Integrated Monarch Monitoring Program 2018 Guidebook

Protocols available for download at: https://monarchjointventure.org/get-involved/mcsp-monitoring/field-activities

Introduction

Monarch butterflies have declined more than 80% during the past 20 years, in both the eastern and western migratory populations, requiring decisive and rapid conservation to increase their numbers and decrease their risk of extinction. Sustaining monarchs requires a broad coalition of conservationists who are knowledgeable and passionate about creating, protecting, and enhancing habitat throughout the monarch range. Although monarchs have been the focus of many citizen science and other monitoring programs for decades, this program aims to provide additional data to address limitations of existing datasets, including:

1) People frequently monitor high quality habitat where they are likely to encounter monarchs, making extrapolating information across the landscape difficult.

2) Spatially representative data are needed to improve models so conservation efforts can use limited resources to have the greatest possible impact.

3) As conservation actions take place over time, ongoing monitoring will help determine if these actions are having the desired outcomes.

What is the Integrated Monarch Monitoring Program (IMMP)?

The Monarch Conservation Science Partnership (MCSP), a group of scientists from federal agency, university, and non-governmental organizations, has been active since 2009 in modeling monarch population trends, assessing potential threats, establishing population targets and habitat goals, and developing conservation tools. In 2015, the group began designing an *Integrated Monitoring Strategy*. The concept of integration was to work with protocols from long-standing, successful monitoring programs and to collect both habitat and monarch data together, representative both spatially and across land use types.

The primary goal of the IMMP is to monitor monarch butterflies and evaluate habitats to inform conservation efforts. To gather this information on the vast scale used by this migratory species, we need a monitoring effort that engages a broad network of citizens, biologists, resource managers, students, landowners, and other conservationists.

To accomplish this, the IMMP has these primary objectives:

- Acquire and share information about how habitat conservation actions affect monarchs and their habitat
- Provide geographically and ecologically representative information to update population and habitat models
- Track long-term changes in the distribution and abundance of monarchs and their habitats

Program Activities

IMMP field activities are designed to collect various types of monarch and habitat data. The modular nature of the activities allows individuals or groups to choose one or more activities that pertain to their interests and/or information needs. In attempt to collect all types of data for each monitoring site, multiple teams could be used to conduct the various activities. If a site does not have data from a particular activity, the information it does have will still be valuable for broader scale analyses. Field monitors will interact with a monitoring coordinator to select monitoring sites, be trained in field

methods, and delineate monitoring plots.

Activity	Required?	Frequency
Site Description	Required	Yearly
Activity 1: Milkweed and Blooming Plant Survey	Optional	Monthly
Activity 2: Monarch Egg and Larva Survey	Optional	Weekly
Activity 3: Adult Monarch Survey	Optional	Bi-weekly
Activity 4: Monarch Survival and Parasitism	Optional	Daily (care)
Activity 5: Red Imported Fire Ant Survey	Optional	Yearly

Monitoring Sites

The IMMP is designed to accommodate data from any location, but there are two main ways in which the program identifies sites for monitoring. First, a spatially balanced set of randomly chosen sites is used to obtain data from across the country and among land use types (e.g. grassland, agriculture, rights-of-way, urban) to provide general information about habitat availability and monarch use. Secondly, sites managed for monarchs or places of interest to landowners or land managers may contribute monitoring data, building our understanding of the importance of conservation sites in providing monarch habitat. Thus, sites may be randomly selected or specifically chosen by the monitor.

Application of the Program

The IMMP standard set of procedures may be used as a whole or in parts by natural resource partners to meet their own information needs or research objectives. The procedures may be used in more focused research projects such as studying how conservation practices like mowing, integrated vegetation management, pollinator habitat planting, or agricultural practices affect monarchs and their habitats. For instance, the milkweed density procedure may be used to provide acre-specific information to be catalogued in the USFWS Monarch Conservation Database. When paired with tools like the Environmental Defense Fund's Habitat Quantification Tool, these procedures may also be used to document or assess relative values of habitat.

This program is eager to engage stakeholders of all kinds involved in the conservation of monarchs through improved monitoring of monarch habitat and use. Participating in the IMMP provides a deeper connection with the monarch butterfly, the diversity of the habitats it requires, and the challenges it faces.

Site Description

Overview

Regardless of the activities conducted at a site, the first visit to the monitoring plot, the site description must be completed, including identifying the plot boundaries, describing the site type, ecology, disturbances, and other features of the habitat in the plot, and recording any miscellaneous monarch observations.

NOTE: Make sure permission is granted from the landowner or land manager before visiting the site (this may be done by monitoring coordinator or monitor) and contact them prior to each visit (if requested). Please consider safety when conducting your field visit (see Appendix G).

Attributes Measured or Assessed

- Site type and subtype (if applicable) (Table 1)
- Site location information
- Relative amount of woody plants
- Dominant vegetation types
- Land ownership and categorization
- Site disturbances
- Miscellaneous observations of monarchs

Supplies (* = optional)

- ☐ GPS or mobile device with geolocation and time-keeping functionality
- ☐ Compass (or mobile device with compass app)
- ☐ Data sheets, clipboard, writing utensil
- ☐ 100 m measuring tape, marked rope, or known distance by pace
- ☐ Markers for plot boundaries (e.g., wire flags, poles)*
- ☐ Camera*

Identify Plot Boundaries

1) Identify plot boundaries from the information provided by the monitoring coordinator. This will typically include the GPS coordinates for the starting location, a bearing in which to orient the monitoring plot, and the dimensions of the plot.
2) Walk the boundaries of the plot to verify that the monitoring plot will fit inside the target site type (given by monitoring coordinator), with no more than 10% of the area taken up by other types (e.g., water, forest, or other site types). If 90% of the plot does not fit within the appropriate type, pivot the box from

NOTE: If the landowner has granted permission, it may be helpful to mark the corners of the plot to relocate the plot during subsequent activities and visits. Wooden survey stakes, colored wire flags, or bamboo poles with bright flagging can be used as markers. Avoid objects that may be hazardous and become hidden when the vegetation grows, and remove markers at the end of the survey season.

the starting point until it does so. Record the new field coordinates of the plot boundaries from a GPS unit or mobile device.

Table 1. Site types and subtypes.

Site Type	Site Type Code	Subtypes	Description
Protected grassland	PRG	(No subtypes)	Grasslands, savanna, and shrubland; herbaceous plants dominant; public or private land under easement
Unclassified grassland	UCG	(No subtypes)	Grasslands, savanna, and shrubland; herbaceous plants dominant; public or private land with no known easement
Agriculture	AGC	Crop fields, edge habitat	Crop fields: row crops, orchards, vineyards, etc. Edge habitat: fence row, in-field habitat strips, etc.
Rights-of-way	ROW	Roadsides, railways, power corridors, transmission lines	Roads with right-of-way habitat ≥ 4 m wide and safe parking/accessibility; some rail corridors.
Developed	DEV	High density, medium density, low density, open space	Areas associated with residential, commercial, industrial, or public development; rural, exurban, suburban, or urban
Agricultural Conservation Land[1]	ACL	(No subtypes)	Land enrolled in an agricultural conservation program (e.g. Conservation Reserve Program, etc.)

1. Agricultural Conservation Land, such as those enrolled in the Conservation Reserve Program, are not currently classified as a site type in the draw of sampling points due to privacy concerns, but are of interest to the program. These lands have been classed as either agriculture or unclassified grassland depending on the land use at the time of the satellite imagery classification. When discovered as such, these lands should be re-classified in the "verified site type" field as "Agricultural Conservation Land."

Describe the Plot

Describe the plot according to the categories in the following sections and walk through the plot to make these determinations.

Plot Classification

1) Verified Site Type: Record the site type (in the ***Verified Site Type*** field) to verify the GIS classification (see Table 1). There are occasionally classification errors in the GIS; if a plot was misclassified, record the correct type and monitor the plot accordingly. Let the monitoring coordinator know of this misclassification as well, so they can allocate new sampling points accordingly.

2) Site Subtype*:* Many site types will have subtypes to choose from (see Table 1). Record the appropriate subtype for your plot in ***Site Description Notes*** if applicable.

Site Ecology

1) Woody Species – Estimate the percent of the monitoring plot covered by woody species (< 5%, 5-50%, or > 50%, or unknown) and write in dominant species if known.

2) Invasive Species/Noxious Weeds – Estimate percent of plot covered by invasive species and noxious weeds (< 5%, 5-50%, > 50%, or unknown). List any known invasive or noxious species present.

3) Forbs – Estimate percent of the plot covered by forbs (0, 1-10, 11-25, 26-50, >50, unknown).

4) Wetland – Estimate the percent of the plot covered by wetland features (0, 1-10, 11-25, 26-50, >50, unknown). If present, identify the wetland feature types that occur within the plot (regardless of whether they are currently wet). Choose the types that best depict what is present from the list provided. If 'other' is selected, provide a description in ***Site Description Notes*** and specify if the wetland is currently wet (has standing water).

Wetland Type	**Description**
Ditch	Human-built linear depression for water conveyance
Lake/Pond edge	Mesic area adjacent to a pond or lake
Marsh/Wet Meadow	Herbaceous area at least periodically wet (includes fen, bog, swale)
Riparian corridor	A linear habitat area within the flood plain of a river, stream, or creek, even if intermittent
Storm water Basin	Human-built depression for holding runoff (retention pond)
Other	

5) Adjacent Land Use (within 100 m of plot): Record the top two dominant land use types (strata) that are present within 100 m of the monitoring plot (Protected Grassland, Unclassified Grassland, Grassland (type unknown), Agriculture Crop Fields, Agriculture Conservation Lands, Roadside or other type of Right-of-Way, Developed area, Wetland/Water, Forest/Woodland).

6) Red Imported Fire Ants (where geographically appropriate): Indicate if there is evidence of red imported fire ants within the monitoring plot.

Site Disturbance/Management

On each site visit, document all disturbances, either natural or by management that may have changed the structure or composition of the vegetation *on at least 10% of the site* (see Table 2)**.** Disturbances should be detectable by the observer during the site visit; most observable disturbances will have occurred within the last two years. Some, such as tree blow-downs, may be visible much longer than two years and should still be documented. 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 *Site Description Notes*.

Table 2. Disturbances that may affect the structure and composition of vegetation within a sampling plot.

Disturbances	
1. No disturbance	6a. Grazed - cattle
2. Mowed	6b. Grazed - sheep
3a. Hayed-residual remains	6c. Grazed - horses
3b. Hayed-hay removed	6d. Grazed - other*
4a. Chemical: fertilizer	6e. Grazed - unknown
4b. Chemical: herbicide	7a. Burned: wildfire
4c. Chemical: insecticide	7b. Burned: prescribed
4d. Chemical: unknown	7c. Burned: unknown
4e. Chemical: other*	8. Plowed or disked
5a. Construction: structure	9. Flooded
5b. Construction: road	10. Tree harvest / woody species removal
5c. Construction: trail	
5d. Construction: other*	11. Other*

Site Type Specific Information

1) Roadside rights-of-way:
   a. Road type: Classify the roadside as a large highway (greater than 4 lanes), medium highway (4 lanes), small paved (2 lanes), small unpaved (2 lanes, gravel or dirt), with lanes counted in total for the entire roadway (both directions and regardless of whether the road is divided).
      i. Width of roadside: Measure the right-of-way habitat area width (in meters), at each end of your plot. If the roadside does not have a consistent width and does not measure the same on both ends (within a few meters), take several more measurements throughout the plot to calculate an average width (maximum of ten measurements). If monitoring for milkweed and blooming plants, this can be done while laying the transects for sampling vegetation. The width of the roadside habitat begins where the vegetation starts near the road (regardless of mowing), and extends to where the land use dominantly changes. For instance, if there is encroachment by adjacent landowners into the road right-of-way (such as by mowing), we will measure and limit our sampling to the area that is vegetated like roadside habitat rather than the other land use strata. However, we do allow smaller inclusions (less than 25% of site) of woody vegetation, bare ground (such as from a driveway or pullout), wetland (such as around a culvert), etc. within the roadside habitat.

b. Agricultural edges: Record the width of the habitat edge at least twice (up to 10 times for sites with variable edge habitat width).

c. Developed sites: Record the percent of the monitoring plot that is inaccessible and therefore, not sampled (e.g. behind a fence, unable to gain permission for access, etc.). This does **not** include accessible paved areas; paved areas are part of the developed landscape. It may be helpful to work with a printed map of the site, mark the areas that cannot be sampled, and then delineate their area with a mapping tool or visually estimate it to fill in these estimates.

Miscellaneous Monarch Observations

If monarchs of any stage are observed outside the described activities, record them as *Miscellaneous Monarch Observations*. The behavior should also be recorded if adult monarchs are observed. These include: flying through site, flying high over site (greater than 5 m high), resting (including roosting), ovipositing (laying eggs), mating, or nectaring. If nectaring, record the associated plant species. For additional egg, larvae, or pupae observations, simply check whether or not those stages were observed in the plot. See Appendix E for behavior definitions and information on how to distinguish among them.

Optional Site Photographs

Photos may be taken at monitoring sites to document the plot interior, site conditions and disturbances, or to assist with plant identification. Photos may be attached to the Survey123 online data entry form.

Activity 1: Milkweed and Blooming Plant Survey

Overview

Participants lay transects and subplots to record milkweed density and blooming plant frequency within a monitoring plot. The Site Description must be completed before conducting this activity. Depending on the desire and skills of the observer(s), blooming plants may either be identified to species or just noted as the presence of a blooming plant. After the subplots are read using the plant sampling frame, participants walk throughout the monitoring plot to record any additional blooming plant species. An alternate census method is also presented for smaller, agricultural, and some developed sites. Any miscellaneous monarchs encountered within the monitoring plot are also recorded. Optionally, participants may also monitor for monarch eggs and larvae in conjunction with this activity. Activity 1 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.

Supplies (* = optional)

- ☐ GPS or mobile device with locating functionality (to locate plot; not necessary if plot was marked during *Site Description*)
- ☐ Plant sampling frame (Figure 1)
- ☐ Datasheets, clipboard, writing utensil
- ☐ Compass or mobile device with compass app
- ☐ 100 m measuring tape (recommended; alternative is to use paces)
- ☐ Monarch identification materials (Appendix E)*
- ☐ Plant identification materials*

Habitat Characteristics Measured

- Milkweed density by species
- Blooming plants
  - *Level A: Frequency and species composition*
  - *Level B: Frequency*
- Miscellaneous observations of all monarch life stages, including adult behaviors

Note about Blooming Plant Identification: There are two levels of difficulty for the blooming plant portion of this activity. Those who can identify most species using existing knowledge or field guides should participate in *Level A,* identifying blooming plants to species. Those who cannot identify most blooming plant species should participate in *Level B*, where only the presence or absence of blooming plants is noted.

Sampling Frequency

This activity should be performed once per month during the season(s) in which monarchs are present, but data recorded at any interval will be accepted. Typically, each monitoring visit takes 2-4 hours, but depends on site conditions and number of activities conducted per visit.

