assessed

- Adjacent site type
- Site size estimate
- Site disturbances
- Vegetation structure
- Miscellaneous observations of monarchs

Frequency

Each time the sampling plot is visited.

The Plot Description section must be completed prior to conducting any surveys (see ***Activity 1***, ***Activity 2*** and ***Activity 3***).

Instructions

Navigate to the sampling plot. If the plot markers are not left in the field, place them as per the ***Monitoring Plot Setup*** in order to complete this section and the other monitoring activities.

There are no steps to follow per se. However, surveyors must describe the plot according to the categories in the following sections.

Supplies Needed

- **GPS device (to locate monitoring plot)**
- **Plot Description Form, clipboard, and pencils**
- **Compass***
- **Long-distance measuring instrument or device (measuring wheel, transect tape, distance tracking device, etc.)***
- **Plot boundary markers (stakes, pin flags, etc.)***

***If plot markers are removed at each visit**

STEP 1

Recording Observations

Adjacent Site Type

Record the dominant site type(s) that are within 100 metres of the monitoring plot (see *Table 2*).

Site Size Estimate

Record the estimated size of the site on which the monitoring plot is located. Estimated areas can be recorded in hectares (1 ha = 10,000 m^2) or in the number of 1 ha monitoring plots that fit within the site.

Information Specific to Certain Site Types

For Rights-of-Way (ROW) sites, record:

- ROW Type: Indicate whether you are monitoring a roadside, railway, transmission/power line.
- Road Type: If monitoring a roadside, classify the road as unpaved (gravel/dirt), paved (2 lanes), a medium highway (4 lanes), or a large highway (> 4 lanes).

For Agriculture (AGC) sites, record:

- Plot Type: Indicate the type of agricultural plot you are monitoring: row crop, orchard, tree farm, or vineyard. If row crop, indicate the type in the space provided (e.g., corn/soy, canola).

Site Disturbances

Site disturbance is defined by any natural or anthropogenic site alteration that may have changed the structure or composition of the vegetation on **at least 10% of the site.**

On each visit, indicate any recent (since the start of the growing season) or new (new from last visit) disturbances (Table 5) within the sampling site.
If there is no observable disturbance, select 'no disturbance.'
If the site is disturbed, estimate the percentage affected and, if possible, estimate time since disturbance in the Plot Description Notes. It is possible to record more than one disturbance.

Table 5 Possible disturbances and corresponding codes

Code	Disturbance
1	No disturbance
2	Mowed (10 inches or less)
3a	Hayed-residual remains
3b	Hayed-hay removed
4a	Chemical: fertilizer
4b	Chemical: herbicide
4c	Chemical: insecticide
4d	Chemical: unknown
4e	Chemical: other
5a	Construction: structure
5b	Construction: road
5c	Construction: trail
5d	Construction: other

Code	Disturbance
6a	Grazed - cattle
6b	Grazed - sheep
6c	Grazed - horses
6d	Grazed - other
6e	Grazed - unknown
7a	Burned: wildfire
7b	Burned: prescribed
7c	Burned: unknown
8	Plowed or disked
9	Flooded
10	Tree harvest / woody species removal
11	Other

Vegetation Structure in the Plot

On each visit, estimate percent of the plot covered (0%, 1-10%, 11-25%, 26-50%, 51-75%, 76-100%) by the vegetation structure categories detailed below.

- **Woody plants**
  - **Conifer trees:** Needle and cone-bearing trees. Record dominant species if known.
  - **Deciduous trees:** Broad-leaf bearing trees generally composed of a single trunk. Record dominant species if known.
  - **Shrubs:** Persistent woody plants shorter than 8 m and composed of multiple trunks with dense foliage reaching the ground.
- **Herbaceous plants**
  - **Forbs:** Non-grass, herbaceous flowering plants. Forb species do not have to be in bloom for this estimate.
  - **Graminoids:** Herbaceous plants with grass-like morphology, including grass, sedges, and rushes.

Wetland Features in the Plot

On each visit, estimate percent of the plot covered (0%, 1-10%, 11-25%, 26-50%, 51-75%, 76-100%) by the wetland feature categories detailed below.

- **Peatland:** Moss-dominated wetland that may include isolated and stunted trees (includes bogs and fens).
- **Marsh:** Wetlands that are periodically inundated by standing or slowly moving water. They are characterized by an emergent vegetation of reeds, rushes, or sedges and the absence of woody vegetation.
- **Swamp:** Wetlands within which standing or gently moving waters occur seasonally or persist for long periods, leaving the subsurface continuously waterlogged. The vegetation may consist of dense coniferous or deciduous forest, or tall shrub thickets.
- **Shallow open water:** Relatively small bodies of standing water, representing a transition stage between lakes and marshes (also known as ponds or sloughs). The surface waters impart an open aspect, free of emergent vegetation, but floating, rooted, aquatic macrophytes may be present.
- **Ditch:** Human-built linear depression for water conveyance. The vegetation may consist of cattails and other reeds.

STEP 2

Miscellaneous Monarch Observations

If monarchs of any stage are observed outside pertinent monarch-related surveys (see ***Activity 2***, ***Activity 3***), record them as Miscellaneous Monarch Observations at the end of the ***Plot Description Form***. Record dead monarchs as well, but note them separately in parentheses "( )".

For immature stages (eggs, caterpillars, chrysalis), simply tally the stages observed in the plot.

For adult monarchs, also record their behaviour. If butterflies are observed nectaring or ovipositing, record the associated plant species.

ACTIVITY 1
MILKWEED AND BLOOMING PLANT SURVEY

Overview

The Site Description must be completed before conducting this activity.

Activity 1 can be conducted simultaneously with *Activity 2* (see additional options on page 35).

Surveyors establish transect routes and lay subplots (while using a plant sampling frame) to record milkweed density and blooming plant frequency within the monitoring plot. Data will be used to quantify the availability of food plants (milkweed for reproduction and nectar plants for adult nutrition) as an indicator of habitat quality.

The following section presents survey options (A and B) adapted to the surveyor's plant identification skills, the steps to follow in order to survey milkweed and blooming resources, and an alternative census adapted to particular sites.

Miscellaneous monarch observations may also be recorded.

Attributes Measured or Assessed

- Milkweed density and species composition
- Blooming plants

  Option A: Frequency, species composition, and diversity
  Option B: Frequency
- Miscellaneous observations of monarchs

Frequency

Refer to *Table 1*

Supplies Needed

- **GPS device (to locate monitoring plot)**
- **Compass**
- **Long-distance measuring instrument or device (measuring wheel, transect tape, distance tracking device, etc.)**
- **Plant sampling frame (see Figure 4)**
- **Plant identification materials (option A)**
- **Milkweed identification materials**
- ***Activity 1 Form (Option A or B)*, clipboard, and pencils**

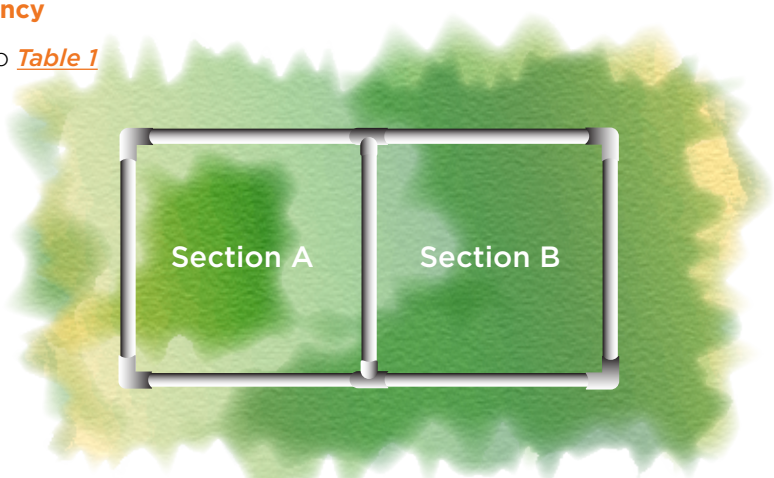

Figure 4 Plant sampling frame of 1.0 x 0.5 m with two (2) 0.5 x 0.5 m sections.

