Monarch Larva Monitoring Project

Need help? Contact us at info@mlmp.org and visit our website at 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.

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.

Example of rearing set-up by Ilse Gebhard

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 refrigerate it in a plastic bag.
4. 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.
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.

DATASHEET #3: ESTIMATING PARASITISM RATES

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

Larva ID #	Location of Collection (if different from site)	Date of Collection	Larval Instar at Collection	Adult Monarch		Date of Sampling for OE (if applicable) and result (if known)		Result: parasitized by fly, parasitized by wasp, dead from another cause (accidental or disease), adult monarch	Number of parasitoids – date emerged from monarch		Notes (e.g. cause of monarch death – accidental, disease or unknown; did adult flies emerge from fly pupae; other observations)
				Date Emerged	Male/ Female	Date	Infected (yes/no)		#	Date	

Please remember to enter your data online through the green 'Data Portal' button in the top-right hand corner of the screen at www.mlmp.org. If you're new to MLMP, you'll first have to register to create an account and then provide details about your site before you can enter this data.
Contact us at info@mlmp.org with any questions!

DATASHEET #3: ESTIMATING PARASITISM RATES

INSTRUCTIONS FOR TESTING ADULTS FOR OE (OPTIONAL)

1. Obtain a sampling kit from Project MonarchHealth (see address below). You will send the sampling card plus a copy of your data sheet to the University of Georgia. You will still enter your data on the MLMP website for the monarch larvae you collected, and whether they died for an unknown reason, turned into monarchs, flies, or wasps.
2. Butterflies should not be handled for the first four or five hours after they emerge, and can be kept in the cage until the next day. To sample adult monarchs for the Oe parasite, wear gloves to prevent contamination. While the parasite is not harmful to humans, it is easily spread from one monarch to another.
3. Remove the butterfly from its rearing container. Hold firmly as shown in the picture below, using a gloved hand. Be sure not to use your other hand to touch the butterfly because that hand will be used to hold the tape sticker and sample for Oe spores. **It is critical that your bare hand NOT touch the butterfly!**


4. Pick up a piece of transparent tape or sticker with your other hand. Gently, but firmly place the sticky side of the piece of tape to the abdomen of the monarch. Press down so that it wraps around and sticks to the sides of the abdomen.
5. Gently peel the tape off and stick it to the index card. You will remove scales in the process, but it will not harm the monarch. Label the tape sample with a number that corresponds to the datasheet entry.


6. Sanitize your working surface with bleach solution. Thoroughly sterilize container with 20% bleach solution and clean all supplies and tools with bleach before rearing another wild monarch.
7. After you've entered your MLMP data, send a copy of the MLMP Activity 3 datasheet and the index card to:

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