Laying Transects

Within a monitoring plot, the surveyor will establish 500 m of transects and collect data within 100 subplots along the transects (see Figure 1). Transect layout methods vary depending on the site type, initially determined by the monitoring coordinator and verified during the first site visit. Once identified, follow the instructions specific to your site type as described in Appendix F. Monitoring coordinators will provide: the delineated monitoring plot, GPS coordinates for the first transect starting location, and a directional bearing.

To place a transect, locate the sample point (by field markers or GPS; see below) and affix the transect measuring tape with a stake (or have one person hold the end). Use a compass or mobile device to move in the direction provided, unwinding the measuring tape as you move. To ensure the transect line is straight, walk out the entire length of the transect before returning to lay subplot frames. Alternatively, a known pace can be used to judge distance between subplots.

Figure 1. Example transect layout. The sample point is indicated by the red dot; transects are indicated by yellow lines, and subplots are in black.

Note: If the transect placement is such that it crosses into a different site type, shift the transect over a few meters so that you monitor within the desired type.

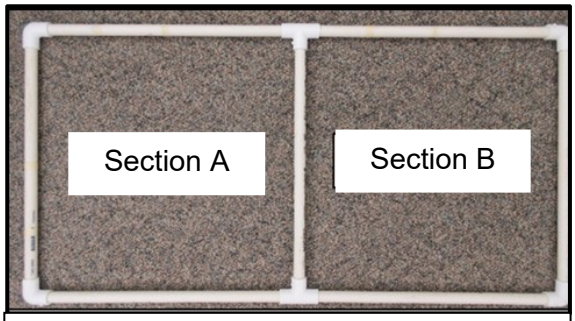


Figure 2. The plant sampling frame measures 1.0 m by 0.5 m and is formed from ¼ inch or ½ inch PVC-pipe. It is either marked or divided down the center to create two equal sections of 0.5 m by 0.5 m. The sampling frame is flipped over the transect line to create one full subplot.

Placing Subplots and Recording Data

Data recording can be done using a mobile device or paper (*Activity 1 Datasheet).* While sampling each subplot (Figure 2), measure the attributes described above simultaneously. Follow these steps for subplot placement and data recording:

1. Indicate which attributes were collected in the boxes at the top of the data form.
2. At the beginning of each transect, place the plant sampling frame (Figure 2) at the zero meter mark on the left side of the transect tape (so that the bottom right corner is aligned with the zero mark) to forms sections A and B (Figure 3). To create section C and complete the subplot, flip the frame over to the right side of the tape so that the bottom left corner now aligns with the zero mark.
3. Measure milkweed density within each subplot. Milkweed is recorded by species for the entire subplot (sections of the subplot are not recorded for milkweed). Count and

record the number of milkweed plants by species that are rooted in each subplot. For each multi-stemmed milkweed plant, also count and record the number of stems. If a plant has multiple stems and falls on the edge of the frame, record the plant and the number of stems that are rooted within the frame (see Figure 5).

Counting Milkweed Plants

In the IMMP, we define a milkweed plant as all above-ground stems originating from a visually-identifiable, common central point in the ground. For some species, such as *A. syriaca* (common milkweed) or *A. verticillata* (whorled milkweed), a single plant may grow several ramets that are connected below ground. Without excavating roots, it is impossible to tell if ramets are from the same or different plants. For single-stemmed milkweeds, we consider each stem that is separated by soil a distinct plant (Table 1).

On the other hand, plants of some milkweed species, such as *A. viridis,* green antelopehorn milkweed, or *A. tuberosa*, butterfly weed, have multiple **milkweed stems,** all originating from the same base. For multi-stemmed species we count each group of stems that come from a common central location in the soil as one plant.

Table 1. Classification of prevalent milkweed species as single- or multi-stemmed

Species	Classification
A. syriaca (common)	Single
A. tuberosa (butterflyweed)	Multi
A. incarnata (swamp)	Multi
A. verticillata (whorled)	Single
A. speciosa (showy)	Single
A. viridiflora (green)	Single
A. viridis (green antelopehorn)	Multi

If a plant has multiple stems and falls on the edge of a frame, record the plant only if it is rooted within the plot and the number of stems that are growing from soil within the subplot frame (see Figure 5).

Counting Stems

For multi-stemmed species, we will also record the number of milkweed stems per plant. Stems do not need to be counted for single-stemmed species.

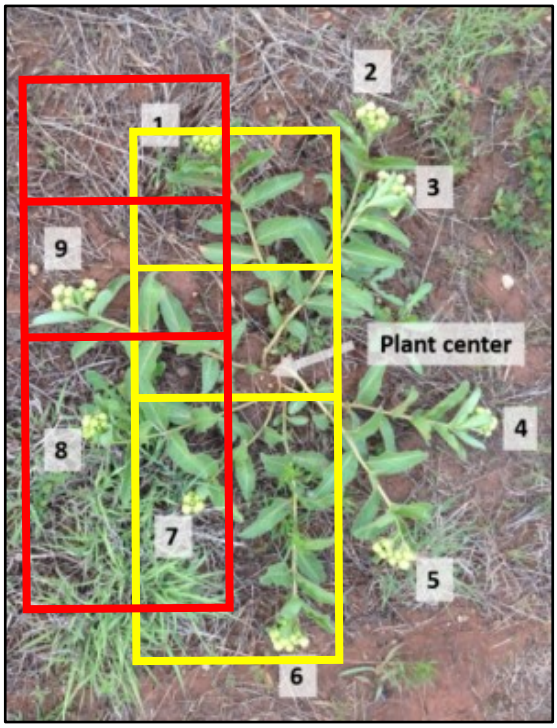


Figure 5. *A. viridis* plant with multiple stems. If the frame was placed as indicated by the yellow lines, the plant would be recorded as: 1 plant, 9 stems, since the milkweed plant is rooted within the sampling frame. If the frame was placed as indicated by the red lines, the plant would NOT be recorded in the subplot. Although there are stems that are bent into the frame, the plant is rooted outside of the frame, so is not included in the milkweed count (Photos courtesy of Kristen Baum).

4. Record blooming plants in the subplot sections; data are recorded according to each section with the plant sampling frame (A, B, and C) (Figure 2). If you are identifying plants to species, follow the Level A instructions. If you are not, follow Level B. If there were not any blooming plants in any section of the subplot, record a zero.

Level A Instructions:

a. Check section A of the sampling frame for blooming plants (with at least one open flower accessible to a butterfly). Record each blooming species you observe.
b. Next, look in section B and record any <u>new</u> blooming plant species. Do not record a species that was already found in Section A.
c. Flip the frame across the transect line to form section C and observe it for new blooming plants. Again, only record <u>new</u> species observed in section C. Do not record a species that was already found in A or B.

Level B Instructions:

a. Check section A of the sampling frame for blooming plants (with at least one open flower accessible to a butterfly). Record "A" on the datasheet if any blooming plants are present in section A. If present in A, record it and move to the next subplot, there is no need to look in sections B or C.
b. If blooming plants were not observed in section A, look in section B. Record a "B" if a blooming plant was present in B (but not in A).
c. If blooming plants were not observed in sections A or B, flip the frame across the transect line to form section C. Record "C" if blooming plants were observed in section C (but not in A or B).

5. Move along the transect and place subplots every 5 meters, repeating steps 3 and 4 for each until **100 subplots** are monitored.
6. After completing the subplot sampling, walk throughout the plot to record any additional blooming nectar plant or milkweed species that were not observed in the subplots as ***Additional Plant Species***. Walk systematically through the plot to ensure as much coverage as possible. You can also record additional species as you see them while monitoring subplots.

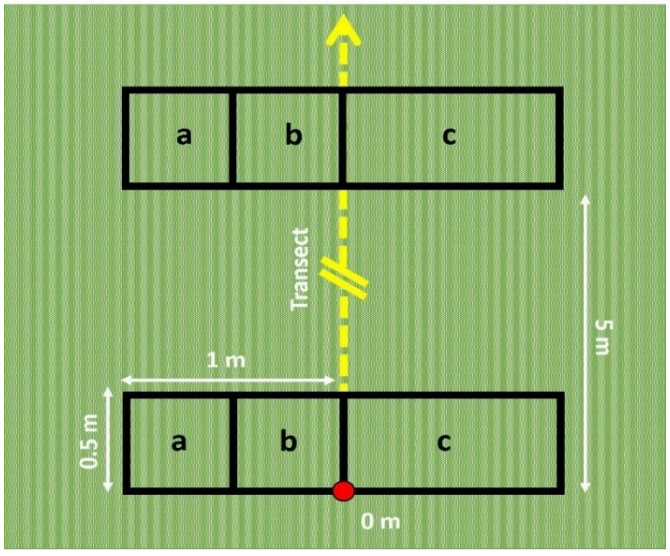


Figure 3. First two subplots on a transect starting at 0 meters (the red dot). The subplot (or plant sampling frame) is first placed to the left of the transect line (in yellow), representing subplot sections 'a' and 'b'. Then the frame is flipped to the right side of the transect, creating section 'c'. The total subplot sampling area (sections a+b+c) measures 1 square meter. The second subplot is placed at the 5 meter mark.

NOTES: If milkweed is blooming in a subplot, it should be recorded twice, once in the milkweed section and once in the blooming nectar plant section.

Some plants have a variety of different common names and scientific names in field guides and on websites. To find the correct listing, we use the USDA PLANTS Database. If using paper data forms in the field, monitors may write whatever naming they prefer, as long as they make the proper species selection when entering data.

If along the transect you encounter unsuitable habitat within a subplot, such as a thicket or water, please collect the data for that subplot. Do not be tempted to move the location of the subplot in order to capture particular plants.

Alternative (Census) Sampling for Small (< 1 ac), Developed (non-open space), and Narrow Agricultural Edge Sites

In developed sites (excluding open space), very small sites (generally less than 0.4 ha or 1 ac), or agricultural edge sites less than 4 m wide, transects and subplots will **not** be used. Instead, participants will comprehensively survey the entire area. Walk a systematic pattern through the plot (weaving back and forth) and record blooming plant species and the number and species of milkweeds. Cover the entire area of the plot thoroughly, and avoid walking through the same area twice. In developed sites (excluding open space), record the species of blooming plants that you are able to identify. For those you cannot identify, tally the number of different species you observe. Taking photos of each new species you encounter may be helpful in remembering which you have already tallied.

Miscellaneous Monarch Observations

If monarchs of any stage are observed outside the described activities, record them as *Miscellaneous Monarch Observations*. The behavior should also be recorded if adult monarchs are observed. These include: flying through site, flying high over site (greater than 5 m high), resting (including roosting), ovipositing (laying eggs), mating, or nectaring (probing flowers with proboscis). If nectaring, record the associated plant species. For additional egg, larvae, or pupae observations, simply check whether or not those stages were observed in the plot. See Appendix E for behavior definitions and information on how to distinguish among them.

Option to Combine with Monarch Egg and Larva Survey

If participants choose to survey for monarch eggs and larvae (Activity 2) during the same monitoring session, it is efficient to monitor the milkweed for eggs and larvae while completing Activity 1. As monitors record milkweed and blooming plants in each subplot, they will also record the number and stages of monarchs found on each milkweed plant. Participants may also monitor milkweed plants between subplots for eggs and larvae. See Activity 2 directions.

Field Methods Summary

Follow these steps for each field survey:

1. Record start time (indicate AM or PM) when you begin walking toward the first transect.

2. If you are monitoring for monarch eggs and larvae, record the temperature (in the shade, indicate Fahrenheit or Celsius).
3. Record any new disturbances at the monitoring plot (see description of this data field in Site Description).
4. Lay out the first transect.
5. Place the first subplot at 0 m, and record data for milkweed and blooming plants, (and monarch eggs and larvae optionally).
6. Continue along the transect placing subplots every 5 m. Record milkweed and blooming plants in each subplot. (If monitoring for monarch eggs/larvae, also record monarchs in each subplot and examine milkweeds between subplots for monarch eggs and larvae.)
7. Once you have monitored 100 subplots, walk through the plot area and record any additional blooming nectar plant or milkweed species that were not encountered in the subplots but are within the plot boundaries. Record these in the ***Additional Plant Species*** data field.
8. At the end of the survey, record in the ***Plant Survey Type*** field whether you: a) completed 100 subplots, b) completed fewer than 100 subplots, or c) used a comprehensive survey. If b) was selected, explain the reason why in the ***Notes*** section.
9. Record any ***Miscellaneous Monarch Observations*** of adults (including behavior) or eggs/larvae observed within the monitoring plot but not recorded in the primary activity.
10. Record any other pertinent information in the ***Notes*** section.
11. Record your end time when you've completed the survey.

Activity 2: Monarch Egg and Larva Survey

Overview

Participants examine milkweed plants in the monitoring plot to count how many monarch eggs and larvae (caterpillars) are found per number of milkweed plants observed. Data will be used to examine how monarch densities vary within a year, between years, and among different sites and locations. This protocol was adapted from the Monarch Larva Monitoring Project, and data will be compatible for expanding that data set. This activity may be done in conjunction Activity 1: Milkweed and Blooming Plant Survey, or it may be done separately.

Sampling Frequency

Weekly sampling to monitor for monarch eggs and larvae is recommended, although data recorded at any interval will be accepted. Begin monitoring for eggs and larvae when milkweed first emerges at the site, throughout the growing season.

Supplies (* = optional)

- ☐ GPS or mobile device with locating functionality (to locate plot; not necessary if plot was marked during *Site Description)*
- ☐ Datasheets, clipboard, writing utensil
- ☐ Field thermometer or mobile device with local weather data
- ☐ Hand lens*
- ☐ Monarch identification materials (Appendix E)*
- ☐ Plant identification materials*

Selecting Milkweed Plants to Monitor

There are different ways to determine which milkweed plants within the monitoring plot to examine for monarch eggs and larvae. Here are three options:

1. Examine as many milkweed plants as possible within the monitoring plot, keeping track of the number of plants examined and the number of monarch eggs and larvae of each instar found. If it is reasonable to monitor every milkweed plant within the monitoring plot, this is preferred.

2. If there are too many milkweed plants in the plot to monitor all of them, you should randomly select a subsample to monitor (it does not need to be the same subset each week). When subsampling, it is important to avoid bias; do not just look at milkweed plants thought to be more likely to have monarchs on them as this may overestimate the monarch density at the site. Avoid this by following one of the random plant survey options below:
    - <u>Large sites with evenly distributed milkweed:</u> Walk one or more random, straight-line transects, or paths, through your site.
        1. First, choose a random direction to walk. You can do this by tossing a pencil or stick, and walking in the direction it points, or using some other random sampling method.

2. After choosing a direction, hold your arms out to your sides as you walk. Stop and examine every milkweed plant that falls along your path between your fingertips. As you examine these plants, record the number of plants and whether they have monarchs.
3. When you reach the perimeter of your site, generate another random direction to conduct the next transect, or simply turn 90 degrees back into your site and continue monitoring.
4. Continue running these transects through the site until you feel you have adequately sub-sampled the site, or have run out of time. We recommend monitoring a minimum of 200 milkweed plants, if subsampling.