ACTIVITY 1
MILKWEED AND BLOOMING PLANT SURVEY

STEP 1

Lay Transects

Within a 1 ha monitoring plot, the surveyor will lay out 500 m of transects and collect data within 50 subplots along them (see *Figure 5*). Transect layouts will vary based on the plot configuration, as described below.

Standard Rectangle Plot (200 x 50 m)

Refer to the Monitoring Plot Setup section for coordinates, directions, and distances between each boundary of the monitoring plot (P1 to P4).

Information between parentheses "( )" are guidelines to adapt/calculate walking distances if the monitoring plot is not 1 ha (standard).

R1. Navigate to P1.

R2. Align yourself with P2.

R3. Walk 200 m (the length of the monitoring plot) to reach P2 while following the starting direction. This is the first transect (T1).

R4. Turn 90 degrees to face P3.

R5. Walk 25 m (half the distance between P2 and P3). ***This IS NOT part of the transect***

R6. Turn 90 degrees again. This is the opposite direction (180 degrees) of point R2.

R7. Walk 200 m (same distance as in R3). This is the second transect (T2).

R8. At the end of the second transect (T2), turn 90 degrees to face P4.

R9. Walk 25 m (same distance as in R5) to reach P4. ***This IS NOT part of the transect***

R10. Turn 90 degrees to face P3. This is the same direction as in R2.

R11. Walk 100 m (half the distance of R3). This is the third transect (T3).

This is the transect layout to follow for surveying a standard rectangle (see *Figure 5a*), which includes two (2) 200 m transects (T1 and T2) and one 100 m transect (T3), for a total transect distance of 500 m.

NOTE

This section only details the transect route to use. Further details on subplot installation (with the plant sampling frame), distances between subplots (based on plot size), and data recording will be given in *STEP 2: Place Subplots and Record Data*

Distances mentioned are for standard 1 ha monitoring plots. If the monitoring plot is smaller than 1 ha but greater than or equal to 0.25 ha, surveyors will need to adjust transect lengths and subplot spacing to fit 50 subplots. If the monitoring plot is smaller than 0.25 ha (only possible if monitoring a non-random site), subplot spacing will be at 5 m along the total transect length.

ACTIVITY 1
MILKWEED AND BLOOMING PLANT SURVEY

Standard Square Plot (100 x 100 m)

Refer to the *Monitoring Plot Setup* section for coordinates, directions, and distances between each boundary of the monitoring plot (P1 to P4).

S1. Navigate to P1.

S2. Align yourself with P2. This is the starting direction.

S3. Walk 100 m (the length of the monitoring plot) to reach P2 while following the starting direction. This is the first transect (T1).

S4. At P2, turn 90 degrees to face P3.

S5. Walk 25 m (one-fourth of the distance between P2 and P3). ***This IS NOT part of the transect***

S6. Turn 90 degrees again. This is in the opposite direction (180 degrees) of your starting direction (S2).

S7. Walk 100 m (same distance as in S3). This is the second transect (T2).

S8. At the end of the second transect (T2), turn 90 degrees to face P4. If you turned 90 degrees to the right at S4, turn 90 degrees to the left at S8, and vice versa.

S9. Walk 25 m (same distance as in S5) towards P4. ***This IS NOT part of the transect***

S10. Turn 90 degrees again. This is in the same direction as your starting direction.

S11. Walk 100 m (same distance as in S3). This is the third transect (T3).

S12. Repeat steps S4 to S11 to walk the fourth (T4) and fifth (T5) transects.

This is the transect layout to follow for surveying a standard square (see ***Figure 5b***), which includes five (5) 100 m transects (T1 to T5), for a total transect distance of 500 m.

Standard Linear Plot (500 x 2 m)

Refer to the *Monitoring Plot Setup* section for coordinates, directions, and distances between each boundary of the monitoring plot (P1 and P2).

L1. Navigate to P1.

L2. Align yourself with P2. This is the starting direction.

L3. Walk 500 m (the length of the monitoring plot) to reach P2 while following the starting direction.

This is the transect layout to follow for surveying a standard linear plot (see ***Figure 5c***), which includes a single 500 m transect.

ACTIVITY 1
MILKWEED AND BLOOMING PLANT SURVEY

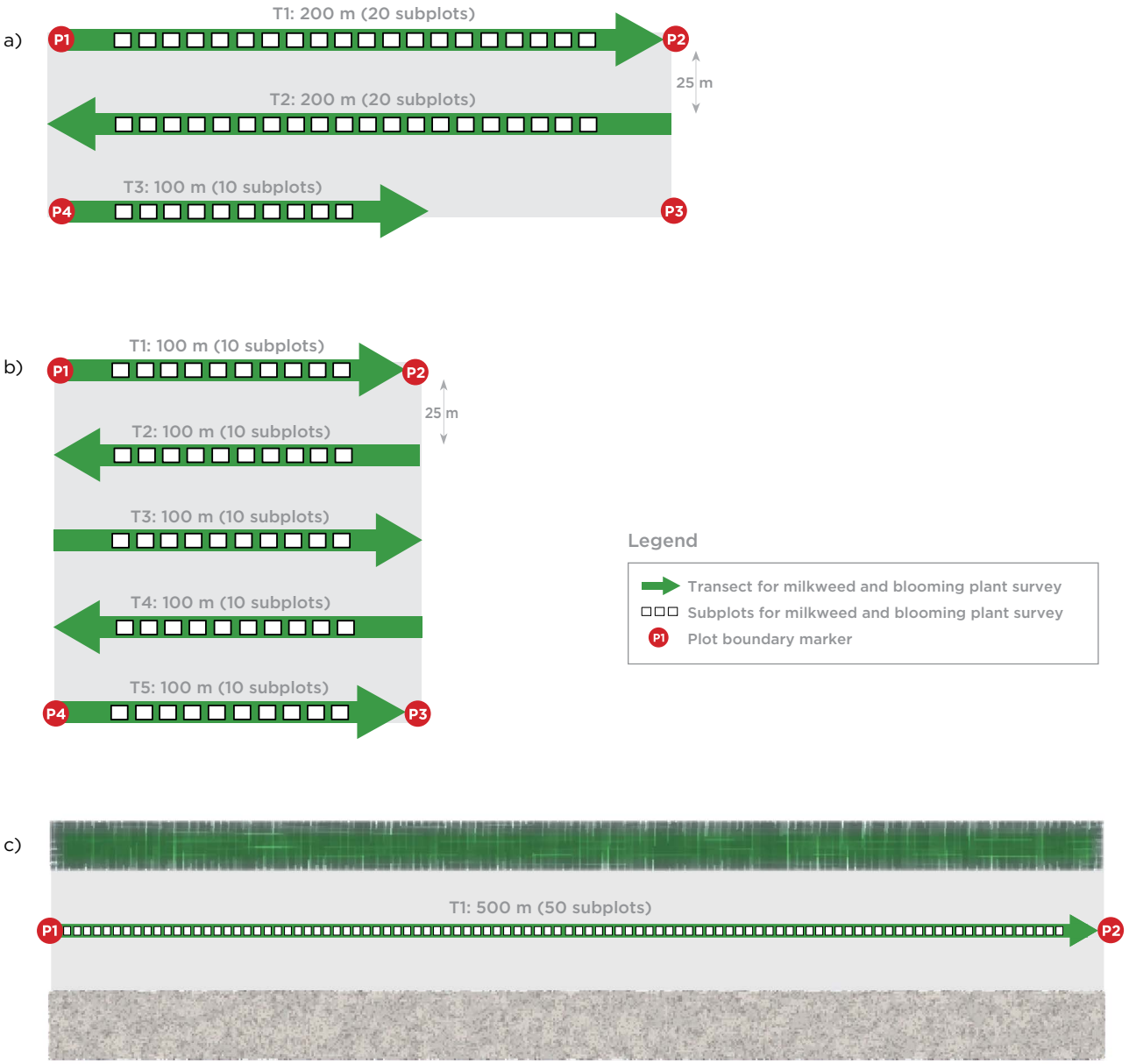

Figure 5 Standard transect layout for Activity 1. a) Rectangle, b) square and c) linear plot.

