

Monarch Butterfly Monitoring Handbook

Annex: Protocols for selected monitoring activities

October, 2008

Commission for Environmental Cooperation



Introduction

Welcome to the Monarch Butterfly Monitoring Handbook Appendix! Whenever possible, we recommend that individuals or organizations interested in setting up monarch monitoring programs obtain up-to-date information from the websites listed in the Monarch Butterfly Monitoring Handbook, since protocols may change slightly from year to year. This book is written to be a resource for individuals and organizations without access to internet resources or for whom English websites are not useful, and for use during monarch monitoring workshops.

Useful Monarch Monitoring Materials

We suggest that you have the following materials available for each participant during monarch monitoring workshops.

- Life cycle cards (produced by Monarchs in the Classroom and the Monarch Butterfly Sanctuary Foundation, available in Spanish and English)
- Monarch Larva Monitoring Project Monitoring Kit (obtained from Monarch Larva Monitoring Project, materials in kit only available in English)
- Field guide to caterpillars (produced by Monarchs in the Classroom, available in Spanish and English)
- Butterfly nets
- Monarch Watch tags and data sheets (obtained at www.monarchwatch.org, data sheets and directions in English)

Authors

Karen Oberhauser and Wendy Macziewski, University of Minnesota, and Elizabeth Howard, Journey North. Protocols for monitoring projects were obtained from the websites listed with each project, and in most cases, were edited for this handbook format.

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BREEDING POPULATION MONITORING

The Monarch Larva Monitoring Project

Contact Information

Monarch Larva Monitoring Project
Univ of MN
Dept of FWCB
1980 Folwell Ave
St Paul, MN 55108
Phone: 612-624-8706
Fax: 612-625-5299
Email: oberh001@umn.edu

General Directions for Participation

There are two ways to participate in the Monarch Larva Monitoring Project:

1. If you have access to a site that you can monitor regularly, you can monitor this site on a weekly basis during the time that milkweed is present. You will register as a Monarch Larva Monitoring Project volunteer, and provide detailed information about your site.
2. If you don't have access to a site that you can monitor regularly, you can submit anecdotal observations of monarch eggs or larvae. Regular volunteers who observe monarchs at locations other than their regular monitoring site can also submit anecdotal observations.

Data Accuracy: The accuracy of the MLMP database depends on the accuracy of individual data sets. Since most of the MLMP sites have too many milkweed plants to check, volunteers estimate the actual monarch densities at their sites. These hints will make the estimates as accurate as possible:

1. Choose the plants you check randomly. If you only look at plants that look "good," there's a good chance that you'll overestimate monarch density.
2. Look at as many plants as you have time for. The more plants you check, the better your estimate.
3. Check the plants carefully. If you miss tiny first instar larvae, or call a milkweed latex dollop an egg, your estimates will be off.
4. Be careful as you identify the different larval instars. The Field Guide to Monarch Caterpillars will help your accuracy.

Overview of Monitoring Activities: Weekly monitoring involves several activities that help us to track monarch populations, understand monarch habitat use, and investigate factors that affect monarch populations. All participants should complete the "Registration for Monarch Larva Monitoring Project," "Description of Monitoring Site" and "Milkweed Density" data sheets. All participants should also do Activity #1, but you may choose whether to do the additional activities. The more activities you complete, the more we learn.

- Registration for Monarch Larva Monitoring Project: Provide background and contact information for yourself and others who will be helping you monitor.
- Description of Monitoring Site: Provide information on characteristics of your monitoring site so that we can correlate features of the environment with monarch abundance and occurrence.
- Milkweed Density: Estimate milkweed density so that per plant monarch densities can be translated to overall density.
- Activity #1: Estimating monarch densities each week at the site: Obtain a count of monarch eggs and larval instars at the site by examining a known number of milkweed plants. The result will be a per plant density measurement of the monarch population at the site. We use this measurement to see how monarch densities vary within a year, between years, and among different sites and locations.
- Activity #2: Recording weather data: Obtain measurements of rainfall and temperature at site.

- Activity #3: Estimating parasitism rates in monarchs: Obtain an estimate of monarch parasitism rates by collecting larvae and rearing them, recording whether they survive to adulthood, and, if not, what caused their death. These data will help us measure the importance of parasitism by insect parasitoids.

General rules

1. Be confident. You have read the instructions and you have the ability to make good decisions in the field.
2. Keep excellent notes. Document what you do. Fill out all fields on the data sheets, or explain why you can't. If you need to deviate from the protocol, keep track of what you did and why.
3. Ask for help when you need it. You can use email, the website or snail mail to contact us or other monitoring project participants.
4. Monitor regularly. Missing a week or two will not make your results useless or invalid--don't let it be a reason to not participate in the project at all. However, if you need to miss a week or two due to illness or travel, you might try convincing a friend or neighbor to monitor for you. If you are short on time one week, you can just do Activity #1 and skip the other activities that week.

Supplies List:

- The Monarch Larva Monitoring Kit is recommended. It contains the life cycle card set; thermometer; meter stick; pocket apron for carrying supplies; clipboard with a summary of directions; hand lens; Milkweed, Monarchs and More Field Guide; pencils; and a welcome letter.
- Data sheets and Field Notebook: You will need at least one copy of each data sheet, and possibly more depending on the number of weeks you monitor. A field notebook (journal type or spiral-bound) is useful for recording observations that don't fit on the data sheets.
- Hand lens: A hand lens or magnifying glass is useful for identifying eggs and small larvae.
- Meter stick or measuring tape: You will need a meter stick to measure milkweed density once during the season.

- Rain gauge (for Activity #2): If you can, mount an inexpensive rain gauge at your site. Try to find one that is designed to prevent the water from evaporating.
- Thermometer (optional): Mount an inexpensive outdoor thermometer at your site, or bring one when you visit the site to monitor each week.
- Field guides (optional): To identify monarch eggs and instars, use our Field Guide to Monarch Caterpillars or the life cycle card set. A guide to wildflowers is useful for identifying the blooming plants at your site.
- Containers for rearing larvae (for Activity #3): See the instructions for rearing larvae in activity 3 instructions for more information.

If you have access to internet, please register online (www.mlmp.org). Click Data Entry and then the new member link in the left hand margin. All registered participants will be put on an e-mail list, and will receive project updates from us throughout the year. This page and linked pages contain step-by-step instructions for following the monitoring protocol. You should read through these instructions before you decide to register!

Send hard copies of data to the address above. We use these to check for accuracy. If you do not have access to a computer, please send us the hard copies of your completed data sheets. Retain copies of all the data sheets!

MLMP Data Sheet: Registration for Monarch Larva Monitoring Project:

Name of participant(s):

Primary Mailing Address:

Secondary Mailing Address:

E-mail address, if available:

How many years have you collected data for the Monarch Larval Monitoring Project?

When was the last year you participated?

Are you participating as part of an organization (school, nature center, etc.)? If so, what organization?

Will anyone (family, friends, etc) be helping you with the monitoring? If so, please list their names (and ages if they are students/children).

We like to know a little about our volunteers. If you would like, tell us about your profession, other interesting information about you, whether you have helpers while you monitor, or anything else you think we would like to know.

MLMP Data Sheet: Monarch Larva Monitoring Project Site Information:

Information on your monitoring site will help scientists understand how the environment in and around your site influences monarch presence and abundance. If you cannot answer any of the questions, it is OK to skip them.

1. If you have collected MLMP data in the past, is this the same site you monitored in previous years? If not, why not?

2. Where is your site located?

City of Township _____ County _____ State _____
Geographical coordinates, if known _____

3. What type of site is it? (only one)

- a. CRP land (Conservation Reserve Program)
- b. Other “old field” (not currently used for crops)
- c. Pasture
- d. Restored prairie
- e. Natural prairie
- f. Nature preserve
- g. Roadside (ditch or strip next to a road)
- h. Garden (planted milkweeds that are watered and maintained)
- I. Agricultural area (cornfield, soybean field)
- j. Other

4. We would like to know the size of your site. This means the entire contiguous area in which milkweed is growing, not just where you monitor. You may either give us the area, dimensions, or estimate the area.