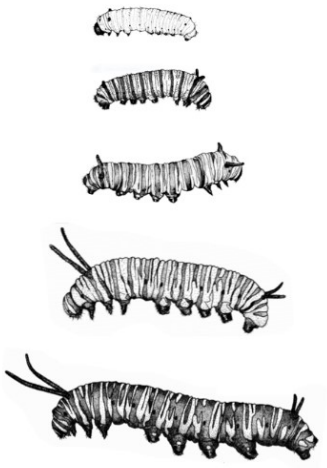
- Sites with patchy milkweed: Use a systematic approach to subsample milkweed.
  1. Estimate the total number of milkweed plants at the site, and determine how many you are capable of observing each week. Use this number to calculate the sub-sampling method. For example, if a site has 900 milkweed plants, and time allows monitoring 300 of them each week, a good rule would be to observe and record every third milkweed plant observed.
  2. Once rule is established, move from patch to patch observing accordingly.

3. Monitor milkweed for monarch eggs and larvae while doing Activity 1 (Milkweed and Blooming Plant Survey). Note: following transects is an option even on dates when Activity 1 is not conducted.
   a. As you record milkweed and blooming plants in each subplot, also record the number and stages of monarchs you find on each plant. When a plant contains a monarch egg or larva (or multiple), record the plant in its own row with the number of eggs/larvae it contains (identified to instar).
   b. As you continue along the transect, search all milkweeds between subplots for monarch eggs and larvae. Monitor all milkweed plants within 1 meter (width of your sampling frame) on each side of the transect line (for a total swath of 2 meters). Label these milkweeds as 'between subplots' in the subplot number column. If milkweed is very sparse, you may widen this swath (or reduce if milkweed is very dense).
   c. Continue examining milkweeds for monarch eggs and larvae until you've reached a minimum sample size of 200 milkweeds searched (if there are that many!). If you've reached this minimum, you can stop monitoring milkweed between subplots but keep monitoring within subplots for the remainder of your session.

NOTE: It is important not to stray from the selected sampling method to include plants that are just outside your reach, plants on which you happen to notice a monarch, or plants you feel might be more likely to have monarchs on them. These would introduce bias and skew data. Record information only for the plants searched, including ones with zero monarchs.

Monitoring Milkweeds for Eggs and Larvae

1. Record the date, temperature in the shade (indicate Fahrenheit or Celsius), and start/stop times on the Activity 2 datasheet.
2. Locate the boundaries of plot (if not marked during ***Site Description***) and examine milkweed plants within the plot for monarch eggs and larvae using a comprehensive survey of all milkweeds or a random plant survey method described above.
3. Record the number of milkweed plants examined by species and the number of monarch eggs and larvae that found on each plant. Be sure to identify the larvae to instar (see Appendix E).



NOTE: If you see a monarch larva 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 larvae sometimes crawl off of plants to molt or cool down.

Tips for observing monarch eggs and larvae on milkweed plants:

- 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.
- Look for caterpillar clues such as chew marks on the leaves or frass.
- Handle the plants carefully to avoid knocking off any eggs or larvae.
- See Appendix E for guidance on distinguishing monarchs from other insects, and for help distinguishing the five monarch caterpillar instars.

Miscellaneous Monarch Observations

If you observe adult monarchs while monitoring for eggs and larvae, record them as *Miscellaneous Monarch Observations*. Adult behavior should also be recorded; these include: flying through site, flying high over site (greater than 5 m high), resting (including roosting), ovipositing (laying eggs), mating, or nectaring. If nectaring, record the associated plant species. See Appendix E for behavior definitions and information on how to distinguish among them. If subsampling for eggs and larvae, you may record any additional monarch eggs or larvae that you observe on plants outside your subsample.

Activity 3: Adult Monarch Survey

Overview

Participants establish an adult monarch survey route within the monitoring plot and count adult monarchs within defined spaces along the route, documenting their behaviors. They also record the species of plants on which monarchs are nectaring. Activity data will be used to track the abundance and chronology of adult monarchs throughout breeding and migration periods.

This activity uses a modified Pollard (1977) walk, in which the surveyor slowly walks along a designated **adult monarch survey route** (typically 500 m or 0.31 mi). During the walk, all monarch adults observed are recorded as well as their activity or behavior within a set vertical and horizontal distance from the survey route (see Figure 1). This vertical and horizontal distance from the route is hereafter referred to as the **adult monarch counting box**. This box extends 5 m beyond the observer (who is fixed on the survey route) to the right, left, in front, and above along the survey route (see Figure 1). Counting adult monarchs in this three-dimensional area permits a fixed-area count and produces a time-specific index of adult monarch abundance (number/ha).

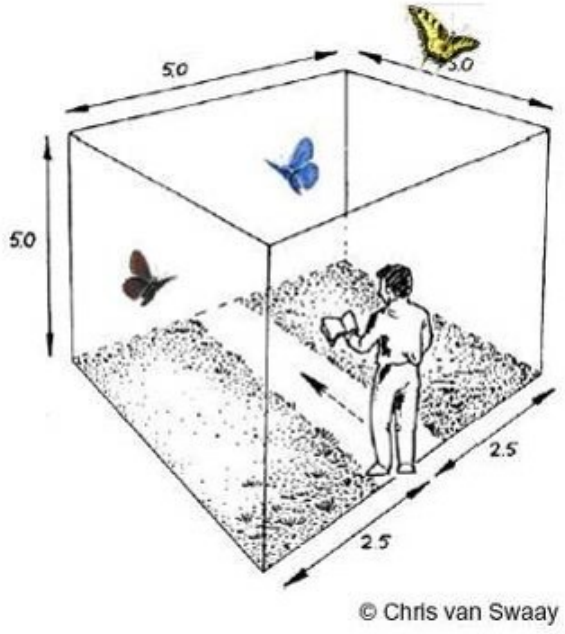


Figure 1. Adult Monarch Counting Box, courtesy of Chris van Swaay.

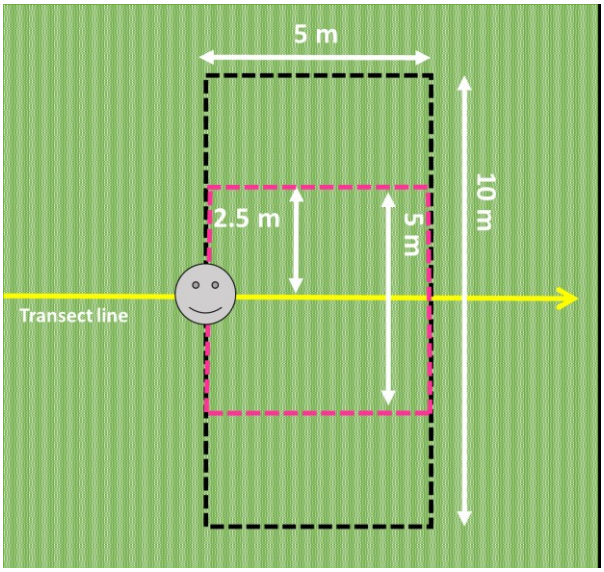


Figure 2. Adult monarch counting box dimensions.

To estimate if the detectability of monarchs decreases with distance from the observer, and to create compatible data with other butterfly counting programs, this activity also defines a narrower box within the adult monarch counting box (hereafter the **inner box**) which is 2.5 m to the left and right of the observer on survey route (but still maintains 5 m in front and above the observer/survey route).

Characteristics Measured

- Time of day and duration of time conducting the survey
- Length of adult monarch survey route walked (m)
- Number of adult monarch butterflies observed
- Location of each monarch within the adult monarch counting box (in or out of inner box)
- Monarch behavior (flying, resting, ovipositing, mating, or nectaring)
- Plant species from which adults are nectaring
- Temperature (ambient ºF)

Supplies (= optional)*

- ☐ GPS or mobile device with locating functionality and clock
- ☐ Data sheets, clipboard, pencil
- ☐ Compass or mobile device with compass app
- ☐ Field thermometer or mobile device with local weather data
- ☐ Rope/cord (marked for 2.5 and 5.0 m) for estimating distances*
- ☐ Monarch identification materials (Appendix E)*
- ☐ Plant identification materials*

Survey Route Location

An adult survey is conducted by walking a predetermined 500 m long route defined for each site, usually around the monitoring plot periphery or down the middle of a linear plot (such as a roadside). Survey routes vary depending on the site type being monitored (indicated by the monitoring coordinator). Follow the appropriate section below according to your site type.

- Grasslands and Agricultural Conservation Program lands:
  - Standard rectangle (200-m by 50-m): Walk the site boundaries (see Figure 2a).
  - Standard square plot (100-m by 100-m): Walk the site boundaries and then diagonally through the interior of the site, until reaching 500 meters (Figure 2b).
- Rights-of-Way: Walk a linear route located midway between the right-of-way edge and the opposite habitat edge, oriented against oncoming traffic (Figure 2c).
- Developed and Irregular Plots: Establish a walking route that is 500 m in length. The route may follow pathways and accessible areas and should correspond as best it can to the site with minimal tight turns.
- Agriculture:
  - Crop fields: Walk the boundaries of the rectangular or square plot.
  - Edge habitat: Walk a linear route along the agricultural field edge, midway between the crop field edge and habitat edge (similar to a right-of-way).

Note: If the survey route is such that it crosses into a different site type, shift the route a few meters into the plot so that the adult monarch counting box fits within the desired type.

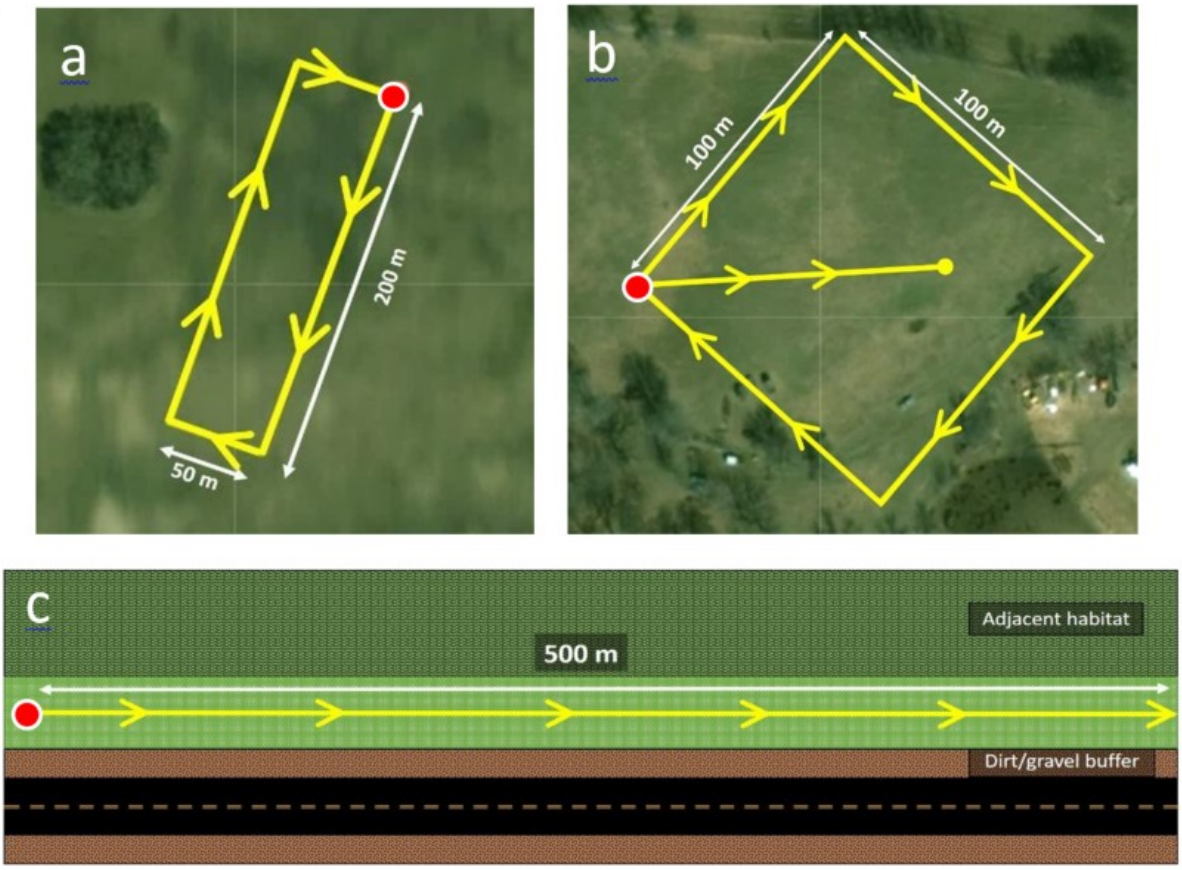


Figure 2. Routes for conducting adult monarch surveys: a) Standard rectangle, b) Standard square, c) Right-of-way. The monitor starts at the red dot and walks along the yellow line in the direction of the arrows.

NOTE: Regardless of site type, an adult count should **not** be conducted at sites that are too small to accommodate a 500 m route with minimal twists and turns. In those cases, adult presence may be reported as Miscellaneous Monarch Observations.

If the plot is less than 10m wide, the full adult monarch counting box will not fit. In this case, restrict observations to the inner box (within 2.5 m to each side of the survey route) and indicate this using the check box on the data form for '**Inner Box Only**.'

Survey Conditions

Surveys are conducted under weather conditions when butterflies are most active; ideal days are sunny, warm, and with little or mild winds. The timing of these conditions can vary among regions and time of year. Ideal conditions are outlined in the Table 1.

Table 1. Ideal and allowable weather conditions for conducting adult monarch surveys.

	Ideal conditions	Allowable conditions
Time	10:00AM-4:00PM	Any daylight times
Temperature	70-87 °F (21–30 °C)	60-95 °F (16–35 °C)
Wind	Less than 10 mph (16 km/hr)	Up to 24 mph (38 km/hr)
Precipitation	None	None

Conditions outside of those outlined above may decrease adult activity at the site and would result in a non-representative sample. Do not conduct this activity if it is raining, if wind speeds are continuously above 24 mph, or if temperatures are below 60 °F; these are not good conditions for viewing butterflies. Since strong winds occur frequently in open prairies and are sometimes unavoidable, wind conditions up to 24 mph are allowable, but lower wind speeds are preferred for spotting flying adults. For reference, according to the Beaufort scale, small trees in leaf begin to sway at 19-24 mph.

Frequency and Timing of Surveys

Frequency: Because adult monarch abundance varies across seasons at a given location, repeated surveys are needed from spring to fall. Sampling every two weeks during the time when monarchs are present in the area is recommended. If time permits, and particularly during peak migration periods, weekly or daily surveys are even more informative and are welcomed. To determine when to conduct adult surveys, consult Journey North (https://www.learner.org/jnorth/maps/monarch.html) or watch for monarch arrival 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.

In southern locations, such as Oklahoma or Texas, monarchs may be more prevalent in spring for breeding and fall during migration, with a period of time during the summer when monarchs are rarely present. In these areas, the recommended frequency for conducting the Adult Monarch Survey is every two weeks while monarchs are more abundant, and once per month during the summer when monarch numbers are low. For average dates of the migration south, visit https://www.monarchwatch.org/tagmig/peak.html.

Timing of Survey Relative to Other Activities: If multiple activities will be conducted at a site on the same day, the Adult Monarch Survey should be conducted first, with the exception of the first site visit of the year (during which the Site Description should be first). Conducting the Adult Monarch Survey first minimizes the effect of your presence on the behavior and detection of the butterflies.

