

## OPTIONAL ACTIVITY

Participate in a separate community science project investigating the natural rates of parasitism in monarch caterpillars.

**Note: These results do not get sent to Project Monarch Health!**



# Monarch Larva Monitoring Project

**Need help?** Contact us at [info@mlmp.org](mailto:info@mlmp.org) and visit our website at [www.mlmp.org](http://www.mlmp.org)

## 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 elektroschirrha*). If you choose to participate in the *Oe* study (last page), 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.

**Disclaimer:** California requires a scientific collecting permit for handling monarchs, which includes any type of collection for scientific research, teaching, or rearing. Canada and Mexico also have notable restrictions on handling monarchs. There may be rules and regulations not presented here that may apply to you, so please check local regulations before handling monarchs.

## 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.



*Example of rearing set-up by Ilse Gebhard*

3. Give larvae fresh milkweed daily. You can pick several days' worth of milkweed, wash it, and refrigerate it in a plastic bag.
4. The 4<sup>th</sup> and 5<sup>th</sup> 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.
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. 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 their 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.
6. 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 larva in your rearing container; they may mate and parasitize new hosts.
7. Remove diseased larvae from any container with other larvae to avoid spreading the disease.



*Monarch pupae with silk-like thread from tachinid fly parasitoids, by Sonia Altizer*



*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.*