ACTIVITY 1
MILKWEED AND BLOOMING PLANT SURVEY

STEP 2

Place Subplots and Record Data

Subplots of 1 square metre (1 m^2) are placed every 10 m along each transect in standard 1 ha monitoring plots for a total of 50 subplots. If the monitoring plot is less than 1 ha, adapt the distance between each subplot in order to monitor a total of 50 plots (see instructions below). In order to delimit the subplot, surveyors will use a plant sampling frame (see ***Figure 4***) that will be flipped from either side of the transect (see ***Figure 6***). Information about both **blooming plants** and **milkweed** inside the subplot will be recorded. This allows accurate characterization of the site without having to survey every single plant within the entire plot.

When recording blooming plants, you may choose between:

Option A: Identifying blooming plants to species. It's possible to record plants to genus, or as unknown species (no need to know every species on site).

Option B: Recording presence or absence of blooming plants.

Recording Sequence

1. On the first transect (T1), place the sampling frame at the starting point (0 m) on the left side of the transect tape, so the bottom right corner is aligned with the zero mark (see ***Figure 6***).
2. Record blooming plant data as instructed below, according to the sampling option you have selected.

   Option A: Identifying blooming plants to species (see ***Activity 1-A Form***)

   - Look in **Section A** of the sampling frame and record each blooming species rooted within this section. Record "A" next to each species.
   - Next, look in **Section B** and record any blooming plant species not already observed in Section A. Record "B" next to the newly recorded species.
   - Flip the frame to the other side to form **Section C** (Section A + B), and record any blooming plant species not already observed in Section A or B. Record "C" next to the newly recorded species.
   - If no blooming species are present in any of the sections, enter "0" on the first row and draw a line through the subplot column

ACTIVITY 1
MILKWEED AND BLOOMING PLANT SURVEY

Option B: Recording presence or absence of blooming plants (see *Activity 1-B Form*)

- Look in **Section A** of the sampling frame. If blooming plants are present, record "A" for this subplot and go to **3.** If there are none, go to ii.
- Look in **Section B**. If blooming plants are present, record "B" for this subplot and go to **3.** If there are none, go to iii.
- Flip the frame to the other side to form **Section C** (Section A + B). If blooming plants are present, record "C" for this subplot. If there are none, record "X."

3. Record **milkweed** data as instructed below:
   a. Record each species of milkweed rooted within the entire subplot (Sections A, B and C together).
   b. For each species of milkweed observed, count the number of stems.
4. Pick up the sampling frame, walk forward 10 m on the transect using a measuring instrument or device, and place the sampling frame at the 10 m mark on the left side of the transect tape (next subplot).
5. Repeat steps 2 to 4 until 50 subplots are sampled. A total of 50 subplots must be monitored for random sites. Non-random sites may have fewer than 50 subplots sampled.

Tips and Warning

Adapt walking distance between subplots if the monitoring plot is less than 1 ha, but greater than or equal to 0.25 ha, using the following equation:

Total transect length (m) ÷ 50

For example, in a square plot of 50 x 50 m, the total transect length is 250 m (5 transects of 50 m according to Activity 1 - Step 1); therefore, the walking distance between subplots is 5 m (250 m ÷ 50 = 5 m).

If the monitoring plot is less than 0.25 ha (only possible with a non-random site), use a walking distance of 5 m between each subplot until the total transect length is surveyed.

ACTIVITY 1

MILKWEED AND BLOOMING PLANT SURVEY

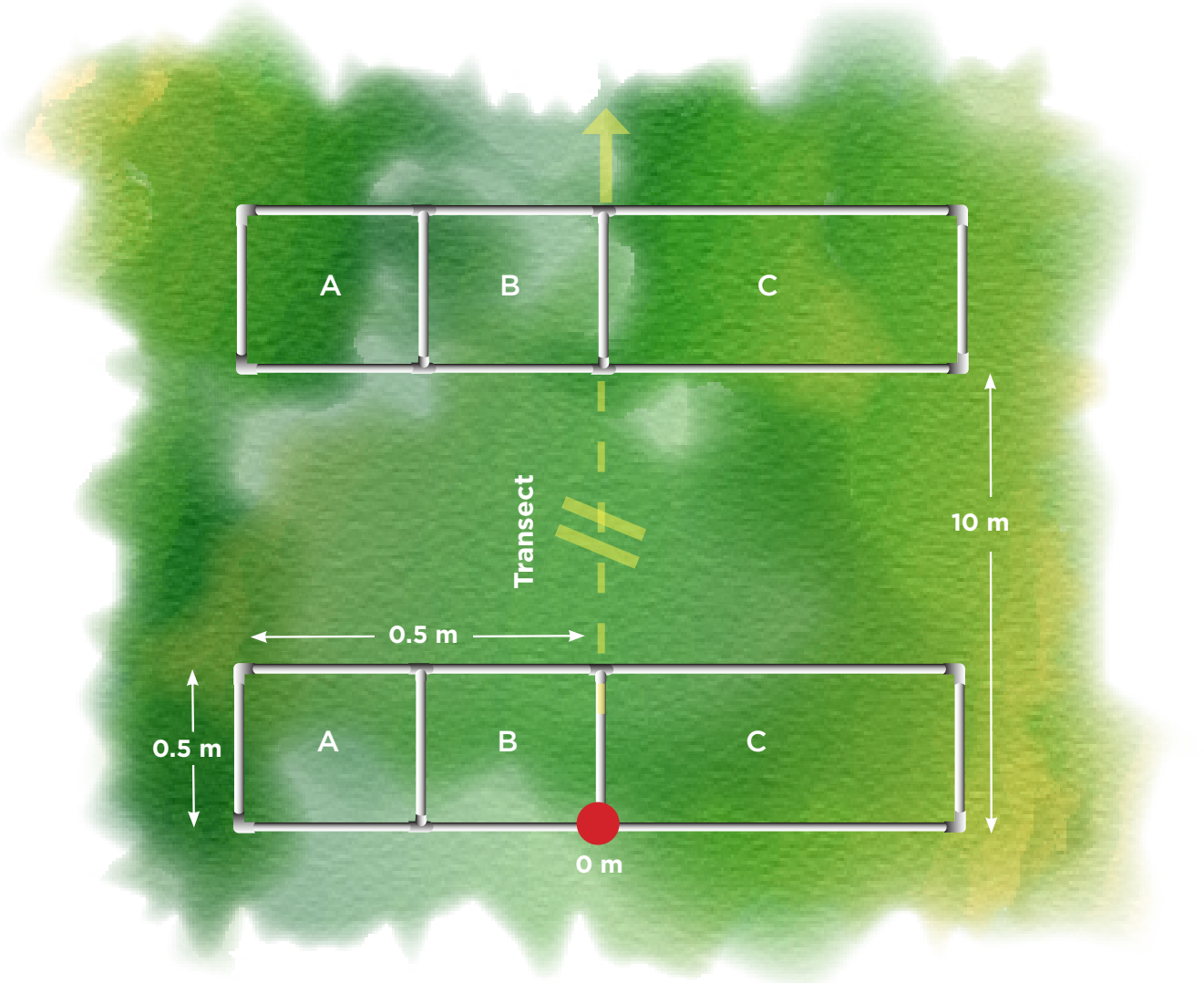

Figure 6 Plant sampling frame positioning along transect routes.
Red dot marks the starting point of the transect.

ACTIVITY 1
MILKWEED AND BLOOMING PLANT SURVEY

Tips and Warning

For a plant to be recorded, **it has to be rooted inside the plant sampling frame**. Plants with aerial parts that fall within the sampling frame but are rooted outside of the frame should not be recorded.

A **blooming plant** is any plant that has at least one (1) flower that is open and accessible to a pollinator.

*When recording blooming plant species (Option A), there is no need to rewrite the name of previously recorded species. Simply record the observation in the appropriate subplot column.

*If you encounter blooming milkweed species, record them in both the **Blooming plants section** and the **Milkweed plants section** (for stem counts) within the ***Activity 1 Form (Option A or B)***.

*Although blooming plant and milkweed recordings are presented separately in the protocol, we strongly suggest that you record both blooming plants and milkweed simultaneously.

A **milkweed stem** is any milkweed main structural axis that emerges from the soil.

A **ramet** is any structure that branches above the soil from the main structural axis (meaning the junction of both structures is visible above ground). Ramets should never be recorded as a stem. For example, if a ramet visibly branches from a stem above the ground, this will be recorded as one (1) single stem.

When no blooming and/or milkweed plant is recorded within the subplot, this observation should be recorded as zero (0) observations. This means that there was a sampling effort that resulted in 0 observations, not the absence of a sampling effort at a given subplot.

For plant names, MMx uses the ***Database of Vascular Plants of Canada (VASCAN)*** to track them. You may record either the common or scientific name, as they are linked together in the data entry system. If you do not find the name you are looking for, check the VASCAN database in case the name has been changed.

ACTIVITY 1
MILKWEED AND BLOOMING PLANT SURVEY

Special circumstances

Inaccessible subplots

Thick patches of vegetation: Some monitoring plots will contain thick patches of vegetation that cannot be traversed. If you cannot reach the subplot and lay the plant sampling frame on the ground, **look into the thicket** and try to estimate the blooming plants and milkweed that would fall within the subplot. If you see either, estimate their location within the subplot (Section A, B or C) and record accordingly. If none are present, record zeros (0). Continue the transect on the other side of the thicket.

If you cannot view or safely access an area of a suitable habitat: Skip this subplot and continue the transect on the other side, but start again with a subplot number that wasn't accessible. For example, if you absolutely cannot view the area where subplot #24 should be, move to the opposite side of the thicket and record the next accessible subplot as #24.

Unsuitable habitat

If along the transect you encounter habitat that appears unsuitable for monarchs within a subplot, such as a particularly dense thicket forming a canopy or water, please collect the data for that subplot as usual (as no habitat present). Do not be tempted to move the location of the subplot in order to capture particular plants.

ACTIVITY 1
MILKWEED AND BLOOMING PLANT SURVEY

STEP 3

Miscellaneous Monarch Observations

If monarchs of any stage are observed outside pertinent monarch-related surveys (Activity 2 and 3), record them as Miscellaneous Monarch Observations at the end of the ***Activity 1 Form (Option A or B)***. Record dead monarchs as well, but note them separately in parentheses "( )".

For immature stages (eggs, caterpillars, chrysalis), simply tally the stages observed in the plot.

For adult monarchs, also record their behaviour. If butterflies are observed nectaring or ovipositing, record the associated plant species.

Additional options

Activity 2: It is possible to conduct Activity 2 (immature monarch survey) while conducting Activity 1 if you select the ***sampling option B or C*** detailed on page 36. As you record milkweed and blooming plants in each subplot during Activity 1, also record appropriate data for Activity 2. However, milkweed observations for both activities must be recorded in their respective field form while following the instructions of their respective protocol. In other words, field forms should be filled out as if each survey has been completed independently and separately.

ACTIVITY 2
IMMATURE MONARCH SURVEY

Overview

The Site Description must be completed before conducting this activity.

Activity 2 can be conducted simultaneously with *Activity 1* (see additional options on page 36).

Surveyors examine milkweed plants in the monitoring plot to count the number of monarch eggs, caterpillars, and chrysalises per milkweed stem observed. Data will be used to examine how monarch densities vary within a year, between years, and among different sites and geographies.

Attributes Measured or Assessed

- Species of milkweed observed
- Number of milkweed stems examined
- Number and stage of monarchs found on a specific milkweed species
- Miscellaneous observations of monarchs

Frequency

Refer to ***Table 1***

Supplies Needed

- GPS device (to locate monitoring plot)
- Compass (if done with activity 1 simultaneously)
- Long-distance measuring instrument or device (measuring wheel, transect tape, distance tracking device, etc.)
- Magnifying glass
- Milkweed identification materials
- Monarch Identification materials
- *Activity 2 Form*, clipboard, and pencils

STEP 1

Select Milkweed Stems to Monitor

Best practice would be to monitor at least 100 random milkweeds stems when possible. Depending on the density and the distribution of milkweed within the sampling plot, the protocol proposes three sampling options to avoid bias when selecting stems to monitor. Milkweed distribution and density evaluated during STEP 1 (Site exploration) of the *Monitoring Plot Setup* activity will help determine the most appropriate sampling option (A,B,C).

A. Monitor every milkweed stem in the monitoring plot.

This is the most appropriate option if milkweed density is low (< 100 stems within the 1 ha monitoring plot).

Walk a systematic pattern through the entire monitoring plot (weaving back and forth) and monitor every milkweed stem encountered. Avoid walking through the same area twice in order not to monitor the same stem twice.

ACTIVITY 2
IMMATURE MONARCH SURVEY

B. Sample all milkweeds along Activity 1 transect layouts.
This is the most appropriate option if milkweed density is estimated at around 2,000 stems within the 1 ha monitoring plot (~ 2 stems/10 m^2).

Monitor every milkweed stem rooted within the plant sampling frame on both sides of the transects (1 m to the right and 1 m to the left) or stop when you reach 100 stems monitored.

This can be done with Activity 1 separatly or simultaneously. If done separately, refer to Activity 1, Step 1 for transect layouts. If done simultaneously with Activity 1, examine every milkweed stem within and between the subplots.

If fewer than 100 stems have been monitored after completing all the transects, add transects between the ones originally planned (see figures 5, 6 and 7) and continue until 100 stems are monitored.

C. Sample a systematic subset of milkweed along Activity 1 transect layouts.
This is the most appropriate option if there are several thousands stems (> 2,000) within the 1 ha monitoring plot.

Monitor every milkweed stem rooted within the subplots.

If there are at least two (2) stems within the subplot, proceed to the next subplot without monitoring the milkweed stems between the subplots.

If there are fewer than two (2) stems within the subplot, walk towards the next subplot and randomly monitor two (2) milkweed stems rooted within the plant sampling frame on both sides of the transect (1 m to the right and 1 m to the left).

Tips and Warning

It is important not to stray from the selected sampling options presented above to include milkweed stems that have or may have monarchs on them. This would introduce bias and skew data.

Record information only for the stems monitored, including the ones with zero (0) monarchs, since absence of monarchs is relevant information.

If the sampling option selected during the first visit did not allow you to monitor the right number of milkweed stems (under or way over 100), choose another sampling option on your next visit. The ideal number of milkweed stems monitored is one hundred (100).

ACTIVITY 2
IMMATURE MONARCH SURVEY

STEP 2

Record Milkweed and Monarch Data

After selecting the milkweed sampling options (STEP 1), carefully examine the selected stems for monarch eggs, caterpillars, and chrysalises. Then, using the ***Activity 2 Form***:

1. Record the date and start time.
2. Record each milkweed species encountered in separate sections.
   MMx tracks monarch activity by milkweed species so it is important to record data in the appropriate species section.
3. Examine each milkweed stem from top to bottom, including the upper and undersides of leaves, stems, buds, and flowers.
4. Record the number and stage (including caterpillar instars) of monarchs found on each stem separately. Record dead immature stages as well, but note them separately in parentheses "( )".
5. If no monarchs are found, record that as well.
   It is very important to record data for every milkweed plant examined, even if no monarchs are found.
6. Record the stop time once the survey is completed.

NOTE

You may stop sampling after examining 100 milkweed stems since this sample size provides a robust picture of monarch activity in your plot. However, you may monitor beyond this number if desired.

Tips and Warning

Tips for monitoring immature monarch stages on milkweeds

- Look carefully at all parts of the plant including the tops and bottoms of the leaves, the area within the small leaves at the top of the plant, stems, and buds/flowers if they are present. Handle the plants carefully to avoid knocking off any eggs or caterpillars.
- Look for caterpillar clues such as chew marks on the leaves or frass (excrement). If you see a monarch caterpillar that is not on a milkweed plant but it is in the area surrounding the milkweed plant you are observing, record it for that milkweed. Mature caterpillars sometimes crawl off of plants to molt or cool down.
- Consult the "*Monarch identification sheet*" and the "*Commonly confused species identification sheet*" for guidance on distinguishing monarchs from other insects. For help distinguishing among the five monarch caterpillar instars, consult "*A Field Guide to Monarch Caterpillars*" from Oberhauser and Kuda (1997).

ACTIVITY 2
IMMATURE MONARCH SURVEY

STEP 3

Miscellaneous Monarch Observations

If monarchs of any stage are observed outside pertinent monarch-related surveys (Activity 2 and 3), record them as Miscellaneous Monarch Observations at the end of the ***Activity 2 Form***. Record dead monarchs as well, but note them separately in parentheses "( )".

For immature stages (eggs, caterpillars, chrysalis), simply tally the stages observed in the plot.

For adult monarchs, also record their behaviour. If butterflies are observed nectaring or ovipositing, record the associated plant species.

Careful not to duplicate monarch observations from the previous steps of this activity. These are monarchs encountered outside of the steps listed above.

ACTIVITY 3
ADULT MONARCH SURVEY

Overview

The Site Description must be completed before conducting this activity.

If conducting other activities during the visit, conduct Activity 3 first to minimize the effect of your presence on the behaviour of butterflies or wait at least one hour prior to any activities and Activity 3.

Surveyors establish an adult monarch survey route within the monitoring plot, count adult monarchs within defined spaces, document their behaviours, and record the species of plants on which monarchs indulge their behaviour (nectaring and ovipositing). Data will be used to track the abundance and chronology of adult monarchs throughout breeding and migration periods, but also throughout their range.

Attributes Measured or Assessed

- Time and duration of the survey
- Temperature (ambient °C)
- Length of survey route walked (m)
- Number of adult monarch butterflies observed
- Monarch behaviour(s) (flying, resting, ovipositing, mating, or nectaring)
- Plant species on which monarchs indulge their behaviour
- Miscellaneous observations of monarchs

Frequency and Timing

Refer to *Table 1*

Because adult monarch abundance varies across the season at a given location, repeated surveys are needed from spring to fall. If time permits, and particularly during peak migration periods, weekly or daily surveys are even more informative than the bi-weekly recommendation.

To determine when to start adult surveys, consult *Mission Monarch* or *Journey North* for recent sightings in your area. Otherwise, simply be aware of when monarchs arrive in your area. Northbound or southbound arrival dates can be shifted earlier or later depending on weather patterns, so watching for initial monarch arrivals (either live or online) is important.

- **Thermometer (or approximative temperature reported from meteorological sources)**
- **GPS device (to locate monitoring plot)**
- **Compass**
- **Long-distance measuring instrument or device (measuring wheel, transect tape, distance tracking device, etc.)**
- **Monarch identification materials**
- **Plant identification materials**
- ***Activity 3 Form*, clipboard, and pencils**

ACTIVITY 3
ADULT MONARCH SURVEY

Survey Conditions

Best practice is to conduct surveys during weather conditions and times when monarch butterflies are most active. Ideal conditions include warm, sunny days with little or mild winds (Table 6). Surveys conducted under unaccepted conditions may result in a non-representative sample (due to potentially low or no adult activity based solely on weather conditions or times only).

Since strong winds occur frequently in open prairies and are sometimes unavoidable, wind conditions up to 38 km/h are allowable, but lower wind speeds are preferred for spotting flying adults.

Table 6 Ideal and accepted conditions for conducting Activity 3

	Ideal	Accepted	Unaccepted
Time	10:00 a.m. - 4:00 p.m.	Any daylight times	Outside daylight times
Temperature	21-30°C	16-35°C	Below 16°C
Wind	Less than 16 km/h* leaves/twigs in motion	Up to 38 km/h* Small trees with leaves begin to sway	Over 38 km/h* Large branches in motion
Precipitation	None	None	Yes

* Refer to *Table 7* (Wind codes) for visual cues to estimate wind speed

Pollard Walk Survey Design

Pollard walks (Pollard, 1977) are the most widely used field protocol for monitoring butterfly populations. MMx uses the standard Pollard Walk survey design that consists of counting all monarchs within a "counting box" (cube area) that extends **2.5 m on each side** and **5 m in front and above** the surveyor (Figure 7), while following a predetermined route (see STEP 2 - Define Survey Route).

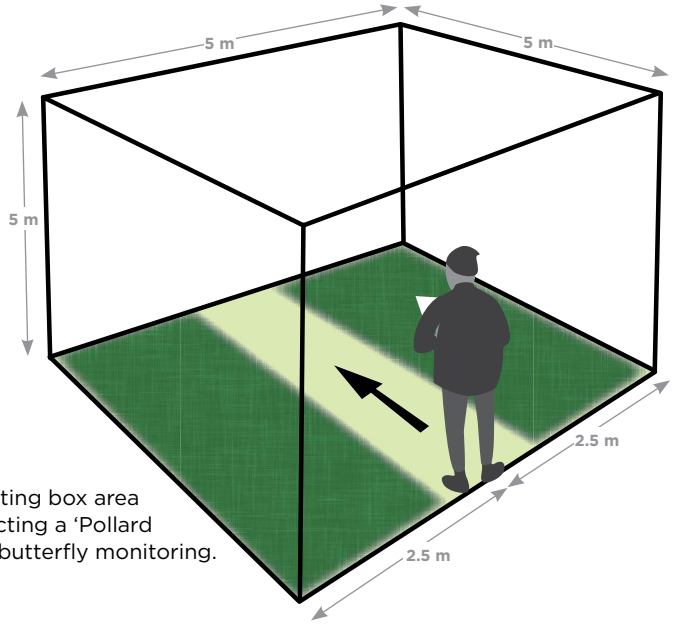

Figure 7 The counting box area used when conducting a 'Pollard walk' linetransect butterfly monitoring.

ACTIVITY 3
ADULT MONARCH SURVEY

STEP 1

Record General Survey Information

Record general survey information in the *Activity 3 Form*, including:

- Plot ID
- Observer
- Date
- Temperature
- Wind speed code (see *Table 7*)
- Sky code (see *Table 8*)

While recording general survey information, make sure that all Accepted conditions are met (see *Table 6*). If one of the weather or time conditions falls within the Unaccepted categories, surveyors should not proceed to the next step.

Table 7 Wind codes (based on the Beaufort scale). Green represents ideal conditions. Yellow indicates accepted conditions. Red indicates unaccepted conditions (no surveying).

Code	Wind speed (km/h)	Description
0	< 1	Smoke rises vertically.
1	1-5	Direction of wind shown by smoke drifts, but not wind vanes.
2	6-11	Wind felt on the face. Leaves rustle. Ordinary vane moved by wind.
3	12-19	Leaves and small twigs in constant motion. Wind extends light flag.
4	20-28	Raises dust and loose paper. Small branches are moved.
5	29-38	Small trees with leaves begin to sway. Crested wavelets form on inland waters.
6	39-49	Large branches in motion. Whistling heard in telephone wires. Umbrellas used with difficulty.

Table 8 Sky codes

Code	Description
0	Clear / few clouds
1	Partly cloudy / variable sky
2	Cloudy / overcast
3	Fog or smoke

ACTIVITY 3
ADULT MONARCH SURVEY

STEP 2

Define Survey Route

Adult surveys are conducted by walking a predetermined 500 m long route. Survey routes and length depend on the monitoring plot size and shape. Follow the appropriate instructions based on the shape of the monitoring plot.

Refer to the Site Description section for coordinates.

Rectangle plot: Starting at P1, walk the site boundaries (see ***Figure 8a***).

Square plot: Starting at P1, walk the site boundaries and then diagonally through the interior of the site, a distance equal to the side of the square plot. (see ***Figure 8b***).

For example, for a square plot of 100 x 100 m, walk 100 m diagonally through the interior.

Linear plot: Starting at P1, walk a linear route to P2 (see ***Figure 8c***).

NOTE

If the monitoring plot is less than 1 ha, follow the instructions but record the survey route length.

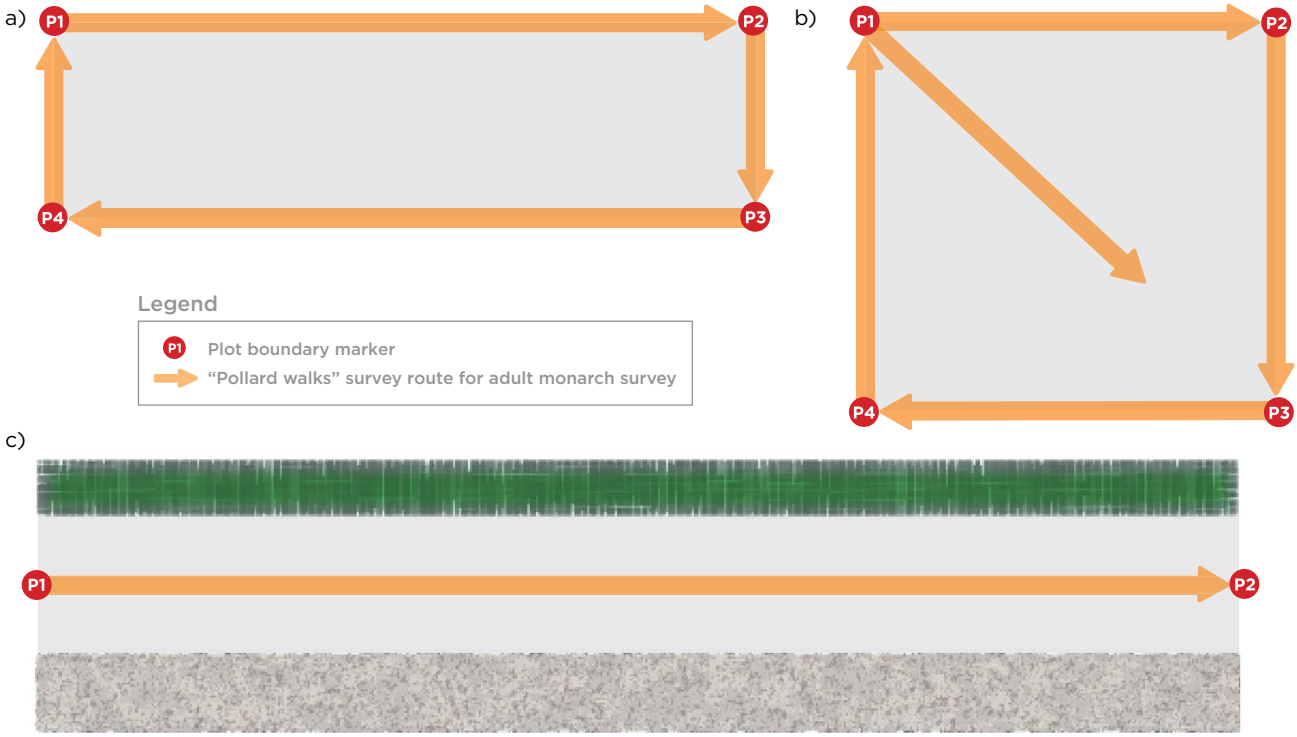

Figure 8 Survey routes for the "Pollard walks" according to the different plot shapes : a) rectangle, b) square and c) linear.

ACTIVITY 3
ADULT MONARCH SURVEY

STEP 3

Record Monarch Butterflies

Only one surveyor should count monarch butterflies during this step. Other participants may help with data recording but should **walk 5 m behind the surveyor** to avoid disturbing adults ahead on the survey route.

1. Start walking the survey route at a constant, slow pace, optimally one metre per three seconds, similar to a "wedding walk" down the aisle.

   At this pace, it will take approximately 25 minutes to complete the 500 m survey; however, in practice, it may take longer since there will likely be brief pauses to record data.

2. Record the behaviour of each monarch observed within the counting box as well as the associated plant species, when applicable (see ***Table 9***). Record dead butterflies as well, but note them separately in parentheses "( )" under the behaviour "Resting".

Table 9 Adult monarch behaviours and corresponding description

Behaviour	Code	Description
Flying	F	In flight
Mating	M	Male and female with abdomen tips stuck together (copulating)
Nectaring[1]	N	Drinking nectar from a flower (extended proboscis)
Ovipositing[2]	O	Female adult monarch arching abdomen and depositing eggs
Puddling	P	Standing on the ground and drinking from damp soil (e.g., mud, water hole edge) or feces
Resting	Re	Standing on some surface, showing no evidence of other behaviours
Roosting[3]	Ro	Resting on trees, in large groups, during migration

[1] Also record the species on which the butterfly is drinking nectar (if possible)
[2] Also record the species on which the butterfly is laying eggs
[3] Also record the species on which the butterflies are roosting

ACTIVITY 3
ADULT MONARCH SURVEY

3

Tips and Warning

Monarch host plant

Monarch butterflies normally lay their eggs on their host plant: milkweeds. However, studies have shown that female monarchs can and will lay eggs on non-milkweed plants (e.g., black swallow-wort *(Cynanchum louiseae)*, pale swallow-wort *(Cynanchum rossicum)* and Himalayan balsam *(Impatiens glandulifera)*). Therefore, it is crucial to also report ovipositing behaviour on plants that aren't milkweed.

Various butterfly species observation

Surveyors can use *STEP 3* to survey butterfly species other than monarchs if they feel confident with their identification. However, the MMx database does not support butterfly observations other than monarchs. Thereby, surveyors can share their observations to the ***eButterfly project***, an international, data driven project dedicated to butterfly biodiversity, conservation, and education.

