



# Monarch Larva Monitoring Project

## ACTIVITY #3: ESTIMATING MONARCH SURVIVAL

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. Here, we provide information on how to collect data on parasitoids and a protozoan parasite called *Oe* (*Ophryocystis elektroscirrha*). If you choose to participate in the *Oe* study, you'll need to contact the coordinators at the University of Georgia to obtain a sampling kit (see instructions below).

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

**Summary of Method:** Collect any 4th or 5th instars each week as you complete **Activity #1 Monarch Density**. If you would like to collect earlier instars as well, you can do this; just be sure to note that you are collecting earlier instars on the online **Site Information** form. You may collect larvae from your monitoring site or other locations. If you collect them from your site, enter their information under your monitoring site profile; if you collect them from other locations, use the data entry form under the "Enter/Edit Data on Monarchs You Rear from Other Locations" section. Rear larvae indoors and record whether they survive to adulthood, and, if not, what caused their death (parasitized by flies, parasitized by wasps, dead for an unknown reason, etc.). If you choose to test butterflies for the *Oe* parasite, do this before releasing them back at the site. Additionally, we would like to identify parasitoids that you rear from monarchs (or other butterflies or moths); please see #8 below for information on how to send the adult parasitoids to us.

## DETAILED INSTRUCTIONS FOR REARING LARVAE TO ESTIMATE SURVIVAL

1. Larvae can be kept in an aquarium, large jar, ice cream bucket, or another container. 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 a car in summer). High temperatures can kill the larvae. It is best if you keep only one larva in each container, as this will help combat disease and allow you to track individual larvae accurately, since you'll want to know the stage at which they were collected.
2. Cages must be cleaned daily. Empty out the caterpillar frass (poop) and old milkweed. Wash your container frequently (at a minimum every time a new larva is introduced) using a 20% bleach-water solution.
3. Give larvae fresh milkweed daily. You can pick several days' worth of milkweed, wash it, and keep it in a plastic bag in a refrigerator. It stays fresher if you put a damp piece of paper towel on the bottom of your container.



Example of rearing set-up. Photo by Ilse Gebhard.

- The 4th and 5th instars that you collect will likely pupate within a week. When they are ready to pupate, they'll crawl to the top of their cage and form a pre-pupal "J" before shedding their skin for the last time. 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 larvae are pupating.
- 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. Their wings will be soft, flexible, and wet when they emerge, but they'll be ready to fly in about 4 hours. 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. Within a day of an adult butterfly emerging, release it back at the site from which it was collected after recording information on the Activity 3 datasheet.



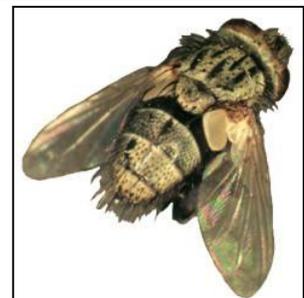
Above: Monarch pupae with silk-like thread from tachinid fly parasitoids. Photo by Sonia Altizer

- Larvae that have been parasitized by flies will often not pupate successfully, but will hang limply and die, although some flies emerge from the pupa. Fly maggots come out of the host larva or pupa on a silk-like thread, and pupate on the bottom of the container. The adult flies emerge about 7-10 days later. Wasps emerge as adults from their host pupa. In both cases, be sure to remove the wasps or fly pupae if there are living monarch larvae in your rearing container; they may mate and parasitize new hosts. Please consider sending the specimens to us to identify after the adults have emerged (see #8).



Above: Parasitized monarch larva with three tachinid larvae (maggots). Below: Soon after emerging, the flies pupate, turning reddish-brown; adult tachinid fly. Above by Jaap de Roode, below by Sonia Altizer and Monarch Lab.

- Remove diseased larvae from any container with other larvae to avoid spreading the disease.
- Please send us adult parasitoid flies or wasps that come from monarchs you rear. After adult flies emerge from their pupae (7-10 days after they emerge from the monarch), let them harden for a day or two, freeze them to kill them, and then put them in small hard containers (e.g. pill bottles, Ziploc-style plastic storage containers), with a piece of tissue to prevent them from bouncing around. Do not use plastic bags or tissue inside a flat envelope. Each container should hold all of the parasitoids that emerged from ONE monarch. If possible, please include the pupal casings from the flies as well as any pupae with flies that did not emerge. DO NOT include the dead monarch. Keep the adult flies in a freezer until you have several or until the end of the season. Label each container with the date of collection of the monarch, the stage at which the monarch was collected, the milkweed species on which the monarch was collected, the location of collection, the number of flies emerged with date(s), and anything else you think is relevant (see sample label below). You may number the containers, and write this information on a separate table that you send with the containers. Contact [info@mlmp.org](mailto:info@mlmp.org) if you need assistance shipping the flies to us, and notify us when a package of flies should be expected. Send the specimens to:



**Monarch Larva Monitoring Project**  
**University of WI Madison Arboretum**  
**1207 Seminole HW**  
**Madison, WI 53711**

Location collected: \_\_\_\_\_  
 Date collected: \_\_\_\_\_  
 Instar collected as: \_\_\_\_\_  
 Date and # flies emerged: \_\_\_\_\_  
 Date adult flies: \_\_\_\_\_

