

## **California volunteer sampling protocol for salvaging dead monarch butterflies for genomic analysis**

### **Sampling goals**

<b>CA, W, or E</b>	<b>Range</b>	<b># sites</b>	<b>Target indiv/site (min-max)</b>	<b>Total N</b>	<b>Season(s)</b>
CA	Breeding	10+	10 (1-10)	100+	Summer (April – August)
CA	Overwintering*	10+	20+ (1-50)	200+	Winter
CA	Year-round (e.g., gardens w/ tropical milkweed)*	20+	10+ (1-20)	200+	Year-round
<b>TOTAL</b>				<b>500+</b>	

**\*NOTE:** In California, we will focus sampling of overwintering and residential/year-round sites along three “transects” in the following areas: (1) San Francisco Bay area; (2) Monterey Bay area (including Santa Cruz); and (3) Los Angeles Basin. We are still interested in samples outside of these areas, but we will focus sampling along these three transects.

### **Permits/regulations**

*Before volunteers may salvage dead monarch butterflies in California, they must be added as a volunteer to Chris Funk’s California Department of Fish and Wildlife (CDFW) Scientific Collecting Permit (SCP).* In order to be added to Chris’ SCP, you must first read this sampling protocol, watch the associated training video, and pass the quiz. Chris Funk or Hillary Sardiñas (CDFW) will email you to let you know once you have been added to Chris’ SCP. **Volunteers may not sacrifice caterpillars.** The one exception is that CDFW employees are allowed to collect without permits following guidance provided by the CDFW Pollinator Coordinator. Also, *you are responsible for checking with the local land managers of public lands or private reserves on which you plan on sampling.*

### **Processing dead monarch butterfly adults:**

1. Required equipment/materials:
  - a. Glassine envelopes (can keep ~20 in a metal band-aid box, which can be kept in a shirt pocket and keeps them from getting squished)
  - b. Fine point Sharpie pen
  - c. GPS unit or smart phone for recording GPS coordinates
  - d. [Freezer boxes](#)
  - e. Large plastic Ziploc bags
  - f. Cooler
  - g. Ice (normal ice or ice packs)

2. When you find a dead adult monarch butterfly, label a glassine envelope with the following information:
  - a. Species name (“Monarch butterfly” is fine)
  - b. Locality name (e.g., “Natural Bridges State Beach, Santa Cruz, CA”)
  - c. Latitude/longitude (in degree decimal format, e.g., “36.9503, -122.0576”)
  - d. Individual # (use unique number for each individual butterfly so that we can distinguish all tissue samples from each other; e.g., for the first butterfly caught at Natural Bridges, CA, in 2021, by W. Chris Funk the code could be something like “WCF-NBCA-2021-01”)
  - e. Date
  - f. Your name
3. Put butterfly in glassine envelope, and then put envelope in freezer box. Then, put freezer box in **two** plastic Ziploc bags (to keep box dry; you DON’T want water to seep in and soak the samples), which can then be temporarily stored in a cooler with ice (normal ice or ice pack are both fine) while traveling in the field.
4. Record data for each butterfly on Excel datasheet (if you prefer, you can print out datasheet and write on them in the field). In the end, will need both printouts of data (hardcopies) AND filled out Excel spreadsheets.
5. When you return from the field, keep samples in a freezer.

**Monarch storage and shipping to the Funk lab at Colorado State University:**

1. Label tissue storage boxes with species name, locality(ies), lat/long coordinates, collector names, and date(s)
2. Tissue should be stored in a freezer for long-term storage
3. Before shipping samples to Chris Funk at Colorado State University, please email both Chris ([Chris.Funk@colostate.edu](mailto:Chris.Funk@colostate.edu)) and Hillary Sardiñas ([hillary.sardinias@wildlife.ca.gov](mailto:hillary.sardinias@wildlife.ca.gov)) to let them know that you have collected samples and when you plan on shipping your samples.
  - a. Also, *please email Chris and Hillary the Excel datasheet(s) for tissues in the same email*
4. ***Please follow the Packaging details and Shipping information specified below.***
5. ***Packaging details:***
  - a. Make sure each glassine envelope is labeled as described above.
  - b. Put glassine envelopes with adult specimens in a labeled cardboard freezer box (label the box with your name, date, species, and locality info). Put freezer box in two Ziploc bags (double-bagged).
  - c. Put this freezer box in a polystyrene (Styrofoam) cooler with regular ice packs to keep cool during shipment.
  - d. Styrofoam cooler should then be put in a cardboard box for shipment (you cannot ship the Styrofoam cooler without first putting it in a cardboard box).
  - e. Put hardcopy of printed datasheet in box with specimens (also email Excel spreadsheet datasheet to Chris Funk; see contact info above).
  - f. Then add a piece of paper that says “Scientific research specimens, not restricted, Special Provision A180 applies.” Add this inside the shipping container. You can also tape this label to the outside of the box.
6. ***Shipping details:***
  - a. Ideally ship with a courier such as FedEx. Ship overnight or maximum of two-day shipping.

- b. Make sure you **ship at the beginning of the week** (ideally Mon or Tues, maybe Wednesday) to make sure they don't get stuck in transit over the weekend.
- c. Address:  
Chris Funk  
Department of Biology  
Colorado State University  
1878 Campus Delivery  
Fort Collins, CO 80523-1878
- d. Email: [Chris.Funk@colostate.edu](mailto:Chris.Funk@colostate.edu)
- e. Phone number: 970-491-3289