STEP 4

Miscellaneous Monarch Observations

If monarchs of any stage are observed outside pertinent monarch-related surveys (Activity 2 and 3), record them as Miscellaneous Monarch Observations at the end of the ***Activity 2 Form***. Record dead monarchs as well, but note them separately in parentheses "( )".

For immature stages (eggs, caterpillars, chrysalis), simply tally the stages observed in the plot.

For adult monarchs, also record their behaviour. If butterflies are observed nectaring or ovipositing, record the associated plant species.

Careful not to duplicate monarch observations from the previous steps of this activity. These are monarchs encountered outside of the steps listed above.

References

Pollard, E. 1977. A Method for Assessing Changes in the Abundance of Butterflies. *Biological Conservation*, 12(2), 115-34. ***https://doi.org/10.1016/0006-3207(77)90065-9***.

MMx

MISSION MONARCH-EXPERT

The Canadian Monarch Monitoring Protocol & Sampling Grid

This monitoring protocol and sampling grid was developed by the Mission Monarch team, being run by the Montréal Space for Life Insectarium.

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Photo credits

Pages 4-7, 9-11, 12-20 and 21-24 :
Espace pour la vie (André-Philippe Drapeau Picard)
Pages 8, 25-34, 35-38 and 39-44 :
Espace pour la vie (André Sarrazin)

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APPENDIX

APPENDIX A

PROGRAM CONTEXT, GOALS AND OBJECTIVES

Context

In Canada, the Monarch butterfly (*Danaus plexippus*) has been listed as a Special Concern species since 2004 under the Species at Risk Act (SARA). In 2016, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) recommended its status be changed to Endangered (COSEWIC 2016) based on long-term surveys showing an ongoing decline of the overwintering grounds in Mexico.

The Management Plan for the Monarch (*Danaus plexippus*) in Canada (ECCC 2016) underlines the importance of addressing knowledge gaps in order to quantify and qualify monarch habitats to allow for targeted conservation efforts. Knowledge gaps include effects of habitat loss and degradation, milkweed distribution and abundance across fine spatial scales and land cover types, and monarch population size in Canada during the summer (CEC 2008).

Existing community science programs like ***eButterfly*** and ***Mission Monarch*** help to address knowledge gaps by providing relevant data regarding monarch and milkweed distribution. However, participants of those programs often visit high-quality habitats where monarchs are expected to be observed, as well as locations close to human population centres, creating bias in the data. Indeed, butterfly surveys conducted in high-quality patches overestimate butterfly abundance. Low-quality habitats with fewer or no milkweed plants or monarchs must also be sampled to avoid such bias. Therefore, spatially balanced surveys stratified by land cover types are needed to detect long-term changes in populations and habitats. Also, more comprehensive surveys, i.e., including habitat description as well as monarch and milkweed monitoring, are needed to locate and quantify priority breeding and nectaring habitats.

To this end, the USGS Monarch Conservation Science Partnership (MCSP), a group of scientists, managers, and conservation organizations from across North America led by the U.S. Geological Survey (USGS), developed the Integrated Monarch Monitoring Program (IMMP) (Cariveau et al. 2019, MJV 2021). This program was designed with a spatially balanced sampling grid in order to collect robust data on monarchs and their habitats that are representative across land uses and geographies in the United States. While field testing and protocol refinement spanned from 2016 to 2018, the Monarch Joint Venture (MJV) is the organization that led program implementation efforts across the U.S. since 2017, engaging community scientists, conservation professionals, and researchers in standardized data collection.

In order to have compatible information on a continental scale, the Montreal Space for Life's Mission Monarch team decided to draw heavily on the IMMP when creating Mission Monarch - Expert (MMx). Although some differences can be noted between the two programs, the proposed methodology and the MMx program data remain compatible in every way with the IMMP.

The approach presented in this document makes it possible to collect data in a standard way while suggesting random sampling sites that will ultimately allow unbiased estimation of milkweed availability, nectar resources, and monarch abundance at a national and trinational (Canada, Mexico and the United States) level.

Goal and objectives

The main goal of MMx is to characterize potential monarch habitats and monitor monarch presence and activities in order to implement efficient conservation actions in Canada. To achieve this goal, MMx seeks to quantify the following at the national level:

- Milkweed species distribution and density to:
  - Document distribution of potential monarch breeding areas
  - Document milkweed density by land-use sector
  - Detect changes in milkweed density by species over time (seasonally and inter-annually)
  - Track regional changes in milkweed phenology (chronology) by species over time
  - Explore the relationship of milkweed species or density to the presence of monarchs of all life stages
- Relative frequency and chronology of blooming plants to:
  - Describe available nectar sources and blooming phenology by land-use sector and region over time (seasonally and inter-annually)
  - Determine if the presence of monarchs in any life stage is related to nectar plant composition, diversity, or frequency
- Density, chronology, and survival of monarch eggs and caterpillars on milkweed plants to:
  - Document geographic distribution and seasonal and inter-annual dynamics of monarch eggs and caterpillars by region
  - Evaluate density of eggs and caterpillars by land-use sector, milkweed species, and region
  - Improve understanding of how habitat characteristics may affect monarch egg and caterpillar density and survival
- Abundance and chronology of adult monarchs throughout breeding and migration periods to:
  - Assess changes in adult monarch presence by land-use sector and region over time
  - Describe resource use (e.g., nectaring, ovipositing)

References

Cariveau, Alison B., Holly L. Holt, James P. Ward, Laura Lukens, Kyle Kasten, Jennifer Thieme, Wendy Caldwell, et al. 2019. The Integrated Monarch Monitoring Program: From Design to Implementation. *Frontiers in Ecology and Evolution*, 7. ***https://doi.org/10.3389/fevo.2019.00167***.

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COSEWIC. 2016. COSEWIC assessment and status report on the Monarch *Danaus plexippus* in Canada. Ottawa: Committee on the Status of Endangered Wildlife in Canada.

Environment and Climate Change Canada. 2016. Management Plan for the Monarch (*Danaus plexippus*) in Canada. *Species at Risk Act* Management Plan Series. Environment and Climate Change Canada, Ottawa. ***https://publications.gc.ca/site/eng/9.814146/publication.html***.

Monarch Joint Venture. 2021. Integrated Monarch Monitoring Program. Version 3.0.

APPENDIX B

MMx SAMPLING GRID

Context

The MMx Sampling Grid was strongly inspired by the **Generalized Random Tessellation Systematic (GRTS)** sampling framework used by the IMMP, where sites were randomly selected at two levels. First, 10 x 10 km grid squares (hereafter, Blocks) were placed across Canada and randomly numbered. Second, thousands of points (hereafter, Sampling Points), within the randomly selected blocks, were also randomly selected.

First Level: 10 x 10 km Block

The first step used a master sample developed by the North American Bat Monitoring Program (Loeb et al. 2015) from a 10 x 10 km grid placed over the conterminous U.S., Canada, and Mexico. Each 10 x 10 km block was assigned a GRTS rank by NABat. Since monarchs and milkweed are not present across the entire Canadian territory, only blocks where monarch and milkweed (all milkweed species) distribution was expected based on a model distribution of both species (not published), were conserved. Also, blocks with fewer than 95% accepted Site type (see ***Table 2***) were excluded. With the remaining blocks, 5% (399 in total) were selected for the MMx Sampling Grid (see ***Table B1***).