- a. What is the area of your site? _____ (indicate units-square meters, acres, hectares, etc. Measure the length and width of the site and multiply them, or use a quantity that you already know, such as 40 acres.)
- b. Estimate the size by choosing one of the following: (only one)
 - Very small: 0-10 sq. meters (100 sq feet) – e.g. a small garden
 - Small: 11-100 sq meters (1000 sq feet) – up to the size of half a tennis court
 - Medium: 101-1000 sq meters (10000 sq feet)– a little smaller than a football field
 - Large: 1001-10,000 sq meters (2.5 acres)
 - Very large: Over 10,000 sq meters (large fields and bigger)

5. Please list all milkweed species at the site.

6. Was this milkweed planted by humans, or did it grow naturally?

7. On what date this year did milkweed first emerge?

8. Please check any forms of management that occur on your site.
- Mowed 1-2 times per year
 - Mowed more than 2 times per year
 - Fertilized 1 or more times per year
 - Weeded
 - Planted with an agricultural crop (milkweed is a “weed” in this site)
 - Burned every year
 - Burned every 2-3 years
 - Burned with a frequency of less than once every 3 years
9. Which of the following are found within your site? This includes the entire contiguous area that contains milkweed, not just the part of it that you monitor.
- Flowering plants
 - Native Grass
 - Lawn grass
 - Shrubs (less than 3 m tall)
 - Trees (more than 3 m tall)
 - Natural body of water (pond, lake, or river)
 - Human-provided water (birdbath, pond, etc.)
10. Which of the following border your site? This includes the entire contiguous area that contains milkweed, not just the part of it that you monitor.
- Lawns
 - Agricultural fields
 - Residential buildings
 - Industrial or commercial buildings
 - Roads
 - Body of water (lake, pond, river)
 - Deciduous woods
 - Evergreen woods
 - Schoolyard
 - Park
 - Other
11. How would you describe most of the area immediately surrounding your site? (only one)
- Undisturbed (forest, prairie, or other natural vegetation)
 - Rural agricultural
 - Small town
 - Suburban
 - Urban
12. If your site is in a city, suburb, or town, what is the population?
- Less than 5000
 - 5001-25,000
 - 25,001-100,000
 - Over 100,000
13. Are either of the following within 1 km (0.6 miles) of your site?
- Another small to medium area with milkweed (0-1000 sq meters)

- Another large to very large area with milkweed (over 1000 sq meters)

14. What is the elevation of your site?

- 0-750 m (0-2500 ft)
- 751-1500 m (2501-5000 ft)
- 1501-2250 m (5001-7500 ft)
- 2251-3000 m (7501-10000 ft)
- Over 3000 m (over 10000 ft)

15. Do you ever release adult monarchs at this site?

- Yes
- No

If yes, how often (choose one)

- Once
- 2-3 times
- More than 3 times

And how many (choose one)?

- 1-5
- 6-10
- More than 10

16. Do you collect monarchs at this site? If so, what stages and approximately how many?

Stage	Check if this stage is collected	Check how often		
		A few	Every once in awhile	Most or all that I see
Egg		<input type="checkbox"/> A few	<input type="checkbox"/> Once in a while	<input type="checkbox"/> Most or all that I see
L1		<input type="checkbox"/> A few	<input type="checkbox"/> Once in a while	<input type="checkbox"/> Most or all that I see
L2		<input type="checkbox"/> A few	<input type="checkbox"/> Once in a while	<input type="checkbox"/> Most or all that I see
L3		<input type="checkbox"/> A few	<input type="checkbox"/> Once in a while	<input type="checkbox"/> Most or all that I see
L4		<input type="checkbox"/> A few	<input type="checkbox"/> Once in a while	<input type="checkbox"/> Most or all that I see
L5		<input type="checkbox"/> A few	<input type="checkbox"/> Once in a while	<input type="checkbox"/> Most or all that I see
Pupa		<input type="checkbox"/> A few	<input type="checkbox"/> Once in a while	<input type="checkbox"/> Most or all that I see
Adult		<input type="checkbox"/> A few	<input type="checkbox"/> Once in a while	<input type="checkbox"/> Most or all that I see

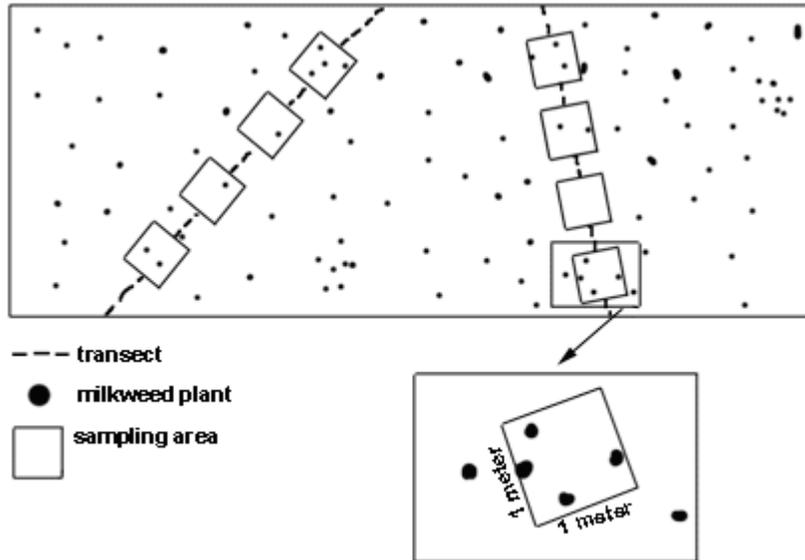
MLMP Data Sheet: Milkweed Density

All participants must complete the “Milkweed Density Data Sheet.”

Since the milkweed may not all be above ground at the beginning of the season, you should wait to do the density measurements until the middle of the season. You only need to complete this activity once.

If you can easily count all of the milkweed plants at your site, record the actual number of milkweeds at the site and the size of your site.

If your site has too many milkweed plants to count, you will need to randomly sample points to obtain plant density data, counting the number of milkweed plants in several 1 meter squares. To do this, randomly choose a direction and walk one or more transects, or paths, through the site. Stop every 5-10 paces, and use a meter stick to delineate a square that is 1 meter on each side. Count all of the milkweed plants within that 1 m² square. The number of paces will vary with the size of your site, but must be consistent for all of your samples. Record the data on the Milkweed Density Data Sheet. Continue with this method until you have sampled up to 100 squares (the more squares you sample the more accurate your density estimate will be, but more than 100 is not necessary). The goal is to sample randomly and obtain data that are representative of the whole site. It is very important not to let the presence or absence of milkweed influence your choice of samples.



MLMP Milkweed Density Data

Observers _____ **Year** _____

You only need to do this once during the season.

If can count all of the milkweed plants at your site, record the number of milkweed plants and area of your site (you can record the area in square meters, square feet, or acres).

Number of milkweed plants _____ Area of Site _____

If your site has too many milkweed plants to count, use the sampling procedure described above and complete the table below.

Point #	# of milkweed plants in 1x1 meter square	Point #	# of milkweed plants in 1x1 meter square	Point #	# of milkweed plants in 1x1 meter square	Point #	# of milkweed plants in 1x1 meter square
1		26		51		76	
2		27		52		77	
3		28		53		78	
4		29		54		79	
5		30		55		80	
6		31		56		81	
7		32		57		82	
8		33		58		83	
9		34		59		84	
10		35		60		85	
11		36		61		86	
12		37		62		87	
13		38		63		88	
14		39		64		89	
15		40		65		90	
16		41		66		91	
17		42		67		92	
18		43		68		93	
19		44		69		94	
20		45		70		95	
21		46		71		96	
22		47		72		97	
23		48		73		98	
24		49		74		99	
25		50		75		100	

MLMP Data Sheet: Activity #1 - Monarch Density

Objective: Obtain a count of monarch eggs and larval instars at the site by examining a known number of milkweed plants. The result will be a per plant density measurement of the monarch population at the site. We can use this measurement to see how monarch densities vary within a year, between years, and among different sites and locations.

Method: Examine as many milkweed plants as possible, keeping track of the number of plants examined. Record the number of eggs and monarch larvae of each instar that you find. Try to monitor on the same day and at the same time each week throughout the time that milkweed is growing in your area. It is critical that you record the number of milkweed plants that you examine. It is also important to examine an unbiased sample of milkweed plants. You can avoid bias by following the directions below.

- 1. Walk one or more straight-line transects, or path, through your site.** Hold your arms out to your sides as you walk. Stop and examine every milkweed plant that falls along your path between your fingertips. **Keep track of the number of plants you look at**, whether they have monarchs or not. Record the total number of plants you examine on Date Sheet #1 (which has rows for several days) or data sheet #1A (which only has rows for one date). *You do not need to use the transect method if you examine **all** of the milkweed plants at your site.*
- 2. Search for monarch eggs and larvae on each plant.** Look carefully at all parts of the plant, including the bottoms of the leaves and the area within the very small leaves at the top of the plant. Keep an eye out for caterpillar clues, such as chew marks on the leaves. Try not to handle the plants roughly, to avoid knocking any larvae off the plant. Remember, not all eggs and caterpillars that you find on milkweed are monarchs; use **A Field Guide to Monarch Caterpillars** to help you distinguish monarchs from other insects.
- 3. Keep track of the number of monarch eggs and larvae that you find, and the instar of each monarch larva.** Note that there is a space to record the number and stage of any dead monarch eggs or larvae that you find.
- 4. Scan for adult monarchs.** Note any adult monarchs you observe, and their gender, if known. To avoid counting individuals more than once, count the maximum number of adults that you observe at any one time.
- 5. Note what plants are blooming each week.** This information will help us know something about the diversity of plants at your site and tell us if there were any nectaring plants there to attract adult monarchs. You do not need to record the numbers of each blooming plant type, just the species.
- 6. Note any disturbances at the site.** Record the date and type of disturbance on the bottom of Data Sheet #1. Disturbance might include mowing, herbicide spraying, haying, or other activities that would temporarily or permanently damage the milkweed plants.
- 7. Record the rest of the required data on Data Sheet #1.** Record the date you monitored, the temperature (indicate Fahrenheit or Celsius), start and stop times, etc.



Images, from left to right:

Monarch egg on milkweed leaf—The egg is a little more than 1 millimeter tall.

Close-up of monarch egg—Note the pointed shaped, the glossy color, and the vertical striping.

Monarch first instar consuming eggshell—Note the dull greenish-grey color, and the size (not much bigger than the egg).

First instar feeding damage—This circular feeding pattern is an indication that a monarch first instar was on the plant at some point.

Monarch fourth instar—Older monarch larvae have bright yellow, black and white striping and 2 pairs of tentacles (on front and back ends).

MLMP Data Sheet #1A: Weekly Monarch Density

Use this information to fill in Data Sheet 1.

Observers: _____ Date: _____ Site Name: _____

Start time: _____ Stop time: _____ Temp in sun: _____ Temp in shade: _____

Eggs	1 st instars	2 nd instars	3 rd instars	4 th instars	5 th instars	Adults f = female m = male u = unknown	Dead (# and stage)	Number of milkweed plants observed (use tick marks to represent 1, 5, 10 or 20 plants and record total at end of session)

Plants in bloom at site (species, not numbers of plants!) _____

Note any disturbances that occurred at the site over the past week (mowing, herbicide spraying, haying, etc.) _____

Other Notes:

MLMP Data Sheet: Activity #2 - Weather

Objective: Obtain measurements of weekly rainfall at the site.

Method: Mount a rain gauge at or near the site and record rainfall amounts.

We are interested in following the weather patterns at your site, especially rainfall. If possible, mount an inexpensive rain gauge at your site. Check the gauge regularly and record rainfall amounts on **Data Sheet #2**. More ambitious data collectors could also keep track of the daily weather in your area: High and low temperatures, humidity, and rain (yes or no), and record these data on the second part of **Data Sheet #2**.

MLMP Data Sheet: Activity #3 - Parasitism Rates

We are interested in learning about the natural enemies that may affect monarchs. Some of these enemies are parasitoids, organisms whose young develop inside the monarch larvae, eventually killing them. This activity will help us learn how common this kind of parasitism is at your site.

Objective: Obtain an estimate of parasitism rates in monarch larvae collected at your site. These data will help us measure the importance of this mortality factor in populations of different densities and at different times and locations.

Method: Collect any 4th or 5th instars found at the site each week as you complete Activity #1. You can collect earlier instars as well; just be sure to note that you are collecting earlier instars on the site information form. Rear these larvae indoors and record whether they survive to adulthood, and, if not, what caused their death (parasitized by flies, parasitized by wasps, etc.). Release butterflies back at the site. You can release the parasites too, but you should put them back as pupae, so that they are not given an advantage in surviving. You can also send samples of the parasites to us to identify.

Instructions for Rearing Larvae to Estimate Parasitism Rates

1. Larvae (caterpillars) can be kept in an aquarium, large jar, ice cream bucket, bug cage, or another relatively large cage. The container should be easy to open, since you need to clean it every day. It should have a screen covering or holes for air flow and should allow you to see the larva inside. Unless you plan to move the pupae, the cage should be large enough for the adult to expand its wings when it emerges. Keep the cage out of the sun or other hot places (like car trunks in summer). High temperatures can kill the larvae.
2. Cages must be cleaned daily. Simply empty out the caterpillar frass and old milkweed. Wash out your container periodically, using a 20% bleach-water solution.
3. Larvae must be provided with fresh milkweed daily. You can pick several days worth of caterpillar food, and keep it in a plastic bag in a refrigerator. It stays fresher in the cage if you keep the end moist by wrapping it in a wet paper towel and covering the towel with aluminum foil, or use the plastic containers in which single cut flowers are sold by florists.
4. The 4th and 5th instars that you collect will likely pupate within a week. When they are ready to pupate, the larvae will crawl to the top of their cage, attach themselves with silken thread, and form a prepupal “J” before shedding their skin for the last time. This process is fun to watch, but it happens quickly. You can tell that they will shed their larval skin soon (within minutes) when their tentacles hang very limply and their bodies straighten out a little. Be careful to not jostle the container while the larvae are pupating.
5. The pupa stage lasts nine to fourteen days. Pupae turn darker the day before butterflies emerge, and look black on the day they emerge. At this point, the wings are visible. The butterflies usually emerge in the morning, and watching this happen is also fun, but hard to catch. Their wings will be soft, flexible, and wet when they emerge. If they fall, carefully pick them up by holding the thorax, and hold their legs next to the top or side of the cage. They need to hang with their wings pointed down. A pupa that has been very dark for more than a few days is almost always dead.
6. Larvae that have been parasitized by flies will not pupate successfully, but will hang limply and die (see pictures below). Fly maggots will come out of the larval body and pupate on the bottom of the container. Larvae that have been parasitized by wasps may pupate successfully, but wasps will emerge from the chrysalis instead of butterflies. In both cases, be sure to remove the wasps or fly pupae from your container. Ideally, you should return them to your monitoring site in the stage they emerge from the monarch (larva, pupa or adult). You can also send specimens to us to identify.
7. Remove diseased larvae from any container with other larvae to avoid spreading the disease.
8. 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, when they should be released. They can also be released the day they emerge, especially if

it is warm and sunny. Hold the butterflies carefully with their wings closed when you release them, or simply open their cage to let them fly free.

9. Record your data on Data Sheet #4: Estimating Parasitism Rates. Make sure you write the date of collection, the stage of the larva at collection, and the outcome (parasitized by fly – how many flies emerge; parasitized by wasp – how many wasps emerge; healthy monarch – gender of monarch; dead from another cause – is the cause accidental or disease).



The tachinid fly life stages you might observe include the larva (maggot), the pupa, and the adult fly. The pupa is hard, brown, oval, and about one centimeter long. The adult fly looks similar to a house fly.



When the tachinid larva emerges from the monarch larva or chrysalis, it drops down on a white thread. The remaining thread is one indicator that the monarch was parasitized by a tachinid.

POPULATION CENSUSES

Butterfly Monitoring Networks

This section describes a general butterfly monitoring network protocol. These ideas should be helpful in establishing or improving a monitoring network. The contacts listed below can provide information on how they conduct monitoring networks in their states.