Survey Methods

Surveyors: Only one person conduct the Adult Monarch Survey. Assistants should help with data recording but should walk about 5 m behind the observer to avoid disturbing adults ahead on the survey route. If an assistant sees butterflies missed by the primary surveyor, he/she is not to alert the primary surveyor or add those butterflies to the Adult Monarch Survey (his/her sightings may be recorded as *Miscellaneous Monarch Observations*).

Weather Conditions: Record the wind speed code and the sky code before conducting the Adult Monarch Survey.

Table 2: Wind speed Codes

0	<1mph (smoke rises vertically)
1	1-3mph (wind direction shown by smoke drift)
2	4-7mph (wind felt on face; leaves rustle)
3	8-12mph (leaves/twigs in motion)
4	13-18mph (raises dust/loose paper)
5	19-24mph (small trees in leaf sway)
6	25-31mph (large branches in motion)
7	32-38 mph (whole trees in motion, effort needed to walk against wind)

Table 3: Sky Codes

0	Clear/few clouds
1	Partly cloudy/variable sky
2	Cloudy/overcast
4	Fog or Smoke

Monarch Identification: For help identifying and distinguishing adult monarchs from other butterflies, see Appendix E and reference regional butterfly field guides; practicing spotting and identifying adult monarchs prior to conducting this activity is recommended.

Estimating Distance: To estimate the 2.5 m and 5 m distances, practicing prior to conducting the survey is recommended. Using a meter stick, tape measure, or measured rope, practice walking while scanning the appropriate distance to either side. Find a habitat area (such as a sidewalk or field edge) that is of appropriate width and practice walking and observing within that space. To help visualize in the field before beginning the survey, bring a 10-m or 5-m length of small-diameter cord/rope to illustrate the appropriate width of the boxes.

Behaviors: For each monarch observed, record its behavior (Table 2); more than one behavior may be recorded per butterfly.

Table 2. Behaviors to record during adult monarch survey.

Flying	Butterflies in flight within the monitoring plot and within 5 m of the ground
Flying High	Butterflies flying more than 5 m high over the plot, may or may not be using the habitat within the monitoring plot
Resting	Alighted on a plant with no sign of mating, ovipositing, or nectaring. Also includes roosting: resting during migration, often by groups in woody vegetation
Ovipositing	Female adult monarch arching abdomen and depositing eggs (on milkweed)
Mating	Male and female adults observed copulating
Nectaring	Adults alighted on a plant and actively extending proboscis into a flower to obtain nectar

Conducting the Survey and Recording Data:

1. Complete the general survey information on the data sheet, including site ID, disturbances, observer, date, temperature, wind speed, and sky code. Note whether using the full width of the survey box or if you are doing a survey of the inner box only.
2. Locate the beginning of the route using a mobile geolocation device and orient yourself in the correct direction to begin the survey (find a target in the distance, such as a tree, that lines up with the compass bearing that you are to walk).
3. Record the time started, start timer, and begin slowly walking the route. The optimal pace is about one meter every two seconds, similar to a "wedding walk" down the aisle. At this pace, it will take approximately 20 minutes to complete the 500-m survey. In practice, it may take a bit longer as you pause to record data.
4. Record all adult monarchs observed within the adult monarch counting box (5 m (16.4 ft) area in front of, above, and to each side (Figure 1). Record each butterfly according to its distance from the survey route within two categories: within the inner box (2.5 m to the sides) or within the outer or full box area (2.5-5 m). Please note that the inner box area includes 5 m above and in front of the observer, the same as in the full box, but is just limited in the side distances (perpendicular to the route). Each butterfly should be recorded in its own row. If you encounter mating butterflies, make a row for each butterfly and indicate that each was mating.
5. If a monarch is first counted in the outer counting box area (2.5 - 5 m away from you to the side), but then moves within the inner area of 2.5 away from the survey route (or 5 m in front or above), check the box: "**moved into inner box**." This enables us to understand where the monarch was first observed. For each monarch observed, record its behavior(s): **flying**, **resting (includes roosting)**, **ovipositing (egg laying)**, **mating**, or **nectaring** (Figure 3).
6. If a monarch is nectaring, record the plant species it is on. If it cannot be readily identified, take a photo or mark the location, and once the survey is completed direct attention to identifying the plant.
7. If pausing to record data, pause facing the same direction of the survey route. Once data have been recorded, continue in that direction at the same pace.
8. Only count adult monarchs within the adult monarch counting box and do not count ones behind you. Track butterflies that have already been counted so they aren't counted twice.
9. If the entire adult monarch survey is conducted with no detections, but adult monarchs were seen farther away but within the boundaries of the site, record them as *Miscellaneous Monarch Observations* (see below). This is only necessary if you have no detections within your survey, as it is important to maintain your focus within the survey area rather than on the miscellaneous observations.

Miscellaneous Monarch Observations

If monarchs of any stage are observed outside the described activities, record them as *Miscellaneous Monarch Observations*. The behavior should also be recorded if adult monarchs are observed. These include: flying through site, flying high over site (greater than 5 m high), resting (including roosting), ovipositing (laying eggs), mating, or nectaring. If nectaring, record the associated plant species. For additional egg, larvae, or pupae observations, simply check whether or not those stages were observed in the plot. See Appendix E for behavior definitions and information on how to distinguish among them.

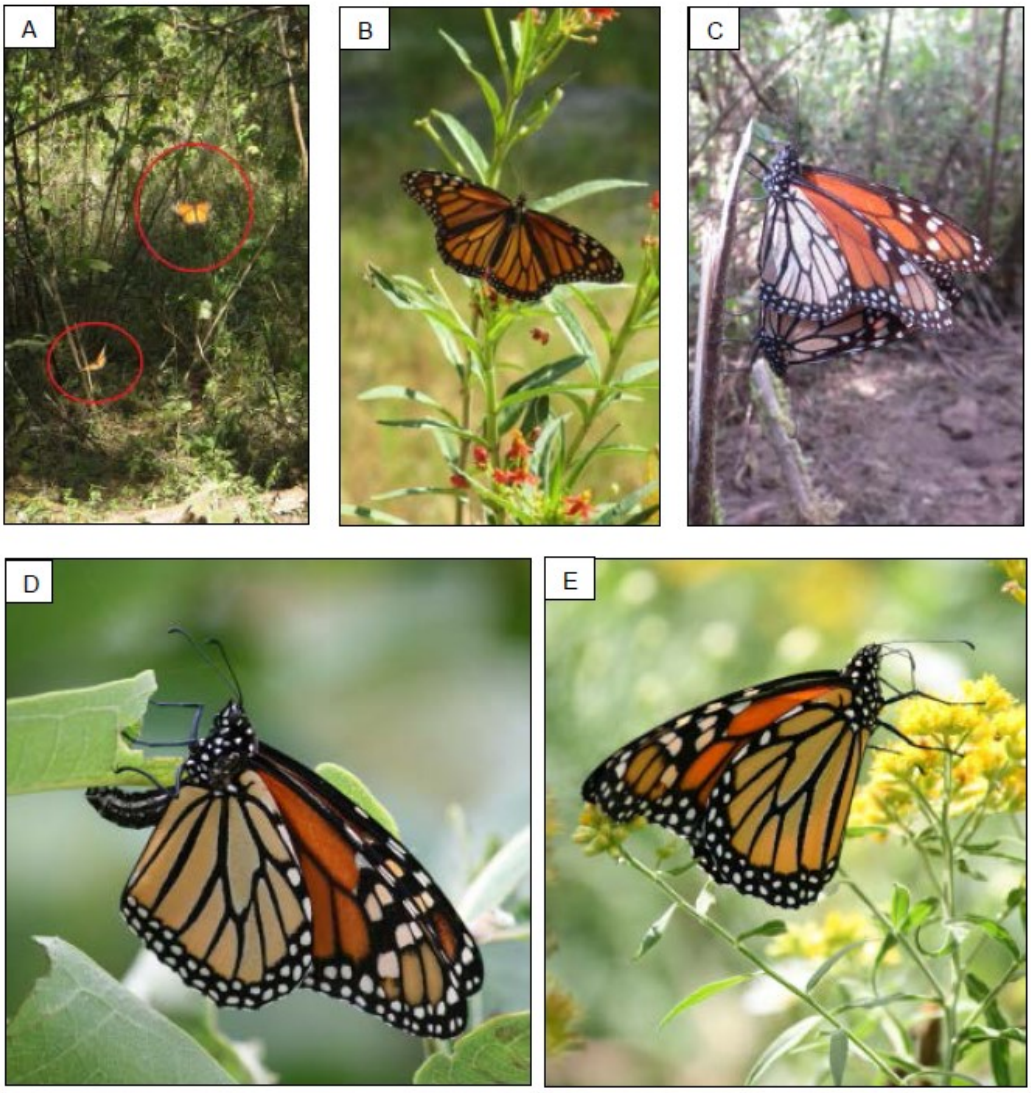


Figure 3. Monarch behavior: (A) Two monarchs flying, (circled in red; photo courtesy of Holly Holt), (B) Resting (photo courtesy of Chuck Patterson), (C) Mating (photo courtesy of Holly Holt), (D) Ovipositing (photo courtesy of Candy Sarikonda), and (E) Nectaring (note that monarch's proboscis is extended into flower; photo courtesy of Candy Sarikonda).

Activity 4: Monarch Survival and Parasitism

Overview

Monarchs have many natural enemies, including predators, parasitoids, and parasites. Some of these are not noticeable until the final stages of larval (caterpillar) development. In this activity, participants collect 4th or 5th instar larvae from within monitoring plots and rear them indoors to track whether they survive to adulthood, and if not, what caused their death. Parasitoids (such as flies or wasps), whose young develop inside the monarch larvae, eventually killing the developing monarch may be detected. Participants also screen newly emerged monarchs for a protozoan parasite called Oe (*Ophryocystis elektroscirrha*). Data collected in this activity help to estimate larval survival and measure the importance or mortality factors in populations of different densities and at different times and locations. Rearing protocols were adapted from the Monarch Larva Monitoring Project and Project Monarch Health and data are compatible for expanding those data sets.

NOTE: Public lands and some states often require a scientific collector's permit to collect live organisms, so this activity cannot be conducted until necessary permits are secured. Check your state regulations and site requirements before conducting this activity.

Supplies (= optional)*

Rearing monarchs:	***Collecting parasitoid flies and/or wasps:***
☐ Containers for rearing larvae	☐ Container labels (Figure 5)
☐ Disinfectant (20% bleach-water solution)	☐ Small containers (e.g. pill bottles)
☐ Markers or tape for labeling containers	☐ Tissues
☐ Paper towels or filter paper	***Testing for Oe:***
☐ Screen-sized mesh, cloth, or other porous material for lids	☐ Oe testing kit
☐ Milkweed to feed larvae	☐ Disposable gloves
☐ Cooler for collecting larvae on hot days*	☐ Disinfectant (20% bleach solution in spray bottle)

Methods for Collecting and Rearing Larvae

Collect 4th and 5th instars from within the monitoring plot. Only collect as many as can be cared for daily.

If it is over 85ºF during monitoring, avoid collecting monarch larvae, or bring an insulated cooler for them. Even at lower temperatures, plastic or glass containers act as small greenhouses, so containers with monarchs should be kept out of the sun, in a cooler, cloth bag, or other opaque carrier. As you collect monarchs, also collect the milkweed leaf on which you find them, and enough additional milkweed to last the rest of the day. If you are interested in collecting additional milkweed for the next few days, take care to collect it from beyond the official monitoring plot and from areas that are not likely to have been sprayed with pesticide.

Rearing tips:

- Keep individual larvae in a jar, quart-sized deli container, or another container. When they are small, they can be kept in smaller containers (Figure 1). Containers should be easy to open, have screen or cloth coverings or holes for air flow, and be large enough for the adult to expand its wings without touching the container when it emerges. Keep only one larva per container to combat disease spread, and to allow accurate tracking.

Figure 1. Example of rearing set-up (Photo courtesy of Ilse Gebhard).

- Clean jars daily. Empty out the caterpillar frass (excrement) and old milkweed. Wash your container before using it for another caterpillar using a 20% bleach-water solution. To do this, soak the container(s) in the bleach solution for at least ten minutes. Afterward, rinse the container(s) thoroughly with water to remove any bleach residue.
- Give larvae fresh milkweed daily. Placing a damp piece of paper towel on the bottom of the rearing container prevents the milkweed from drying out quickly. Pick several days' worth of milkweed, wash it, and keep it in a plastic bag with a damp paper towel in a refrigerator. If possible, collect milkweed leaves from an area outside the monitoring plot to avoid altering the milkweed density of the plot.
- 4^{th} and 5^{th} instars will likely pupate within a week. Before shedding their skin for the last time, they will crawl to the top of their cage and form a pre-pupal "J" (Figure 2). Be careful to not jostle the container while larvae are pupating. If the pupa becomes detached, carefully tie dental floss or thread around the stalk of the pupa (cremaster) and tape it to the top of the cage or tie it around something solid.

Figure 2. Pupation sequence (Photos courtesy of Siah St. Clair).

- The pupal stage lasts 9-14 days. Wing scales gain color and become more visible the day before a butterfly emerges, causing the pupa to darken just before emergence (Figure 3). Butterflies typically emerge in the morning, and their wings are soft and flexible. After about 4 hours of drying, they are ready to fly. If they fall, carefully pick them up by the thorax, and hold their legs next to the top or side of the cage to hang with their wings pointed down. Within a day of an adult butterfly emerging, test it for OE and release it after recording information on the Activity 4 Datasheet.
- A pupa that has been very dark for more than a few days is almost always dead.
- Parasitized larvae die as larvae, pre-pupae, or pupae. They will often not pupate successfully, but will hang limply and die. Tachinid fly maggots may emerge one to several days after the monarch death, come out of the host on a silk-like thread (Figure 4A), and pupate on the bottom of the container. Adult flies emerge 7-10 days later.

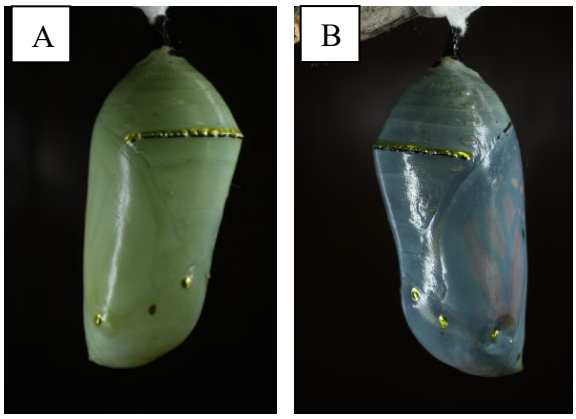


Figure 3. Monarch pupae; (A) early stage chrysalis/pupa and (B) mature monarch chrysalis/pupa about to eclose. Note that wings are visible through the chrysalis wall (Photos courtesy of Siah St. Clair).

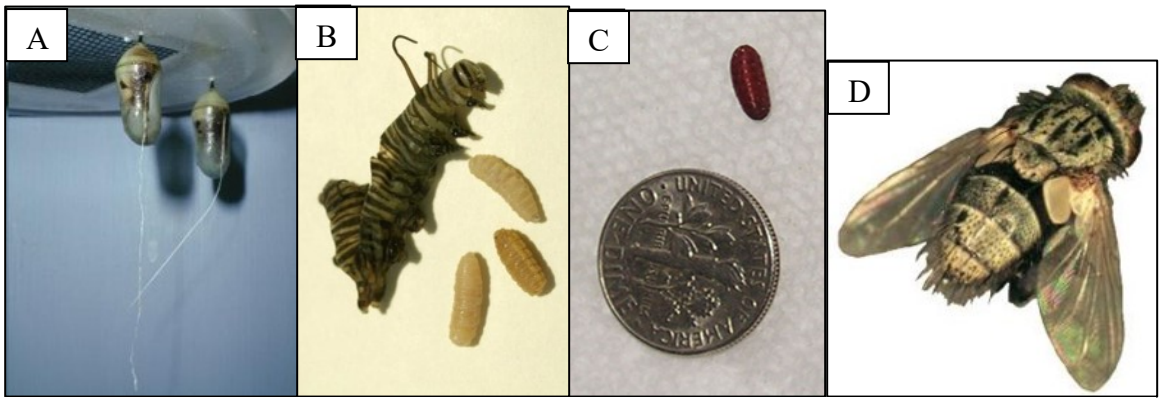


Figure 4. Tachinid fly parasitoids of monarchs. (A) Monarch pupae with silk-like thread from tachinid fly parasitoids (Photo courtesy of Sonia Altizer). (B) Three tachinid fly larvae (maggots) from parasitized caterpillar (Photo courtesy of Jaap de Roode). (C) Soon after emerging, the flies pupate, turning reddish-brown (Photo courtesy of Sonia Altizer). (D) Adult tachinid fly (Photo courtesy of UMN Monarch Lab).

- Keep fly pupae in a ventilated container until the adult flies emerge. Depending on the size of screen used for monarch rearing containers, a screen with smaller openings may be needed to prevent flies from escaping (tachinid flies are about the size of house flies). Once the adult flies emerge, follow the instructions below for how to submit specimens to the Monarch Larva Monitoring Project.
- If larvae die, wait a few days to see if parasitoids emerge before disposing them. Wash hands after handling ill or dead larvae and dispose of them in a different room from

where living larvae are kept). Search the dried milkweed leaves for fly pupae. Pupae are small, reddish-brown, and oval-shaped (see Figure 4).

Instructions for Submitting Parasitoids

Send adult parasitoid flies or wasps that come from collected monarchs to the Monarch Larva Monitoring Project (MLMP). After the ***adult flies*** emerge from their pupae (7-10 days after they emerge from the monarch) or after the wasps emerge from the monarch pupa, put the emerged adult parasitoids ***and*** their pupal cases in small containers (e.g. pill bottles, small boxes, Ziploc storage containers) and place them in the freezer. It is best to pack them with tissue to prevent them bouncing around. Do not use cotton balls, as the cotton filaments become attached to parts of the fly that are used for identification purposes. If fly larvae emerge from the monarch but no adult flies emerge from the pupal casings, send the pupae (with the same information on the label) because they may still be identified. ***Each container should hold all of the parasitoids that emerged from ONE monarch.*** Keep specimens in the freezer until several are accumulated or until the end of the season. Label each container with the information noted on the container label below (Figure 5), or number the containers and write the information on a separate table sent with the containers. Contact info@monarchlab.org to notify when a package of parasitoids should be expected. Send the specimens and labels to:

Monarch Larva Monitoring Project
University of Minnesota
Dept of FWCB
20003 Upper Buford Circle, 135 Skok Hall
St. Paul, MN 55108

Date monarch was collected:

Stage of monarch at collection:

Milkweed species on which monarch was collected:

Location of collection:

# of flies and date emerged from monarch*:

Other notes:

**NOT date adult fly emerged from fly pupal*

Figure 5. Label for container.

Instructions for Testing Monarch Adults for Oe:

Follow these steps:

1. Obtain a Project Monarch Health sampling kit; send the sampling card plus a copy of your datasheet to Monarch Health (see address below).
2. Sanitize work surface and sampling equipment with 20% bleach-water solution.
3. Butterflies should not be handled for the first four or five hours after they emerge, and can be kept in the cage until the next day. To sample adult monarchs for Oe, wear laboratory-style gloves to prevent contamination and change them frequently (after checking every adult monarch). While the parasite is not harmful to humans, it is easily spread from one monarch to another.

4. Remove the butterfly from its rearing container. Hold firmly as shown in the picture below, using a gloved hand (Figure 6A). Be sure not to use your other hand to touch the butterfly because that hand will be used to hold the tape sticker and sample for Oe spores.
5. Pick up a piece of transparent tape or sticker with your other hand. Gently, but firmly place the sticky side of the piece of tape against the abdomen of the monarch. Press down so that it wraps around and sticks to the sides of the abdomen (Figure 6B).
6. Gently peel the tape off and stick it to the index card (Figure 6C). You will remove scales in the process, but it will not harm the monarch. Label the tape sample with a number that corresponds to your datasheet entry (Figure 6D).
7. After you've entered your data, send a copy of your datasheet and the index card to:

Project Monarch Health
c/o Sonia Altizer
Odum School of Ecology
University of Georgia
Athens, GA 30602

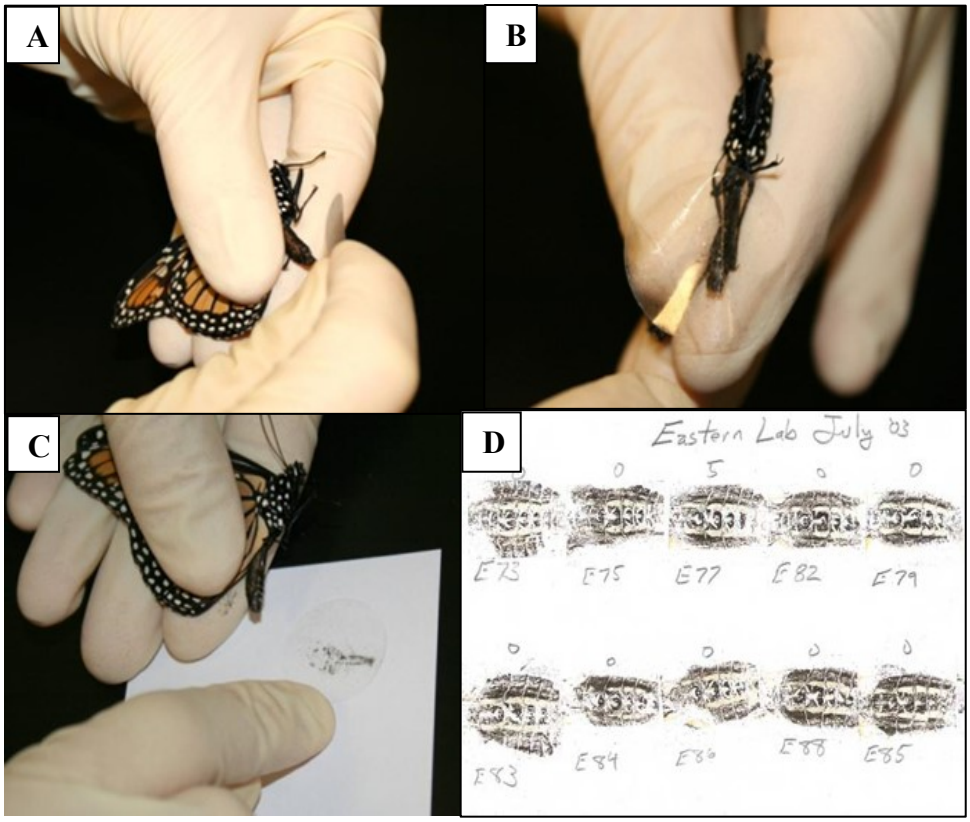


Figure 6. Sampling adult monarch for Oe. (A) Hold the monarch gently, but firmly, with gloved hands (B) Wrap tape around the abdomen (C) Gently peel the tape off and stick it to the Oe index card (D) Example of labeled Oe index card (Photos courtesy of Sonia Altizer).

Appendix A. Program Goals and Objectives

The Integrated Monarch Monitoring Program (IMMP) is a national initiative to monitor monarch butterflies and their habitats throughout their breeding and migratory range. The primary goal of the IMMP is to monitor monarch butterflies and evaluate habitats to inform conservation efforts. The IMMP has the following primary objectives:

- To acquire and share information about how habitat conservation actions affect monarch butterflies and their habitat
- To provide geographically and ecologically representative information to update population and habitat models
- To track long-term changes in the distribution and abundance of monarch butterflies and their habitats

More specifically, the IMMP seeks to quantify the following at the national level:

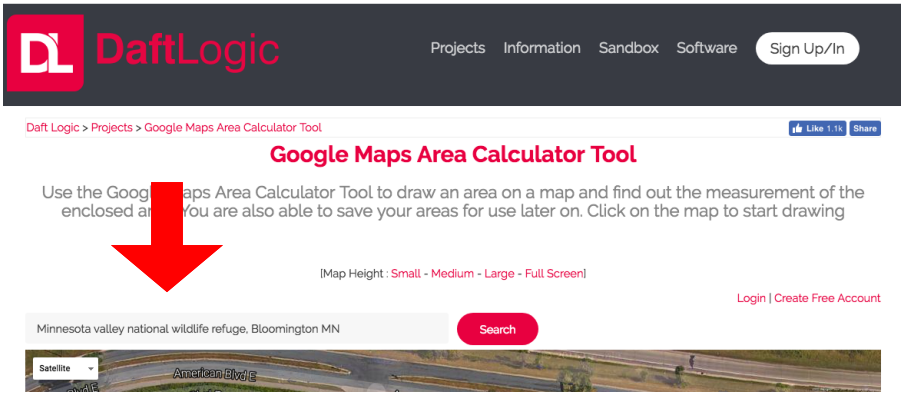
- Milkweed species distribution and density to:
    - Document distribution of potential monarch breeding areas
    - Document milkweed density by land use sector
    - Detect changes in milkweed density by species over time (seasonally and inter-annually)
    - Track regional changes in milkweed phenology (chronology) by species over time
    - Explore the relationship of milkweed species or densities to the presence of monarchs of all life stages
- Relative frequency and chronology of blooming plants to:
    - Describe available nectar sources by land use sector and region over time (seasonally and inter-annually)
    - Describe blooming phenology of nectar sources by 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 larvae on milkweed plants to:
    - Document geographic distribution and seasonal and inter-annual dynamics of monarch eggs and larvae by region
    - Evaluate densities of eggs and larvae by land use sector, milkweed species, and region
    - Improve understanding of how habitat characteristics may affect monarch egg and larval densities 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 resources use (e.g., nectaring, ovipositing)
- Estimated monarch survival to adulthood by rearing caterpillars in the 4^{th} and 5^{th} instars, to contribute to a greater understanding of monarch parasitism and disease.
- Abundance of red imported fire ants within their range to:
    - Document distribution of red imported fire ants
    - Evaluate effect of fire ants on monarch densities and egg and larval survival
    - Obtain information pertinent to fire ant control

Appendix B. Instructions for Delineating Areas of Interest for Self-selected Sites

To map self-selected sites, follow the directions below for using the Google Maps Area Calculator. You will generate a KML file to send to the coordinator. They will use that file to delineate a monitoring plot within your area of interest.

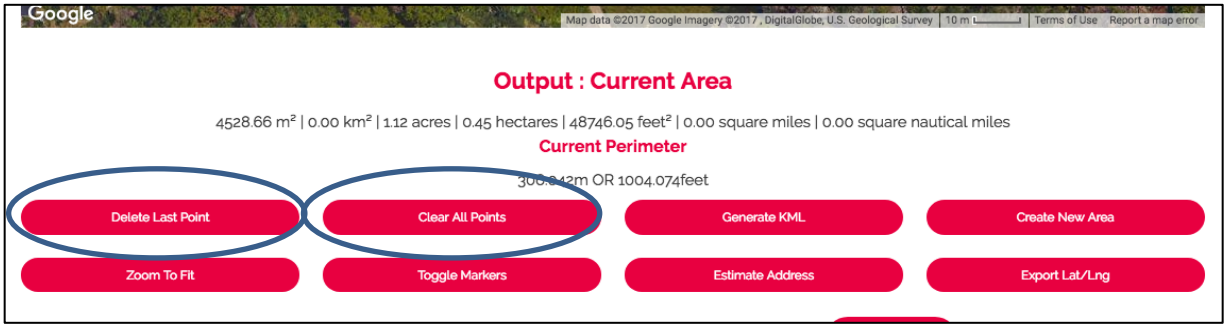
Using the Google Maps Area Calculator:

1. Navigate to the following webpage: https://www.daftlogic.com/projects-google-maps-area-calculator-tool.htm.
2. Using the search bar, enter an address or coordinates for the location of your site.

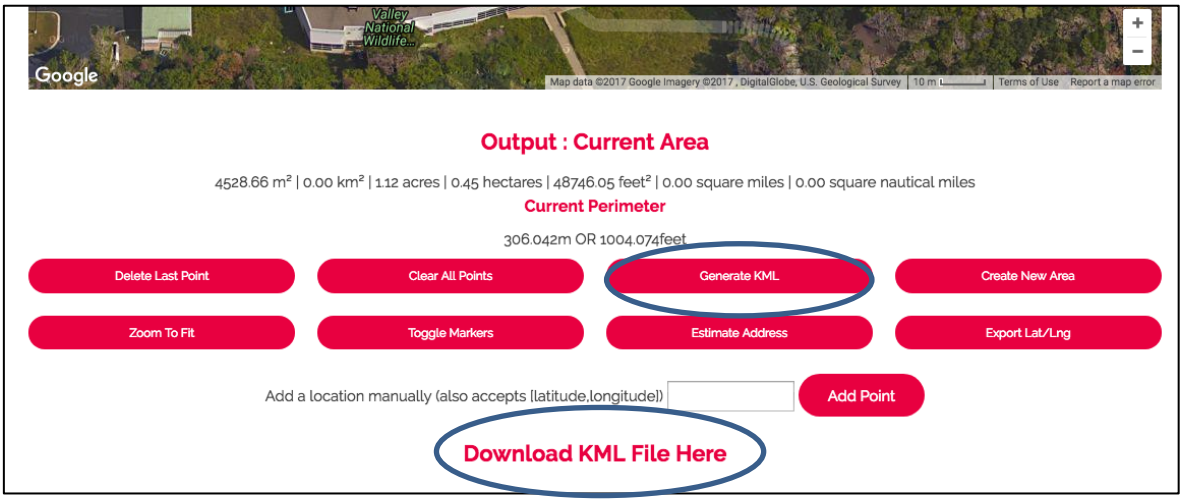


3. Using your mouse, click around the edges of your site to create a polygon or an outline of the site area (see image example).
    a. If there is a pond (or another large obstacle) in the middle of your site, first outline the full site, then follow the remaining steps. Next, go back, outline the obstacle, and send us a both files.