Second Level: Sampling Points

For the sampling points selection, the *2015 Land Cover of North America at 30 metres* produced by the North American Land Change Monitoring System (NALCMS) was used for site type classification. The initial site types of interest (based on monarch habitat) were reclassified into five (5) accepted site types: Agriculture, Developed, Grassland, Rights-of-way, and Wetland (see ***Table 2***).

Data from other sources were used to complete the site type classification. The "Protected land" file was used to convert Grassland, Wetland, and Agriculture site types to their respective Protected land categories: Protected agricultural land, Protected grassland, and Protected wetland (see ***Table 2***). The "Canada Highway," "Canada Railway," and "Canada Transmission Line" files were used to convert other site types into Rights-of-way site types from the *2015 Land Cover of North America at 30 metres* file.

When the reclassification of the accepted site types was completed, 20 sampling points for each site type, with a minimal distance of 300 metres between each sampling point, were randomly selected (34 321 in total) for the MMx Sampling Grid (see ***Table B1***). Blocks with fewer than 20 sampling points were excluded from the MMx Sampling Grid.

Table B1 Sampling block and sampling point count per province (from west to east)

Province	Block Count	Point Count
British Columbia	3	243
Alberta	38	2,995
Saskatchewan	73	7,256
Manitoba	47	4,337
Ontario	130	10,288
Quebec	53	4,424
New Brunswick	32	2,686
Nova Scotia	22	1,975
Prince Edward Island	1	117
Total	399	34,321

References

Loeb, Susan C., Thomas J. Rodhouse, Laura E. Ellison, Cori L. Lausen, Jonathan D. Reichard, Kathryn M. Irvine, Thomas E. Ingersoll, et al. 2015. A Plan for the North American Bat Monitoring Program (NABat). *Gen. Tech. Rep. SRS-208. Asheville, NC: U.S. Department of Agriculture Forest Service, Southern Research Station*. 208: 1-100. *https://doi.org/10.2737/SRS-GTR-208*.

APPENDIX C

INFORMING AND GAINING PERMISSIONS FOR ACCESS

Context

Before monitoring a site, the landowner or land manager must be informed of the surveyors' intention **AND** grant permission to access and collect data at the site . Even if the land is in public ownership, the managing authority should be contacted prior to accessing the site for the first time. Contact the most local person affiliated with the site (e.g., private landowner, Federal or Provincial Department, park manager) to let them know that surveyors would like to visit their land to collect monarch butterfly and habitat information as part of a national effort (see *Table C1*).

Table C1 Suggestions for finding landowner and acquiring permission to survey

Landowner/Site Type	Suggestions
Publicly accessible land (e.g., government parks, natural reserve)	1. Search for the person in charge of granting permission to access the site and contact them. 2. Provide a description of the area to be surveyed, such as the coordinates of the plot or an aerial photo from the mapping application. 3. Ask about special considerations, such as hunting seasons, or other safety concerns (e.g., scheduled prescribed fire). 4. Ask if they'd like to be contacted prior to each visit and provide approximate dates of surveys.
Rights-of-way	1. Search for the person in charge of granting permission for the roadside, railway, or transmission line to survey. 2. Ask about safety measures such as where to park, what to wear, and any other requirements for surveys. 3. Ask if they need to be contacted each time prior to your survey, or just once at the beginning of the season. 4. Make sure that monitoring activities will not interfere with scheduled management actions.
Private land	1. Obtain landowner or land manager information with the help of municipal authorities, online resources of your region, or an MMx coordinator. 2. Visit the site in person to directly get in contact with the landowner or land manager of the site. 3. When in contact with the person in charge, use the **Outreach script** to present the MMx program and the activities that you wish to carry out on the site. 4. Get the person in charge to sign the **Waiver and Release of Liability**.

Also, feel free to use the outreach script presented below when contacting the landowner or land manager in order to inform them about the goal and the monitoring activities of this project.

> *Hello, my name is _____*
> *and I am a participant/community scientist of Mission Monarch - Expert.*
>
> *The goal of this project is to monitor monarch butterflies and their habitat across the North American breeding range. I'm contacting you to ask permission to survey in _____ (specific area) of _____ (name of park/unit).*
>
> *This involves walking through the site and recording the abundance of milkweed, blooming plants, and monarchs of all stages. No specimens will be removed and nothing will be left behind* **[or I propose only to place four small pin flags that will be removed on my final visit].**
>
> *I would like to make my first visit on _____ (date) and would also make several repeat visits over the course of the summer. Would you like for me to contact you or someone else prior to each visit?*
>
> *Please let me know if you have any questions or concerns. I can provide you with my contact information for easy follow-up if anything additional comes up. Thank you so much for considering this opportunity to assist us in the conservation of monarch butterflies across North America.*

APPENDIX D

MONITORING SAFETY INFORMATION

Context

Surveyor safety should always be the highest priority. The surveyor should be aware of the general safety concerns related to field work, and also those related to specific activities, such as roadside surveys. Proper clothing is essential – light-coloured, lightweight, long-sleeved shirt, long pants, brimmed hat, and sturdy shoes entirely covering the feet (preferably boots). In roadside areas, reflective vests are typically required and sometimes hats or helmets, too (consult the managing road authority for details). Always let someone know where you will be and when you intend to return. Also, always park in a safe location. During the summer months, be sure to always carry a sufficient quantity of water to avoid dehydration.

Listed below are some safety considerations to keep in mind before and during monitoring:

- It is best to work in pairs for safety. If you plan to survey alone, it is best to alert a friend/relative of your location and expected time of return. Check in with this person upon completing data collection. If possible, keep a cell phone with you while on site.
- End monitoring with sufficient daylight to return to your vehicle.
- Never enter a property if you are uncomfortable with the landowner/manager or situation. Communicate any concerns about safety with the Mission Monarch staff.

Consult table D1 for specific hazards you may encounter in the field and the measures you can take to help prevent injury or illness in those instances. Be aware that it is not possible to list every potential hazard you may encounter. Please use caution while doing field work.

Table D1 Potential hazards encountered in the field and preventive measures

Potential Hazard	Preventive Measures
Overexposure to sun	• Use sunscreen • Wear brimmed hat, long sleeves, and pants • Limit midday exposure
Dehydration **3 heat-related disorders** **- Heat cramps** **- Heat exhaustion** **- Heat stroke*** *(Life-threatening)	• Wear light-coloured, loose-fitting, breathable clothing • Drink water frequently • Rest frequently • Seek shade • Learn to recognize the symptoms of each heat-related disorder AND the first aid treatment necessary
Poisonous vegetation	• Learn to identify • Avoid contact • Wear long-sleeved shirt and long pants • Wash/shower after exposure - use soap and water, or specialized wash or scrub to remove plant oils
Animals stings/bites	• Apply repellent periodically (follow label instructions) • Wear light-coloured clothing • Wear long-sleeved shirt (tucked into pants) and long pants (tucked into socks or boots) • Watch for bee/wasp nests in trees on ground • Carry allergy meds as needed • Examine body for ticks after monitoring, then shower
Rough/uneven terrain	• Take your time • Be aware of surroundings • Be certain of footing • Watch for changes in vegetation • Use stick for added stability or probing
Roadside safety	• ALWAYS look both ways • Wear reflective vest or conspicuous clothing • Park in designated area or as far off the road as can be done safely • Park a safe distance from hills or curves • Set up reflective triangle(s) (when available) • Work facing traffic • Be aware of driver visibility; hills, curves, shrubs/trees, parked vehicles, fog, sunlight • Store equipment and supplies in an area safe to access and away from traffic • Be aware of changes in traffic volume • Beware of trip and/or fall hazards such as culverts, erosion gullies, trash, stumps, downed fence, etc. • Walking the length of a steep incline is inevitable – sturdy boots are strongly recommended