Contact Information

Ohio

Curator of Entomology
The Cleveland Museum of Natural History
1 Wade Oval Drive
Cleveland OH 44106
216-231-4600 ext. 315
Fax 216-231-5919

Illinois

Illinois Butterfly Monitoring Network
c/o Mel Manner
41W039 Bowes Bend Dr
Elgin IL 60124-8325

Florida

Florida Butterfly Monitoring Network
Attn: Jaret C. Daniels
P.O. Box 110620
University of Florida
Gainesville, FL 32611-0620
Fax: (352) 392-0190

Indiana

John Henry Drake
Restoration Coordinator
The Nature Conservancy
Southern Lake Michigan Rim Project
5690 Chase Street
Merrillville, Indiana 46410
219-981-9183
jhdrake@tnc.org

General Directions for Participation

Establish a Transect or Census Route

- The transect route should be reasonably representative of your locality. However, it is interesting to include areas which are managed differently or, perhaps, have more species present than others, or contain a population of a particularly interesting or local species. Routes will ideally:
 1. Cross a variety of habitats and/or management units
 2. Take advantage of existing paths or trails whenever possible
 3. Be easy to locate and repeat in subsequent years
 4. Require anywhere from 1/2 hour to 2 hours to complete
- The transect should be only as long as is easily manageable, bearing in mind that:
 1. It must be walked at least once a week by one monitor for consistency.
 2. At the height of the season, when there are many butterflies on the wing, counting will take longer than earlier in the year.
 3. Someone else may take over the transect or substitute for a week in the absence of the regular recorder.
- It is better to use paths with obvious boundaries. In more open habitats, established paths may be used and the butterflies counted within limits judged by eye. The precise width is not important, but recording becomes more difficult if the width is over about 5 meters (2.5 meters to either side). A fixed route can be marked out to ensure that the same path is followed. If some sections are covered twice due to overlapping paths, butterflies should be recorded only on the first occasion that the section is covered. A loop is ideal.
- Divide the transect into different sections according to the features they contain. A transect with about 15 sections is ideal for monitoring.
- Make a detailed map of the route with information about the landscape and features with an appropriate scale so that another person would be able to follow it without confusion.
- Monitor and record consistently throughout the season. It is important to find the correct or practical timeframe for monitoring in your area.
- Between 10AM and 5PM is ideal for conducting census routes. Choose a day with less than 50% cloud cover, light to moderate winds, and is at least 70°F (21° C) for best results. The transect should be walked at an even pace and only the butterflies which come within ~5 meters from the recorder should be counted. Imagine yourself walking along in a box, 5 m wide, 5 m tall, and extending 5 m ahead of you. Only butterflies in this volume should be counted. Note butterflies seen by 'scoring' in the appropriate square. Make sure that the correct total is clearly legible in the appropriate square as each section is completed. Do not record a butterfly flying more than 5 m ahead, even if it is identifiable, as it may have moved away by the time you reach the spot. It is okay to follow a butterfly if you need to have a better look at it for identification purposes. Begin counting again when you return where you left off.
- Carry a net, so that you can examine some butterflies more closely, to be sure of identification. Retain one or more voucher specimens of any species of which you are uncertain so identifications can be verified. If you can't collect unknown butterflies, take a picture of them for future identification.

Safety

- Consider using the "buddy system" when monitoring. If you twist an ankle or fall, you will want someone with you who can get help. The second person can be a note taker, and record butterflies that you see on the field form.

- If you do go out alone, set up a check-in system with someone. Call this person before you went into the field and check in with them once you were done. If they didn't hear from you within a specified length of time, they would come to find you, or notify the authorities, or both.
- **EXTREMELY IMPORTANT:** If you are allergic to bee stings, carry a bee sting kit.
- Carry drinking water and take precautions against heat exhaustion.

Paying Attention to Site Details

- Field notes are very important and can include butterflies that could not be included in the regular record because they were outside the survey area, or observations of other animals and interesting plants.
- Comments concerning variation within a season may be particularly valuable. Such information could include the appearance of different growth stages of plants such as buds and flowers, and weather events such as storms, sustained unusual weather, or lack of rain on preceding days.
- Record any disturbances of the habitats, including deliberate management actions, floods, horse and deer damage, treefalls, turkey scratches, *etc.* The effects of management are easily overlooked if not noted regularly. Even such routine management as path cutting may have an effect if, for example, stands of nectar-producing flowers are cut. Use the recording forms to note changes which take place.
- A short description of the different habitat types in each section is useful for the transect records. It is also useful to have a short list of the most abundant plant species in each section. Particular attention should be given to larval host plants and popular nectar sources. The aim of these records is not to acquire quantitative information on the abundance of plants but to help with the interpretation of results.
- Weather conditions have a considerable effect on the numbers of butterflies seen. To ensure that the counts are standardized as much as possible:
 1. Do not walk a transect when the temperature is below 60°F.
 2. Between 60-65° F, a transect may be walked if it is sunny for at least 75% of the sections.
 3. Between 65-70° F, a transect may be walked if it is sunny for at least 50% of the sections.
 4. Above 70° F, a transect may be walked in any conditions, if it is not actually raining.
- Wind speed should be estimated using the Beaufort scale (see below), at the beginning and end of the walk. Wind direction is the direction from which the wind is blowing. For example, SW wind direction means the wind is blowing from the southwest toward the northeast. Don't count butterflies when windspeed is over 19-24 mph (30-40 km per hour).

<1 mph	no perceptible movement
1-3 mph	leaves barely move
4-7 mph	leaves rustle/wind felt on face
8-12 mph	leaves and twigs move
13-18 mph	small branches move
19-24 mph	small trees sway/large branches move
25-31 mph	large branches move continuously/wind begins to whistle
- Record shade temperature at the beginning and end of the walk.
- The percent cloudiness should be estimated by examining the sky from horizon to horizon. Ten percent clouds mean that 10% of the sky is covered in clouds, and 90% of the sky has no clouds.
- Sunshine should be recorded section by section in the Weather Codes section at the bottom of the form. As the transect progresses, fill in the boxes. If a shadow is cast, then record the condition

as S for sunny. If no shadows are cast, then record the condition as O for overcast. If rain commences, record the condition as R for rain.

- Note some of the common energy sources the butterflies are currently using. This may include both nectar sources and other sources such as sap or feces.
- Identify common flowers which are currently blooming. They need not be nectar sources used by butterflies, and can include trees. These data help gauge the progression of the growing season.

Photography and Other Activities. Many people use photography to document identification. We encourage this practice, but monitors should minimize photography during data collection unless they are photographing an unknown butterfly for identification. Do not remove any butterfly from the site to photograph it. Do not do any other activities during monitoring, such as monitoring other animals or plants, walking your dog, or taking someone on a site tour. These activities will distract from your monitoring, causing you to miss some butterflies. Your sole focus should be looking for butterflies.

Before monitoring, fill out all of the information possible

- If more than one person is monitoring, note which person is the monitor (the person spotting the butterflies), but list everyone. No more than two or three is recommended.
- Round your starting and ending times to the nearest 5 minutes.
- Get the temperature from an outside thermometer if you have one, or another source such as a weather website or radio.
- Estimate the wind conditions and circle the appropriate choice. Note that there is no choice for very windy. If it is very windy, do not monitor.
- Estimate the cloudiness of the sky and circle the appropriate choice. Note that there is no choice for very cloudy (more than 50%). If it is very cloudy, do not monitor.
- List each habitat type next to the appropriate transect letter corresponding to that column. For example, if your first area on your route is a wet prairie, list Transect A as "Wet Prairie" and record all individuals seen in that type of habitat in column A.

