



Monarch Larva Monitoring Project

ACTIVITY #1: MEASURING MONARCH DENSITY

Objective: Obtain a count of monarch eggs and larvae at the site by examining a recorded 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 densities vary within a year, between years, and among different sites and locations.

You can choose one of two different datasheets to record your monarch density data for Activity 1.

- **Datasheet #1 SUMMARY**

- This datasheet asks for the total numbers of plants and monarchs you observe at the site—for example, 4 eggs on a total of 100 plants observed. If you use this datasheet, you will need to print a new copy each time you monitor your site. It is the simplest datasheet for recording and is great for new volunteers or working with kids.

- **Datasheet #1 DETAIL:**

- This datasheet asks you to keep track of the number of monarchs you observe on each individual plant you observe, instead of just reporting the total numbers of plants and monarchs that you observe. For example, with Datasheet #1 DETAIL you could record seeing 2 eggs on 1 plant, 1 larva on another, and zero monarchs on a third. This information will be useful in understanding potential impacts of crowding on monarch survival. Because you will also record the milkweed species, we'll be able to assess how female monarchs make egg-laying decisions when they have more than one milkweed species in a single site. Your data will be added to overall monarch densities, but will also be analyzed separately.
- We introduce datasheet 1 DETAIL in our training workshops as it gives us better quality data to work with.

Method: Regardless of what datasheet you use, the basic monitoring method remains the same. You will examine as many milkweed plants as possible, keeping track of the number of plants examined, and recording the number of monarch eggs and larvae of each instar that you find. Follow the specific instructions below: Try to monitor on the same day and at about the same time each week, throughout the time that milkweed is growing in your area. It is okay if your timing is slightly off from one week to the next, or if you have to miss weeks.

1. **Record the required basic monitoring event details.** Record the date you monitored, the temperature in the shade (indicate Fahrenheit or Celsius), start and stop times, etc.
2. Always record the number of milkweed plants that you examine. The result will be a weekly estimate of monarch density at your site, measured as a proportion of milkweed plants with monarchs.
3. Examine as many plants as possible. **If you can monitor every milkweed plant at your site, please do so.** If there are too many for you to monitor all of them, randomly select plants to monitor during each monitoring session (it does not need to be the same subset of plants each week). It is important to monitor an unbiased sample of milkweed plants. In other words, you should not just look at the milkweed plants that you think are most likely to have monarchs on them because you will overestimate the monarch density at your site. You can avoid bias by following the directions below.

- For large sites where milkweed is more evenly distributed, **walk one or more random, straight-line transects, or paths, through your site.** 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. 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, **keep track of the number of plants you look at**, whether they have monarchs or not. Record the total number of plants on one of the Activity 1 datasheets. When you reach the perimeter of your site, you can generate another random direction to conduct the next transect, or simply turn 90 degrees back into your site and continue monitoring. Continue running these transects through the site until you feel you have adequately sub-sampled the site, or have run out of time.
- For sites where milkweed density is patchy, a systematic approach to sub-sampling may be easier. To do this, estimate the total number of milkweed plants at the site, and determine how many you are capable of observing each week. You can use this number to calculate your sub-sampling method. For example, if your site has 900 milkweed plants, and you have time to monitor 300 of them each week, your method would be to observe and record every third milkweed plant that you observe. Again, be sure not to bias your sample by choosing plants you feel are more likely to have monarchs present..

Note: you do not need to use the transect method if you are able to examine all of the milkweed plants at your site.

4. **Search for monarch eggs and larvae on each plant that you examine.** Remember that monarch eggs and larvae can be hard to find! To examine a milkweed plant, look carefully at all parts of the plant, including the bottoms of the leaves, the area within the very small leaves at the top of the plant, and buds and flowers if they are present. Keep an eye out for caterpillar clues, such as chew marks on the leaves or frass. Handle the plants carefully, to avoid knocking any larvae off the plant. Remember, not all eggs and caterpillars that you find on milkweed are monarchs; use the pictures of each instar in this packet along with our [Field Guide to Monarch Caterpillars](#) to help you distinguish monarchs from other insects.

Note: Sometimes it is difficult to distinguish one “plant” from another; many species of milkweed have different growth forms. A rule of thumb is that if stems are separated by dirt, you can call them separate plants. We know that they might not be separate plants; common milkweed stems all over a field might actually be from the same “plant” and all be connected underground, and the stems in a clump of swamp milkweed (which is one plant) are often separated by dirt. If there are uncertainties, please email us first for clarification. Always write what you are doing in the notes section of your site information page, and be consistent from year to year.