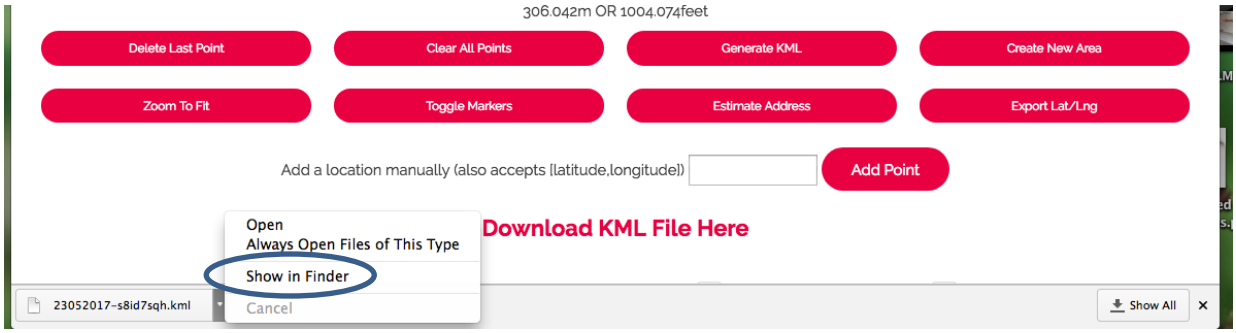
4. If you make a mistake, you can use the buttons “Delete Last Point” or “Clear All Points” beneath the map.



5. After you draw the polygon, click “Generate KML” (pink button under map).
6. Click the “Download KML File Here” link that appears below the pink buttons.



7. You probably won’t be able to open the KML file on your computer. Click “Show in Finder” or “Show in Folder” and rename the file using the naming system: lastname_city_state_yyyy.mm.dd
    a. Here is an example: hancock_philadelphia_PA_1776.07.04



8. Email the saved KML file to your monitoring coordinator. The coordinator will determine the number for your site and delineate a monitoring plot.

Appendix C. Example Landowner or Land Manager Outreach Script

Hello my name is _____ and I am a participant/citizen scientist with the Integrated Monarch Monitoring Program (IMMP). The goal of this project is to monitor monarch butterflies and their habitat across the breeding and migratory range. I'm calling 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 monarch adults, eggs, and larvae. No specimens will be removed [or I propose to collect a sample of fourth and fifth instar caterpillars to determine parasitism and disease] and nothing will be left behind [or I propose only to place four small pin flags that will be removed on my final visit]. The data will be stored in a database hosted by the USFWS. 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. Access, Permission, and Landowner Release of Liability

2018 Waiver and Release of Liability

In consideration for allowing _____("Permittee"), and his/her associates and agents, to enter_____("Landowner") property located at _____, (the "Property") for the purposes of [weekly/ bi-weekly / monthly (circle one)] monitoring of milkweed, nectar plants, and/or monarchs through the month of _____. Permittee hereby waives all claims against Landowner, its officials, employees, and agents for injuries or damages incidental to, connected with, or arising out of Permittee's access to the Property. Each party shall be responsible for its own acts and behavior and the results thereof.

Permittee understands that entry and access to the Property may be hazardous and that injuries or damages may occur in the normal course of accessing the Property. Permittee assumes all risks and hazards incidental thereto by Permittee's employees and agents.

Landowner agrees to allow monitoring data collected from the Property to be released for public use. No personal contact information will be associated with the monitoring data.
Data will only be associated with a [GPS coordinate / 10x10-kilometer cell / county (circle one)] where the land is located.

Dated:_____

PERMITTEE

Signature:

_____

Name:

_____

Organization:

_____

LANDOWNER

Signature:

_____

Name:

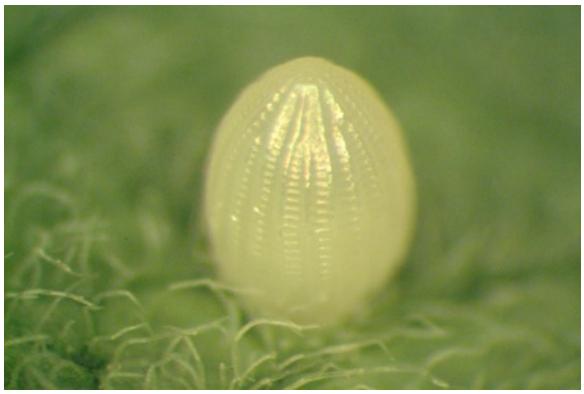
_____

Appendix E. Identification of Monarch Eggs, Larvae, and Adults

Eggs and Larvae (caterpillars)

Early life phases of the monarch butterfly occur as eggs and larvae (caterpillars) as up to 5 instar stages of development. Eggs and caterpillars are found on milkweed plants (*Asclepias* spp.).

Monarch egg on milkweed leaf — The egg is a little more than 1 millimeter tall *(Photo courtesy of Lynda Andrews).*



Close-up of monarch egg — Note the pointed shape, the glossy off-white/yellow color, and the vertical striping *(Photo courtesy of Michelle Solensky).*

Monarch egg (left) and latex drop (right) *(Photo courtesy of Anurag Agrawal).*

Live monarch egg about to hatch *(Photo courtesy of Valerie Evanson).*

Dead monarch egg. Note the "puddle" of dead larva in the bottom of the egg *(Photo courtesy of Valerie Evanson).*

Monarch first instar consuming eggshell. Note the dull greenish-grey color and the size (not much bigger than the egg). First instar larvae have a dark, black head capsule and do not develop colored striping until they begin eating milkweed. They have tiny front tentacles but lack a set in the back *(Photo courtesy of Mary Holland).*

First instar feeding damage. This circular feeding pattern is an indication that a monarch first instar was on the plant at some point *(Photo courtesy of Tom Collins).*

Monarch second instar. Second instar larvae have a distinct pattern of black, white, and yellow bands, and the body no longer appears transparent and shiny. They now have two sets of tentacles (front and back), and the front tentacles only extend about halfway to the tip of the head (they are not yet long enough to reach the tip) *(Photo courtesy of Monarch Lab).*

Monarch third instar. This third instar monarch has just molted. As monarch larvae develop, they increase in size and their stripes become more distinct. The tentacles also increase in length, now extending to the tip of the head (if bent forward). Third instar larvae usually feed using a unique cutting motion on leaf edges *(Photo courtesy of Monarch Lab).*

Monarch fourth instar. Fourth instar monarchs front tentacles extend beyond the tip of the head. Internal changes, including the development of reproductive structures, begin to occur in late instar monarchs *(Photo courtesy of Monarch Lab).*

Monarch fifth instar. Older monarch larvae have bright yellow, black and white striping. Both sets of tentacles are longer than a fourth instar monarch, and the front set often droop at the tips *(Photo courtesy of Richard Hicks).*

Monarch instars*.* The entire larval stage in monarchs lasts from 9-14 days under normal summer temperatures. The speed of monarch development is temperature dependent; higher temperatures lead to faster development *(Photo courtesy of Monarch Lab).*

Adult Monarchs

Male and female monarchs can be distinguished easily. Males have a black spot (indicated by the red arrow) on a vein on each hind wing that is not present on the female. The ends of the abdomens are

also shaped differently in males and females, and females sometimes look duller than males and have slightly wider veins on their wings.

Male Monarch Butterfly
(Photo courtesy of Michelle Solensky)

Female Monarch Butterfly
(Photo courtesy of Barbara Powers)

Monarch Butterfly Mimics

Watch out for mimics! There are a few butterfly species that look very similar to monarchs in the larval, pupal, and/or adult stages.

Black swallowtail, *Papilio polyxenes*

Black swallowtail larvae have yellow spots and lack the white stripes of monarch larvae (monarch larvae do not have spots). They eat plants in the carrot family (parsley, dill, carrots, etc.) (*Photo courtesy of Carl Stenoien*).

Viceroy, *Limenitis archippus*

Viceroy adults (left) look very similar to monarch adults (right) (the larvae look very different). They have the same color and similar wing patterns. To differentiate between them, look at the lower wing. Viceroys have a horizontal line across the bottom of their hindwings (as shown above). Viceroy flight patterns also tend to be faster and more erratic than monarchs *(Photos courtesy of Diane Rock, Candy Sarikonda, and Wendy Caldwell).*

For more practice identifying monarchs and their mimics, take this quiz by the National Wildlife Federation (http://blog.nwf.org/2015/03/quiz-will-these-monarch-look-alikes-fool-you/) or download the Monarch SOS app for apple devices.

Queen, *Danaus gilippus*

Both queen and monarch caterpillars feed and oviposit on milkweed. You can tell adults apart by looking at the forewings. Queen adults (left) lack black veins on their upper forewing and instead have white spots scattered in that area. Monarch adults (right) have black veins on the upper forewing and only have white dots within the back borders.

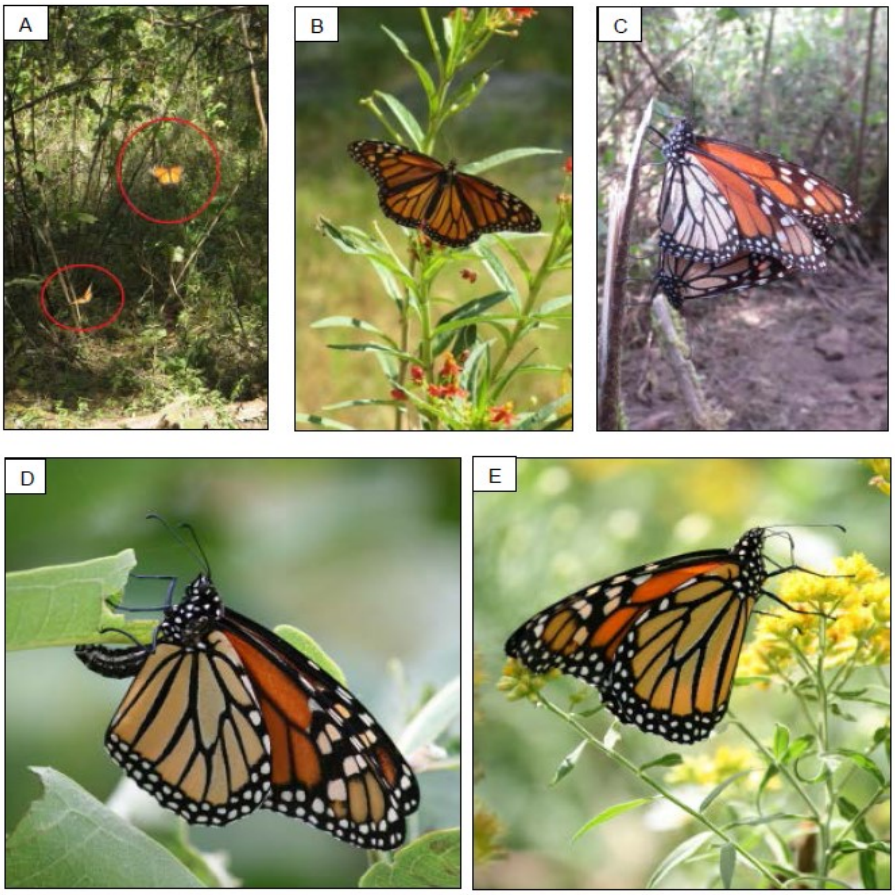
(Photos courtesy of Kristen Baum and Wendy Caldwell).

Queen larvae have three sets of tentacles (left), whereas monarchs only have two (front and back, no middle set) (right) *(Photos courtesy of Wendy Caldwell and Hayley Schroeder).*

Adult Monarch Behaviors

Monarch behaviors as noted in *Adult Monarch Surveys* or *Miscellaneous Monarch Observations*.

Flying	butterflies in flight within the monitoring plot and within 5 m of the ground
Flying High	Butterflies flying more than 5 m high over the plot, may or may not actually be using the habitat within the monitoring plot
Resting	Alighted on a plant with no sign of mating, ovipositing, or nectaring. Also includes roosting: resting during migration, often by groups and in woody vegetation.
Ovipositing	A female adult monarch arching the abdomen and depositing eggs (on milkweed plants)
Mating	Male and female adults are observed copulating
Nectaring	Adults alighted on a plant, actively extending proboscis into a flower to obtain nectar



Adult Monarch Behaviors: (A) Two monarchs flying, (circled; photo by Holly Holt), (B) Resting (photo by Chuck Patterson), (C) Mating (photo by Holly Holt), (D) Ovipositing (photo by Candy Sarikonda), and (E) Nectaring (note that monarch's proboscis is extended into flower; photo by Candy Sarikonda).

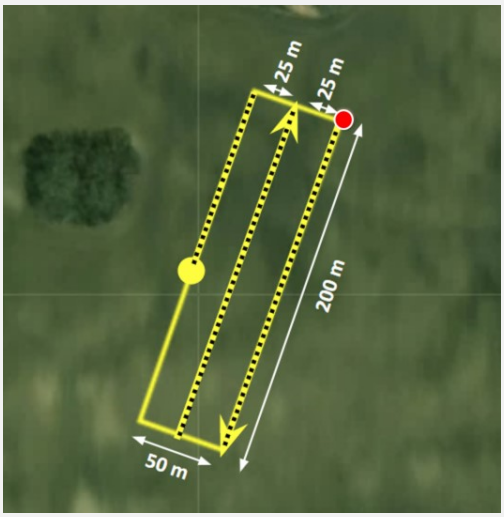
Appendix F. Transect Layout for Activity 1

To collect data in Activity 1, participants will establish multiple transects (and place subplots along each) within a monitoring plot. Transect layout methods vary depending on the monitoring site-type. Once the site-type is determined by the monitoring coordinator (or by using the site mapping tool), follow the instructions below.

Standard Rectangle (200 m x 50 m):

Three transects are placed across the monitoring plot, one on each edge and one through the center. The first two transects measure 200 m, accommodating 40 subplots each, and the last 100m holds the remaining 20 subplots.

1. Place the first transect at the starting location and walk the measuring tape in the appropriate direction, for a total of 200 m. Note that most measuring tapes are not longer than 100m, so you will need to run the tape out at least a second time.
2. Move 25 meters over in a perpendicular direction (90 degrees from the first direction) to locate the starting point for the second transect. Extend the second transect so that it runs parallel to the first (your directional bearing will be 180 degrees different from the first transect), for 200 m.
3. Move another 25 m over and extend the transect for 100 m and complete the remaining 20 subplots.



The starting point is indicated by the red dot; transects as dashed yellow lines; subplots in black.

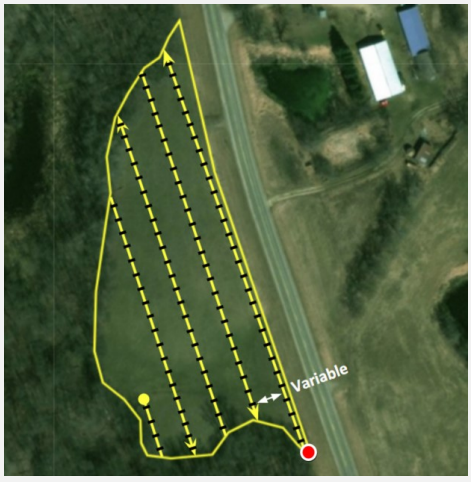
Standard Square (100 m x 100 m):

The starting point is indicated by the red dot; transects as dashed yellow lines; subplots in black.