During monitoring, fill out the following information

- If something is unusual or you feel it needs explanation, use the comment section. For example, if you stop for more than two or three minutes for a rest or to identify a butterfly, note the length of time you stopped monitoring in the comment section. If there is a disturbance to an area along your route, such as brush clearing or off-road vehicle damage, note that in the comment section. Anything that you believe might influence your data should be noted here.
- Use one line for each butterfly species seen along your route. (You may want to use a separate sheet of paper on the route, then transcribe the counts to the field form. Be careful to transcribe exactly.) Make a hatch mark for each individual of a species in the column that represents the habitat transect in which the individual was seen. For example, if you see a monarch in the wet prairie, make one hatch mark in column A on the row for *Danaus plexippus* (Monarch). As you see more monarchs in that same habitat, make additional hatch marks for each individual. Once you move into Transect B, mark any monarchs seen in that area in column B on the same row.
- If you see a species that is not already listed on the Field Form, fill the name in on the next blank line.
- If you are not positive of identification, do not guess. Mark it down as well as you can with certainty. For example, if you know it was either a Question Mark or a Comma, list it as *Polygonia sp.* (Question Mark/Comma) on a blank line. If you know for certain that another is a

Question Mark, list it on the line for *Polygonia interrogationis* (Question Mark). If you can only say for certain that it was a skipper, list unknown skipper on a blank line and record the number seen in the appropriate column.

- If you see butterfly larvae, or butterflies nectaring or getting energy from other sources in any of the transect sections, make a note of this using codes from the bottom of the data sheet.

After monitoring, fill out the following information

- Fill in your ending time in the top portion of the form. Round off to the nearest 5 minutes.
- Total each species in each row in the right-most column. Note that there are no totals at the bottom. You do not need to total the individuals seen in each transect.

Censuses at Fall Migration and Stopover Sites

There is no centralized data collection program for censuses at fall migration and stopover sites, and thus no pre-designed data sheets. However, if you would like to conduct a fall census, please read the following protocols to determine how you could set up a program at your location. Feel free to contact organizers of other programs for helpful hints. A good contact is Andy Davis at the University of Georgia (listed below), who has worked with several of these programs to analyze and publish their findings.

Contact

Monarch Migration Association of North America (Overview of Migration Monitoring Programs):
<http://www.mmana.org/>

Chincoteague (Virginia USA)
Denise Gibbs
Email: monarch301@verizon.net

Peninsula Point (Michigan, USA)
Gina Badgett
Email: gnb_43@yahoo.com

Cape May Bird Observatory (New Jersey, USA)
Dick Walton
Email: rkwalton@earthlink.net

For more information:
Andy Davis
University of Georgia
Athens GA 30602-2202
<http://www.arches.uga.edu/~akdavis>
akdavis@uga.edu

General Directions for Participation

Migration monitoring methods include the driving census, walking census and roost count. Items you will need for these methods include a hand tally (i.e. a clicker), clipboard and perhaps a pair of binoculars if you are conducting a walking census.

The same census method must be used each year for long term observations.

The Driving Census: This method involves driving slowly along a standardized route a number of times each day during the fall migration (usually throughout September and October) and counting all monarchs you see using a hand tally (a clicker). Some observers use two clickers and record monarchs flying and not flying, to differentiate between actively migrating and 'bivouacked' monarchs. This method is used in Chincoteague and Cape May.

The Walking Census: This is a variation on the driving census. The observer walks a standardized route several times daily during the migration season, counting all monarchs seen. As with the driving census, the walking census should be conducted 2 or 3 times daily; 9 am, 11 am and 3 pm are good times. The trail should cover as many habitats as possible. Ensure that the trail passes known roosting or nectaring spots so that these monarchs are censused. Only one person should do the counting

The slower pace of a walking census means seeing more monarchs, and getting more accurate counts. Also, the observer can look for monarchs near the ground or flying high above. However, not as much ground is covered as with the driving census. This method is used at Peninsula Point.

The Roost Count: This method is not widely-used, but if done properly and at the right location, it can yield important data. It can only be conducted at a location where monarchs are *known to roost* every year during their fall migrations. Thus, some advanced knowledge of the area is needed before this method can be used.

The observer visits the roost area once or twice daily during the fall, either at dawn or at dusk (or both), and records the number of roosting monarchs seen. Roosting monarchs are great for counting, and also for catching (and tagging) monarchs. However, keep in mind that monarchs roost at night because the temperature drops too low for them to fly. For this reason, do not capture monarchs in the evening as they will not be able to fly back up to their roost tree when they are released. Save the netting for the mornings, when monarchs can easily warm themselves with sunlight. This method is used at Peninsula Point.

For the best results it may be necessary to combine more than one migration census method. Both the Roost Count and the Walking Census are used at Peninsula Point

MIGRATION

Monarch Watch Tagging

Contact Information

Monarch Watch
University of Kansas
1200 Sunnyside Ave.
Lawrence, KS 66045-7534
1.888.TAGGING (toll-free)
1.785.864.4441
monarch@ku.edu

General Directions for Participation

Importance of Tagging: Many questions remain unanswered about the fall migration of the monarch population east of the Rocky Mountains. Only through the cooperative efforts of volunteer taggers will we be able to obtain sufficient recoveries and observations of the migration to answer questions about monarch pathways and how they vary from year to year.

When to Tag: As the daylength shortens in mid August and September, monarchs in northern latitudes, i.e. near the Canadian border, begin to migrate. Monarchs farther south will begin their journey a few weeks later. Tagging and monitoring should begin in late August in all regions, with a concentrated effort made in September and early October. A GOOD RULE: when the wild asters, especially *A. novae-angliae*, goldenrod and Joe Pye weed are in bloom, the monarchs are migrating.

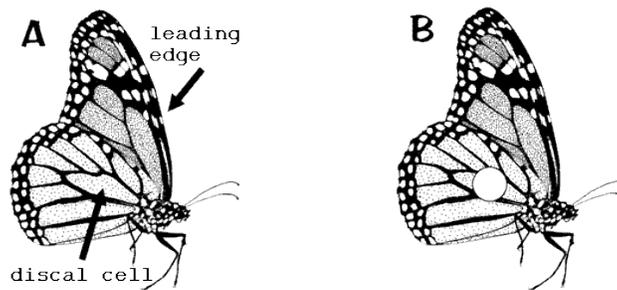
Peak Migration Dates: The following is a general, not a specific, guideline for when you are most likely to encounter good numbers of monarchs at different latitudes. The record at specific locations for a given year may differ from this overall pattern but it has proven to be remarkably consistent when viewed as a large-scale phenomenon. This pattern is likely to be modified by weather patterns that slow or advance the migration, such as strong southwesterly winds or a series of rapidly moving cold fronts arriving from the northwest. Similarly, the pattern of the migration is likely to be modified along the coasts due to strong head winds or storms that have the effect of sweeping monarchs toward the coast on the backside of fronts.

Latitude	Midpoint	Peak in monarch abundance
49	26 August	18-30 August
47	1 September	24 August -5 September
45	6 September	29 August - 10 September
43	11 September	3 – 15 September
41	16 September	8 – 20 September
39	22 September	14-26 September
37	27 September	19 September - 1 October
35	2 October	24 September - 6 October
33	7 October	29 September - 11 October
31	12 October	4-16 October
29	18 October	10-22 October
27	23 October	15-27 October
25	28 October	20 October - 1 November
23	4 November	27 October -8 November
21	11 November	3-15 November
19.4*	18 November	10-22 November

*This latitude represents the general vicinity of the overwintering colonies.

Tagging Method: Purchase a Monarch Watch tagging kit at shop.monarchwatch.org. Each Monarch Watch Tagging Kit includes a set of monarch butterfly tags (you specify quantity), a datasheet, tagging instructions, and additional monarch/migration information. Tagging Kits are shipped beginning August 1st, in plenty of time for the start of the monarch migration. Please note tags are only good for the year they are issued. Please only order enough tags for your tagging activities this year.

The tag is placed over the large, mitten shaped cell (discal cell) on the underside of the hindwing of the monarch (see figure). The discal cell position is close to the center of lift and gravity for the butterfly and will not impede flight.



The 9mm diameter polypropylene tags are numbered specifically for each tagging season. The tagging method is quite simple - remove a tag from the backing, place it over the discal cell and position the balls of your thumb and forefinger over the discal cells on both side of the butterfly, press firmly for two seconds and release the butterfly after recording the tag number and other information on the datasheet.

Capturing a Butterfly: You can purchase a good butterfly net or make one. The net should be at least 24 inches deep, allowing you to trap the butterfly in the deep end of the net without harming it. Good quality nets are available from the Monarch Watch Shop at 1-800-780-9986 or online at: Shop.MonarchWatch.org.

When in flight, monarchs are difficult to catch. To maximize the number of monarchs collected for tagging, it's best to locate them feeding on flowers or while they are on the roosts late in the day or early in the morning. If you do collect butterflies from roosts late in the day, be sure to place back in the roosts, or store them in a safe place (see below), as they do not fly when it's dark. Approach each butterfly slowly (from behind if possible) as sudden movement will startle it into flight. Sweep the net forward quickly and flip the end of the net bag over the net handle. With one hand holding the handle, use the other hand to collapse the end of the net. There should be enough space at the deep end to prevent damaging the butterfly.

Flatten the net bag so the wings of the butterfly are closed over its back (thorax) and place thumb and forefinger over the leading edge of the wings (from outside of net). Next, with the thumb and forefinger of your other hand, reach in to the net and firmly grasp the thorax. Remove the butterfly for tagging.

Storing Live Monarchs: If you collect more monarchs than you can tag immediately, you can store them in a paper triangle or glassine envelope (stamp envelope). If you need to store the butterfly for more than

a few hours, i.e. overnight or up to 2 days, place the envelope in a plastic box or zip lock bag in a refrigerator. They'll be fine! Be sure to keep the butterflies out of the sun before you refrigerate them and to keep the butterfly from drying out and dying, place a damp paper towel in the container.

Journal: Some of our most useful information has been obtained from individuals who maintain a monarch journal, diary or calendar, keeping records of the first appearance of migratory monarchs (those showing directional rather than local flight), and the numbers seen each day, particularly at a roost or roosts, or on flowers in a garden, etc. Many Monarch Watchers obtain very good quantitative data by counting the numbers of monarchs passing a given observation site each hour. It would also be useful to record the numbers of mating pairs seen along with the dates and circumstances of these observations.

All 11,000+ Monarch Watch tag recoveries 1994 to date are accessible via a searchable database.

Journey North

Contact Information

Website: www.learner.org/jnorth/monarch/

E-mail: jnorth@learner.org

Mail: 1321 Bragg Hill Road, Norwich, VT 05055 USA

General Directions for Participation

Spring Migration-Report Sighting of First Adult Monarch Butterfly: Watch for the first monarch butterfly to appear in the spring as you go about your daily activities, and report the date and location of your sighting to Journey North. If you see a monarch butterfly egg before you see an adult monarch butterfly, report the date that the egg was sighted. (The presence of a monarch egg is evidence that adult monarch butterflies are present.) Even if other people have already reported “first” monarchs from your region, please report *your* first sighting. The number of first sightings is an indicator of relative monarch abundance, particularly if you have watched for monarchs regularly and frequently. Plan to collect yearly observations so your data can be compared from year to year.

The only required information about your sighting is date and location. However, additional information, such as weather and habitat conditions at the time of the sighting, adds substantially to the value of your report. In addition, describe how regularly and frequently you watch for monarchs. This information helps scientists interpret the data you and others have collected. See detailed notes on the Spring Migration Data Sheet for the weather, habitat, and behavioral details you should note.

Fall Migration-Report Sightings of Overnight Roosts and Peak Migration: Watch for gatherings of monarchs at an overnight roost in your hometown during fall migration. Report the date the roost formed or was discovered and its location to Journey North. If you read in your local newspaper or elsewhere about the discovery of a roost site, please report its location, too. Spread the word! Help gather information about when and where monarchs form these stop-over sites by notifying people in your region or in other geographic areas. See detailed notes on Fall Migration Data Sheet #1 for the type of weather, habitat, and behavioral details you should note. After you report the discovery of a roost site we will contact you for more information and ask your help in documenting how large the site is and

how long the monarchs use it. We will also contact you in future years to continue to document if and how monarchs use your stopover site.

To report peak migration, choose a method for watching and counting monarchs during fall migration and follow the protocol regularly. (For example, establish a road census or follow the point-count method as described in the section on fall migration monitoring.) Keep track of the monarchs you see per minute or per hour. When you think monarch activity is at its highest, report your observations as "Peak Migration." Don't be concerned if you're not *sure* you're seeing the peak. Our staff will read your comments, contact you, and adjust if necessary. Your site will be included on a real-time tri-national map to illustrate the timing of peak migration.

First Milkweed Sightings: Long before the migration begins, begin to watch how the monarchs' habitat changes. First, go outside and inspect their habitat in the dead of winter. Then monitor the same site once each month, and later more frequently, leading up to the monarchs' arrival in the spring. On each visit, record the date, photoperiod & temperature; look for milkweed and sources of nectar.

Journey North Spring Migration Data Sheet
Sighting of First Adult Monarch Butterfly

Required Information

Date first adult monarch (or egg*) was sighted: _____

*The presence of a monarch egg is evidence that the first monarchs have arrived.

Location of Sighting

Nearest Town: _____

State/Province: _____

Send to Journey North

Website: www.learner.org/jnorth/monarch/

E-mail: jnorth@learner.org

Mail: 1321 Bragg Hill Road, Norwich, VT 05055 USA

Optional Information

If possible, please include the following information with your report:

About the Observer

How frequently & regularly did you watch for monarchs? Describe.

About the Monarch

Sex of butterfly

Condition of wings (Tattered, faded, or fresh?)

Activity of monarch (Flying, nectaring, laying egg, etc.)

Did you see other monarchs? (If so, when and how many?)

About the Habitat

Flowers in bloom (including trees such as lilacs, apples, etc.)

Milkweed height

Date milkweed emerged (give exact date or estimate)

Milkweed species

About the Weather

Temperature

Wind direction & speed

Sky (sunny, cloudy, precipitation)

Weather: Describe recent weather conditions, *before* the sighting

Journey North Fall Migration Data Sheet (#1)
Sighting of Fall Roost

Required Information

Date roost formed (or was discovered): _____

Location of Roost:

Nearest Town: _____

State/Province: _____

Send to Journey North

Website: www.learner.org/jnorth/monarch/

E-mail: jnorth@learner.org

Mail: 1321 Bragg Hill Road, Norwich, VT 05055 USA

Optional Information

If possible, please provide the following information:

About the Roost

How many monarchs are in the roost? (Try to estimate.)

Describe its location (suburb, coastline, agricultural field, etc.)

If in trees, what kind and on which side did the roost form?

About the Weather

Temperature, wind direction & speed

Weather conditions: Describe recent weather *before* sighting

About the Habitat

Nectar sources: What flowers are blooming near the roost site?

What is blooming in your region at this time?

About the Monarchs' Behavior

Try to watch the roost regularly and find out: What time does the roost form in the evening?

What time do the monarchs become active in the morning? How does the size of the roost change from day to day? When did the last monarchs leave? What were the weather conditions at the time? etc.

Journey North Fall Migration Data Sheet (#2)
Sighting of Peak Migration

Required Information

Date of peak migration: _____

Location of Sighting

Nearest Town: _____

State/Province: _____

Number of monarchs observed: _____

Number of minutes (or hours) observing: _____

Migration rate: ____ monarchs per hour (or minute).

Send to Journey North

Website: www.learner.org/jnorth/monarch

E-mail: jnorth@learner.org

Mail: 1321 Bragg Hill Road, Norwich, VT 05055 USA

Journey North First Milkweed Sightings

Required Information

Date of first milkweed: _____

Location of Sighting

Nearest Town: _____

State/Province: _____

Species of milkweed observed: _____

Height of milkweed plant: _____

Send to Journey North

Website: www.learner.org/jnorth/monarch

E-mail: jnorth@learner.org

Mail: 1321 Bragg Hill Road, Norwich, VT 05055 USA

Optional Information

Pre-emergence observations

List other dates that you observed the site, and found no milkweed.

Describe conditions during these observations (temperature, presence of snow, frost, etc.)

List other plant species that are present in the site.

When milkweed is up

Temperature

Nectar sources: What flowers are blooming in and near the site?

When did you first spot monarch eggs or adult monarchs at the site?

About the site

Describe the location of the site (garden, open field, etc.)

Describe the microclimate of the site (exposure to sun, proximity to buildings, etc)

Correo Real

Contact Information

Correo Real: <http://www.profauna.org.mx/monarca/>

E-mail: correo_real@prodigy.net.mx

Telephone: (844) 414-4997

Mailing address:

Rocío Treviño Ulloa

Nueva Vizcaya 480

General Directions for Participation

Correo Real participants record the number of butterflies they observe, the time of their observations, the butterflies' behavior (such as flying, feeding or resting), the locations and plants on which the butterflies feed or rest, and climate conditions. The protocol matches that of Journey North as described above, and the same data sheets can be used.

INDIVIDUAL MONARCH ASSESSMENTS

Project Monarch Health

Contact information

Dr. Sonia Altizer

Odum School of Ecology

University of Georgia

Athens, GA 30602 USA

saltizer@uga.edu

Telephone: 706-542-9251

Fax: 706-542-4819

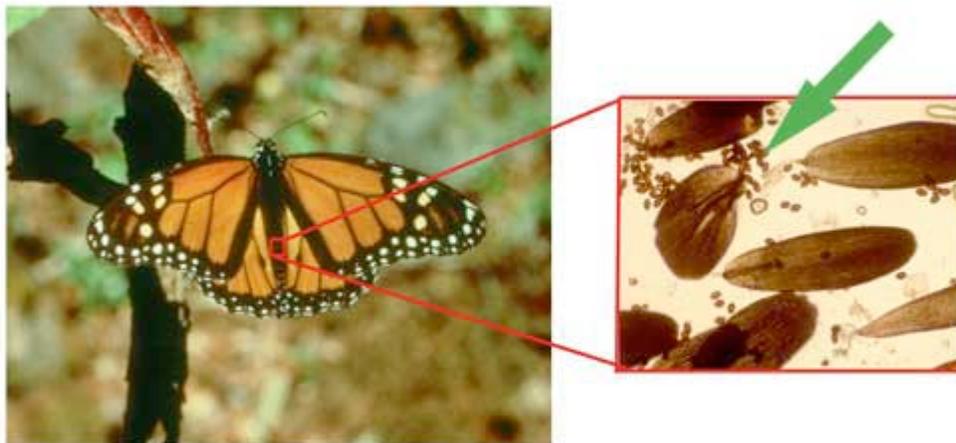
Disease/Parasite/Parasitoid information

Fewer than 10% of all monarch eggs laid by females in the wild will survive to become adult butterflies. Natural enemies are an important source of monarch mortality; many predators, parasites, and parasitoids attack monarch eggs, larvae, and adults. Predators such as spiders and fire ants attack eggs and young larvae feeding on milkweed, and birds and wasps prey on adults. Although predators can be highly visible, other natural enemies are hidden inside the monarchs' own bodies. These include parasitoids, which are parasitic insects that feed within their host's body, and parasitic microorganisms such as viruses, bacteria, and fungi. Some parasites can become extremely problematic when monarchs and other insects are held in captivity and reared under close quarters.

Parasitoids: Parasitoids are specialized insects such as small flies and wasps that lay eggs on other insects. Parasitoid larvae then eat their prey from the inside out, usually emerging from the prey carcass as a pupa or adult. A few species of parasitoids lay their eggs on monarch larvae, including tachinid flies and braconid wasps. Tachinid fly larvae feed on monarch caterpillars, but usually don't kill their hosts

until just before the caterpillars pupate. When a parasitized caterpillar hangs upside down in the pre-pupal "J"-shape, one or more fly maggots will emerge from the dorsal anterior end and drop to the ground on long, gelatinous tendrils. Braconid wasp parasitism is less common, but as many as 32 tiny adult wasps may emerge from a single monarch carcass. Very little is known about variation in rates of parasitism and predation by invertebrates throughout the monarchs' range.

Parasites and infectious diseases: Parasites are small organisms that complete most or all of their life cycle within a host, and many are capable of a high degree of within-host replication. Parasites can be unicellular microbes such as viruses and bacteria, or larger organisms like mites and nematodes. Not all parasites kill their hosts, but they almost always have negative effects on host survival and reproduction. Many parasites and pathogens attack insects, including viruses, bacteria, fungi, protozoans, nematodes, and mites. Several viral and bacterial pathogens can infect monarchs, including a nuclear polyhedrosis virus and *Pseudomonas* bacteria. Protozoan parasites such as *Ophryocystis elektroscirrha* and a microsporidian *Nosema* species have also been identified in wild and captive monarchs. The infective stages of most insect parasites must be consumed orally, although some can invade through pores or membranous joints in the insect cuticle.

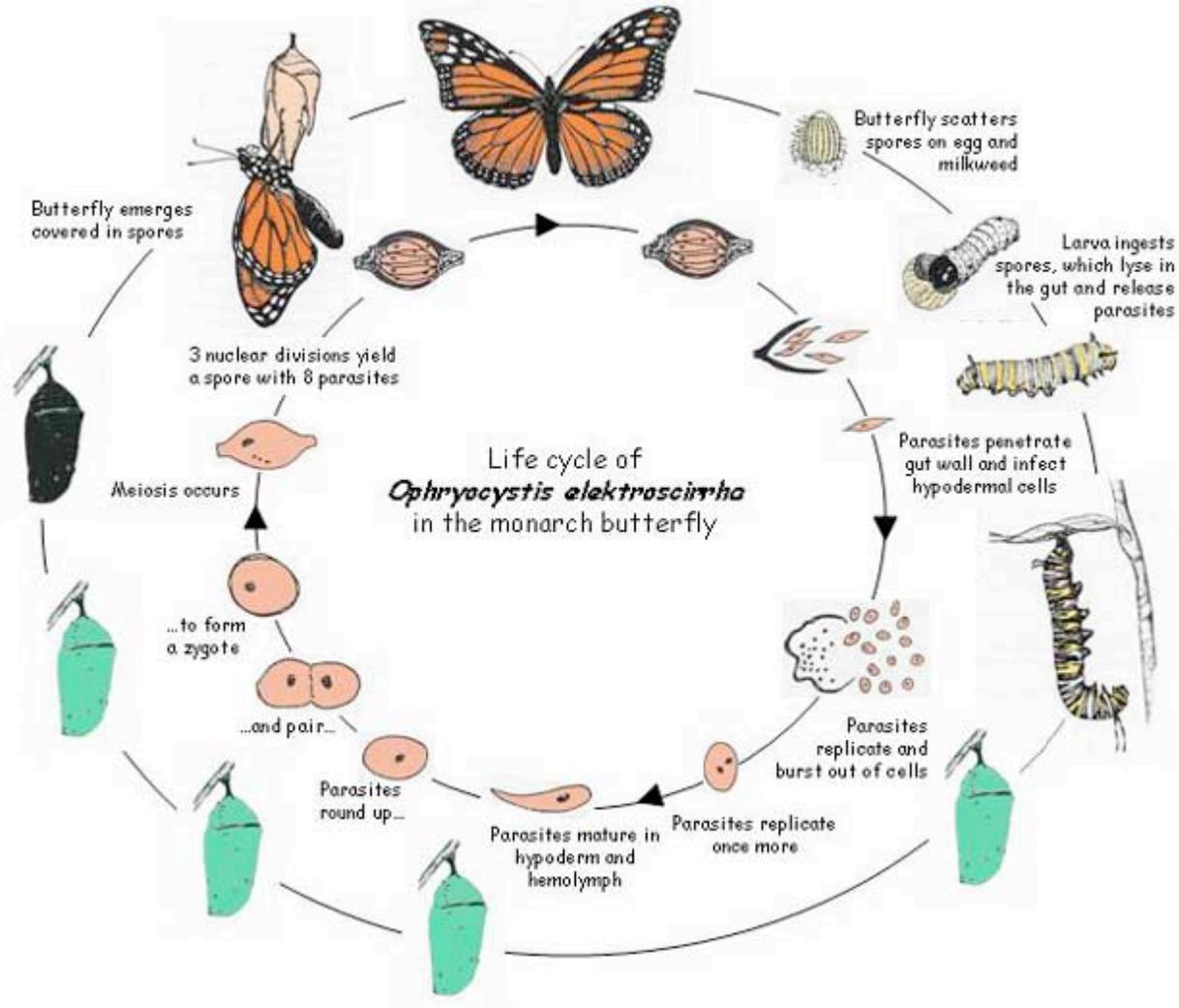


Ophryocystis elektroscirrha (*Oe*) is an obligate, neogregarine protozoan parasite in the phylum Apicomplexa that infects monarch and queen butterflies. This parasite, shown above (green arrow), was first discovered infecting monarch and queen butterflies in Florida in the late 1960s (McLaughlin and Myers 1970). There are no known other hosts. It has since been found in all other monarch populations world-wide. Because of this world-wide range, all indications are that this parasite has coevolved with monarchs (i.e. it is a *naturally occurring* parasite).

Dormant spores of this parasite occur on the exterior of the cuticles of infected butterflies, sandwiched between the butterfly's scales. When viewed under a light microscope at 40 to 100x, they appear as small, brown or black lemon-shaped objects about 1/100th the size of a butterfly scale as shown in the image above. Here is a picture of spores at 400X.

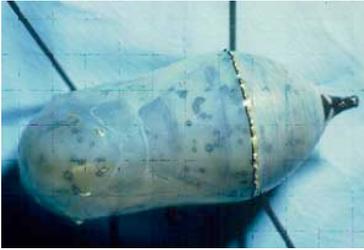


The life cycle of *Oe* is closely related to the developmental cycle of its host (see figure below). Parasites are transmitted from females to their offspring when the females scatter dormant spores of this disease on milkweed during oviposition. Spores are then eaten by larvae, germinate in gut, migrate to hypoderm, and undergo 2 phases of vegetative reproduction. After the host pupates, the sexual phase of reproduction is initiated. About 3 days before the adults emerge, spores can be seen forming through the pupal integument. Infected adults emerge covered with spores, with the highest densities on their abdomens. *Once hosts are infected, they do not recover.* By the time adults emerge with parasite spores, all physical damage by the parasite has been done. The parasites do not continue replicating on adults, and these dormant spores on adult monarchs must be eaten by another larva to continue development.



General Directions for participation

Detecting Oe Infection: Pupae infected with *Oe* show dark spots or blotches about 3 days before adults eclose. These are developing parasite spores, and lesions of many thousands of these spores can be seen through the pupal integument before any pigment is laid down on the butterfly scales. A monarch dissected from its pupal case at this stage will be covered with parasite spores (everything black in the image below aside from the background). Spores form on the eyes, antennae, wing veins, but by far the greatest number of spores form on the abdomen.



Adults that are severely infected with *Oe* often have difficulty emerging from their pupal cases, and can be too weak to cling to their pupal case to fully expand their wings. These heavily infected adults either fail to eclose fully or fall to the ground, leading to severe wing deformities and relatively rapid death.



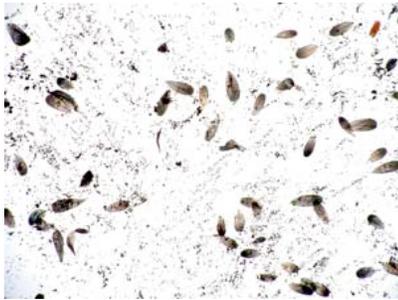
The abdominal cuticles of heavily infected adults are damaged by the large numbers of parasite spores that disrupt the integument, and this causes the adults to lose weight at a faster rate than healthy individuals.

Heavily infected adults can also be smaller than healthy butterflies, weigh less upon eclosion, and have a shortened adult lifespan. On the other hand, many parasitized monarchs do eclose normally, show no signs of deformity, and can be nearly impossible to distinguish from healthy butterflies without looking directly for the parasite spores.

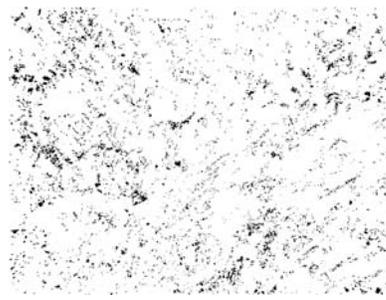
Sampling Monarchs for Parasites: Monarchs can be easily assessed for parasite loads by pressing a piece of ultraclear Scotch™ tape on their abdomens and counting the number of spores in a 1-2cm area. This figure shows how spores appear relative to abdominal scales under a light microscope at 200x.



Scientists use this method to categorize parasite loads on an approximate logarithmic scale of 0-5, with 5 being the most heavily infected class, and 0 being butterflies with no detectable spores. This method allows for rapid classification of disease status and the severity of infection. Researchers in Dr. Sonia Altizer's lab also use digital image analyses to get more refined measures of spore densities.



Digital picture of scales and spores obtained from an infected monarch. *Oe* spores look like dust particles in this picture.



Same picture after digitally removing scales. Computer then does the rest. There are approximately 3600 spores in this picture!

If you are interested in examining your own butterflies for the *Oe* parasite, you can follow these steps. Alternatively, you can have the Altizer lab test your butterflies by participating in project MonarchHealth.

Supplies

1. Disposable gloves
2. Clear scotch tape
3. Blank index cards
4. 1 pair of fine forceps
5. A standard light microscope or handheld 30X mini-scope

Procedure I (Sampling your own butterflies)

1. Put on your gloves!
2. Cut out a 1cm square piece of scotch tape.
3. Position the adult monarch in your fingers with its abdomen exposed.

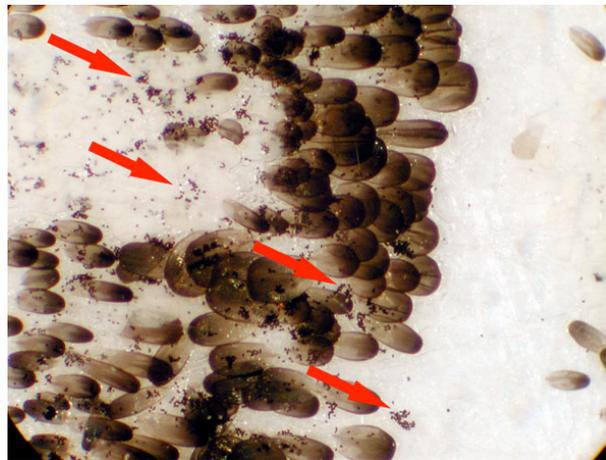


- Using your forceps, gently place the sticky side of the piece of tape to the abdomen of the monarch. Press down so that it wraps around and sticks to the sides of the abdomen.
- Gently peel the tape off and stick it to the index card. You will remove some scales in the process, but this will not harm the monarch. Label the tape sample on the spore card with the identity of the monarch. Continue these steps until you have sampled all of your monarchs. In the end, your index cards should look something like this:



The numbers on the bottom of each sample refer to the number of the butterfly, and the numbers above refer to the parasite 'spore load' on each monarch. Notice that one monarch from this samples was infected and was given a 5 score (heaviest infection category).

- To determine if your monarchs are infected with *Oe*, look at each tape sample under your microscope at 30-40X. Tape samples from monarchs infected with *Oe* look something like this:



The red arrows indicate the parasite spores in this image. The big objects are the monarch scales. The spores look like tiny lemon-shaped objects, often clumped together. Under a good scope they'll have a reddish tinge. This monarch would be considered heavily infected. Keep in mind that many infections are mild so you may only see 20 spores in the entire tape sample. If you do not find any parasite spores the monarch is healthy! If you do find an infected individual, it should be isolated from your other monarchs and then destroyed. Also, change your gloves after handling any infected monarch so you will not spread the spores around your workspace.

Be Sure to dispose of your gloves and sterilize your work surface when you finish, especially if you found (and handled) an infected monarch!

Procedure II (Sending samples to Altizer lab): To participate in the MonarchHealth citizen science project, write to monarch@uga.edu to receive directions, a free monitoring kits, and relevant data sheets. You will test monarchs that you capture as adults or raise from the caterpillar stage in separate containers. In either case, you will gently swab each butterfly's abdomen with a Q-tip to collect the *Oe* spores. Next, you will send the sample, along with a simple data sheet for each butterfly, back to the scientists at the Altizer lab where they will analyze the sample. After the data are compiled, they'll send you the results of your sampling contribution.