5. **Keep track of the number of milkweeds, monarch eggs, and larvae that you find, and the instar of each monarch larva.** Use one of the Activity 1 datasheets to record your observations of eggs, larvae, and milkweed. Be sure to identify the larvae to instar (see photos below, MLMP Life Cycle cards, Field Guide to Monarch Larvae, or illustrations on MLMP clipboard). Note that you can record the number and stage of any dead monarch eggs or larvae that you see.
6. **Scan for adult monarchs.** Note any adult monarchs you observe, and their sex, if known. To avoid counting individuals more than once, count the maximum number of adults that you observe at any one time by scanning the entire site when you see an adult. Record this number on the datasheet, indicating how many are males, females, or unknown.
7. **Note what plants are blooming each week.** This information will help us learn about the diversity of blooming plants at your site and tell us if there were any nectar plants there to attract and feed adult monarchs. You do not need to record how many of each kind of blooming plant,, just the species.
8. **Note any disturbances at the site.** Record the date and type of disturbance, which might include mowing, herbicide spraying, haying, or anything else that might affect the milkweed plants or monarchs.

9. **Note *Aphis nerii* presence or absence (optional).** Note if you saw any of the bright yellow Oleander Aphids (*Aphis nerii*) while you were monitoring. You don't need to record numbers of aphids or plants with aphids, just whether they were there or not. If you didn't look, just check "didn't look".

ADULT MONARCHS

Male and female monarchs can be distinguished easily. Males have a black spot (indicated by a 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 often look darker than males and have wider veins on their wings.



Male Monarch Butterfly
(photo courtesy of Michelle Solensky)



Female Monarch Butterfly
(photo courtesy of Barbara Powers)

EGGS AND CATERPILLARS



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 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).
(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 band, and the body no longer appears transparent and shiny.
(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. Third instar larvae usually feed using a unique cutting motion on leaf edges.

(Photo courtesy of Monarch Lab)



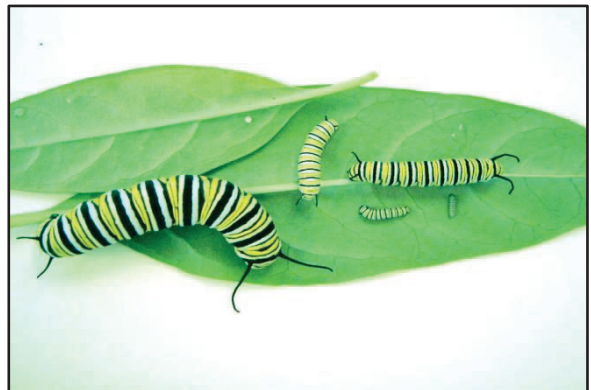
Monarch fourth instar — Fourth instar monarchs front tentacles extend beyond the tip of the head. Internal changes, including the development of reproductive structure, begins to occur in late instar monarchs.

(Photo courtesy of Monarch Lab)



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

(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.

(Photo courtesy of Monarch Lab)

APHIS NERII (APHID)



Aphis nerii – the only bright yellow aphid found on milkweed.

(photo courtesy of Anurag Agrawal)



Aphis nerii – hundreds of aphids on one milkweed plant.

(Photo courtesy of Grant Bowers)

DATASHEET #1 SUMMARY: MONARCH DENSITY (WEEKLY SUMMARY)

Date: _____ **Observers:** _____ **Site Name:** _____
Start Time: _____ **Stop Time:** _____ **Temp in Shade:** _____ **City, State:** _____

Eggs	1 st Instars	2 nd Instars	3 rd Instars	4 th Instars	5 th Instars	# of Adults (F = female M = male U = unknown)	# Dead (egg or larval stage)	# 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.):

Did you see any *Aphis nerii* at your site this week? Circle one: Yes No Didn't look

Other Notes:

ACTIVITY #1 DETAIL: MONARCH DENSITY (WEEKLY DENSITY PER MILKWEED PLANT)

Objective: Activity #1 DETAIL, like the simpler Activity #1 SUMMARY, results in an estimate of monarch density at your site. The difference is that you will keep track of the number of monarchs you observe on individual plants, instead of just reporting the total numbers of plants and monarchs that you observe. Whereas in the simpler activity you would report seeing, for example, 4 eggs on a total of 100 plants observed, this protocol will allow you to report if these eggs were on 4 separate plants, or if they were all on the same plant. This information will be useful in understanding potential impacts of crowding on monarch survival. Because you will also be recording the milkweed species, we'll be able to assess how female monarchs make egg-laying decisions when they have more than one milkweed species in a single site. Your data will be added to overall monarch densities, but will also be analyzed separately.