Five 100 m transects are placed across the monitoring plot, with 25 m separating each. On each transect, monitor 20 subplots.

1. Place the first transect at the starting location and stretch the measuring tape in the appropriate direction for 100 m.
2. Move 25 m over in a perpendicular direction (90 degrees from the first direction) to locate the starting point for the second transect.
3. Extend the second transect so that it runs parallel to the first (your directional bearing will be 180 degrees different from the first transect), for another 100 m.
4. Place the third, fourth, and fifth transects in the same fashion, for five parallel, evenly spaced transects.

Irregular Plots (0.4 – 1 ha) (1-2.4 ac)



The starting point is indicated by the red dot; transects as dashed yellow lines; subplots in black.

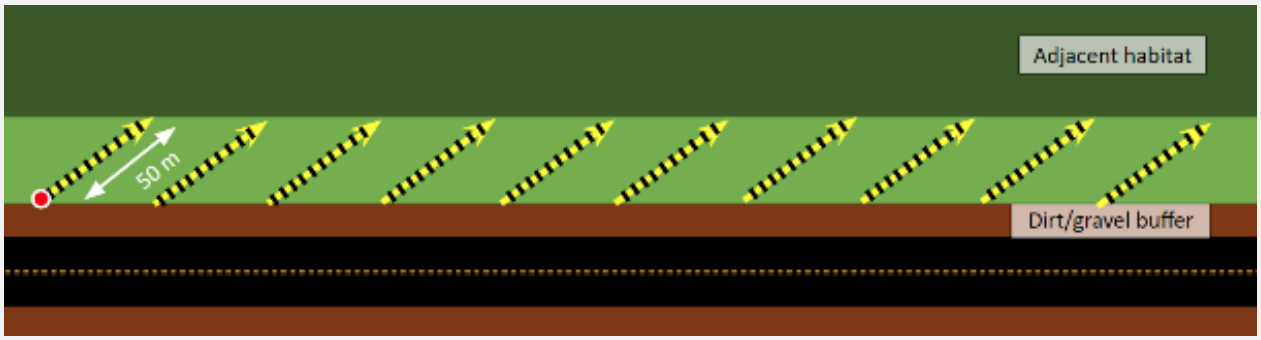
For sites smaller than 1 ha (2.4 ac) or irregularly shaped, a custom plot will be made (see Figure). The number of transects will vary based on the size and shape of the plot.

1. Place the first transect at the starting point and stretch the measuring tape in the direction that provides the longest axis, extending it until you reach the site boundary.
2. When you reach the opposite boundary, move over the number of meters specified (transect spacing varies by the size of your plot). Walk out your second transect so that it is parallel to the first.
3. If you cannot fit 100 subplots with transects spaced at the minimum 7 m, use the comprehensive (census) survey method instead (as described in Activity 1).

Rights-of-Way

Ten 50 m long transects are placed diagonally across a rights-of-way (ROW) plot, with 10 subplots along each (see Figure). Typically, transects are placed end-to-end. (If the ROW is 30 – 40 m wide, then add 10 m after the end point of each transect, so that the plot extends further along the ROW. For ROWs 40 – 50 m wide, add 20 m at the end point of each transect.)

1. From the sample point, move to the closest ROW edge to begin the first transect. The transect then angles diagonally away from the ROW edge. For a roadside, start at the road edge (where vegetation begins) and angle the transect against the flow of traffic.
2. Walk out the transect tape 50 m and pull it tight so that it runs diagonal from one ROW edge to the other edge, or edge of the adjacent site type. The angle of the diagonal varies with the ROW width.
3. After recording the 10 subplots along the transect, either walk straight across back to the opposite edge and place the next transect – OR – move up the ROW 20 m and then place the next transect.
4. Proceed until you have finished 10 transects.



Transect layout in a roadside right-of-way. The starting point is indicated by the red dot, transects are shown as yellow lines, and subplots are indicated by black hash marks.

Agriculture: Crop Fields

The starting point is indicated by the red dot and transects are shown as dashed yellow lines.

1. Begin in the field corner (or field edge if the field is not rectangular) closest to the sample point.
2. In the nearest crop row, walk out the measuring tape (in the direction of the longest crop rows) for 200 m, sampling 40 subplots. (You may need to run the tape out at least a second time.)
3. Move 25 m over from the first transect and in the nearest crop row, walk along the row for another 200 m and another 40 subplots (in the direction opposite of the first transect, back toward where you started.)
4. Move another 25 m over and run the last transect along the nearest row for 100 m and 20 subplots.

NOTE: If the 200 m by 50 m rectangle does not fit in the field, substitute the 100 m by 100 m square (see 'Standard Square' above). If the field is devoid of vegetation, you may complete a comprehensive survey instead (see Activity 1).

Agriculture: Edge Habitat

If the habitat adjacent to or within an agricultural field (i.e. fencerow, in-field grassland strip) is at least 4 m wide and there is at least 500 m of collective habitat (may round a curve or corner), place ten diagonal transects with ten subplots each (similar to rights-of-way). Typically, transects are placed end-to-end. If the habitat is 30 – 40 m wide, then add 10 m between transect start points so that they extend further along the plot. For ROWs 40 – 50 m wide, add 20 m between each transect start point. *Habitat patches narrower than 4 m will be sampled with a comprehensive survey (see Activity 1).*

1. Locate the corner (or edge) of the field closest to the sample point.
2. Walk out the transect tape 50 m and pull it tight so that it runs diagonal from the crop field edge to the outer boundary of the habitat edge. The angle of the diagonal will vary with the width of the habitat.
3. Sample ten subplots along each transect.
4. Walk perpendicularly back to the crop edge and repeat step 2. (If needed, move 10 or 20 m along edge.)
5. Proceed until you have monitored 10 transects.

The starting point is indicated by the red dot; transects are shown as yellow lines, subplots are indicated by black hash marks.

Alternative (Census) Sampling for Small (< 1 ac), Developed (non-open space), and Narrow Agricultural Edge Sites

In developed sites (excluding open space), very small sites (generally less than 0.4 ha or 1 ac), or agricultural edge sites less than 4 m wide, transects and subplots will **not** be used. Instead, participants will comprehensively survey the entire area. Walk a systematic pattern through the plot (weaving back and forth) and record blooming plant species and the number and species of milkweeds. Cover the entire area of the plot thoroughly, and avoid walking through the same area twice. In developed sites (excluding open space), record the species of blooming plants that you are able to identify. For those you cannot identify, tally the number of different species you observe. Taking photos of each new species you encounter may be helpful in remembering which you have already tallied.

Appendix G. Monitoring Safety Information

Monarch monitoring can be like a walk in the park, but even a walk in the park can have inherent hazards. Surveys are conducted throughout the season, in many different plant communities, covering various terrains. Surveyors will encounter heat and humidity, tall and dense vegetation, deep holes and ruts, steep slopes, wild parsnip and poison ivy, mosquitos, bees and ticks, black berries, prickly ash, and honey locust among other things; sometimes all in the same day. Our highest priority is the safety of staff and volunteers. 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. The place to start in preventing injury or illness from field activities is to wear proper clothing – light-colored, lightweight, long sleeved shirt, long pants, brimmed hat, and sturdy shoes (preferably boots). Always let someone know where you will be and when you intend to return. Park in a safe location. Listed below are some specific hazards you may encounter in the field and measures you can take to help prevent injury or illness in those instances.

Potential Hazard	Preventive Measures
Over exposure to sun	• Use sunscreen SPF30 or higher (follow label instructions) • Wear brimmed hat, long sleeves and pants • Limit mid-day exposure
Dehydration – 3 heat-related disorders • Heat cramps • Heat exhaustion • Heat stroke (Life-threatening)	• Wear light-colored, loose-fitting, breathable clothing • Drink water frequently • Rest Frequently • Seek shade • Learn to recognize the symptoms of each disorder and the first aid treatment necessary
Poisonous vegetation • Poison ivy, oak, sumac Plant oils = allergic reaction • Wild Parsnip Plant sap + sunlight = blisters	• Learn to identify • Avoid contact • Wear long-sleeved shirt and long pants • Apply barrier cream to exposed skin • Wash/shower after exposure - use soap and water, or specialized wash or scrub to remove plant oils • Wild parsnip - wash and cover exposed area
Insect stings/bites • Mosquitos	• Periodic application of repellent (follow label instructions) • Wear long pants (tucked into socks or boots), long-sleeved shirt (tucked into pants), hat

• Ticks • Bees/wasps • Fire ants	• Light-colored clothing • Examine body for ticks after monitoring, then shower • Watch for bee/wasp nests in trees and on ground • Carry allergy meds as needed and advise coworkers of response requirements • Avoid fire ants (southeastern US)

Poisonous snakes	• Wear heavy boots or snake chaps • Use stick to disturb the brush in front of you • Watch where you place your hands and feet • Back away from the snake and allow it to proceed
Rough/uneven terrain • Burrowing animals • Steep ditches • Erosion • Flood zones	• 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 • Contact DOT office for state-specific requirements	• ALWAYS look both ways • Wear reflective vest or conspicuous clothing • Park in designated area if available, or as far off the road as can be done safely • Park a safe distance from hills or curves • Set up reflective triangle(s) and Monarch Monitoring sign (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.

It is not possible to list every potential hazard you may encounter, or all of the in-depth details about those listed above. Please use caution while doing field work. Knowledge of first aid is advised. You can find more information about these and other safety topics through the following resources:

https://www.conezonebc.com/wp-content/uploads/2015/04/Workers_Roadside_Worker_Safety.pdf

www.grounds-mag.com/mag/grounds_maintenance_roadside_safety/

https://www.mtu.edu/ehs/docs/field-safety-guide-school-of-forestry.pdf

https://www.dot.state.mn.us/adopt/documents/wild-parsnips.pdf

www.osha.gov

www.americanredcross.org – How to perform First Aid – FREE mobile apps available for download

(Information contributed by Tenlea Turner, USFWS.)

Glossary

Flying: behavior when butterflies are in flight during adult monarch counts or miscellaneous observations within the monitoring plot and within 5 m of the ground.

Flying high: behavior recorded when observed monarch is flying more than 5 m high over the plot and it is unknown whether the monarch has used or will use plot resources.

Mating: behavior during adult monarch counts or miscellaneous observations within the monitoring plot when male and female adults are observed copulating.

Milkweed plant: all above-ground stems or stalks of milkweed originating from a visually-identifiable, common central point in the ground. A single milkweed plant (as defined in this program) may be composed of multiple stems or single stalks depending on the species.

Milkweed stem: stalk growing from a point in the ground, joined to a centralized part of a plant or location above ground. In multi-stemmed species, stems from the same plant can be separated by a bit of soil (e.g. *Asclepias tuberosa*), but still emanate from the same central location. In single-stemmed species, milkweed plants grow as single stems (or ramets) that are separated by soil (e.g. *Asclepias syriaca*). Even though these plants may be connected underground by rhizomes, for these species it is impossible to tell which ramets are connected without digging them up so each stem is considered an individual plant for this program.

Monarch adult counting frame: a 3-dimensional space used to count adult monarch butterflies projected 5 m on either side of and in front of an observer walking a survey route as well as 5 m above the observer.

Monarch block: 10 km by 10 km cell within a national grid that is numbered; forms the first stage of the spatially balanced random sampling framework for the project.

Monitoring plot (plot): the area delineated for monitoring, anchored by the sample point; default design is 200 m by 50 m, alternative plot layouts depend on sample strata and site size or shape.

Nectaring: a behavior when adult monarch butterflies are perched on a plant and actively extending their proboscis into a flower to obtain nectar.

Online sampling map: the interactive map that is used to select sample points and establish monitoring plots.

Ovipositing: a noted behavior of a female adult monarch arching the abdomen and depositing eggs (most typically on milkweed plants).

Plant sampling frame: A (0.5 m x 1 m) frame constructed of PVC pipe that is divided through the center and placed on both the right and left side of a transect line to form a 1 m^2 subplot (for a total area that measures 0.5 m x 2 m). The frame is used in vegetation surveys to measure nectar plant frequency and milkweed density.

Adult Monarch Survey: a modified Pollard walk, a technique of slowly walking a transect (1 m per 2 sec), counting adult monarch butterflies according to perpendicular distances from the transect line and recording their behaviors.

Resting: a noted behavior of an adult monarch when perched on a plant or other surface with no sign of mating, ovipositing, or nectaring. Term also includes roosting, a behavior commonly used to describe resting during migration, often by groups and in woody vegetation.

Roadside width: a distance measured from a roadway edge where vegetation begins perpendicular to the road until an obvious change in vegetation or ownership occurs.

Sample stratum(a): the land cover types that form the subdivisions for the allocation of points in the second stage of the sampling framework.

Sample point: a point location from which a monitoring plot is established

Subplot: The area defined by the plant sampling frame, a 1 m^2 (0.5 m x 2 m) rectangle, delineated on two sides of a transect using a subdivided measuring frame and used to measure frequency of blooming plants and milkweed density.

Survey: inventory or monitoring activity usually to record the distribution or abundance of a natural resource or organisms.

Transect: a line within a monitoring plot, often with intervals for placing subplots to monitor milkweed and blooming plants. Transects are typically laid out with a 100-meter measuring tape, but in some instances could be placed between field markers and paced.

Datasheet entered into ☐ **Survey123** or ☐ **Excel** on (Date)_____ by (Full Name)_____ sd.v1

Sampling Site Description Form

Site Identification Number

Original Site Type	Verified Site Type	Monarch Block #	Sampling Point #
☐ PGS ☐ UGS ☐ AGC	☐ PGS ☐ UGS ☐ AGC		
☐ ACL ☐ ROW ☐ DEV	☐ ACL ☐ ROW ☐ DEV		

Visit Date:	Start Time:	End Time:	Observer Name(s)

Site Disturbances: From the list below, enter up to five *recent (within last year)* disturbances observed on ≥10% of the plot that may affect the structure and composition of the vegetation.

Disturbance Type: (Code)	% Disturbed nearest 10%	If,"Other" list the disturbance
1)		
2)		
3)		
4)		
5)		

Disturbance Type Codes:

1. No Disturbance	**6a.** Grazed-cattle
2. Mowed	**6b.** Grazed-sheep
3a. Hayed-residual remains	**6c.** Grazed-horses
3b. Hayed-hay removed	**6d.** Grazed-other
4a. Chem-fertilizer	**6e.** Grazed-unknown
4b. Chem-herbicide	**7a.** Burned-wildfire
4c. Chem-insecticide	**7b.** Burned-prescribed
4d. Chem-other	**7c.** Burned-unknown
4e. Chem-unknown	**8.** Plowed or disked
5a. Construction-structure	**9.** Flooded
5b. Construction-road	**10.** Tree harvest/woody species removal
5c. Construction-trail	
5d. Construction-other	**11.** Other

Site Description Notes (e.g., additional information about site conditions or access):

Vegetation in the Plot

Herbacious Plants	**% Cover of Grasses**	**3 Most Common Forbs**
	☐ 0% ☐ 1-10% ☐ 11-25% ☐ 26-50% ☐ > 50%	1)
	% Cover of Forbs (non-grass, herbacious flowering plant)	2)
	☐ 0% ☐ 1-10% ☐ 11-25% ☐ 26-50% ☐ > 50%	3)
Woody Plants	**% Cover of Shrubs**	**3 Most Common Woody Plants**
	☐ 0% ☐ 1-10% ☐ 11-25% ☐ 26-50% ☐ > 50%	1)
	% Cover of Trees	2)
	☐ 0% ☐ 1-10% ☐ 11-25% ☐ 26-50% ☐ > 50%	3)

Noxious Weeds	Name of noxious weeds present in plot. *Refer to your state NOX list.*	Percent Cover
	1)	☐ 0% ☐ 1-10% ☐ 11-25% ☐ 26-50% ☐ > 50%
	2)	☐ 0% ☐ 1-10% ☐ 11-25% ☐ 26-50% ☐ > 50%
	3)	☐ 0% ☐ 1-10% ☐ 11-25% ☐ 26-50% ☐ > 50%
	4)	☐ 0% ☐ 1-10% ☐ 11-25% ☐ 26-50% ☐ > 50%
	5)	☐ 0% ☐ 1-10% ☐ 11-25% ☐ 26-50% ☐ > 50%

Wetland Features: Check all features found within the plot.	☐ None	☐ Marsh/wet meadow	☐ Other _____
	☐ Riparian	☐ Lake/pond edge	_____
	☐ Ditch	☐ Stormwater basin	
Percent Cover:	☐ 0% ☐ 1-10 %	☐ 11-25 %	☐ 26-50 % > 50%

Plot Vegetation Notes:

Dominant Adjacent Land Use(s) within 100m of Monitoring Plot: Rank top 2

____ Protected Grassland ____ Unclassified Grassland ____ Grassland (type unknown) ____ Ag Crop Field
____ Ag Conservation Land ____ Forest/Woodland _____ Wetland/Water ____ Roadside or other ROW
____ Developed Area _____Other_____

Information Specific to these Site Types

Roadside

Count all lanes of the road:

☐ Large highway (>4 lanes)
☐ Medium highway (4 lanes)
☐ Small paved (2 lanes)
☐ Small unpaved (2 lanes, gravel/dirt)

Agricultural

Agriculture Plot Type:

☐ At the Edge of Field
☐ In Field-Crop ☐ Orchard/Trees Present
Type of Crop: _____
☐ In Field-Tilled, Not Planted
☐ In Field-Not Tilled (w/o rows or furrows)

Width of Right-of-Way (m): Measure at start, stop, and each time road width changes. Then average.

1)	6)
2)	7)
3)	8)
4)	9)
5)	10)

Average Roadside (ROW) Width=

Width of Ag Edge (if surveyed) (m): Measure at start, stop, and each time width changes. Then average.

1)	6)
2)	7)
3)	8)
4)	9)
5)	10)

Average Ag Edge Width=

Developed Areas

Dominant Development Type

☐ Residental Single-family ☐ Residental Multi-family

☐ Recreational Park ☐ Natural Area ☐ Industrial ☐ Vacant Lot

☐ Community (e.g., hospital, school) ☐ Other _____

Estimate the % of the plot that you did not survey because you could not see/access it: _____%

Miscellaneous Monarch Observations (Tally for each observed and list nectar plant species)

# Adults					# Immatures						
Ovipos-iting	Resting/ roosting	Flying	Flying high	Mating	Eggs	1sts	2nds	3rds	4ths	5ths	Pupae

***List Nectar Plant(s):**

Southern U.S. regions only:

Did you see evidence of fire ants at this site (ants/mounds)? **YES/NO.**

How many mounds did you see with in the plot? _____

ACTIVITY 1: MILKWEED & BLOOMING PLANTS (Level A)		**Start Time:**	**Observer(s):**	**page 1 of 5**
Site ID:	**Date:**	**End Time:**		
Notes:				

BLOOMING PLANTS

	Subplot #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
Blooming species																										

MILKWEED

Subplot #	**Milkweed Species**	**# Plants**	**# Stems**	**Subplot #**	**Milkweed Species**	**# Plants**	**# Stems**	**Subplot #**	**Milkweed Species**	**# Plants**	**# Stems**

Miscellaneous Monarch Observations (Tally for each observed and list nectar plant species)

# Adults								**# Eggs and Caterpillars**					
Ovipositing	Resting	Flying	Flying high	Mating	*Nectaring	*List Nectar Species	Eggs	1sts	2nds	3rds	4ths	5ths	Pupae

ACTIVITY 1: MILKWEED AND BLOOMING PLANTS

Notes:

BLOOMING PLANTS

	Subplot #	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Blooming species																										

MILKWEED

Subplot #	Milkweed Species	# Plants	# Stems	Subplot #	Milkweed Species	# Plants	# Stems	Subplot #	Milkweed Species	# Plants	# Stems

Miscellaneous Monarch Observations (Tally for each observed and list nectar plant species)

# Adults								# Eggs and Caterpillars					
Ovipositing	Resting	Flying	Flying high	Mating	*Nectaring	*List Nectar Species	Eggs	1sts	2nds	3rds	4ths	5ths	Pupae

ACTIVITY 1: MILKWEED AND BLOOMING PLANTS

Notes:

BLOOMING PLANTS

	Subplot #	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
Blooming species																										

MILKWEED

Subplot #	Milkweed Species	# Plants	# Stems	Subplot #	Milkweed Species	# Plants	# Stems	Subplot #	Milkweed Species	# Plants	# Stems

Miscellaneous Monarch Observations (Tally for each observed and list nectar plant species)

# Adults								# Eggs and Caterpillars					
Ovipositing	Resting	Flying	Flying high	Mating	*Nectaring	*List Nectar Species	Eggs	1sts	2nds	3rds	4ths	5ths	Pupae

ACTIVITY 1: MILKWEED AND BLOOMING PLANTS

Notes:

BLOOMING PLANTS

	Subplot #	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Blooming species																										

MILKWEED

Subplot #	Milkweed Species	# Plants	# Stems	Subplot #	Milkweed Species	# Plants	# Stems	Subplot #	Milkweed Species	# Plants	# Stems

Miscellaneous Monarch Observations (Tally for each observed and list nectar plant species)

# Adults								# Eggs and Caterpillars					
Ovipositing	Resting	Flying	Flying high	Mating	*Nectaring	*List Nectar Species	Eggs	1sts	2nds	3rds	4ths	5ths	Pupae

ACTIVITY 1: MILKWEED AND BLOOMING PLANTS

Meandering Walk: After you've finished monitoring 100 subplots, walk through the plot and note any blooming plant species or milkweed species that were missed by your transects.

Blooming Plant Species		Milkweed Species

Was there a disturbance at your monitoring plot since your last visit? ☐ **Yes** ☐ **No** ☐ **Unknown**

If yes, please describe below.

Notes:

ACTIVITY 1: MILKWEED & BLOOMING PLANTS (Level B)		**Start Time:**	**Observer(s):**
Site ID:	**Date:**	**End Time:**	
Notes:			

Subplot #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
(Section A, B, C)																									
Subplot #	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**
(Section A, B, C)																									
Subplot #	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**	**71**	**72**	**73**	**74**	**75**
(Section A, B, C)																									
Subplot #	**76**	**77**	**78**	**79**	**80**	**81**	**82**	**83**	**84**	**85**	**86**	**87**	**88**	**89**	**90**	**91**	**92**	**93**	**94**	**95**	**96**	**97**	**98**	**99**	**100**
(Section A, B, C)																									

Subplot #	**Milkweed Species**	**# Plants**	**# Stems**	**Subplot #**	**Milkweed Species**	**# Plants**	**# Stems**	**Subplot #**	**Milkweed Species**	**# Plants**	**# Stems**

Miscellaneous Monarch Observations (Tally for each observed and list nectar plant species)

# Adults								**# Eggs and Caterpillars**					
Ovipositing	Resting	Flying	Flying high	Mating	*Nectaring	*List Nectar Species	Eggs	1sts	2nds	3rds	4ths	5ths	Pupae

Activity 1: Developed/Small (<1ac)/Narrow Ag Edge Sites	Date:	Start time:	End time:	Page_1_of ____
	Observer(s):		Site ID:	

Notes:

Blooming Plants: Record each species of blooming plants present within the monitoring plot.		

Milkweed: Record each milkweed species present within the monitoring plot. Tally the number of plants by species and the number of stems for each milkweed plant.	
Species 1:	
# Plants	**# Stems**
Total # plants:	Total # stems:
Species 2:	
# Plants	**# Stems**
Total # plants:	Total # stems:
Species 3:	
# Plants	**# Stems**
Total # plants:	Total # stems:

Tally the number of blooming plant species that you cannot identify here.

Miscellaneous Monarch Observations (Tally for each observed and list nectar plant species)

# Adults							# Eggs and Caterpillars						
Ovipositing	Resting	Flying	Flying high	Mating	*Nectaring	*List Nectar Species	Eggs	1sts	2nds	3rds	4ths	5ths	Pupae

Tally plants for which 0 monarchs are observed. When monarchs are found on plants, record the stage of the monarch(s) in each box.
e = egg | 1 = 1st instar | 2 =2nd instar | 3 = 3rd instar | 4 = 4th instar | 5 = 5th instar | p = pupa

ACTIVITY 2: MONARCH EGGS & LARVAE	Date:	Start Time:	End Time:	Site ID:	page ___of___
Observer(s):	Notes:				

Milkweed Species:

0 monarchs/plant	1 monarch/plant	2 monarchs/plant	3 monarchs/plant	4 monarchs/plant	>4 monarchs/plant

Milkweed Species:

0 monarchs/plant	1 monarch/plant	2 monarchs/plant	3 monarchs/plant	4 monarchs/plant	>4 monarchs/plant

Miscellaneous Monarch Observations (Tally for each observed and list nectar plant species)

# Adults							# Eggs and Caterpillars						
Ovipositing	Resting	Flying	Flying high	Mating	*Nectaring	List Nectar Specie	Eggs	1sts	2nds	3rds	4ths	5ths	Pupae

Tally plants for which 0 monarchs are observed. When monarchs are found on plants, record the stage of the monarch(s) in each box.
e = egg | 1 = 1st instar | 2 =2nd instar | 3 = 3rd instar | 4 = 4th instar | 5 = 5th instar | p = pupa

ACTIVITY 2: MONARCH EGGS & LARVAE	Date:	Start Time:	End Time:	Site ID:	page ___of___
Observer(s):	Notes:				

Milkweed Species:

0 monarchs/plant	1 monarch/plant	2 monarchs/plant	3 monarchs/plant	4 monarchs/plant	>4 monarchs/plant

Milkweed Species:

0 monarchs/plant	1 monarch/plant	2 monarchs/plant	3 monarchs/plant	4 monarchs/plant	>4 monarchs/plant

Miscellaneous Monarch Observations (Tally for each observed and list nectar plant species)

# Adults							# Eggs and Caterpillars						
Ovipositing	Resting	Flying	Flying high	Mating	*Nectaring	List Nectar Specie	Eggs	1sts	2nds	3rds	4ths	5ths	Pupae

ACTIVITY 3: ADULT MONARCHS	Date:	Start Time:	End Time:	Temp in Shade (ºF):	Page 1
Site ID:	Distance walked (if not 500m):		Sky code:	Wind speed code:	☐ Site too narrow; only inner box surveyed
Observer(s):					

Wind speed codes:	0 = <1mph (smoke rises vertically)	1 = 1-3mph (wind direction shown by smoke drift)	2 = 4-7mph (wind felt on face; leaves rustle)
	3 = 8-12mph (leaves/twigs in motion)	4 = 13-18mph (raises dust/loose paper)	5 = 19-24mph (small trees in leaf sway)
	6 = 25-31mph (large branches in motion)	7 = 32-38 mph (whole trees in motion, effort needed to walk against wind)	
Sky codes:	0 = Clear/few clouds	1 = Partly cloudy/variable sky	2 = Cloudy/overcast 4 = Fog or Smoke

Directions: Walk at a pace of 1 m per 2 seconds following your 500 m transect route. For each monarch you see, record the distance box it is first observed in and the behaviors it is engaging in within the box. If a butterfly was observed in the outer box but moves into the inner box, check "moved into inner box."

Ideal Conditions for Monitoring

Time
Ideal: 10am-4pm
Allowable: During daylight

Temperature
Ideal: 70-87 °F
Allowable: 60-95 °F

Wind
Ideal: <10 mph
Allowable: <24 mph

Precipitation
Ideal: None
Allowable: None

	Distance (m)			Behavior					
Monarch	Inner box, 0-2.5 m	Outer box, 2.5-5m	Moved into inner box	Flying	Resting	Ovipositing	Mating	Nectaring	List Nectar Plant Species
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									

Miscellaneous Monarch Observations (Tally for each observed)

# Adults							# Eggs and Caterpillars						
Ovipositing	Resting	Flying	Flying high	Mating	*Nectaring	*List Nectar Species	Eggs	1sts	2nds	3rds	4ths	5ths	Pupae

ACTIVITY 3: ADULT MONARCHS

Notes:

Ideal Conditions for Monitoring

Time
Ideal: 10am-4pm
Allowable: During daylight

Temperature
Ideal: 70-87 °F (21–30 °C)
Allowable: 60-95 °F (16–35 °C)

Wind
Ideal: <10 mph
Allowable: <24 mph

Precipitation
Ideal: None
Allowable: None

	Distance (m)			Behavior					
Monarch	Inner box, 0-2.5 m	Outer box, 2.5-5m	Moved into inner box	Flying	Resting	Ovipositing	Mating	Nectaring	List Nectar Plant Species
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									
32									
33									

Miscellaneous Monarch Observations (Tally for each observed)

# Adults							# Eggs and Caterpillars						
Ovipositing	Resting	Flying	Flying high	Mating	*Nectaring	*List Nectar Species	Eggs	1sts	2nds	3rds	4ths	5ths	Pupae

ACTIVITY 4: MONARCH SURVIVAL & PARASITISM

Observer(s):

Site ID:

Notes:

Larva ID #	Location of Collection (if different from site)	Date of Collection	Larval Instar at Collection	Adult Monarch		Date of Sampling for Oe (if applicable) and result (if known)		Result: parasitized by fly, parasitized by wasp, dead from another cause (accidental or disease), adult monarch	# of Parasitoids and Date Emerged from Monarch		Notes (cause of death - accidental, disease, unknown, did adult flies emerge from fly pupae, etc.)
				Date Emerged	Male/ female	Date	Infected (Y/N)		#	Date	

ACTIVITY 4: MONARCH SURVIVAL & PARASITISM	Observer(s):	Site ID:
Notes:		

Larva ID #	Location of Collection (if different from site)	Date of Collection	Larval Instar at Collection	Adult Monarch		Date of Sampling for Oe (if applicable) and result (if known)		Result: parasitized by fly, parasitized by wasp, dead from another cause (accidental or disease), adult monarch	# of Parasitoids and Date Emerged from Monarch		Notes (cause of death - accidental, disease, unknown, did adult flies emerge from fly pupae, etc.)
				Date Emerged	Male/ female	Date	Infected (Y/N)		#	Date	