Method: Follow the directions for Activity #1 SUMMARY, with these modifications. When you look at a plant with no monarchs, place a tick mark in the box labeled "0 monarchs/plant." When you see one monarch on a plant, write "e," "1st," "2nd," "3rd," "4th," or "5th" (depending on whether you see an egg or a first, second, etc. instar) in one of the boxes under the words "1 monarch/plant." When you see 2 monarchs on a plant, write "e,e" or "e,1st," or "1st,1st," etc., depending on the stage of the two monarchs you see. Do the same in the boxes under the words "3 monarchs/plant," "4 monarchs/plant," and ">4 monarchs/plant" as needed. Note if an individual is dead; for example, if you see a dead first instar and a live first instar on a plant, you would note "dead 1st, 1st". At the end of the session, add up the total number of plants (including those with and without monarchs), eggs, and larvae you observed, and tally this in the appropriate section in the summary below below the table.

If you have only one species of milkweed on your site, you'll use one table per monitoring session. If you have more than one milkweed species, you should keep a separate table for each species you observe. There are two tables per sheet.

DATASHEET #1 DETAIL: WEEKLY MONARCH DENSITY PER MILKWEED PLANT

Year: _____ Observers: _____ Site Name: _____ City, State: _____

Date: _____ Start Time: _____ End Time: _____ Temp in Shade: _____

Key: e = egg | 1 or 1st = 1st instar | 2 or 2nd = 2nd instar | 3 or 3rd = 3rd instar | 4 or 4th = 4th instar | 5 or 5th instar | p = pupae

EXAMPLE OF DATA COLLECTION PROCESS AND DATA ENTRY. Record each plant you observe, using a separate table for each milkweed species. If the plant has no monarchs, make a tick mark under 0 monarchs/plant. If it has one or more monarchs, use the key above to record their stage(s) in a cell under the correct column.

Milkweed Species: Common

0 monarchs/plant	1 monarch/plant				2 monarchs/plant		3 monarchs/plant		4 monarchs/plant	>4 monarchs/plant
	e	2 nd	2 nd			1 st , 2 nd		e, e, 3 rd	e, e, 3 rd	

SUMMARY TABLE FOR DATA ENTRY	
0 monarchs	6
1 egg	1
1 2 nd instar	2
1 1 st & 1 2 nd instar	1
2 eggs & 1 3 rd instar	2

When finished monitoring, fill out the **SUMMARY TABLE FOR DATA ENTRY**. Create a row for each unique configuration of monarchs on a plant, i.e., the exact same stages and numbers of eggs and larvae. E.g., In the bolded row to the left, you saw 2 plants with one 2nd instar each.

In the **data portal**, enter each row of this summary table. The portal will ask: "Did you see any monarch eggs or larvae on this plant?" For the bolded row, you'd enter **yes** and then record the stages: "Live 2nd instars" = 1. It will then ask: "How many plants *exactly like this one* did you see?" You'd enter 2: you saw two common milkweed plants with one 2nd instar each. Click "save and add another plant" and continue entering the rest of your summarized data.

As a final step, summarize the week's monitoring findings in the **SITE TOTALS FOR DATA CHECKING** table to double check the data you've entered in the data portal.

SITE TOTALS FOR DATA CHECKING	
Total plants: 12	Total eggs: 5
Total 1 st s: 1	Total 2 nd s: 3
Total 3 rd s: 2	Total 4 th s:
Total 5 th s:	
Dead (list stages):	

1st Milkweed Species: _____

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

SUMMARY TABLE FOR DATA ENTRY (1 st milkweed species)			
0 monarchs			

SITE TOTALS FOR DATA CHECKING	
Total plants:	Total eggs:
Total 1 st s:	Total 2 nd s:
Total 3 rd s:	Total 4 th s:
Total 5 th s:	
Dead (list stages):	

SEE TOP OF OTHER SIDE for additional milkweed species in your site

SEE BOTTOM OF OTHER SIDE for additional data fields (adult monarchs, blooming plants, disturbance, etc.) for this monitoring session

2nd Milkweed Species (if observed): _____

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

SUMMARY TABLE FOR DATA ENTRY (2 nd milkweed species)			
0 monarchs			

Be sure to add these plants to your SITE TOTALS FOR DATA CHECKING table on the first page.

Plant species blooming this week: _____

Note any disturbances at your site this week: _____

Did you see any adult monarchs this week? Number of females _____ Number of males _____ Number of unknown sex _____

Did you see any oleander aphids (bright yellow *Aphis nerii*) at your site this week? Circle one: Yes No Didn't Look

Other notes or observations:
