



Monitoring

Monarch Butterflies

and Their Habitat across North America

Inventory and Monitoring
Protocols and Data Standards
for Monarch Conservation

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For more information:



Commission for Environmental Cooperation

393, rue St-Jacques Ouest, bureau 200

Montreal (Quebec)

H2Y 1N9 Canada

t 514.350.4300 f 514.350.4314

info@cec.org / www.cec.org



TRINATIONAL
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List of Abbreviations and Acronyms

AGOL	ArcGIS (Geographic Information System) Online
C	carbon
CEC	Commission for Environmental Cooperation
CMCSP	Canadian Monarch Conservation Science Partnership
Conabio	<i>Comisión Nacional para el Conocimiento y Uso de la Biodiversidad</i> (National Commission for the Knowledge and Use of Biodiversity) (Mexico)
Conanp	<i>Comisión Nacional de Áreas Naturales Protegidas</i> (National Commission of Protected Natural Areas) (Mexico)
CRP	Conservation Reserve Program (US)
ECCC	Environment and Climate Change Canada
GRTS	generalized random tessellation stratified
H	hydrogen
IA	Iowa
IUCN	International Union for the Conservation of Nature
LIDAR	light detection and ranging
MCSP	Monarch Conservation Science Partnership (focused on eastern monarch population) (US)
MI	Michigan
MJV	Monarch Joint Venture
MLMP	Monarch Larva Monitoring Project
MW	Monarch Watch
NFWF	National Fish and Wildlife Foundation (US)
NRCS	Natural Resources Conservation Service (US)
NWR	National Wildlife Refuge (US)
Oe	<i>Ophryocystis elektroscirrha</i>
PNAMP	Pacific Northwest Aquatic Monitoring Partnership
PROFAUNA	<i>Protección de la Fauna Mexicana AC</i> (Protection of Mexican Fauna AC)
SD	South Dakota
Semarnat	<i>Secretaría de Medio Ambiente y Recursos Naturales</i> (Ministry of the Environment and Natural Resources)
SOP	standard operating procedure
Sr	strontium
SSA	species status assessment
SWMS	Southwest Monarch Study
TAP	Texas Advisory Panel
TMCSP	Trinational Monarch Conservation Science Partnership
TX	Texas
UMN	University of Minnesota
UNAM	<i>Universidad Nacional Autónoma de México</i> (National Autonomous University of Mexico)
UNC	University of North Carolina
US FWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
WI	Wisconsin
WMCSP	Western Monarch Conservation Science Partnership (focused on western monarch population)
WMTC	Western Monarch Thanksgiving Count
WSSU	Winston-Salem State University
WWF Mexico	World Wildlife Fund Mexico

Abstract

As part of the Commission for Environmental Cooperation's project *Monarch Butterfly Flyway: Communication, Participatory Conservation, and Education Programs throughout the Migratory Route*, a workshop entitled *Monitoring Monarch Butterflies and their Habitat across North America* was held in Toluca, Mexico, on 4–5 March 2017. Monitoring experts from Canada, Mexico and the United States gathered to identify trilateral inventory and monitoring priorities. The main objectives were to: 1) identify shared monitoring priorities for Canada, Mexico and the United States, 2) discuss inventory and monitoring protocols for monarch butterflies and their habitat that are currently being implemented, compare, and where appropriate, outline steps for aligning monitoring activity methodology and reporting standards, and 3) establish shared monitoring action plans and set timelines. Shared monitoring priorities were identified and challenges, recommendations and next steps discussed.

Acknowledgements

We are grateful to all workshop attendees whose dedication to monarch conservation made this workshop a success. In particular, we would like to thank Gloria Antúnez and Teresa Flores for expert interpretation services. All participants are deeply appreciative of the ejidos San Antonio and San Francisco for their generosity in sharing the Las Palomas Colony with workshop attendees. Words cannot express the depth of our gratitude for this transformative experience. We also would like to thank Felipe Martínez for a field demonstration of measuring overwintering colony area, and Alfonso Alonso, Marco Castro, Greg Mitchell and Maxim Larrivée for their contributions. Finally, we wish to acknowledge the many citizen scientists who have contributed invaluable data to numerous monitoring programs; these data have formed the foundation of national monitoring efforts and analyses in progress.

Executive summary

Monitoring both habitat availability to a species and its response to threats enables conservation professionals to evaluate and improve their conservation actions. However, establishing the status and population trends of a species requires capturing complex information, at large temporal and spatial scales. Therefore, it is a challenge to develop comprehensive monitoring strategies for migratory species when those species traverse ecological and political boundaries.

In North America, monarch butterflies (*Danaus plexippus*) embark on an annual migration cycle that spans up to three countries, involving four generations of the species. We owe much of our understanding of monarch life-history to a rich array of citizen-science and other monitoring programs that have collectively unraveled this migratory phenomenon. Data gathered by these programs indicate that monarchs have significantly declined in the last 20 years. This decline has sparked trilateral concern and highlighted the need for additional monitoring data to generate science-based conservation recommendations. To address this need, topic experts from Canada, Mexico and the United States gathered in Mexico City (February 2016) and formed the Trilateral Monarch Conservation Science Partnership (TMCSP), which is focused on shared research and monitoring priorities, as part of the Commission for Environmental Cooperation's (CEC) 2015–2016 project *Monarch Butterfly Flyway: Communication, Participatory Conservation, and Education Programs throughout the Migratory Route*.

Following its inaugural meeting, a subset of the TMCSP convened in Toluca, Mexico (March 2017) to discuss shared monitoring priorities. Workshop goals included: 1) identifying trilateral monitoring priorities, 2) discussing inventory and monitoring protocols for monarch butterfly life stages, and monarch habitat, and 3) determining monitoring action plans across North America.

This report compiles products of the workshop, including trilateral monitoring priorities, an inventory of monitoring protocols produced to date, initial steps toward coordinating trilateral monitoring efforts, and recommendations for anticipated challenges.



1

Summary of monarch reproduction and migration

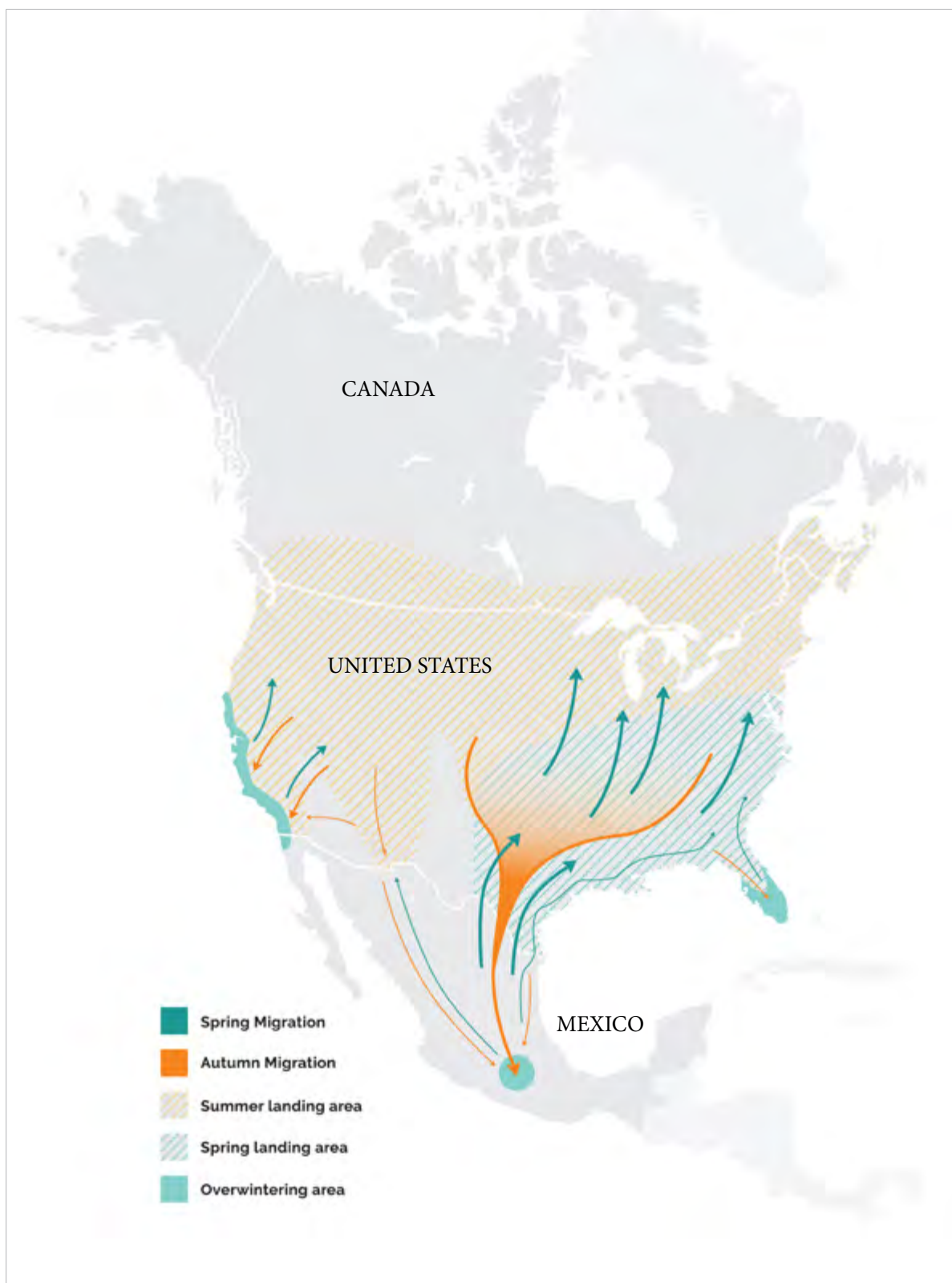
Monarch butterfly reproduction and migration in North America are tightly interwoven. After emerging as adults, non-migratory monarchs mature for 3–8 days before mating (with the exception of the generations that overwinter in Mexico). Mated female monarchs will immediately seek out and begin laying their eggs on milkweed plants. To avoid competition between offspring, a female usually lays only one egg per milkweed plant. Females lay an average of ~700 eggs during their adult lifespan (2–5 weeks). After 3–8 days (depending on temperature), the eggs hatch and the caterpillars feed on the milkweed plant tissue, passing through five growth stages (instars), with a molt between each stage. After completing its 5 instar stages (9–14 days), each monarch caterpillar generally crawls away from its host milkweed plant and seeks a sheltered location (frequently the underside of a leaf or stem of another plant) to pupate. Leaving their host plant to pupate may offer some protection against predators and parasitoids, though this requires further study. After approximately 1–2 weeks as a chrysalis, the adult butterfly emerges. Butterflies feed on nectar from a variety of blooming plants, and their adult life-span and migratory behavior depend on the time of year (UMN Monarch Lab 2016).

Though genetic studies demonstrate that the North American monarch population is panmictic (Lyons et al. 2012), monarchs can generally be partitioned into eastern and western groups (segregated by the Rocky Mountains). For the purposes of this report, we will refer to them as eastern and western populations (Figure 1).

East of the Rocky Mountains, monarch butterflies overwinter in dense colonies in the oyamel fir forests of central Mexico (UMN Monarch Lab 2016). In the spring, butterflies depart from their overwintering grounds and migrate into the north of Mexico and southern United States, seeking milkweed. The subsequent generation migrates into the northern half of the United States and southern regions of Canada. There, the monarch population is amplified by a 3rd generation, and finally a 4th generation, which emerges in the late summer to early fall. Shortening days and other cues trigger physiological and behavioral changes in these adults, which do not mate but instead begin migrating south. On their approximately 4,000-kilometer (2,500-mile) flight to Mexico, these butterflies feed on nectar plants, building the majority of their fat reserves near the end of their migration (Brower et al. 2006). Upon reaching overwintering grounds in Mexico in the fall, butterflies form dense colonies that expand and contract with temperature (Thogmartin et al. 2017). Monarchs do not feed while overwintering, but clusters may temporarily disband when butterflies seek water. Lengthening days and warmer temperatures signify the arrival of spring and availability of milkweed, releasing monarchs from their overwintering state and beginning the annual cycle anew (UMN Monarch Lab 2016).

West of the Rocky Mountains, monarchs overwinter in groves along the California coastline, with spring and summer breeding activity in areas to the north, east and south (Pelton et al. 2016). Studies indicate that a small percentage of monarchs from the southwest United States may migrate to Mexico as opposed to California, but additional research is needed (Morris et al. 2015).

Figure 1. Monarch migration



2

Inventory and monitoring priorities established for North America

Newly established in response to declining monarch populations, the Trinational Monarch Conservation Science Partnership (TMCSP) held its first meeting in February 2016, in Mexico City. Recognizing that monarch biology and ecology vary according to spatial and temporal factors, topic experts established seven shared priorities to inform trinational conservation efforts: five priority research topics and two operational priority actions.

2.1 Trinational Research Priorities

1. Estimate overwintering monarch density/abundance
2. Identify monarch natal origins
3. Evaluate threats posed by climate change
4. Evaluate threats posed by pesticide exposures
5. Evaluate the availability of nectar resources

2.2 Trinational Operational Priorities

1. Implement integrated or compatible monitoring protocols, using appropriate sampling schemes to address data gaps
2. Design compatible databases to host and share data

Monitoring efforts have the potential to inform, directly or indirectly, all research priorities. Furthermore, both operational priorities are germane to establishing integrated monitoring practices that ensure collected data are compatible across geographic regions and are accessible to all parties.

In 2017, a subgroup of the TMCSP met in Toluca, Mexico, to discuss ongoing monitoring efforts that relate to priority research topics and operational actions. Two research topics were discussed: estimating overwintering monarch density, and identifying monarch natal origins. These monitoring efforts require access to state-of-the-art technology. Operational actions necessary to share monitoring protocols and to set data standards for these research priorities are relatively straightforward. Additional monitoring efforts to evaluate the status and trends of monarchs and their habitat across their trinational range, including estimation of summer monarch breeding population sizes, were also discussed. Because gathering spatially representative data about monarchs and their habitat across their North American range requires the participation of many partners, including citizen scientists, operational actions required to standardize monitoring protocols and sampling designs and to develop compatible data management and sharing of practices are more complex. Key meeting decisions along with anticipated challenges and recommendations for next steps and timelines are included in Appendix A.



Monarch butterfly experts during a visit to an overwintering site in Mexico in February 2016.

2.3 Key workshop decisions

- **Monitoring protocols will be shared.** The three countries will share existing monitoring protocols and identify points of contact for protocols.
- **Common data standards will be adopted and data will be made available.** As protocols are further developed and data collection infrastructures are built, common data standards must be adopted for shared monitoring attributes, to ensure data compatibility across monitoring efforts. The Darwin Core standards developed by Biodiversity Information Standards are recommended (Biodiversity Information Standards 2009). Properly formatted data should be uploaded to publically accessible databases or made available for download from program websites. Additional communication between protocol developers from each country will be necessary for establishing these standards and ensuring database compatibility.
- **A trinational, spatially balanced sampling scheme will be made available.** A prioritized, spatially balanced sampling framework (generalized random tessellation stratified [GRTS] draw) is important for interpreting collected monitoring data. The United States Geological Survey (USGS) will work with identified topic experts from each country to identify appropriate monarch strata and expand the current US-based GRTS draw to the monarch's full trinational range.
- **A trinational "Monarch Data Blitz" will be launched.** To address data gaps, Canada, Mexico and the United States will promote a citizen science-based "Monarch Data Blitz" campaign (see Appendix B). Intensively gathering additional breeding data at critical windows of time across the monarch's range will provide a valuable snapshot of monarch population status, something that has never been done at a continental scale. It will also engage thousands of citizens, reconnecting them with nature and raising awareness of the migratory monarch's situation in North America. Additional communication among identified parties will be necessary to coordinate this effort.
- **Mexico will form a joint venture:** Mexico is currently evaluating opportunities to form a joint venture.

3

Estimating monarch overwintering density

Monarchs annually migrate to overwintering grounds in central Mexico and along California's coastline (see Section 1 and Figure 1). There, monarchs seek out forested microclimates and form dense clusters or colonies on trees. This annual gathering presents not only a beautiful visual spectacle but also a regular opportunity to estimate monarch population size, since individuals formerly scattered across vast North American tracts coalesce in small and generally predictable locations. Starting in 1993, the *Comisión Nacional de Áreas Naturales Protegidas* (Conanp) and World Wildlife Fund Mexico (WWF) began estimating colony sizes in Mexico by measuring the area (hectares) occupied by each colony (Appendix C). Likewise, the citizen science-driven Western Monarch Thanksgiving Count (WMTC) began estimating California colony sizes in 1997 (Xerces Society for Invertebrate Conservation 2017). WMTC participants estimate colony size by counts because monarchs overwinter in smaller numbers in California than in Mexico.

Records from both overwintering monitoring programs indicate that monarchs have declined over the last 20 years, and these data have significantly contributed to published and ongoing population viability assessments (PVAs) for eastern and western monarchs (Pelton et al. 2016; Schultz 2016; Semmens et al. 2016). However, while extremely valuable, these data have limitations. Since eastern colony sizes are estimated by measuring occupied areas, these values do not provide density information. Over the course of a single winter, monarch colonies may expand or contract depending on abiotic factors such as temperature. Furthermore, as the monarch population declines, it is unknown whether density within colonies has remained constant (Thogmartin et al. 2017). Potential interactions between monarch colony size and density limit interpretation of existing population extinction probability and habitat conservation targets (Pleasants 2016; Semmens et al. 2016) (Appendix C). Though western colony sizes are estimated by counts, variation in annual monitoring effort also complicates interpretation of results.

To obtain more accurate data on overwintering colony density, the TMCSP has collaborated with researchers from the Winston-Salem State University (WSSU) to explore the use of terrestrial light detection and ranging (LiDAR) and other innovative technologies, like thermography and high-resolution videography, to estimate the volume of monarch clusters and link volume estimates to individual object-based models. LiDAR is a remote sensing technology that scans object surfaces with a pulsed laser. Laser pulses are reflected from target surfaces back to a sensor; temporal delays in laser pulse emission and return can be used to measure distances between the target surface and the laser (Heidemann 2014). Resulting point clouds are used to create three-dimensional digital images of scanned surfaces. These data can be evaluated alongside thermal imagery and slow-motion videography to evaluate behavioral patterns and model complex geometry at various scales.

3.1 Key points

- Feasibility of scanning monarch overwintering colonies with LiDAR was tested at select locations in both Mexico and California in 2016.
- Preliminary results suggest that monarch spectral properties allow digital separation of monarchs from underlying substrate.



- Automated computer vision and metrological analytical approaches will be developed to either estimate colony volume and/or count individual monarchs within clusters.

3.2 Main challenges and recommendations

- In order to obtain accurate LiDAR readings, colonies must be calm. Any disturbance, such as motion caused by wind, distorts data collection. Other environmental conditions (e.g., fog) may also interfere with readings.
 - This challenge is not easily addressed. The best solution thus far has been to scan colonies on windless, clear mornings when monarchs are not active (as temperatures rise during the day, monarchs may become active).
- While individual monarchs can be distinguished from background substrate, the best analytical approach to estimating colony volume and/or individual counts must be developed. Potential mathematical approaches include:
 - *Subtractive volume estimation:* Using this methodology, LiDAR colony scans are compared with reference scans (underlying strata when monarchs are absent) and differences in surface area allow estimation of colony volume. This analytical approach may need to account for different positions of underlying strata in space when monarchs are present versus absent. For example, large clusters of monarchs may weigh down branches, which will rebound to former heights when monarchs depart. Metrics will be needed to convert colony volume estimates to monarch count estimates.
 - *Geometric approach:* Using this methodology, LiDAR colony scans are fused with digital camera imagery, and recognition software is used to count individuals.
- LiDAR equipment and data processing are expensive.
 - Though funding availability was not addressed in this workshop, it was generally felt that if LiDAR could yield high-quality density information, then the expense may be justified. At minimum, LiDAR and other technologies may help to validate assumptions and/or evaluate variability of densities; whether such applications will be feasible as a census tool is unknown.

3.3 Points of Contact

- Ryan Drum (US FWS, United States)
- Nickolay Hristov (WSSU, United States)
- Leonel Ruiz Paniagua (Conanp, Mexico)
- Gloria Tavera (Conanp, Mexico)

4

Identifying the monarch's natal origins

To identify effective conservation actions and strategically allocate limited conservation funds for monarchs, it is important to know which portions of the monarch's breeding range support the largest portion of the overwintering monarch population (see Section 1, above). Historically, two different monitoring methods have been used to determine the natal origins of successful migrants: tagging and isotopes.

Today, there are two major citizen-science tagging programs in North America: Monarch Watch (MW) tracks eastern monarchs, while the Southwest Monarch Study (SWMS) tracks movement of the migratory generation emerging in the southwestern United States (University of Kansas 2017; Southwest Monarch Study 2010). Monarch tagging involves either rearing or capturing a migratory monarch and placing a sticker with a unique code on one of the monarch's wings before release. The location of the tagging event and the sticker code are recorded and reported. If the tagged monarch is sighted or recovered at a later point en route to a colony or at a colony, the monarch's unique code and location are reported. Comparing the number of tagging events in breeding grounds to tag recoveries at overwintering grounds can reveal information about monarch migratory pathways and relative contributions of different breeding regions to overwintering colonies.

Monarch tagging studies have yielded valuable information about successful migratory monarchs' natal origins and migratory corridors and have highlighted knowledge gaps where more information is needed. For example, data from the SWMS indicate that some monarchs from Arizona migrate to California while others migrate to Mexico (Morris et al. 2015). Additional studies are needed to further characterize factors (including wind) that influence individual migrants' trajectories and to quantify the volume of exchange between western and eastern monarchs through southwest migratory corridors.

Isotope analysis offers a complementary, chemical approach to determining monarch natal origins, with the added benefits of only having to capture an adult monarch once and being able to glean information from adult monarchs that have died. Stable isotopes ("atomic versions" of the same element with different numbers of neutrons) occur in documented gradients across North America. Organisms naturally incorporate isotopes of select elements into their biological tissue, imprinting that tissue with that geographic region's isotope signature. Thus, chemical determination of isotopes ratios present in an organism's tissues can reveal information about the geographic origins of where the tissue was grown (Hobson 1999; Rubenstein and Hobson 2004). For example, inert hydrogen isotopes present in monarch wing tissues reflects the hydrogen isotope signature in the plant tissue on which the caterpillar developed and fed, which in turn reflects the hydrogen isotope signature of the ground water, which varies predictably with latitude across North America.

A seminal isotope analysis published in the late 1990s indicated that approximately 50% of monarchs collected from an overwintering colony in Mexico originated from the US Corn Belt region, pointing to the importance of breeding habitat in the north-central United States (Wassenaar and Hobson 1998). However, since Wassenaar and Hobson's analysis, the advent of herbicide-tolerant crops has significantly reduced milkweed prevalence in the north central United States. This loss of milkweed from the Corn Belt landscape has been linked to declines in annual overwintering colony sizes in Mexico (Pleasants and Oberhauser 2013; Flockhart et al. 2015).



In addition to changes in agricultural practices, land development and climate change may have also affected the distribution and availability of monarch habitat (including milkweed and blooming nectar plants) since Wassenaar and Hobson's study. Researchers recently analyzed H and C isotopes from monarchs collected in Mexico over 40 years (Flockhart et al. 2017). Interestingly, their results indicate that overall, despite large-scale changes in land-use practices and land cover, the US Corn Belt region still produces the largest number of monarchs overwintering in Mexico, relative to the number from other breeding regions, and that while there is significant inter-annual variation in the contribution of eastern breeding regions to overwintering colonies in Mexico, annual variation is primarily explained by climate patterns. However, this study was conducted with a limited number of samples. Furthermore, most samples were collected from one colony, limiting inference about natal origins at other colonies and our understanding about potential shifts in the relative contribution of breeding monarchs from different regions on the wintering grounds as a whole. Further research is needed.

4.1 Key points

- Protocols should be established for the annual collection of monarchs at overwintering locations throughout the wintering period.
 - *Questions that can be answered by collecting these samples include the following:*
 - Do the relative proportions of monarch natal origins as determined from monarchs collected at overwintering colonies remain constant through time?
 - Are the relative proportions of monarch natal origins constant across wintering colonies?
 - Is there an interaction between monarch natal origins, sex and mortality?

- *Suggested protocol steps are as follows:*
 - A minimum of 200 dead monarch samples should be collected from the two or three colonies that are monitored for the entire winter by Conanp (i.e., 400 – 600 samples per year).
 - 50 samples (half males and half females) should be collected during the last week of monitoring in each month (50 samples collected once a month * 4 months * 3 colonies = 600 samples).
 - The collection of more than 200 samples is encouraged. For example, collecting 100 samples every month would reduce variability in assignments of breeding origins.
 - Catastrophic mortality events due to weather for example should be exploited opportunistically.
 - Samples can be collected in bulk (i.e., without any individual sorting or in envelopes and later sorted prior to shipment).
 - At all other colonies visited by Conanp for estimates of wintering area, a minimum of 100 samples are collected per year.
 - All analyses of monarch wing tissue for isotope ratios should be conducted using compatible standards (CBS, KHS¹) the comparative equilibration approach to reporting data, and be measured using high temperature glassy carbon combustion (~1350 to 1450°C). If other approaches are used, it will result in non-comparable data across studies.
- Future isotope analyses have the potential to address fundamental questions about monarch natal origins that are germane to developing conservation strategies. These questions include the following:
 - What is the relative contribution of different breeding regions in North America and how have these contributions changed through time?
 - How will the contribution of geographic regions change in the future, due to climate change?
 - Do monarchs' natal origins correspond to other physiological attributes potentially linked to survival (e.g., monarch size, coloration, parasite loads, fat content, sex ratios, genetic traits, etc.)?
- Long-term data collection will support collaborations across Canada, Mexico, and the United States.
- As the relative contributions of different geographic regions to overwintering populations are clarified by future isotope analyses, it may be possible to make inferences about the amount of breeding habitat (milkweed) present on the landscape. Combined with other monitoring procedures, these inferences might aid in the Species Status Assessment (SSA) process.

4.2 Main challenges and recommendations

- Permits are needed in order to collect monarchs from overwintering grounds in Mexico. (Mexico is prepared to help obtain required permits for dead butterflies.)
- Additional communication between Canada and Mexico is needed to devise a representative sampling scheme within and between overwintering colonies and to delineate a collection protocol in the case of severe weather events. In the meantime, Canada will share proposed methodology with Mexico.
- Incorporation of additional stable isotopes (e.g., strontium [Sr]) can improve the resolution of natal origin inferences. (However, before Sr can be incorporated into the analyses, its gradients across the landscape must be established. Furthermore, additional calibration tests must be conducted to determine the relationship between Sr concentration in milkweed and in the tissue of caterpillars feeding on milkweed.)

4.3 Points of contact

- Greg Mitchell (ECCC, Canada)
- Keith Hobson (ECCC and Western University, Canada)
- Gloria Tavera (Conanp, Mexico)
- Ryan Drum (US FWS, USA)

1. CBS (also known as EC-01) – Caribou hoof standard and KHS (also known as EC-02) – Kudu horn standard.



Monterey Bay, California, USA - December 5, 2011.
An orange monarch butterfly caution sign alerts
visitors at the Pacific Grove forest where thousands of
migrating monarch butterflies cluster together.

5

Evaluating the status and trends of monarchs and their habitat, and setting prioritized, spatially balanced site selection methodology and common data standards

Refining estimates of monarch overwintering population sizes by gathering colony density information (see Section 3) and identifying the natal origins of migratory monarchs through isotope analyses (see Section 4) will aid in developing trinational, science-based conservation recommendations. However, both overwintering-population size measurements and isotope analyses focus on the migratory monarch generation produced each year, and as such do not focus on place-based threats throughout migration flyways or on threats and population bottlenecks that might be occurring in earlier generations. To better explain inter-annual variation in monarch overwintering numbers, it is important to understand the distribution and abundance of monarchs and their habitat throughout the monarch's entire annual cycle (see Section 1). Tracking the status and trends of monarchs and their habitat across their North American range, however, is logistically challenging.

Monarchs are iconic pollinators with fascinating life histories, and thus are well represented by citizen-science programs in Canada, Mexico, and the United States. Different programs track different aspects of monarch life history and habitats. Together, these monitoring programs offer a collective portrait of monarch biology and population health and have made significant contributions to peer-reviewed literature (Ries and Oberhauser 2015).



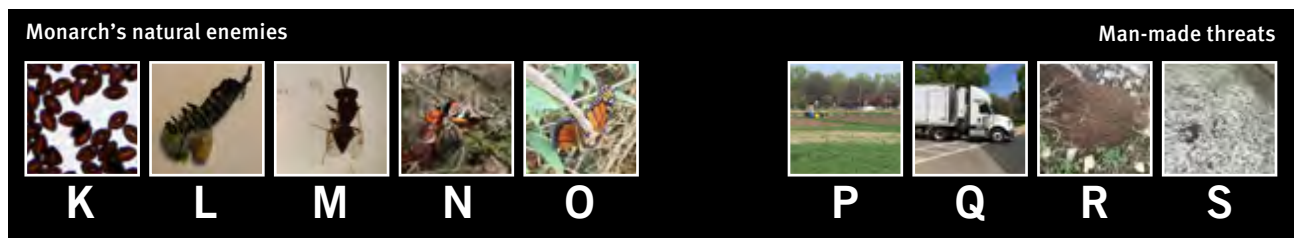
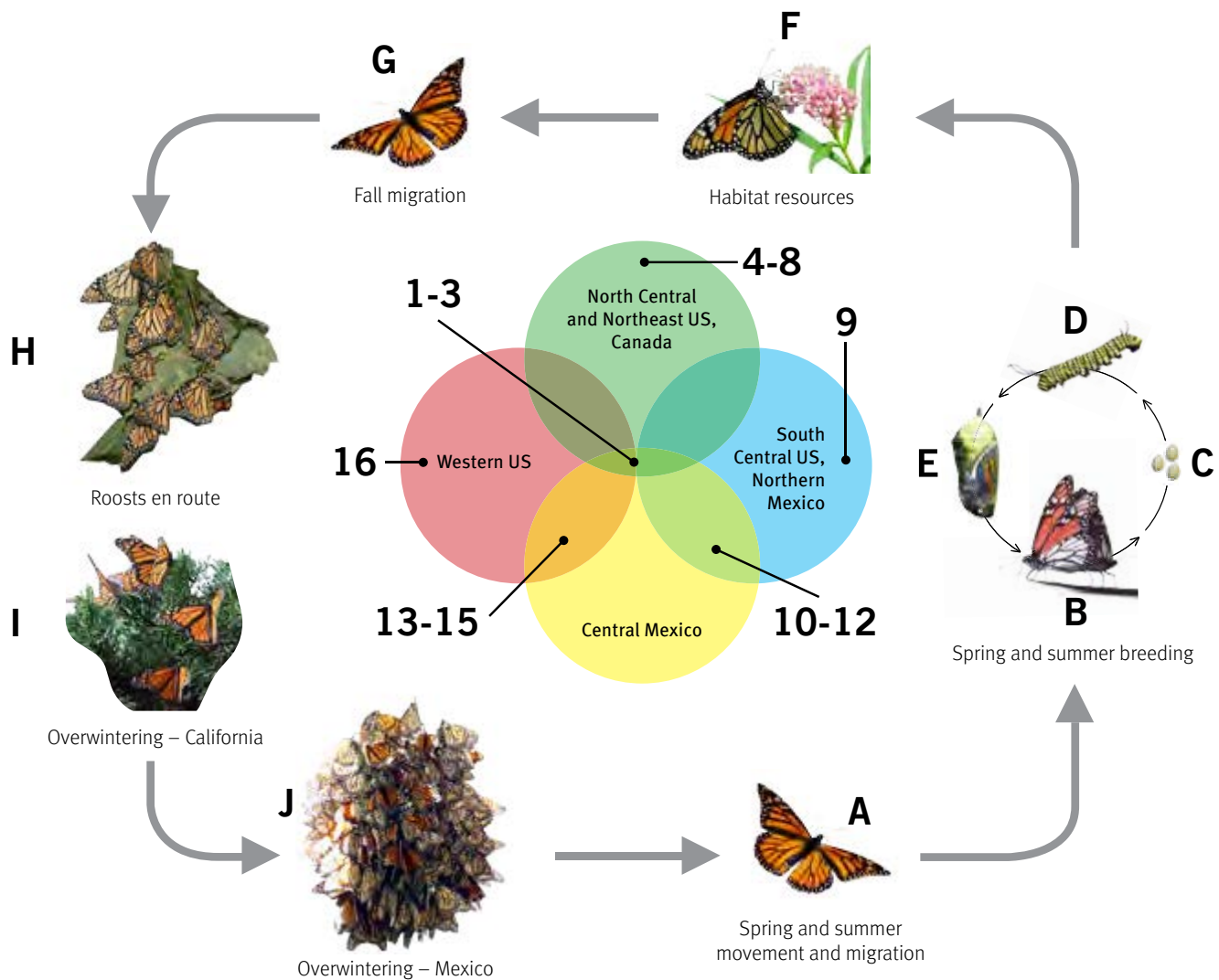
However, there are limitations to collecting data through current programs. First, sampling is not random; participants frequently monitor where they are likely to encounter monarchs, complicating extrapolation of data across relevant geographies that are not subject to equal observational scrutiny. Second, these programs do not monitor all attributes pertinent to monarchs and their habitat. For example, existing citizen-science programs do not rigorously monitor relative abundance of blooming nectar plants, or additional threats such as fire ants. Finally, depending on data collection methods, it may be difficult to combine data across programs, limiting interpretation of results. Canada, Mexico, and the United States have each launched national-level efforts to address these data gaps (see Appendix C). However, since both the biogeographical and the pre-existing citizen-science landscapes vary between and within countries, selected monitoring attributes, associated protocols, and sampling schemes are not always standardized. Furthermore, different means of reporting and storing data are available, depending on the monitoring program. In order to align trilateral monitoring efforts, it is important that the three countries adopt compatible protocols and data standards for shared monitoring attributes, utilize a spatially balanced sampling framework and, finally, maintain compatible and accessible databases.

5.1 Key points

- **Ongoing national monitoring efforts:** During the workshop, a representative from each country made a presentation on the current status of national monitoring programs and associated analyses. Summaries of national monitoring activities and analyses for each country are given in Appendix C. A comprehensive summary focused on the rich contributions of all active monitoring programs and ongoing analyses in each country is beyond the scope of this report. These status summaries focus only on analyses and monitoring activities related to national monitoring efforts.
- **Priority monitoring questions:** During presentations and following conversations, each country identified key questions that could be addressed with additional monitoring data (summarized in Figure 2).
- **Spatially balanced sampling:** The United States is currently testing the application of a spatially balanced, prioritized sampling scheme (GRTS draw) for sampling monarch-relevant strata base on their attributes. Because GRTS draw sampling schemes assign weights to all possible monitoring sites, data from any location designated as habitat can be incorporated into an analysis, though data collected from priority sites will strengthen results. The United States has generated a GRTS draw for the eastern monarch range (incorporating six strata: protected grasslands, unprotected grasslands, CRP lands, cultivated fields/orchards, right-of-way habitats, urban/suburban spaces) and is currently consulting topic experts to identify additional strata for the western monarch range. The GRTS draw can be expanded internationally if relevant land-cover strata are identified; all three countries agreed to explore the possibility of expanding the draw in Mexico and Canada (supported by USGS). Additional communication among the three countries will be necessary to coordinate this effort.
- **Monarch Data Blitz:** Intensively gathering monarch breeding data at critical time windows across their range will provide a valuable snapshot of monarch population status. Canada successfully promoted a Monarch Data Blitz (through the Mission Monarch platform) in 2016 and suggests expanding this effort trilaterally (see Appendix B). Additional communication among identified parties will be necessary in order to coordinate this effort.

The annual monarch life cycle is depicted in the outer ring of Figure 2. In the spring, adult monarchs depart from their overwintering grounds (**A**). Adult monarchs mate either as they leave their overwintering grounds or en route to spring breeding grounds (**B**). Adult females lay eggs on milkweed plants (**C**). Caterpillars emerge from the eggs (**D**) and pupate (develop) into adults (**E**). Additional generations of adult monarchs emerge, depending on habitat resources, climate, and other environmental factors (**F**). In late summer to fall, adult monarchs migrate (**G**), sometimes forming roosts en route (**H**) to their overwintering grounds in California (**I**) and Mexico (**J**). Stressors, including monarch natural enemies [**K**] parasites, [**L, M**] parasitoids, [**N, O**] predators and [**P**] man-made threats (habitat destruction and pesticide application, [**Q**] traffic collisions, [**R**] invasive species, [**S**] climate change may affect the survival and fitness of monarchs across time and space.

Figure 2. Annual monarch life cycle, threats and associated priority trinational monitoring questions



Source: CEC workshop summary notes. Images reproduced with permission. (K) Protozoan parasite (*Ophryocystis elektroscirrha*) spores (Sonya Altizer); (L) Tachinid fly larvae emerging from monarch caterpillar (Cindy Stone); (M) Adult parasitoid wasp (UMN Monarch Lab); (N) Grosbeak eating adult monarch in Mexican overwintering colony (Hayley Schroeder); (O) Praying mantis with captured adult monarch (Charlie Gatchell); (P) Cultivated field (Holly Holt); (Q) Trucks (and other vehicles which may cause adult monarch roadside mortality) (Holly Holt); (R) Red imported fire ant (*Solenopsis invicta*) mound (Kristen Baum); and (S) Climate change (Holly Holt).

The geographic location of priority monitoring questions is indicated within the central Venn diagram in Figure 2, with full questions listed below. This list of monitoring questions is not comprehensive; rather, it reflects priority national and trinational monitoring questions.

1. What is the distribution and abundance of eggs, caterpillars and adult monarchs and how do these vary with season, habitat, climate, landscape context and land-use practices?
2. What is the distribution and phenology of monarch habitat resources (e.g., milkweed, blooming nectar plants, riparian corridors) and how do they affect monarch migration, breeding and survival? Where are habitat-resource limiting factors and when do they occur?
3. What is the prevalence and incidence of monarch natural enemies and man-made threats and how do these factors interact with season, habitat and resource availability, to affect monarch survival and fitness?
4. What are the regional contributions of “Canadian-born” and “US-born monarchs” to overwintering colonies in Mexico?
5. When and where is milkweed a limiting factor for monarch reproduction?
6. How does the abundance and timing of monarch arrival and departure in these regions change on an annual basis?
7. Is there potential for phenological mismatches between habitat resource availability and monarch spring and summer movement, breeding, and fall migration?
8. Is there potential to expand the northern range of milkweeds and monarchs? Is there a northern limit or maximum distance from the overwintering grounds for summer-breeding individuals?
9. What are the distribution, abundance and phenology of milkweed species (native and non-native) and where and when does milkweed contribute to spring and/or fall breeding, especially in Northern Mexico?
10. What are the migratory pathways for spring and fall migrants?
11. Which plant species are used for roosts by fall migrants? What portions of the plants do monarchs roost on?
12. What is the magnitude of predation pressures and man-made threats (e.g., traffic collisions, pollution) in migratory corridors and on the overwintering grounds?
13. How can we estimate overwintering monarch colony size and density?
14. How can we increase monitoring efforts to capture data for new overwintering monarch colonies or colonies that we are not yet monitoring?
15. How much exchange is there between eastern and western populations?
16. What are the natal origins of monarchs overwintering in California?

Based on priority monitoring questions symbolized in Figure 2, each country listed attributes that it is currently monitoring, with associated monitoring organizations and protocols (Table 1). An initial inventory of monitoring protocols and associated materials is included on page 43. Countries agreed to adopt or adapt existing protocols, as appropriate. Attributes of greatest trinational interest included:

- adult monarch density and phenology,
- monarch egg and larval density,
- nectar plant availability (by species), and
- milkweed presence and/or density (by species).

Table 1. Monitoring organizations contributing data to national monitoring efforts

Attribute	Monitoring organizations		
	Canada	Mexico	United States
Adult monarch density and phenology	eButterfly, Bird Studies Canada	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); coordinated by Conanp	MCSP Monitoring Activity 2, North American Butterfly Association 4 th of July Counts, Journey North first occurrence counts, eButterfly
Monarch egg and larval density	Mission Monarch	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); coordinated by Conanp	MCSP Monitoring Activity 3, MLMP
Nectar plant availability and adult feeding preferences	Mission Monarch*	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); coordinated by Conanp, WWF Mexico*	MCSP Monitoring Activity 3, MLMP
Milkweed presence and/or density	Mission Monarch, University of Ottawa	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); coordinated by Conanp (currently records distribution only, interested in density*)	MCSP Monitoring Activity 3
Natal origins (tagging)	Monarch Watch	Conanp*, WWF Mexico* (tagging spring migrants)	Monarch Watch, Southwest Monarch Study
Natal origins (isotopes)	ECCC, University of Guelph, Western University	Conanp, WWF Mexico*	---
Overwintering population area or count	---	Conanp, WWF Mexico	Western Monarch Thanksgiving Count
Overwintering mortality	ECCC, University of Guelph, Western University	Conanp, WWF Mexico	---
Overwintering population density (LiDAR)	---	Conanp, WWF Mexico	University of North Carolina, US FWS, MCSP
Overnight roost observations and preferred host plant	eButterfly	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); coordinated by Conanp	Journey North
Monarch egg and caterpillar survival	MLMP	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); coordinated by Conanp	MCSP Monitoring Activity 4, MLMP
Parasitism (Oe) prevalence	Project Monarch Health	Conanp* (northern Mexico), <i>Correo Real</i> *	MCSP Monitoring Activity 4, Project Monarch Health
Threats (e.g., road collisions, climate, pesticides)	---	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); coordinated by Conanp	---
Predation	---	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); coordinated by Conanp	---
Fire ant density	---	---	MCSP Monitoring Activity 5
Data management	eButterfly	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); coordinated by Conanp	MCSP Monitoring Activity 6

Note: * Indicates an organization that is not currently monitoring designated attribute, but is interested in adapting protocols currently in use in other countries.

5.2 Main challenges and recommendations

- **Sharing existing protocols and setting compatible data standards:** Since monarchs have a complex life history (Figure 2) and many monitoring programs already exist, it is not possible to generate protocols that will be universally applicable. Instead, as each country develops monitoring protocols, we need to ensure that monitoring programs are compatible and to reference each other where appropriate.
 - *Protocols:* Each country will share existing national monitoring protocols in their current form (recognizing that these protocols will evolve with future testing).
 - *Data standards:* Protocol and database developers from each country will identify required data fields and standards for each shared attribute. Adopting Darwin Core standards (TDWG, 2009) is recommended.
- **Developing new, compatible protocols:** Monarchs utilize diverse ecosystems. Thus, existing monitoring protocols may not be equally suited for monitoring in each ecosystem. For example, the importance of riparian corridors has been recognized in the western and southern United States and in Mexico.
 - Representatives from WWF Mexico and the Monarch Conservation Science Partnership (MCSP) have already held preliminary discussions for modifying an existing MCSP protocol, which was developed for use in eastern strata, for use in riparian corridors. As this protocol is developed, it will be shared with all parties, including the Western Monarch Conservation Science Partnership (WMCSPP) and the Texas Advisory Panel (TAP).
- **Establishing a common prioritized, spatially balanced, sampling scheme (GRTS):** Monarchs exploit resources in diverse land strata, and therefore, representatively sampling all relevant strata is a challenge.
 - *Trinational GRTS draw:* The United States will expand its prioritized, spatially balanced GRTS draw to Mexico and Canada. Topic experts have already begun communicating with the USGS to identify appropriate strata and land-cover data for incorporation into the draw.
- **Sharing data:** Data for different programs is currently kept in different databases. Data access will be important for collaborative trinational analyses.
 - Once appropriate data standards have been adopted, formatted data for relevant attributes (e.g., species occurrence) should be uploaded to nationally accessible databases (Mexico National Biodiversity Information System (SNIB) and Mexico National Biodiversity Monitoring Network [SNMB]).
 - Alternatively, appropriately formatted data should be made available for download from an individual program's website (e.g., eButterfly). In the meantime, individuals may request data from program contacts.
- **Implementing monitoring programs:** More data are needed to inform monarch conservation. Gathering these data across the monarch's North American range will require participation of citizen scientists. There are a number of logistical, financial and social challenges to implementing monitoring at this scale.
 - *Participant recruitment and training:* Recruiting participants and providing training require coordination and funding. Each country is working with limited resources and existing recruitment networks.
 - *Participation engagement and retention:* Retaining participants from one season to the next and motivating participants to monitor at key periods is critical. This requires coordination and communication as well as careful identification of critical monitoring periods. For example, asking citizens to monitor at times when they are unlikely to make any observations can lead to program attrition. Some aspects of engagement communication can be automated through app design (e.g., Mission Monarch plans to incentivize data reporting by encouraging participants to reach monitoring targets).
 - *Data integrity and validation:* Submitting photographs and observations to crowd-sourcing and validation by experts can improve data integrity. Explaining to participants the importance of not omitting to report when the finding is "0" is also crucial.

- **Coordinating the Monarch Data Blitz:** Additional communication is needed to coordinate intensive data collection amongst citizen scientists during key monarch breeding windows.
 - Identified parties will communicate about selected monitoring attributes (monarch egg and larval density in a selected milkweed patch, plus random walks to identify additional milkweed plants), timeframes (windows of 3–4 days, as appropriate for each geographic type), data platforms/monitoring programs (likely Mission Monarch, MLMP, Monarch Butterfly National Monitoring Network; *Red Nacional de Monitoreo de la Mariposa Monarca*) and citizen-science engagement (Appendix C).
- **Increasing stakeholder coordination and communication, and addressing misconceptions:** Monarchs have become a flagship pollinator conservation species, drawing the attention of funding agencies, industry, and conservation professionals from federal and state agencies, NGOs and academics. Coordinating research and monitoring, educational outreach, and on-the-ground habitat conservation among veterans and newcomers is challenging.
 - *Joint Ventures:* Coordinating bodies like the Monarch Joint Venture (MJV) for the United States (Appendix C) have an obligation to reduce conservation effort redundancies, and publicize and promote correct information (especially in response to media misinterpretations). Mexico is working on forming its own joint venture and will leverage resources and experiences from existing organizations, including *Red Monarca* (Red Monarca 2017) (Appendix C).
 - *Partner engagement:* When appropriate, the TMCSP should engage representatives from existing citizen-science programs.

5.3 Points of contact

- **Monarch Butterfly National Monitoring Network; *Red Nacional de Monitoreo de la Mariposa Monarca*:** Marco Castro (Conanp, Mexico)
- **MCSP Monitoring Protocol:** Karen Oberhauser (MJV, United States), Wendy Caldwell (MJV, United States)
- **Mission Monarch and eButterfly:** Maxim Larrivée (Montreal Insectarium, Canada)
- **Monarch Data Blitz coordination:** Maxim Larrivée and Sonya Charest (Montreal Insectarium, Canada), Michael Gale (US FWS, United States), Karen Oberhauser (MJV, United States) and Rocío Treviño (*Correo Real*, Mexico)
- **GRTS draw:** Emily Weiser (USGS, United States), Wayne Thogmartin (USGS, United States), Greg Mitchell (ECCC, Canada), Francisco Botello (UNAM, Mexico)
- **Joint Venture formation:** Gloria Tavera (Conanp, Mexico), Rocío Treviño (*Correo Real*, Mexico), Karen Oberhauser (MJV, United States), Wendy Caldwell (MJV, United States)

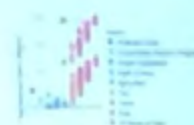
1. Monarch Conservation Target 2. Milkweed Conservation Target 3. Milkweed Storylines Analysis



6 hectares of overwintering monarchs



Add ~1.4 billion stems of milkweed



All hands on deck!

4. Demographic Model



All regions on deck!

5. USGS Conservation Tools



Fractional recommendations

6. Threats Analysis



In progress



Toluca monarch workshop, 4–5 March 2017

Workshop agenda

Monitoring Monarch Butterflies and Their Habitat across North America

4–5 March
Toluca, Estado de México
México

Agenda

As part of the Commission for Environmental Cooperation's (CEC's) project Monarch Butterfly Flyway: Communication, Participatory Conservation, and Education Programs throughout the Migratory Route (Project 13), this workshop will gather monitoring experts from the Trinational Monarch Conservation Science Partnership to identify priorities for the monitoring and inventorying of monarch butterfly life stages and monarch habitat at geographic scales relevant to the conservation of the species.

The objectives of this workshop are as follows:

1. Identify key monitoring priorities in Canada, Mexico and the United States.
2. Discuss inventorying and monitoring protocols for the life stages of the monarch butterfly across North America.
3. Determine inventorying and monitoring priorities and action plans across North America.

Saturday, 4 March 2017

- | | | |
|----|-------------|--|
| | 09:00–09:30 | Coffee and registration |
| 1. | 09:30–10:00 | Introductions, ongoing work and priorities, desired meeting outcomes
Presentation of participants (10 min)
Introduction of CEC projects 12 and 13, and meeting objectives; Georgina O’Farrill (10 min)
Introduction and update, Trinational Monarch Science Partnership; Michael Gale (10 min) |
| | 10:00–10:15 | Coffee break |
| 2. | 10:15–12:30 | Presentations, including brief description of current monitoring protocols and monitoring proposals (2h15 min)
Priorities for monitoring and inventorying monarchs in Canada, Maxim Larrivée & Greg Mitchell (45 min presentation, including questions)
Priorities for monitoring and inventorying monarchs in Mexico, Gloria Tavera & Marco Antonio Castro (45 min presentation, including questions)
Priorities for monitoring and inventorying monarchs in the United States, Holly Holt & Wayne Thogmartin (45 min presentation, including questions) |
| 3. | 12:30–13:00 | Facilitated discussion: Priorities for monitoring and inventorying monarchs in North America (30 min)
Key monitoring and inventorying priorities
Establishing trilateral priorities
Future steps and agreements |
| | 13:00–14:00 | Lunch break |
| 4. | 14:00–15:30 | Facilitated discussion: Priorities for monitoring and inventorying monarchs in North America (1h30 min)
Identifying monitoring goals in North America
Suggested trilateral monitoring protocols
Databases required
Implementation, sampling, communication |
| | 15:30–15:50 | Coffee break |
| 5. | 15:50–17:30 | Conclusions (1h40 min)
Next steps and collaborations
Identification of goals for practical session |

Sunday, 5 March 2017

- | | | |
|----|-------------|---|
| | 07:00–09:00 | Travel to Colonia de Hibernación Oxtotilpan, Temascaltepec, Estado de México |
| | 09:00 | Arrival at Colonia de Hibernación Oxtotilpan |
| 6. | 09:00–13:00 | Practical session at Colonia de Hibernación Oxtotilpan |
| | 13:00–14:00 | Lunch |
| | 14:00–16:00 | Travel to Toluca |
| 7. | 17:00–18:30 | Overview and questions from practical session
General comments
Main challenges
Action plan and timeline
Next steps |



Identifying trinational monitoring priorities during the Monitoring Monarch Butterflies and Their Habitat across North America Workshop, Toluca, Mexico March 2017.

Next steps and timelines identified during the workshop:

- **April 2017:** The Winston-Salem State University (WSSU) LiDAR team will attempt to return to Mexico and California following monarch departure from overwintering grounds, to scan vacated substrate.
- **29 July to 6 August 2017:** Identified parties will communicate and set next steps. These are the projected implementation dates for the Monarch Data Blitz.
- **August 2017:** Greg Mitchell will compile and email to Gloria Tavera recommendations for a monarch collection protocol.
- **May 2017–December 2017:** Gloria and Greg will work with appropriate parties to collaboratively devise a representative sampling scheme for collecting monarchs within overwintering colonies and to delineate a collection protocol in the case of severe weather events. Gloria will obtain necessary sampling permits.
- **January 2018:** Pending analytical results, more LiDAR testing in monarch overwintering grounds may be warranted. If methods are successfully developed, LiDAR and associated high-resolution photography may have future applications not only in annually estimating overwintering density, but also in tracking mortality throughout the course of each winter.
- **May 2017–August 2018:** The WSSU LiDAR team will develop and evaluate analytical approaches (subtractive volume estimation and/or geometric) to estimate monarch colony volume and/or individual counts.
- **GRTS draw:** Identified parties have begun and will continue to exchange information.
- **Monitoring protocol coordination:**
 - *Monarch egg and larval density:* Identified parties (MJV, Maxim Larrivée and Marco Castro) will identify common data fields.
 - *Nectar plant availability:* MJV will share protocols with other identified parties.

Workshop attendees

Attendee	Email	Country	Title	Organization
Maxim Larrivé	maxim.larrivee@ville.montreal.qc.ca	Canada	Head of Entomological Collections and Research	Montreal Insectarium
Gregory Mitchell	gregory.mitchell@canada.ca	Canada	Research Scientist	Environment and Climate Change Canada
Alfonso Alonso	alonsoa@si.edu	Mexico	Field Studies Program Director	Smithsonian Institution
Francisco Botello	franciscobotello@conbiodes.com	Mexico	<i>Investigador, Instituto de Biología</i> (Investigator, Biology Institute)	<i>Universidad Nacional Autónoma de México</i> (National Autonomous University of Mexico)
Marco Antonio Castro Martínez	marco.castro@conanp.gob.mx	Mexico	<i>Coordinador Regional de Iniciativas nacionales e internacionales</i> , Conanp (Regional Coordinator of National and International Initiatives, Conanp)	<i>Comisión Nacional de Áreas Naturales Protegidas</i> (National Commission of Protected Natural Areas)
Diana Caterine Forero Díaz	dcforero@wwfmex.org	Mexico	<i>Coordinadora de Ciencia</i> (Science Coordinator)	World Wildlife Fund Mexico
Felipe Martínez Meza	fmartinez@conanp.gob.mx	Mexico	<i>Director de la Reserva de la Biosfera Mariposa Monarca</i> (Director of the Monarch Butterfly Biosphere)	<i>Comisión Nacional de Áreas Naturales Protegidas</i> (National Commission of Protected Natural Areas)
Eduardo Rendón	erendon@wwfmex.org	Mexico	<i>Coordinador del Programa Mariposa Monarca</i> (Coordinator of the Monarch Butterfly Program)	World Wildlife Fund Mexico
Gloria Tavera	gtavera@conanp.gob.mx	Mexico	<i>Directora, Regional Centro y Eje neovolcánico</i> (Director, Central Region and Neovolcanic Artery)	<i>Comisión Nacional de Áreas Naturales Protegidas</i> (National Commission of Protected Natural Areas)
Rocío Treviño	correo.monarca@gmail.com	Mexico	<i>Coordinador, Programa Correo Real</i> (Coordinator, Royal Mail Program)	<i>Correo Real</i> (Royal Mail), Profauna AC
Michael Gale	michael_gale@fws.gov	USA	Special Assistant, Branch of Policy	US Fish and Wildlife Service
Holly Holt	hholt@umn.edu	USA	Science Coordinator	Monarch Joint Venture
Karen Oberhauser	oberh001@umn.edu	USA	MJV Steering Committee Co-Chair, UMN Professor of Fisheries, Wildlife and Conservation Biology	Monarch Joint Venture, University of Minnesota Monarch Lab
Wayne Thogmartin	wthogmartin@usgs.gov	USA	Research Ecologist	US Geological Survey
Emily Weiser	eweiser@usgs.gov	USA	Biologist	US Geological Survey
Georgina O'Farrill	gofarrill@cec.org		Senior Project Coordinator	CEC

Additional project contacts

Attendee	Email	Country	Title	Organization
Sonya Charest	scharest@ville.montreal.qc.ca	Canada	Staff	Montreal Insectarium
Keith Hobson	khobson6@uwo.ca	Canada	Professor & Research Scientist	Western University, Environment and Climate Change Canada
Leonel Ruiz Paniagua	lpaniagua@conanp.gob.mx	Mexico	Department head	<i>Comisión Nacional de Áreas Naturales Protegidas</i> (National Commission of Protected Natural Areas)
Wendy Caldwell	monarchs@monarchjointventure.org	USA	Coordinator	Monarch Joint Venture
Ryan Drum	ryan_drum@fws.gov	USA	Strategic Habitat Conservation Coordinator	US Fish and Wildlife Service
Nickolay Hristov	hristovni@wssu.edu	USA	Associate Professor	Winston-Salem State University

Appendix B

Monarch Data Blitz



Material developed for the 2017 Monarch Data Blitz.

Why a North American Monarch Blitz?

Data collected by this effort will offer a unique opportunity to estimate the size of the summer breeding population of migratory monarchs across North America. Intensively gathering monarch breeding data at critical time windows across their range will provide a valuable snapshot of the summer monarch population status, something that has never been done at a continental scale.

How to participate in the Blitz?

Go out in as many milkweed patches as possible to count the number of caterpillars you can find on up to 100 milkweed plants.

What's the protocol?

It's easy: Between 29 July and 5 August, anywhere in North America, go to many different types of habitat where you can find milkweed, look for caterpillars on milkweed plants, and tell us how many you found and how many milkweed plants you looked at. Report all your missions on the Mission Monarch website: <www.mission-monarch.org/> (Montreal Insectarium, Ottawa University, et al. 2016).







Current status of national monitoring programs and analyses

C.1 Canada

Introduction

- The Canadian Monarch Conservation Science Partnership (CMCSP) was formed in 2016 and comprises representatives from the academic, nongovernmental, and government sectors across Canada, including the University of Guelph, the University of Ottawa, the University of Calgary, Bird Studies Canada, the Montreal Insectarium, and Environment and Climate Change Canada.
- The CMCSP finalized its list of current Canadian research and monitoring priorities in 2017.
- Monitoring of monarchs in Canada is primarily achieved through citizen-science programs, including Mission Monarch and eButterfly, which are coordinated through the Montreal Insectarium.
- The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) recommended at the 2017 Species Assessment Meeting that monarchs be uplisted from Species of Special Concern to Endangered. ECCC is currently evaluating this recommendation.

Key findings

Major publications describing ongoing analyses and highlighting key findings for Canada's national monarch conservation efforts include: Flockhart et al., 2013, 2015 and 2017; Hobson et al. 1999, 2017; Soto et al. 2017; and Meier-Augenstein et al. 2013 (see bibliography for full references).

Analyses in progress

Comprehensive analyses of the distribution of milkweed and monarch breeding range in Canada are underway, in conjunction with ECCC, the Mission Monarch project (Montreal Insectarium, Ottawa University, et al. 2016) and eButterfly (Montreal Insectarium, Vermont Center for Ecostudies, University of Arizona, and Ottawa University 2016), to identify and map regional and landscape-level breeding hotspots. Analyses of the spatio-temporal and regional changes in monarch migration and reproduction (from citizen-science programs) in relation to co-occurrence patterns of co-occurring local and migrant species are also in progress. Finally, analyses to characterize relationships between summer population size in Canada vs. previous and next overwintering population size in Mexico are underway.

Current monitoring programs

Protocols and sampling design: eButterfly (<www.e-butterfly.org>) and Mission Monarch (<www.mission-monarch.org>) are the two main citizen-science programs gathering georeferenced occurrences of monarch and co-occurring butterflies; milkweed density; and other monitoring-site data in Canada.

Implementation status: The national monitoring program for monarch breeding trends and milkweed density distribution was launched in 2016 through the Mission Monarch project, while the eButterfly project has been monitoring adult monarch distribution in conjunction with co-occurring butterfly species in Canada since 2012. The current focus of both initiatives is to expand the human network of participants and partners across Canada.

Future needs and opportunities for national and trinational collaboration: Major challenges for monitoring program implementation in Canada, and opportunities for trinational collaboration include the following:

- Increasing coordination capacity to standardize data collection across Canada and expand the human network of citizen scientists and partners.
- Dedication resources to analyze and publish monitoring data on breeding habitat productivity and other research priorities as outlined by the Canadian Monarch Conservation Science Partnership (CMCSP).

C.2 United States

Noting declines in the eastern monarch population, the MCSP held its inaugural meeting in 2009 and originally named itself the Powell Center Working Group (after the USGS John Wesley Powell Center for Analysis and Synthesis, which hosted the group's first and subsequent meetings). This consortium of scientists and conservation professionals from government, academia and NGOs focuses on: 1) modelling monarch population trends and assessing the impact of threats, 2) establishing population targets and habitat goals and 3) developing conservation tools to guide decision making. To better reflect research priorities, the group's name was changed to the Monarch Conservation Science Partnership in 2014, the same year that the US FWS was petitioned in a joint report to list monarchs as threatened (Center for Biological Diversity, Center for Food Safety, Xerces Society for Invertebrate Conservation, and Brower 2014). The positive US FWS 90-day finding released at the end of 2014 triggered the SSA process under the Endangered Species Act, and a listing decision is due in June 2019. Because the eastern monarch population is larger than the western monarch population, MCSP studies to date (listed below) have primarily focused on the eastern population (see Section 1). Previous and ongoing MCSP analyses and monitoring efforts continue to contribute to the US FWS SSA process and conservation recommendations.

MCSP Analyses

1. *Eastern monarch population conservation target estimated through a population viability analysis* (Semmens et al. 2016): Using overwintering (WWF Mexico and *Reserva de la Biosfera Mariposa Monarca*) and citizen-science (MLMP) data, this study found that the eastern monarch population stochastically fluctuates, but overall is in decline. Furthermore, according to International Union for the Conservation of Nature (IUCN) standards, the eastern monarch population is endangered (it has a >20% risk of extinction over the next 20 years). To reduce the chances of the eastern population dropping to such a low level that it will be unable to recover, this analysis suggests boosting the eastern overwintering population to a cumulative size of 6 hectares to buffer it against stochastic events. Because loss of milkweed from the United States landscape is a large contributor to monarch declines (Pleasants and Oberhauser 2013; Stenoien et al. 2016), this study recommends adding milkweed to the United States landscape, especially to the north-central and northeastern United States.
2. *Milkweed conservation target estimate:* In aid of meeting the eastern monarch population target of 6 hectares of overwintering monarchs in Mexico, the MCSP has used a variety of methods to estimate how much milkweed needs to be added to the United States landscape to support monarch breeding:
 - a) *Bottom-up approach:* Pleasants (2016) used historical data to estimate how much milkweed used to be on the landscape in the north-central and northeastern United States before widespread adoption of glyphosate and how much milkweed is present now, from current data sets. The difference between the amount that used to be present and current levels reflects how much milkweed should be added to the United States landscape (Pleasants 2016).

- b) *Top-down approach*: Thogmartin et al. (2017) combined data from a variety of studies to estimate how many monarchs are present in a hectare. Other studies have estimated how many milkweeds it takes to produce one overwintering monarch (Nail et al. 2015). Thus, Thogmartin and colleagues multiplied the desired number of hectares covered with monarchs \times number of monarchs/hectare \times number of milkweeds/monarch, to estimate how much milkweed is needed. The USGS also created a land-cover map and applied current estimates of milkweed to the north-central and northeastern regions, to determine how much milkweed is present. Subtracting estimates of existing milkweed from estimates of the total amount of milkweed needed to achieve six hectares of overwintering monarchs yields an estimate of how much milkweed should be added to the landscape.

Though these and other extrapolations have limitations, taking both bottom-up and top-down approaches has helped the MCSP to hone in on estimated number of milkweeds needed to reach the 6-hectare monarch population target. These studies estimate that an additional 1.4–1.8 billion stems of milkweed need to be added to the United States landscape to reach this population target (with 85% of the stems added to the north-central United States, and 15% to the northeast).

3. *Conservation Storylines Analysis (manuscript in progress)*: Researchers created a land-cover map of the United States and applied estimated milkweed densities across different land-cover types (e.g., grasslands, Conservation Reserve Program [CRP] acreage, marginal croplands, urban/suburban spaces, right-of-way habitats), to model how much milkweed is currently present on the United States landscape. Next, different conservation scenarios, or “storylines,” were considered, in which different land-sectors or combinations of sectors added milkweed to the landscape. This analysis had two salient findings: 1) participation from the agricultural sector is germane to meeting the US milkweed target, and 2) even with modest participation from the agricultural sector, if all other sectors participate we can get close to an additional 1 billion stems of milkweed on the United States landscape. Thus, the MCSP advocates for comprehensive conservation participation across sectors—or an “All hands on deck!” approach.
 4. *Demographic model* (Oberhauser et al. 2016): The MCSP developed a spatially explicit model that simulates the annual and multi-generational monarch life cycle in North America. Considering different management actions that address monarch population stressors (increasing floral nectar resources, increasing milkweed, decreasing insecticide use, etc.) across different regions, researchers simulated expected increases in the eastern monarch population. Results indicated that employing regionally-specific conservation actions across the entire eastern population’s continental range would best support the monarch population, as opposed to focusing conservation efforts on select regions.
 5. *Desktop and online support tools to help in conservation planning for the imperiled monarch butterfly* (USGS 2016): These tools were produced by Jason Rohweder and Wayne Thogmartin (USGS), in support of the MCSP. Tools were developed with a python scripting library, with ESRI ArcGIS software version 10.3, and include:
 - a) a *County Ranking Tool*, which can be used for national, regional, or local prioritization of conservation activity;
 - c) a *Milkweed Calculator*, to tabulate the amount of current and expected milkweed in a particular area of interest; and
 - d) a *County Area Adjustment Tool*, which can tabulate consequences of land change (a tool which has utility well beyond monarchs and the resources that sustain them).
- Online and desktop versions of these tools, an instructional video, and an associated user’s manual are available at: www.umesc.usgs.gov/management/dss/monarch.html.
6. *Threats analysis*: Beyond loss of milkweed from the United States landscape (Pleasants and Oberhauser 2013; Stenoien et al. 2016), monarchs are also threatened by climate change, loss of blooming nectar plants, and exposure to agrichemicals and disease. The MCSP is developing a threats model, to better assess the impact of these abiotic and biotic stressors and identify the best strategies to mitigate negative pressures.

MCSP Integrated Monitoring Strategy

Protocols and sampling design: As previously discussed, monarchs are the subject of numerous North American citizen-science programs, and these monitoring efforts jointly provide valuable information about the health of the monarch population. However, information gathered by current monitoring programs has important limitations, including non-random data collection, and data gaps in key monitoring attributes. The MCSP Integrated Monitoring Strategy aims to build on the strengths of existing monitoring programs while addressing programmatic shortcomings in sampling design and monitoring attributes. This Monitoring Strategy will engage multiple partners (citizen-science, federal, state, NGO, etc.) in monitoring key monarch and habitat attributes, using a broad-scale, spatially-balanced sampling scheme (GRTS draw). Resulting surveillance data will be used to populate MCSP models that inform eastern monarch population and habitat targets, identify and quantify the relative magnitude of potential threats, and inform strategies for implementing habitat enhancement. Combined with concurrent monitoring of habitat management, resulting surveillance data may also be used for assessing the effectiveness of local projects on an opportunistic and case-by-case basis, if correct study designs are adopted.

This monitoring framework has six protocols designated as standard operating procedures (SOPs), or activities, that provide instructions on how to:

1. ground-truth sites;
2. monitor adult monarchs through modified Pollard Walks and identify preferred adult nectar plants; estimate milkweed species diversity and density, as well as per-plant density of monarch eggs and caterpillars, and estimate relative abundance of blooming nectar plants;
3. estimate monarch survival to adulthood (this protocol requires monarch caterpillar collection and rearing, testing successfully emerged adults for a protozoan parasite, and recording instances of parasitism);
4. estimate abundance and biomass of fire ants, where geographically appropriate; and
5. enter and review data for quality control and assurance.

Wherever possible the MCSP has used or modified existing citizen-science monitoring protocols. These were developed for programs such as the Monarch Larva Monitoring Project (MLMP) (UMN Monarch Lab 2017), Project Monarch Health (University of Georgia Odum School of Ecology 2015), and the Illinois Butterfly Monitoring Network (IBMN 2004). Tailored instructions for monitoring across different land strata designated as potential monarch habitat (protected grasslands, unprotected grasslands, CRP lands, cultivated fields/orchards, right-of-way habitats, urban/suburban spaces) are provided on page 43 .

Site selection, governed by the GRTS draw, works by incorporating all possible sites within strata into a prioritized list. Data from priority sites (sites near the top of the ranked list) are weighted more heavily than data from lower-priority sites, in the final analysis. This weighting process allows citizens and other monitoring participants to submit data from any eligible location (it must fall into one of the six strata incorporated into the GRTS draw for the eastern monarch range) without introducing bias. However, to increase the impact of submitted data, priority sites should be monitored. While collecting data on all attributes at every site is encouraged, it is not necessary for program participation. Currently the GRTS draw focuses on the eastern monarch range, and the USGS is consulting topic experts to identify additional strata for expansion of the draw to the western monarch range (see Section 5.1, third bullet).

Implementation status: Because executing this project at large scale will require communications, engagement and technological infrastructures, the MCSP aims to implement this project in phases, with each phase building on the progress of previous years. Monitoring protocols, site selection, site access, and data management procedures were pilot-tested in 2016 at selected (non-priority) locations by US FWS biological technicians and other academic, NGO and agency partners. Protocols were edited based on feedback, and the US FWS and other partners, including the MJV, will test revised versions in 2017.

The MJV is a science-based and partner-driven organization that operates in three monarch conservation arenas, namely: 1) research and monitoring, 2) habitat restoration, and 3) education and outreach. The MJV functions as a communications hub servicing diverse stakeholders, and has extensive experience in training broad audiences (citizen scientists, practitioners, researchers, etc.) in citizen-science monitoring protocols. Furthermore, the UMN Monarch Lab, a close MJV partner, offers a “train-the-trainer” program that will serve as a template for building monitoring capacity for the MCSP Monitoring Strategy. Since the MCSP Integrated Monitoring Strategy ultimately aims to engage citizen scientists and other audiences in monitoring priority GRTS sites, the MJV will leverage its connections and training expertise to recruit citizen scientists through a series of workshops in 2017. Participants will be encouraged to monitor priority GRTS sites within self-designated travel radiuses, but may elect to monitor any site of their own choosing (as long as it falls within a GRTS stratum). In addition, the US FWS will hire biotechnicians to monitor priority GRTS sites in the southwest and midwest (US FWS Regions 2 and 3). Results from the 2017 field season will inform future implementation stages. Table 2 summarizes MCSP Integrated Monitoring Program implementation activities to date.

Future MCSP Integrated Monitoring Strategy needs, and opportunities for national and trilateral coordination: Development and implementation of the Integrated Monitoring Strategy has, to date, been funded primarily by the US FWS. To ensure future program success, the MCSP and other partners are working to diversify funding support and have applied for a National Fish and Wildlife Foundation (NFWF) grant (NFWF 2017). In order to successfully expand the Integrated Monitoring Strategy, the United States must build scalable communications infrastructures, accessible databases, and online coordination platforms. The anticipated needs summarized below also present opportunities for national and trilateral collaboration:

1. Protocols (and data standards): All countries agree to adopt compatible monitoring methodology and data standards, where appropriate. Future discussion is needed to align methodology and data standards across North America.
2. Site selection (GRTS): The USGS has agreed to create a trilateral GRTS draw. Future discussions between the USGS and topic experts are necessary, to identify unique strata in Canada and Mexico.
3. Citizen-science coordination: To gather data at the scale necessary to inform analyses, increased capacity is needed to recruit, train, support and retain citizen scientists. In addition to ongoing national efforts to engage citizen scientists in monitoring, Canada, Mexico and the United States will launch trilateral, citizen science-driven monitoring efforts, including a Monarch Data Blitz. Additional communication among identified parties is necessary to coordinate this effort.
4. Other participant coordination: Beyond citizen scientists, the MCSP Integrated Monitoring Strategy envisions engaging multiple entities (NGOs, other federal and state agencies, etc.) in the monitoring of priority GRTS sites. TMCSP partners have a shared obligation to build these strategic partnerships nationally and internationally.
5. Site access: Monarchs use diverse ecosystems, and consequently, GRTS-draw strata reflect a variety of land-use types and ecosystems. Because some strata are privately owned (e.g., unprotected grasslands, CRP lands, agricultural lands and urban-suburban spaces), obtaining permission to monitor at priority sites within these strata is logistically complicated. Furthermore, individuals may need to seek permission from appropriate authorities to monitor on public strata (protected grasslands and right-of-way habitats). Site access will continue to be a challenge to future completion of a trilateral GRTS draw.
6. Data management and access: All countries agree that data should be made accessible to TMCSP parties and partners. However, since different monitoring programs operating at different scales (regional to trilateral) have already developed separate databases, it is unlikely that a single platform or repository will serve all monitoring program needs. Rather, participating parties agree to format data to trilateral standards and either make data accessible from individual monitoring databases or upload properly formatted data to internationally accessible databases (Section 5.2).



7. Data analysis: As data are gathered, TMCSP members will need to analyze results and iteratively update monitoring and conservation recommendations.
8. Online coordination platforms/portals: To realize the long-term vision of the MCSP Integrated Monitoring Strategy, a platform that can automate aspects of site selection and track participant activities across time and space relative to the GRTS draw is needed. This tool would greatly enhance coordination of future monitoring activities. The Pacific Northwest Aquatic Monitoring Partnership (PNAMP) has developed an analogous tool for the North American Bat Monitoring Program (Pacific Northwest Aquatic Monitoring Partnership 2017). The MJV, PNAMP and other partners have applied for 2017 NFWF funding to adapt the existing platform to support MCSP monitoring activities. With completion of the trinational GRTS draw and pending funding, this platform could, potentially, be expanded trinationally.

Western Monarch Conservation Science Partnership

As previously discussed, the MCSP's research and conservation tools have primarily focused on the eastern monarch migratory population. However, the western monarch population is also in decline (Pelton et al. 2016). Accordingly, experts on the western monarch population and collaborators gathered at the Asilomar Conference Grounds, California, in December 2016, and formed the Western Monarch Conservation Science Partnership (WMCSPP). The WMCSPP is developing parallel models and conservation recommendations that take unique aspects of western monarchs into account. As in the east, monitoring data will support development and parameterization of these models. However, since western strata differ from eastern strata, current MCSP monitoring protocols and attributes may not be appropriate for all habitat types used by western monarchs. Evaluating and improving the applicability of MCSP protocols for western monarchs and associated strata is essential if the MCSP Integrated Monitoring Strategy is to be expanded beyond monarchs in the east. To facilitate evaluation of protocol applicability for the western strata, the MJV has sought funds to host a Development Workshop in Boise, Idaho, where MCSP Monitoring Coordinators will demonstrate protocols. Topic experts in attendance will provide feedback and recommendations for adjusting protocols for western strata. Furthermore, topic experts may elect to continue testing and/or implementing MCSP protocols (or relevant variations) as part of their monitoring activities in 2017.

Other recommended United States resources

Monarch Joint Venture.

- *Implementation plan*: The MJV implementation plan summarizes priority research gaps related to monarch conservation (Monarch Joint Venture 2016a). This plan is updated annually by MJV partners.

Table 2. MCSP Integrated Monitoring Framework Implementation activities, by year

Year	Coordinating organizations	Protocols implemented ¹	Site selection methodology	Strata tested	Monitoring participants	Geographic locations	Database ²
2016	US FWS	Activities 1–6	Miniature GRTS draws ³ and convenience	<ul style="list-style-type: none"> Protected grasslands Unprotected grasslands CRP lands Agricultural lands Right-of-way habitats (roadsides) 	Biotechnicians	<ul style="list-style-type: none"> US FWS Region 2: Washita NWR and surrounding area, Oklahoma US FWS Region 3: Neal Smith NWR and surrounding area, IA US FWS Region 6: Waubay NWR and surrounding area, SD 	US FWS AGOL repository
	<ul style="list-style-type: none"> UMN Monarch Lab The Chicago Field Museum 	Activities 1 and 3	Stratified, random	Urban-suburban spaces	Biotechnicians	Minneapolis–St. Paul metropolitan area, MN	Chicago Field Museum repository
	<ul style="list-style-type: none"> UMN Monarch Lab NRCS 	Activities 1 and 3	Convenience	CRP lands	Biotechnicians	IA, MN and WI	UMN Monarch Lab repository
2017	US FWS	Activities 1–4	GRTS draw ⁴	<ul style="list-style-type: none"> Protected grasslands Unprotected grasslands CRP lands Agricultural lands Right-of-way habitats (roadsides) 	Biotechnicians	<ul style="list-style-type: none"> US FWS Region 3: 200-mile radius around Lamoni, IA and Necedah, WI. US FWS Region 2: 150-mile radius around Balconies Canyonlands National Wildlife Refuge, Neches National Wildlife Refuge and Hagerman National Wildlife Refuge, TX. 	US FWS AGOL repository
	MJV	Activities 1–4 and 6	GRTS draw ⁴	<ul style="list-style-type: none"> Protected grasslands Unprotected grasslands CRP lands Agricultural lands Right-of-way habitats (roadsides) Urban-suburban spaces 	Citizen scientists	<ul style="list-style-type: none"> Minneapolis–St. Paul and surrounding area, MN Neal Smith NWR and surrounding area, IA Lansing and surrounding area, MI Madison and surrounding area, WI 	MJV repository

1. See page 43 for current protocols (June 2017).

2. Information from these databases is not yet publically accessible.

3. These GRTS draws drew priority sites from NWR and surrounding lands. A separate draw was conducted for each US FWS Region. Due to limited capacity to obtain permission to monitor in private strata, some sites in unprotected grasslands, CRP lands and agricultural lands were selected based on convenience.

4. This GRTS draw includes 6 strata for the eastern monarch range. Due to limited capacity to obtain permission to monitor in priority private strata, some sites in unprotected grasslands, CRP lands, agricultural lands and urban-suburban strata may be selected based on convenience.

- *Webinars:* In collaboration with the National Conservation Training Center, the MJV periodically produces and publishes webinars related to monarch biology, ecology and conservation, given by leading monarch biologists, conservation professionals and educators (Monarch Joint Venture, and National Conservation Training Center 2016).
- *Handouts:* The MJV has produced several informational handouts, including one summarizing different citizen-science programs. These are available online through the MJV site’s “Resources” tab (Monarch Joint Venture 2016b).

MonarchNet houses general information about monarch biology, recommended conservation actions, and a directory of monarch-related publications and citizen-science programs (<www.monarchnet.org>).

- *Library database:* MonarchNet maintains a searchable library of peer-reviewed literature related to monarch biology, ecology and conservation (MonarchNet 2016a).
- *Citizen-Science Programs:* MonarchNet maintains a directory of citizen-science programs focused on monarchs and other butterfly species (MonarchNet 2016b).
- *US FWS* provides information about monarch butterfly conservation (<www.fws.gov/savethemonarch/>) and updates on the Species Status Assessment (US FWS 2016).

C.3 Mexico

Introduction

The monarch butterfly was listed as Under Special Protection in 2001, according to the Species at Risk Standards of the Ministry of the Environment and Natural Resources (*Secretaría de Medio Ambiente y Recursos Naturales* — Semarnat). Mexico seeks to collect field information about this species throughout its migratory routes and areas used for hibernation. Goals include protecting priority sites important to monarch migration and overwintering, and learning more about the biological and ecological aspects of the species, to benefit its conservation.

During the last four years, monitoring activities have intensified, involving actors from civil society, government and academia. Currently, there are 22 participating states, which constitute the northwest, northeast and center of the country:

- Baja California Norte
- Baja California Sur
- Sonora
- Sinaloa
- Chihuahua
- Coahuila
- Nuevo León
- Tamaulipas
- San Luis Potosí
- Zacatecas
- Aguascalientes
- Jalisco
- Nayarit
- Durango
- Querétaro
- Guanajuato
- Hidalgo
- Tlaxcala
- Puebla
- Estado de México
- Michoacán
- Ciudad de México

Monarch Butterfly National Monitoring Network (*Red Nacional de Monitoreo de la Mariposa Monarca*); coordinated by Conanp in Mexico

Beginning in October 2014, Conanp promoted the consolidation of the Monarch Butterfly National Monitoring Network (*Red Nacional de Monitoreo de la Mariposa Monarca*) in protected natural areas that are part of the migratory route and wintering habitat in Mexico. Currently, this monitoring network includes personnel assigned to 44 Protected Natural Areas, 3 Priority Conservation Regions and 29 State Protected Natural Areas. Personnel represent groups from civil society, federal, state and municipal governments, and academia.

The national network (Figure 3) is organized hierarchically through regional and local networks (Figure 4). Local networks operate at a city or provincial scale, and together, several local networks from the same geographic area constitute a regional network. Each network, either local or regional, is responsible for coordinating monitoring activities in addition to organizing and disseminating information. All actors involved in the monitoring network share standardized monitoring protocols and information, moving toward national coverage.

Key partners that have supported the Monarch Butterfly National Monitoring Network (*Red Nacional de Monitoreo de la Mariposa Monarca*) from its origins are: Conanp, *Correo Real*–Profauna, Conabio, UNAM, and state governments (Coahuila, Tamaulipas, Guanajuato and Baja California, Hidalgo y Estado de México).

Important results

- Over 4,000 records have been submitted along the migratory route.
- In 2017, the Monarch Butterfly National Monitoring Network (*Red Nacional de Monitoreo de la Mariposa Monarca*) registered a new, small, overwintering colony in Tijuana, Baja California.
- The Monarch Butterfly National Monitoring Network (*Red Nacional de Monitoreo de la Mariposa Monarca*) created a National Data Base (Excel file format).

Figure 3. Structure of Monarch Butterfly National Monitoring Network – Mexico
(Red Nacional de Monitoreo de la Mariposa Monarca, México)

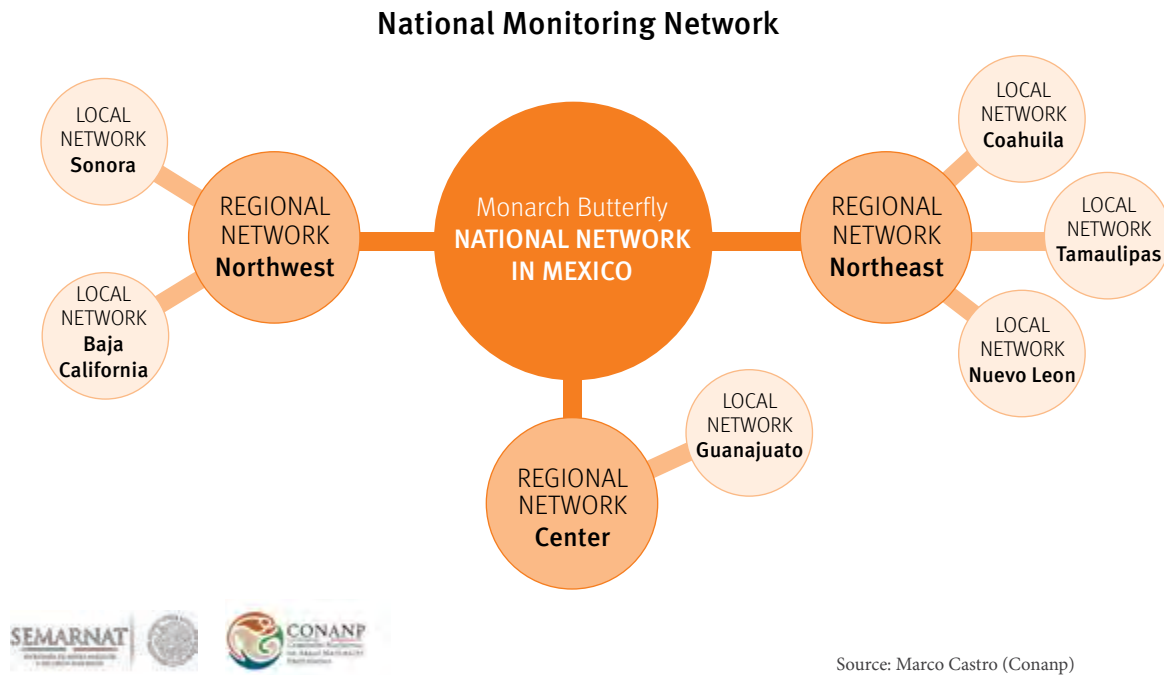
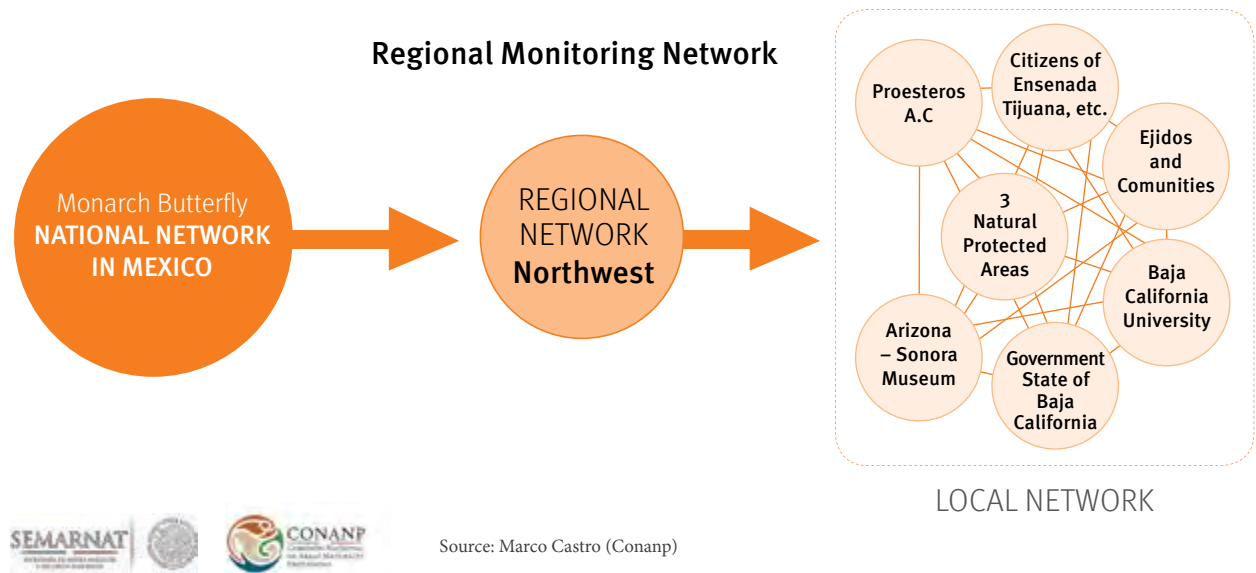


Figure 4. Examples of regional and local monitoring network structures



Key publications

Information for the general public: Dirección General de Vida Silvestre 2010; Galindo-Leal and Rendón Salinas 2005; Galindo-Leal and Rendón-Salinas 2007; Urquhart 1976; Commission for Environmental Cooperation 2008.

Monitoring: Brower et al. 2004; Comisión Nacional de Áreas Naturales Protegidas 2015; García Serrano et al. 2004; Rendón-Salinas and Galindo-Leal 2004; Rendón-Salinas et al. 2006a,b; Rendón-Salinas et al. 2008a; Rendón-Salinas et al. 2008b; Rendón-Salinas et al. 2009; Rendón-Salinas et al. 2010; Rendón-Salinas et al. 2011; Rendón-Salinas and Tavera Alonso 2012; Rendón-Salinas and Tavera Alonso 2013; Rendón-Salinas et al. 2014; Rendón-Salinas et al. 2015; Rendón-Salinas et al. 2016.

Management and Conservation of the Monarch Butterfly Biosphere Reserve: Comisión Nacional de Áreas Naturales Protegidas 2001.

Milkweed: Luna and Dumroese 2013.

Population decline: Barve et al. 2012; Brower 1999; Brower et al. 2011; Flockhart et al. 2015; Honey-Roses et al. 2009; Hoth 1995.

Deforestation: Alvarado-Flores 2009; López-García 2007; Ramírez 2006; Romeau 2000; Ruiz-Lopez 2009; Vidal et al. 2014; WWF 2004, 2006a, 2006b.

Biology and ecology of the monarch butterfly: Alonso-Mejía et al. 1997; Bonan 1997; Brower et al., 2004; Calvert et al. 1986; Calvert 2004; Dingle et al. 2005; Dockx 2003; Dockx et al. 2004; FCMM 2008; Frey and Schaffner 2004; Hobson and Wassenaar 1999; Howard and Davis 2004; Leong et al. 2004; Loveland and Acevedo 2007; Malcolm and Brower 1989; Ramírez and Zubieta 2005; Solensky 2004; US Department of Agriculture 2000; White 1996; Wilkins et al. 2003.

Connectivity and climate change: Sánchez-Cordero et al. 2015.

Tourism: Esquivel-Ríos et al. 2014; Granet and Fonfrede 2005.

Overview of current monitoring programs and select protocols for Mexico

Several organizations have been working together to standardize and nationally implement standardized monarch monitoring protocols (Table 3).

Protocol from the Monarch Butterfly National Monitoring Network (Red Nacional de Monitoreo de la Mariposa Monarca): This protocol implemented by the Monarch Butterfly National Monitoring Network Protocol (*Red Nacional de Monitoreo de la Mariposa Monarca*) was standardized by experts from across the country and collects information on: 1) the life cycle of the monarch, 2) geographic data of priority sites, 3) threats, and 4) types of ecosystems, see page 43.

Monitoring, location, and measurement of monarch butterfly overwintering colonies: Each overwintering season, field expeditions are carried out, beginning 1 December, to locate overwintering colonies. Boundary measurements of the forest surface covered by each colony are recorded. Subsequently, the area occupied by each colony is determined, using ArcView 3.3 spatial analysis software, see page 43.

Monarch Butterfly Mortality in Overwintering Colonies Protocol: The protocol subsamples forest floor quadrants within overwintering colonies. In each quadrant, five 1-m² (square-meter) sampling units are defined and the remains of dead butterflies are collected. Sampling is carried out in selected colonies—which are generally the largest colonies, with the longest occupancy records.

Correo Real: This program is an active initiative that Profauna AC has promoted for over 25 years. *Correo Real* (Royal Mail) works with citizen scientists to monitor the monarch butterfly and with social groups to raise conservation awareness. Sightings were historically documented in letters, and today are recorded in email reports. Observations include numbers of observed butterflies, caterpillars, and site characteristics where monarchs were spotted.

iNaturalist: This virtual platform is coordinated by Conabio to register biodiversity sightings by citizen scientists. iNaturalist currently supports a special user-interface to gather data for monarch butterflies, including observations of monarch life stages and associated geographic data, collector information, and images (Naturalista 2017). At time of writing, the platform houses over 4000 observations for Mexico.

Table 3. Mexican national monitoring activities, by year and program

Year	Coordinating organizations	Protocols implemented	Site selection methodology	Strata tested	Monitoring participants	Geographic locations	Database
2016, 2017	<i>Monarcas en el oeste</i> Conanp – National Park Sierra San Pedro Martir	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); Monitoring Protocol	Random selection	Protected natural areas Urban-suburban spaces	Students Rangers Citizen scientists	Northwest (Baja California, Baja California Sur, Sonora, Sinaloa, Chihuahua)	National database
2015 to 2017	<i>Monarcas en camino</i> Conanp, State Government of Tamaulipas	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); Monitoring Protocol	Random selection	Protected natural areas Urban-suburban spaces	Students Rangers Citizen scientists Communities	Tamaulipas and Nuevo León	National database
2015 to 2017	iNaturalist Conabio, Conanp	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); Monitoring Protocol	Random selection	Protected natural areas Urban-suburban spaces	Citizen scientists	All country	iNaturalist database
1992 to 2017	<i>Correo Real Profauna</i>	Monarch Butterfly National Monitoring Network (<i>Red Nacional de Monitoreo de la Mariposa Monarca</i>); Monitoring Protocol	Random selection	Protected natural areas Urban-suburban spaces	Citizen scientists	Northeast and center of Mexico	<i>Correo Real</i> database
20 years	WWF Mexico, Conanp	<i>Protocolo de hibernación</i>	Random selection	Natural protected areas	Technical personnel	Reserva de la Biosfera Mariposa Monarca	WWF Mexico and Conanp database

Future needs and opportunities for national and trinational collaboration: Challenges for the implementation of monitoring programs in Mexico and opportunities for trinational collaboration include:

- increasing monitoring efforts in northern Mexico;
- designating key, fixed sites to document monarch occurrence changes through time;
- monitoring monarch reproduction along the migratory route and establishing its relationship to availability of native and non-native milkweed plant species;
- adjusting protocols as necessary to meet data standards;
- increasing the area of protected habitats for monarchs;
- restoring winter habitats; and
- establishing permanent financial support for monitoring.

Recommended resources

Additional contacts and online resources are given in Table 4.

Table 4. Additional contacts and online resources for Mexico

Institution	Name	Email	Web Site
<i>WWF Mexico</i>	María José Villanueva	mjvillanueva@wwfmex.org	www.wwf.org.mx/que_hacemos/programas/mariposa_monarca/
<i>Naturaleza y Cultura Internacional</i>	Lydia Lozano	llozano@natureandculture.org	www.naturalezaycultura.org
<i>Conanp Conabio</i>	Carlos Galindo	n/a	http://www.soymonarca.mx/en/index.html



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National monitoring protocols for Canada, Mexico and the United States

Canada

As discussed in Appendix C, Section C.1, eButterfly (Montreal Insectarium, Vermont Center for Ecostudies, University of Arizona, and Ottawa University 2016) and Mission Monarch (Montreal Insectarium, Ottawa University, et al. 2016) are the two main citizen-science programs gathering georeferenced occurrences of monarch and co-occurring butterflies, milkweed density and other monitoring-site data in Canada. Protocols are not attached to this report but are available online (<www.mission-monarch.org/faq/>). The Long-term Monitoring of Natal Origins of Monarchs Overwintering in Mexico: Butterfly Collection Protocol is attached.

Mexico

Mexico, through the Monarch Butterfly National Monitoring Network (*Red Nacional de Monitoreo de la Mariposa Monarca*), has put together the National Monarch Butterfly Monitoring Protocol (*Protocolo de Monitoreo de Mariposa Monarca*), which includes the description of all fields included in the Monitoring Datasheet (*Ficha de Monitoreo*). In addition, Mexico has developed a protocol for the monitoring of monarch butterflies in their overwintering sites (*Monitoreo de colonias de mariposas monarca en sus áreas de hibernación en México*). Protocols are attached and can also be found online at: <www.conanp.gob.mx/acciones/fichas/mariposa/info/info.pdf>.

United States

MCSP protocols for biotechnicians and citizen scientists are accessible. The current versions of protocols for citizen scientists (as of 5 June 2017) follow (see attached Monarch Monitoring Protocols: Monarch Conservation Science Partnership Integrated Monitoring Strategy and MCSP Citizen Science datasheets). This document includes monitoring protocols for activities mentioned in Table 2. As these protocols will continue to evolve over time, for updates and more information about the protocols, please visit: <<http://monarchjointventure.org/get-involved/mcsp-monitoring>>.



Canada

Long-term Monitoring of Natal Origins of Monarchs Overwintering in Mexico: Butterfly Collection Protocol

Greg W. Mitchell, D.T. Tyler Flockhart, Keith A. Hobson

Background

In order to develop effective conservation strategies for migratory animals, understanding migratory connectivity between their breeding and wintering grounds is critical (Webster *et al.* 2002; Runge *et al.* 2014; Mazar *et al.* 2016). For example, knowledge of the proportion of a breeding population that inhabits a specific location on the wintering grounds (i.e., a measure of the strength of migratory connectivity), can provide direct insights into how management strategies on the wintering grounds will affect breeding population densities, and vice versa (Martin *et al.* 2007). Thus, understanding the strength of migratory connectivity has direct implications for allocating scarce resources to conservation actions (Martin *et al.* 2007). In the case of the eastern North American population of monarch butterflies, knowledge of the natal origins of wintering individuals allows the identification of key zones of origin (production), resulting in direct recruitment to the adult population.

Stable isotope analysis provides an effective and proven means for determination of approximate geographic origins of migratory animals at continental scales (Hobson and Wassenaar 2008). Wassenaar and Hobson (1998) first demonstrated that the measurement of stable hydrogen ($\delta^2\text{H}$) and carbon ($\delta^{13}\text{C}$) isotope ratios in monarch wing chitin could be used to infer natal origins of overwintering monarchs in Mexico. That study used monarchs raised throughout their eastern breeding range as well as captive-rearing studies (Hobson, Wassenaar, and Taylor 1999) to create an isotopic basemap, or isoscape, which was used to assign wintering individuals to approximate natal origin. This approach was based primarily on the well-known continental pattern of deuterium (^2H) in precipitation in North America that shows an impressively strong latitudinal gradient with higher concentrations of this heavy isotope at low latitudes and lower values at higher latitudes. The pattern found for $\delta^{13}\text{C}$ was not expected but has added to our ability to resolve origins and is likely based on the isotopic response of milkweed to patterns of relative humidity. Regardless, we now have a well-established isotopic tool to that allows us to infer origins of monarchs and how they might change through time: forming the basis of a powerful monitoring tool in the conservation of eastern monarchs (e.g., Flockhart *et al.* 2017).

An important consideration in the design of an isotopic monitoring tool is to ensure strict laboratory protocols for the measurement of $\delta^2\text{H}$ in wing chitin. This is because a significant

fraction of H in chitin can exchange with ambient water vapor in the laboratory. Because ambient vapor deuterium concentration varies seasonally and with geography, unless this exchange is controlled for, laboratories measuring the same sample or the same laboratory making measurements at different seasons will produce different values (Meier-Augenstein *et al.* 2013). Fortunately, Wassenaar and Hobson (2003) developed a comparative equilibration approach that corrects for this problem by using organic standards of known non-exchangeable H composition. This solves the analytical problem but it is crucial that all measurements be made using an identical protocol that also accounts for other sources of isotopic variation, such as the effects on $\delta^2\text{H}$ of differential wing pigments (Hobson *et al.* 2017).

Purpose of protocol

The original isotopic work of Wassenaar and Hobson (1998) surprisingly demonstrated that the majority of overwintering monarchs at 13 known roost sites in Mexico originated from the American Midwest or “corn belt”. This immediately focused concern on the use of genetically modified crops that could withstand herbicide treatment or which could produce the toxin from the *Bacillus thuringiensis serotype kurstaki* (Btk), which is lethal to lepidoptera. While the controversy is still unresolved, it is important to monitor putative origins of monarchs and, especially, the relative importance of this zone, which historically produced the majority of adult recruitment. Since the original isotope study, populations of monarchs have crashed and there are now far fewer wintering roost sites in which to conduct isotopic monitoring. Recently, Flockhart *et al.* (2017) used monarch wing tissue collected from the wintering colony Sierra Chincua 1976–2014 to examine possible shifts in natal origins through time. They found that on average the Midwest region of the US still supported the largest proportion of overwintering monarchs (38%), however, there was quite a bit of year-to-year variability in relative proportions. While part of this variability was accounted for by temperature and precipitation during the breeding season, sample sizes for many years were extremely low; suggesting further monitoring of natal origins is needed with higher sample sizes per year. Also, because samples were collected from a single colony, there is also a need to understand how the relative proportions of monarchs from different breeding regions changes across different wintering colonies. A long-term monitoring protocol of monarch wing tissues from multiple wintering colonies would allow us to answer the following questions:

- 1) How does the proportion of monarchs from different breeding regions at a wintering colony change through time with respect to different environmental factors, including climate change?
- 2) To what extent does the contribution of a specific breeding region to wintering population densities vary across wintering colonies?

Answering both of these questions will allow the Trinational Monarch Conservation Science Partnership to make informed recommendations regarding conservation actions and remediation both on the breeding and wintering grounds.

Methods

Stable isotope analysis requires that the butterfly is collected and preserved so it can be transferred to the laboratory for preparation. Previous analyses have used natural overwinter mortality and in general this will be appropriate since wing chitin locks in the isotope signatures. Individual (whole) samples should be stored dry, preferably in paper envelopes. Colony location, date of collection, sex (if known), and name of collector/organization should be written on the outside of each envelope. There is no need to freeze or preserve the samples in any way but plastic bags should not be used as they do not allow air movement to keep samples dry.

- A minimum of 200 dead monarch samples should be collected from the two or three colonies that are monitored for the entire winter by Conanp (i.e., 400 – 600 samples per year).
 - 50 samples (half males and half females) should be collected during the last week of monitoring in each month (50 samples collected once a month * 4 months * 3 colonies = 600 samples).
 - The collection of more than 200 samples is encouraged. For example, collecting 100 samples every month would reduce variability in assignments of breeding origins.
 - Catastrophic mortality events due to weather for example should be exploited opportunistically.
- Samples can be collected in bulk (i.e., without any individual sorting or in envelopes and later sorted prior to shipment).
- At all other colonies visited by Conanp for estimates of wintering area, a minimum of 100 samples are collected per year.
- This protocol can also be easily adapted for monitoring natal origins for monarchs collected at staging sites in northern Mexico during fall migration. For example, 50 monarchs per site can be collected (balanced between sexes) from each staging site where migration monitoring is being carried out.
- Samples should be shipped under appropriate permits to the National Wildlife Research Centre, Environment and Climate Change Canada, or to another agreed-upon

government facility, as determined by the Trinational Monarch Conservation Science Partnership.

- All isotopic data should be archived at Environment and Climate Change Canada or at another agreed upon government facility as determined by the Trinational Monarch Conservation Science Partnership.

Ultimately, this long-term monitoring project will provide invaluable information on migratory connectivity for the monarch butterfly and will help with the spatial prioritization of conservations efforts now and into the future.

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Mexico

PROTOCOLO DE MONITOREO

DE MARIPOSA MONARCA



DATOS GENERALES

HOJA DE DATOS SOBRE LA MARIPOSA MONARCA

■ Nombre común	Mariposa monarca
■ Nombre científico	<i>Danaus plexippus</i> L.
■ Categoría de riesgo	No es una especie amenazada. La IUCN reconoce la migración de la monarca como un fenómeno amenazado.
■ Descripción	<ul style="list-style-type: none"> • Mariposa ninfálida grande (con una envergadura alar de 9-10 cm). • Coloración aposemática (de advertencia): anaranjado y negro. • Tóxica para la mayoría de los vertebrados por los glucósidos cardíacos que obtiene de las asclepias —comúnmente llamadas algodoncillo o venenillo (<i>Asclepias spp.</i>)—, de las que se alimenta. • Presenta dimorfismo sexual: las vetas negras son más anchas en las alas de la hembra y el macho tiene pequeños "sacos" en las alas traseras donde almacena feromonas. • Grandes poblaciones pasan el verano en regiones templadas y migran hacia el sur para el invierno. • Hay pequeñas poblaciones residentes en México. • La monarca es una especie de origen tropical.
■ Hábitat	<ul style="list-style-type: none"> • Regiones templadas a tropicales. • Dondequiera que crezcan plantas del género de las asclepias. • Bosques de oyamel, pino, encino y cedro durante la invernación. • Vegetación secundaria. • Hábitats alterados como bordes de carreteras y alrededores de tierras de labranza.
■ Distribución	<ul style="list-style-type: none"> • En América: del sur de Canadá a América Central y del Sur. • En América del Norte: al menos tres poblaciones (oriental, occidental y residente en México). • La población occidental se extiende de Columbia Británica a California. • La población oriental se extiende del sur de Canadá y el este de Estados Unidos (al este de las montañas Rocosas al centro de México (Michoacán y Estado de México). Algunas mariposas continúan su migración hacia el Caribe pasando por Florida. • La población residente en México está diseminada por todo el país. • Por medio de introducciones ocurridas en el siglo XIX, la monarca colonizó sitios en Australia, Indonesia, Islas Canarias y España.
■ Migración	<p><i>Población occidental:</i> Las monarca migran en el otoño de Columbia Británica, Washington, Oregon y otros estados del oeste a sitios de reposo en la costa de California.</p> <p><i>Población oriental:</i> Durante el otoño, las monarca migran hacia el sur, del sureste de Canadá y el este de Estados Unidos a sus sitios de invernación en la región central de México, y en la primavera recolonizan su área de reproducción en Texas. Durante la invernación se congregan en áreas muy reducidas.</p>
■ Etapas de desarrollo	Huevo – Larva, oruga – Pupa, crisálida – Adulto, imago
■ Dieta	Las larvas se alimentan exclusivamente de hojas de asclepias. En este sentido, son especialistas estrictas. Los adultos son generalistas que se alimentan de una amplia variedad de flores, néctar de flores y agua.
■ Longevidad:	La duración de la vida adulta oscila entre menos de un mes y nueve meses: los adultos de las cohortes de primavera y verano viven alrededor de cuatro semanas; en cambio, la generación migratoria puede vivir hasta nueve meses ("generación Matusalén") y hacer el viaje de ida y vuelta. Son la segunda o la tercera generaciones las que alcanzan los límites boreales de la distribución de la monarca.
■ Factores de impacto y amenazas	<ul style="list-style-type: none"> • Destrucción y fragmentación de hábitats a todo lo largo de la ruta migratoria, en especial en los sitios de invernación y reproducción. • Pérdida de hábitat a causa de la urbanización. • Uso de productos agroquímicos tóxicos. • Reducción de las poblaciones de asclepias. • Organismos genéticamente modificados, como la soya, que toleran los herbicidas (en tanto que no ocurre así con las asclepias). • Parásitos (virus, bacterias y protozoarios). • Cambio climático. • Falta de información y de educación ambiental.

ciclo de vida: **ADULTO**



Los **MACHOS** son un poco más grandes que las hembras y tienen en cada ala trasera una mancha negra formada por escamas de androconia que producen feromonas. Estas sustancias se suelen utilizar en especies emparentadas para atraer a las hembras. Además las venas de las alas son más delgadas que en las hembras.



Las **HEMBRAS** no poseen la mancha de androconia, sino escamas ligeramente más marrones en las zonas color naranja de sus alas, y más escamas negras sobre las vetas de las alas, lo que hace que las vetas parezcan más anchas.

ciclo de vida: HUEVO**Características:**

Los huevos de la monarca son de forma cónica con una base plana. Miden aproximadamente 1.2 mm de alto por 0.9 mm de diámetro en la parte más ancha y son de color amarillo-crema claro con bordes o “costillas” desde la punta hasta la base. Las monarca sólo ponen huevos en plantas llamadas **asclepias**.

Las hembras adultas ponen solas sus huevos y secretan una sustancia pegajosa que los adhiere a las plantas. En estado silvestre, las hembras probablemente ponen de 300 a 400 huevos durante su ciclo de vida, aunque las hembras en cautiverio pueden poner un promedio de 700 huevos en un periodo de dos a cinco semanas (Oberhauser, 2004). Normalmente, las larvas emergen dentro de los siguientes tres a cinco días, pero cuanto más altas son las temperaturas, menores los periodos de desarrollo.

Asclepias (algondicillos)

Son plantas (hierbas) perennes que contienen savia lechosa (de ahí su nombre en inglés: milkweed) contiene alcaloides y otros compuestos complejos como los cardenólidos que son tóxicos, de ahí el nombre de venenillo, debido a su naturaleza tóxica y algodoncillo a la apariencia de sus semillas. Se encuentran regularmente en zonas perturbadas como zonas de cultivo.



ciclo de vida: **ORUGA**

Las larvas de la monarca (orugas) son blancas con rayas negras y amarillas; tienen dos pares de filamentos negros en los segmentos larvales dos y once, y pasan por cinco fases larvarias durante un lapso de nueve a trece días. Los patrones de colores vivos en las larvas de monarca probablemente representen una coloración aposemática o de advertencia.

Sus principales características son que solo cuentan con 4 antenas, 2 en la parte de enfrente y 2 en la parte de atrás, además que los anillos de coloración si cubren todo el cuerpo, estas larvas las podemos encontrar desde 5 mm hasta 5 cm que es cuando llega a la fase adulta.



Larvas con menos de 1 cm de longitud. Su coloración no se logra apreciar del todo y tienden a ser muy blancas con delgadas líneas amarillas y negras. Aun no presentan antenas anteriores y posteriores.

Oruga en quinta etapa larvaria alcanzando la madurez. Las larvas en esta etapa abandonan la asclepia hospedera para ir en busca de un sitio elevado y generalmente bien escondido donde efectuar su transformación en crisálidas.



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Proceso de transformación de oruga a crisálida.

ciclo de vida: **PUPA o CRISALIDA**

Las crisálidas de la monarca miden aproximadamente 3 cm de largo y son de color verde turquesa vivo con manchas doradas. Estas manchas de apariencia metálica, típicas de las Danainae, son producto de capas densas y transparentes que se alternan en la endocutícula y que reflejan y transmiten la luz de diferente forma, causando una interferencia constructiva de la luz que es lo que les da una apariencia metálica brillante. La etapa de crisálida dura de nueve a quince días en condiciones veraniegas normales.



Pupa durante el periodo de incubación.



Pupa con mariposa a punto de emerger. En la etapa final de la incubación la crisálida se torna color cristalina, logrando observar las alas de la mariposa.



Proceso de la mariposa saliendo de la crisálida.

OTRAS ESPECIES PARECIDAS

A las monarca adultas se les confunde en ocasiones con especies emparentadas, por ejemplo con *D. gilippus* (la mariposa reina), *D. eresimus* (la mariposa soldado), *D. erippus* (la monarca meridional o de América del Sur) y *Limenitis archippus* (la mariposa virrey de América del Norte).

También podemos encontrar los casos de confusión al momento de observar fases larvarias. Como lo es el caso con la falsa monarca.

Larvas de Falsa monarca

Características: Esta larva presenta tres pares de antenas, dos anteriores, dos centrales y dos posteriores. El patrón de sus colores es similar al de las monarca, sin embargo las distribución y formas son diferentes.



MONARCA (*Danaus plexippus*)



SOLDADO (*Danaus eresimus*)



REINA (*Danaus gilippus*)

Protocolo de Monitoreo de **Mariposa Monarca**
Falsas Monarca:

Estas mariposas son las especies más comunes que se logran confundir con las mariposas monarca:



MONARCA (*Danaus plexippus*)



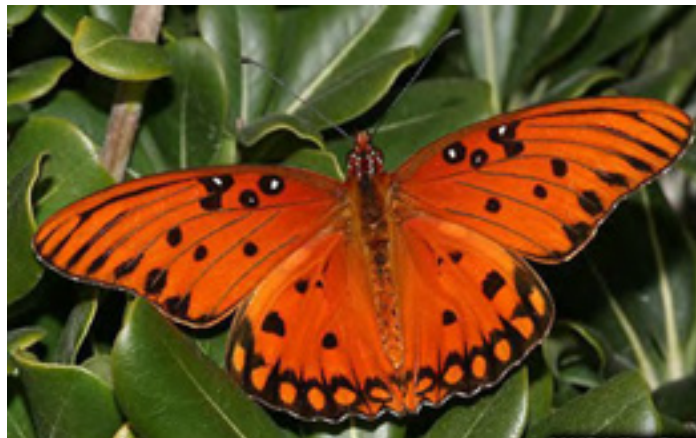
SOLDADO (*Danaus eresimus*)



REINA (*Danaus gilippus*)



VIRREY (*Limenitis archippus*)



FRITILARIA (*Agraulis vanillae*)

Este protocolo ha sido consensuado con diferentes especialistas en el monitoreo de la mariposa monarca a lo largo de la ruta migratoria y el hábitat de hibernación guía tiene la finalidad de describir los campos con los que cuenta la ficha de monitoreo de mariposa Monarca, la información que debe ser incluida en cada uno de ellos y los estándares de monitoreo para la toma de los datos en campo.

ESTÁNDARES DE MONITOREO

Periodo de monitoreo: se recomienda que se haga lo más frecuente posible (al menos una vez a la semana), o bien con el apoyo de las personas de las comunidades que ya tiene identificadas para monitorear.

Área para el sitio de muestreo para el registro de huevos, larvas y pupas: **10 m x 10 m ó 100 m²**

Para el conteo de mariposas volando

Tiempo de observación de mariposas volando: **20 minutos**

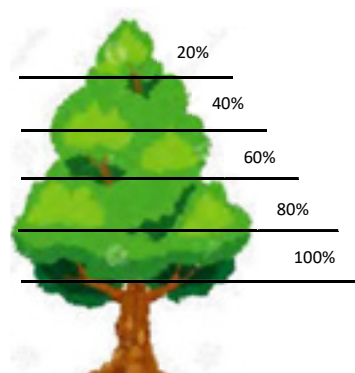
Contar únicamente las mariposas que lleven un vuelo direccional, especialmente en época de migración, por la mañana temprano y en la tarde (5pm) las mariposas dan vuelta en círculos esperando las termas para elevarse y por la tarde buscando un sitio donde pernoctar.

Se recomienda definir una línea entre dos objetos de 30-50 metros y que los observadores, si es que hay más de uno, estén a una distancia uno de otro de por lo menos 100 metros.

Se recomienda que la observación se haga de preferencia en un área grande y abierta sin árboles o edificios cercanos y que revisen con cuidado si las mariposas no les están pasando muy alto, fijando la vista por un momento hacia una nube.

Densidades de árboles ocupados por mariposas:

20 %	Por lo regular solo equivale a la punta cubierta del árbol
40 %	Poco menos de la mitad del árbol cubierto
60 %	Más de la mitad del árbol
80 %	Casi por completo
100 %	El árbol cubierto por completo



Para obtener el promedio:

$$\% \text{ promedio} = \frac{\%A1 + \%A2 + \%A3 \dots}{\# \text{ de árboles}} = \frac{40\% + 20\% + 80\%}{3} = 46.6\%, \text{ lo que se considera como un } 40\% \text{ aprox.}$$

Si es posible, realice el conteo del número de racimos que identifique en el árbol. Un racimo se considera cuando se agrupan 12 o más mariposas. La observación puede anotarla en el apartado de observaciones.

Protocolo de Monitoreo de Mariposa Monarca

FICHA DE MONITOREO



Programa Nacional para la Conservación del Fenómeno Migratorio de la Mariposa Monarca

Ficha De Monitoreo

DATOS DEL OBSERVADOR	Nombre:				Fecha:			
	Lugar:							
COORDENADAS	N:		Referencia:					
	WO:							
HORA DE OBSERVACION	HRS.		CONDICIONES DEL CLIMA	Soleado	Medio nublado	Nublado	Frío	
				Viento	Lluvia	Nevando	Calor	
FASE REPRODUCTIVA	HUEVO	LARVA	PUPA	MARIPOSA				
Actividad	Puestos por planta	Alimentándose por planta	Fijadas por planta	Alimentándose Agua / Flores	Volando (durante 20 minutos)	Perchadas		
Cantidad observada	1 – 5 6 – 10 Más de 10	1 – 5 6 – 10 Más de 10	1 – 5 6 – 10 Más de 10	Número estimado: _____	Número estimado: _____	Número de árboles ocupados: _____ Densidad promedio del grupo de árboles: _____		
	# de plantas: _____	# de plantas: _____						
En que planta o árbol (si aplica)		Nombre común:			Nombre científico:			
TIPO DE ECOSISTEMA	Bosque	Desierto	Matorral	CUERPOS DE AGUA VISIBLES	Río	Arroyo	Lago	
	Pastizal	Selva	Localidad		Laguna	Presa	Charco	
	Otro: _____				Otro: _____			
FAUNA DE COMPAÑIA	Otras mariposas	Libélulas	Que especie o nombre común (si se la sabe):					
	Avispas	Otro: _____						
AMENAZAS	Zona Industrial	Carretera	Indique alguna otra amenaza:					
	Ciudad	Cultivo						
DEPREDACIÓN	Aves	Avispas	Que especie o nombre común (si se la sabe):					
	Hormigas	Otro: _____						
OBSERVACIONES								

CROQUIS DE UBICACIÓN

	<p>Área de muestreo para huevos, larvas y pupas: 100 m2</p> <p>Tiempo de observación de mariposas volando: 20 minutos</p> <p>Densidades de mariposas perchadas por árbol:</p> <table border="1" style="width: 100%;"> <tr> <td>20 %</td> <td>Por lo regular solo equivale a la punta cubierta del árbol</td> </tr> <tr> <td>40 %</td> <td>Poco menos de la mitad del árbol cubierto</td> </tr> <tr> <td>60 %</td> <td>Más de la mitad del árbol</td> </tr> <tr> <td>80 %</td> <td>Casi por completo</td> </tr> <tr> <td>100 %</td> <td>El árbol cubierto por completo</td> </tr> </table> <div style="text-align: right;"> </div> <p>Para obtener el promedio:</p> $\% \text{ promedio} = \frac{\%A1 + \%A2 + \%A3 \dots}{3}$ $= \frac{40\% + 20\% + 80\%}{3}$ $= 46.6\%, \text{ lo que se considera como un 40\% aprox.}$	20 %	Por lo regular solo equivale a la punta cubierta del árbol	40 %	Poco menos de la mitad del árbol cubierto	60 %	Más de la mitad del árbol	80 %	Casi por completo	100 %	El árbol cubierto por completo
	20 %	Por lo regular solo equivale a la punta cubierta del árbol									
	40 %	Poco menos de la mitad del árbol cubierto									
	60 %	Más de la mitad del árbol									
	80 %	Casi por completo									
100 %	El árbol cubierto por completo										

CAMPO	DESCRIPCION
Nombre	Nombre completo de la persona que está realizando el monitoreo
Fecha	Ponerla como en el siguiente ejemplo 25/09/2015
Lugar	Indicar el estado, municipio y localidad (ejido, comunidad, pueblo, ciudad, etc.)
N	Coordenadas geográficas latitud norte (grados, minutos, segundos). Ejemplo: 19° 14.5' 36.8"
WO	Coordenadas geográficas longitud oeste (grados, minutos, segundos). Ejemplo: 100° 22.5' 50.3"
Hora de observación	Deberán estar escritas en formato de 24 horas. Por ejemplo 21:14 hrs.
Condiciones del clima	Indicar si está nublado, soleado, con viento, lluvia.
Huevo	Señalar el rango en el que se contabilicen el total de huevecillos en una planta. Si no se registran huevos al momento del monitoreo, indicar 0 (cero).
Larva	Señalar el rango en el que se contabilicen el total de larvas en una planta. Si no se registran larvas al momento del monitoreo, indicar 0 (cero).
Pupa	Señalar el rango en el que se contabilicen el total de pupas encontradas dentro del sitio de muestreo. Si no se registran pupas al momento del monitoreo, indicar 0 (cero).
# de plantas	Indicar el total de plantas que se identificaron con presencia de huevos y larvas en el sitio de muestreo.
Mariposa alimentándose	Indicar el total de mariposas que se observen alimentándose de agua o flores e indicar cuál de estas dos opciones registran. Si no se registran mariposas al momento del monitoreo, indicar 0 (cero).
Mariposa volando	Indicar el total de mariposas volando durante un tiempo de 10 minutos (ver estándares de monitoreo). Si no se registran mariposas al momento del monitoreo, indicar 0 (cero).
Mariposa perchadas	Indicar el número de árboles que estén ocupando las mariposas dentro del sitio de muestreo. Indicar el promedio de la densidad de árboles ocupados por las mariposas dentro del sitio de muestreo. Por ejemplo 40%
En que planta o árbol (si aplica)	Indicar el nombre (común o científico) de la planta donde se observaron los huevecillos o las larvas. Indicar el nombre del árbol o árboles que están ocupados por las mariposas perchadas.
Tipo de ecosistema	Señalar o indicar (en su caso) el tipo de ecosistema donde se está realizando el monitoreo.
Cuerpos de agua visibles	Señalar o indicar (en su caso) el tipo de cuerpo de agua que se observa en el lugar o cerca (distancia no mayor a 50 metros) del sitio del monitoreo.
Fauna de compañía	Señalar o indicar (en su caso) el tipo de fauna que se observe volando junto con las mariposas durante la migración, donde se está realizando el monitoreo. Por ejemplo: cuando se observan libélulas volando junto a las mariposas.
Amenazas	Señalar o indicar (en su caso) el tipo de amenaza que se observe en el sitio donde se está realizando el monitoreo.
Depredación	Señalar o indicar (en su caso) el tipo de depredación que se observe en el sitio donde se está realizando el monitoreo.
Observaciones	Indicar cualquier otra observación adicional, que no esté mencionada en la ficha de monitoreo. Indicar N/O = no observación , cuando no se haya podido realizar el monitoreo por mal clima, ocupación, etc.



Programa Nacional para la Conservacion del Fenómeno Migratorio de la Mariposa Monarca

FICHA DE MONITOREO

DATOS DEL OBSERVADOR	Nombre:				Fecha:		
	Lugar:						
COORDENADAS	N:		Referencia:				
	WO:						
HORA DE OBSERVACION	hrs.		CONDICIONES DEL CLIMA	Soleado	Medio nublado	Nublado	Frío
				Viento	Lluvia	Nevando	Calor
FASE REPRODUCTIVA	HUEVO	LARVA	PUPA	MARIPOSA			
Actividad	Puestos por planta	Alimentándose por planta	Fijadas por planta	Alimentándose Agua / Flores	Volando (durante 20 minutos)	Perchadas	
Cantidad observada	1 – 5 6 – 10 Más de 10 # de plantas: ____	1 – 5 6 – 10 Más de 10 # de plantas: ____	1 – 5 6 – 10 Más de 10		Número estimado: ____	Número de árboles ocupados: ____ Densidad promedio del grupo de árboles: ____	
En que planta o árbol (si aplica)		Nombre común:			Nombre científico:		
TIPO DE ECOSISTEMA	Bosque	Desierto	Matorral	CUERPOS DE AGUA VISIBLES	Río	Arroyo	Lago
	Pastizal	Selva	Localidad		Laguna	Presa	Charco
	Otro:				Otro:		
FAUNA DE COMPAÑIA	Otras mariposas	Libélulas	Que especie o nombre común (si se la sabe):				
	Avispas	Otro:					
AMENAZAS	Zona Industrial	Carretera	Indique alguna otra amenaza:				
	Ciudad	Cultivo					
DEPREDACIÓN	Aves	Avispas	Que especie o nombre común (si se la sabe):				
	Hormigas	Otro:					
OBSERVACIONES							



Programa Nacional para la Conservacion del Fenómeno Migratorio de la Mariposa Monarca

FICHA DE MONITOREO

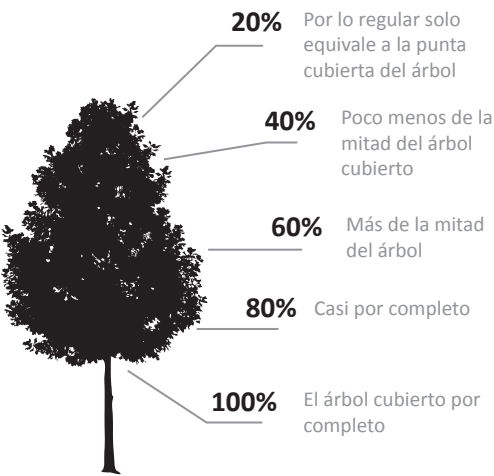
DATOS DEL OBSERVADOR	Nombre:				Fecha:		
	Lugar:						
COORDENADAS	N:		Referencia:				
	WO:						
HORA DE OBSERVACION	hrs.		CONDICIONES DEL CLIMA	Soleado	Medio nublado	Nublado	
				Viento	Lluvia	Nevando	
FASE REPRODUCTIVA	HUEVO	LARVA	PUPA	MARIPOSA			
Actividad	Puestos por planta	Alimentándose por planta	Fijadas por planta	Alimentándose Agua / Flores	Volando (durante 20 minutos)	Perchadas	
Cantidad observada	1 – 5 6 – 10 Más de 10 # de plantas: ____	1 – 5 6 – 10 Más de 10 # de plantas: ____	1 – 5 6 – 10 Más de 10		Número estimado: ____	Número de árboles ocupados: ____ Densidad promedio del grupo de árboles: ____	
En que planta o árbol (si aplica)		Nombre común:			Nombre científico:		
TIPO DE ECOSISTEMA	Bosque	Desierto	Matorral	CUERPOS DE AGUA VISIBLES	Río	Arroyo	
	Pastizal	Selva	Localidad		Laguna	Presa	
	Otro:				Otro:		
FAUNA DE COMPAÑIA	Otras mariposas	Libélulas	Que especie o nombre común (si se la sabe):				
	Avispas	Otro:					
AMENAZAS	Zona Industrial	Carretera	Indique alguna otra amenaza:				
	Ciudad	Cultivo					
DEPREDACIÓN	Aves	Avispas	Que especie o nombre común (si se la sabe):				
	Hormigas	Otro:					
OBSERVACIONES							

CROQUIS DE UBICACIÓN

Área de muestreo para huevos, larvas y pupas: **100 m²**

Tiempo de observación de mariposas volando: **20 mins.**

Densidades de mariposas perchadas por árbol:



Para obtener el promedio:

% promedio =

%A1 + %A2 + %A3....

3

= 40% + 20% + 80%

3

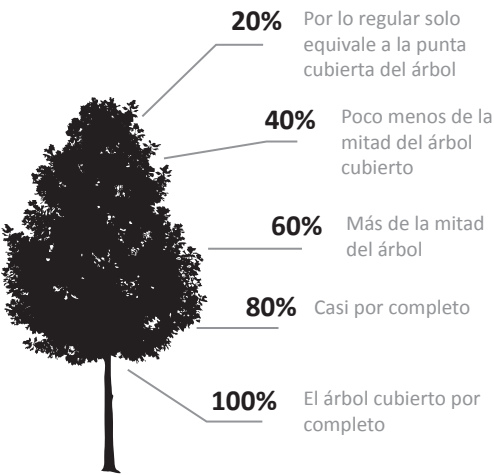
= 46.6%, lo que se considera como un 40% aprox.

CROQUIS DE UBICACIÓN

Área de muestreo para huevos, larvas y pupas: **100 m²**

Tiempo de observación de mariposas volando: **20 mins.**

Densidades de mariposas perchadas por árbol:



Para obtener el promedio:

% promedio =

%A1 + %A2 + %A3....

3

= 40% + 20% + 80%

3

= 46.6%, lo que se considera como un 40% aprox.

Monitoreo de colonias de mariposas Monarca en sus áreas de hibernación en México.

Ubicación y medición de colonias de hibernación de mariposa Monarca:

Durante cada temporada a partir del 1 de diciembre se realizan recorridos de campo, en ocasiones hasta el 31 de marzo del siguiente año. Los recorridos se realizan en 11 microrregiones montañosas, conocidos localmente por la presencia histórica de una o más colonias de hibernación de mariposa Monarca. Al respecto se visitan cada quince días 11 sitios (1 por día con excepción de las colonias de Valle de Bravo, Oxtotilpan y Palomas, ya que su muestreo se hace de manera conjunta, en virtud de su cercanía y para lo cual se conformaban dos equipos de trabajo). Para ubicar geográficamente las colonias, se visitan todos aquellos predios o núcleos agrarios (ejidos, comunidades indígenas y pequeñas propiedades) en donde se han documentado históricamente colonias de mariposa. Las coordenadas geográficas de cada colonia se registran mediante el uso de un geoposicionador Garmin® en proyección UTM y con base en el datum WGS 84, tomando como base el punto periférico superior de la colonia con respecto a la pendiente.

Los recorridos de campo se realizan usualmente al amanecer con hora de conclusión indistinta, toda vez que ello depende de las condiciones ambientales prevalecientes, del número de integrantes participantes y de las distancias de los sitios en que se ubiquen las colonias de mariposas, ya que estas conforme avanza la temporada de hibernación van cambiando de lugar. Usualmente participaron en los recorridos de campo de 2 a 4 gentes de la Reserva de la Biosfera Mariposa Monarca (RBMM) y del Fondo Mundial para la Naturaleza (WWF). Para las colonias de Oxtotilpan, Piedra Herrada y Palomas, se cuenta con el apoyo en campo de personal adscrito a las Áreas de Protección de Flora y Fauna Nevado de Toluca y Protección de Recursos Naturales Valle de Bravo, Malacatepec, Tilostoc y Temascalatepec.

Durante cada visita a las colonias de hibernación registradas, se mide el perímetro de cada una de ellas utilizando brújula y cintas topográficas, usando los árboles periféricos ocupados por mariposas como vértices de un polígono. Como punto inicial se utiliza un árbol georreferenciado (Fig. 1). El perímetro de las colonias se marca con cintas plásticas

de colores, con el número de árbol y la fecha de medición. Posteriormente se determina la superficie ocupada por cada colonia utilizando el software de análisis espacial ArcView 3.3.

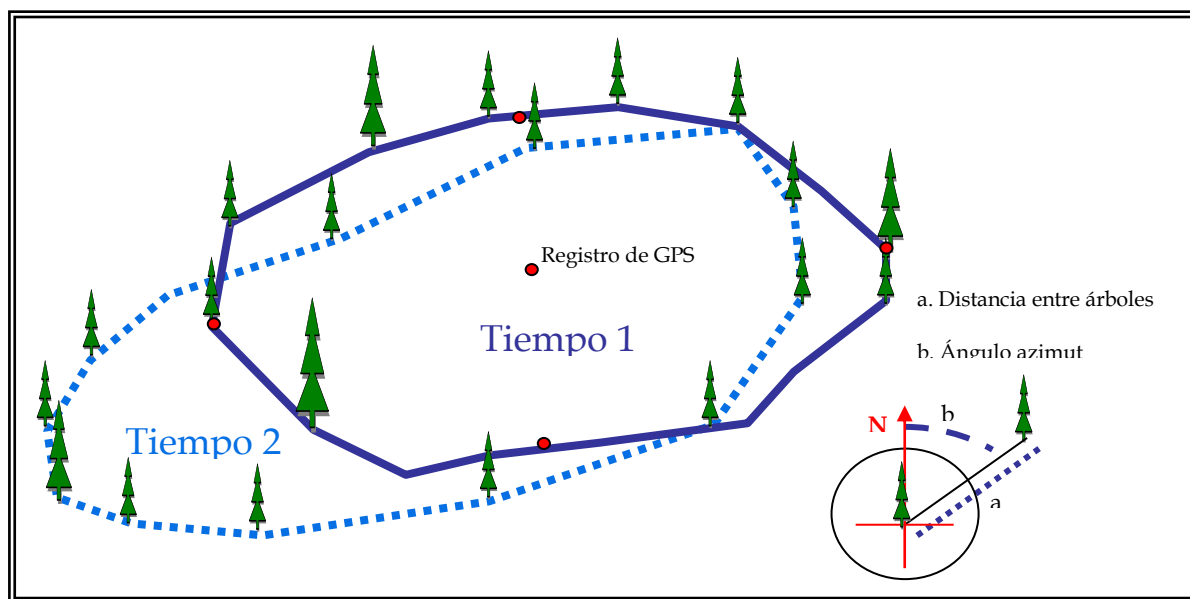


Fig 1.- Medición de superficie forestal ocupada por mariposa Monarca

Mortalidad de mariposas Monarca:

Se determinan de tres a cinco cuadrantes de 10 x 10m dependiendo del tamaño de la colonia. En cada uno de estos cuadrantes se definen cinco unidades de muestra de 1m² c/u. Siempre con orientación norte- sur (Fig. 2). En cada unidad de muestra (1 m²) y cada tercer día, se realizan colectas de los restos de mariposas que se encuentren dentro. Los muestreos se realizan en las colonias más representativas en materia de superficie forestal ocupada y constancia de ocupación. En la colecta de mariposas participan pobladores locales, previa capacitación, quienes se trasladan al amanecer para llevar a cabo la revisión de los cuadrantes establecidos y tomar las muestras respectivas utilizando para ello bolsas de plástico en las que se señala con marcador, la fecha y sitio de colecta. Las muestras, una vez entregadas al personal técnico de la reserva, son analizadas cada 14 días con el objeto de cuantificar y determinar la causa de muerte, por parte de personal adscrito a la Reserva y al Fondo Mundial para la Naturaleza (WWF, por sus siglas en inglés).

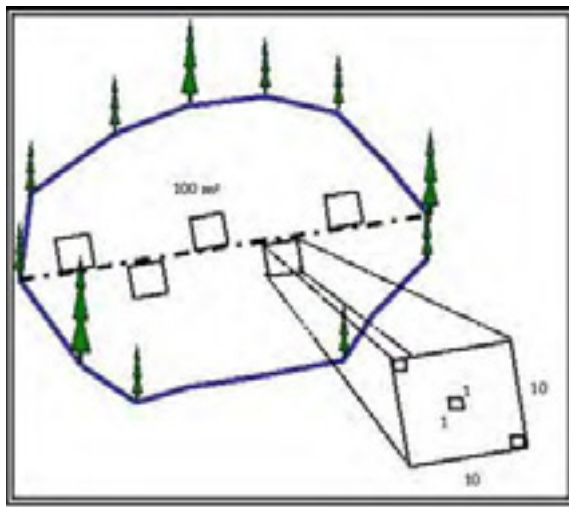


Fig. 2. Muestreo de mortalidad de mariposa Monarca

Las mariposas que presentan rastros claros de haber sido atacadas por aves y ratones son diferenciadas de aquellas que no presentan señales de algún tipo de afectación física, a las que se les denomina Muertas Sin Causa Aparente (MSCA).

Es importante señalar que las colectas realizadas y el manejo de ejemplares, son soportadas con un permiso expedido por la Dirección General de Vida Silvestre. Una vez concluido el análisis de mortalidad, los individuos colectados son regresados a los sitios originales de colecta.

United States



Monarch Monitoring Protocols: Monarch Conservation Science Partnership Integrated Monitoring Strategy

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Introduction

What is the Monarch Conservation Science Partnership?

The Monarch Conservation Science Partnership (MCSP) is a group of scientists and conservation professionals from government, academia and NGOs. They have been working together since 2009 to:

1. Model monarch population trends and assess the impact of threats,
2. Establish population targets and habitat goals, and
3. Develop conservation tools to guide conservation decision-making.

Monarch Monitoring

Monarchs have been the focus of a rich array of citizen science and other monitoring programs for decades. Together, these monitoring programs offer a collective portrait of monarch biology and population health and have made significant contributions to monarch science (Ries & Oberhauser, 2015). However, with the dramatic decline of the monarch population in recent years, more monitoring data are needed for two reasons.

1. People frequently monitor high quality habitat where they are likely to encounter monarchs, making extrapolating information across the U.S. landscape difficult. Spatially representative data are needed to improve models so conservation efforts can use limited resources to have the greatest possible impact.
2. As conservation actions take place over time, ongoing monitoring will help determine if these actions are having the desired outcomes.

The Integrated Monitoring Strategy is a way to address these challenges, and your participation is needed!

What is the Integrated Monitoring Strategy?

The Integrated Monitoring Strategy is a program to monitor key monarch and habitat characteristics across the monarch's U.S. range. Your results will inform monarch population habitat targets, help scientists understand the threats monarchs face, and inform habitat enhancement strategies.

The Integrated Monitoring Strategy consists of three activities:

- Monitoring adult monarchs and identifying preferred adult nectar plants
- Estimating milkweed species diversity and density, per plant density of monarch eggs and caterpillars, and relative abundance of blooming nectar plants
- Estimating monarch survival to adulthood by raising collected monarchs

You can also choose which activities to do. While doing all activities is encouraged, it is not required! Instructions for monitoring in different land types (protected grasslands, unprotected grasslands, CRP (Conservation Reserve Program) lands, cultivated land, right-of-way habitats, urban/suburban spaces) are provided.

Your monitoring coordinator will provide a list of priority (public) sites near you. Priority sites are where monitoring is most needed, however, you may also monitor a site of your own choice.

This is the second year of the project, and your contributions this year will help the MCSP to implement and improve the project in the future. With help from people like you across the U.S., we will be able to address important gaps in our knowledge about monarchs and their habitat!

Steps to Begin Monitoring

1. Select a site to monitor (either adopt a high priority site or choose a site).
2. Delineate your site edges by walking it in the field or by looking at aerial imagery (such as in Google Earth), and then send this site location and boundary information to your monitoring coordinator, along with information about which activities you'd like to partake in.
3. Your monitoring coordinator will send you sampling maps.
4. Start monitoring (return to the site every 3 weeks).

Getting Started: Site establishment

Before you can start sampling, you will need to 1) *Choose a site*, 2) *Delineate site boundaries*, and 3) *Describe the site*.

A site is defined as the area in which you will conduct these monitoring activities. Sites will vary in size, shape, and land cover depending on your location. Sites are contiguous areas of the same type of habitat (such as grassland, park, garden, or roadside right-of-way), bounded by edges of other habitats, or by roads or field edges.

1) *Choose a Site*

You can choose either to *adopt a high priority site* (made available by the monitoring coordinator), or *define a site of your own*.

Adopt a High Priority Site: High priority sites come from a spatially balanced, random sample provided by program coordinators. *Data from randomly selected sites will be weighted more heavily in the analysis than data from sites selected by participants.* Randomly selecting locations is essential in order to obtain an accurate representation of monarch habitat and monarch distribution throughout the US.

To adopt a high priority site, the monitoring coordinator will provide site options based on your location and willingness to travel. Once you've selected a site from the options list, you will visit it to verify its suitability for monitoring and delineate the site boundaries.

Define your own site: A site can be any large open space to which you have access. It could be part of a public location, a property that you own, or a location where you know the owner/manager and can obtain permission. If you have a site in mind already, contact your monitoring coordinator and provide details on the location and approximate size. The following land cover types are appropriate for monitoring:

- **Grassland or prairie:** Restored or remnant prairies are excellent monitoring sites. If you are not the landowner, make sure you have permission to be on the land.
- **Roadsides:** Roadsides should be at least 6 meters wide. Try to select an area without bends or curves, which can make it difficult to lay out your transect. Even though right-of-ways are public lands, bear in mind that you usually need to seek permission to monitor from your local transportation authority.
- **Public Parks:** Many city, county, state, and federal parks have monarch resources such as milkweed and nectar plants. Remember, most milkweed grows in sunny areas! You should reach out to the park's manager or land care office and let them know that you would like to monitor the monarch habitat so that they will expect to see you regularly in the park.

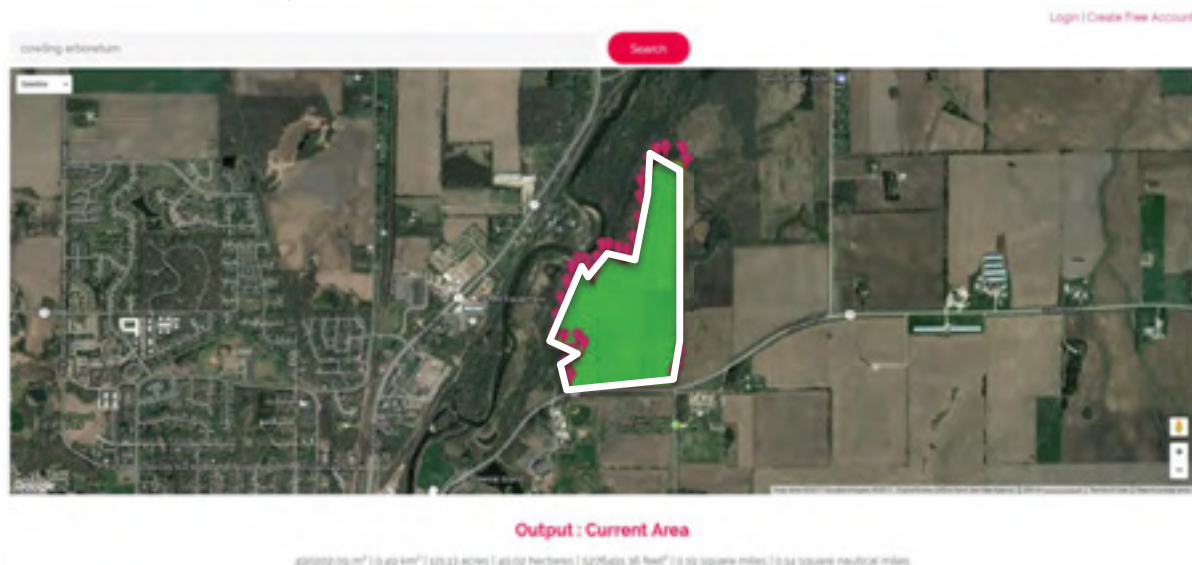
2) Delineate Site Boundaries: Once you have selected a site, you will delineate the site boundaries in order for the monitoring coordinator to provide you with a sampling map.

- a. **Roadsides:** Due to their linear nature, for roadside sites, just send the monitoring coordinator an address or coordinates (latitude and longitude) for the location you'd like to monitor (see Appendix C). Later, after vegetation monitoring, you will provide the coordinates from the end of the stretch of road that you have monitored and that will serve to delineate the site boundaries.
- b. **Grasslands, prairies, or parks:** To generate sampling locations within the site, monitoring coordinators need a map of the site boundaries. Follow these directions to create an outline of your site and then send it to your monitoring coordinator, who will make a sampling map.

You can delineate your site boundaries by using aerial imagery through a Google Maps Area Calculator or by walking the boundaries of the site using a GPS unit.

Using the Google Maps Area Calculator:

1. Navigate to the following webpage: <https://www.daftlogic.com/projects-google-maps-area-calculator-tool.htm>
2. Using the sites search bar, enter an address or coordinates near the location of your site.
3. Using your mouse, click around the edges of your site to create a polygon or an outline of the site area (see image example).
 - a. If you have a pond (or another large obstacle) in the middle of your polygon, first outline the full site, and follow the remaining steps. Then go back, outline the obstacle and send us a both files.



4. After you draw the polygon, click “Generate KML” (pink button under map).

5. Click the “Download KML File Here” link that appears below the pink buttons.
6. Save the file using the naming system: lastname_city_state_yyyy.mm.dd
 - a. Here is an example: hancock_philadelphia_PA_1776.07.04
7. Email the saved KML file to your monitoring coordinator at mjvmonitoring@umn.edu.

Using a GPS unit:

Walk the perimeter of the site using the track function on your GPS to obtain a polygon of your site. Then download the locations and send them to the monitoring coordinator. For information about how to use a GPS unit and how to send the GPS information to your monitoring coordinator, see Appendix C.

3) Describe the Site: Each time you visit your site, your first task will be to describe the site on the Site Summary datasheet. On your first visit write a brief description of the site and surrounding landscape, in particular noting the adjacent land uses (including what type of crops if you can tell, etc). You will also want to carefully document any disturbances (such as mowing, grazing, burning) on your site. If you’d like, take four photos from various angles in order to document as much of the site as possible. You may also take additional photos to document the disturbances on your site.

For each subsequent visit throughout the sampling season, you will note any changes to the surrounding landuse or site such as new disturbances (different from those noted during your last visit) on the Site Summary datasheet. If nothing has changed from your first visit, it is fine to leave the “Site Notes” section of the datasheet blank.

What is considered a disturbance?

Any recent management or natural disturbances that have changed the structure or composition of the vegetation **over at least 10% of the monitoring site**. Most observable disturbances will have occurred within the last year. Some disturbances, such as tree blow-downs, may be visible much longer than two years and should be documented. If there is no observable change to the expected structure or composition of the vegetation (even if records indicate management took place; e.g. burning or grazing), then put a check next to “No Disturbance” on the Site Summary datasheet.

Activity 1: Counting Adult Monarchs

If you are doing both Activity 1 and 2, Activity 1 should always be completed first. This is because monarchs you would count during Activity 1 will be disturbed by the transect and subplot layout required in Activity 2.

Purpose

In this Activity, you will 1) *Count adult monarchs*, and 2) *Document their behaviors*.

Attributes Measured

- Number of adult monarch butterflies
- Adult behavior
- Plant species used by adults for nectaring
- Temperature in the shade (ambient °F)

Equipment and Materials (* = optional)

- Paper map (provided by monitoring coordinator)
- Datasheets, clipboard, pen/pencil
- Compass
- GPS or smartphone*
- 5m-long string*
- Monarch identification materials (see Appendix B)*

Overview

Roadside areas take approximately 20 minutes to monitor. For other sites, this activity takes 30-45 minutes to complete, depending on the size of the site. Monitoring should be completed every three weeks throughout the growing season (May-September in the Midwest). Conducting this activity more frequently, such as once per week, would provide additional data if your schedule allows, but is not required. The data gathered will help evaluate monarch use of the habitat. Ideally, surveys should only be conducted:

- Between 10:00am and 4:00pm,
- On sunny, warm days with temperatures ranging between about 70–87 °F
- During mild winds (less than 30 mph)

Do not conduct this activity if it is raining, if wind speeds are continuously above 30 mph, or if temperatures are below 60 °F; these are not prime conditions for viewing butterflies. Since strong winds occur frequently in open prairies and are sometimes unavoidable, wind conditions up to 30 mph are allowable, but low wind speeds are preferred for spotting flying adults. To give you an idea of how wind speeds compare, the Beaufort wind scale describes a 30 mph winds a “strong breeze” (just under a moderate gale) and notes that, at 30 mph, large branches move and umbrellas are difficult to control.

You will use a modified Pollard walk (Pollard, 1977) to survey adult monarch butterflies. This involves walking the longest straight line (transect) possible across your site; the location of this transect will be provided by the monitoring coordinator (Figure 1). For roadside sites, you will walk a 300 meter transect up the middle of the right-of-way corridor on one side of the road and then repeat on the other side of the road (Figure 2). During each transect walk, you will count all adult monarchs observed within a specific distance relative to the transect and note their behavior. For help identifying and distinguishing adult monarchs from other monarch butterfly mimics, see Appendix B.

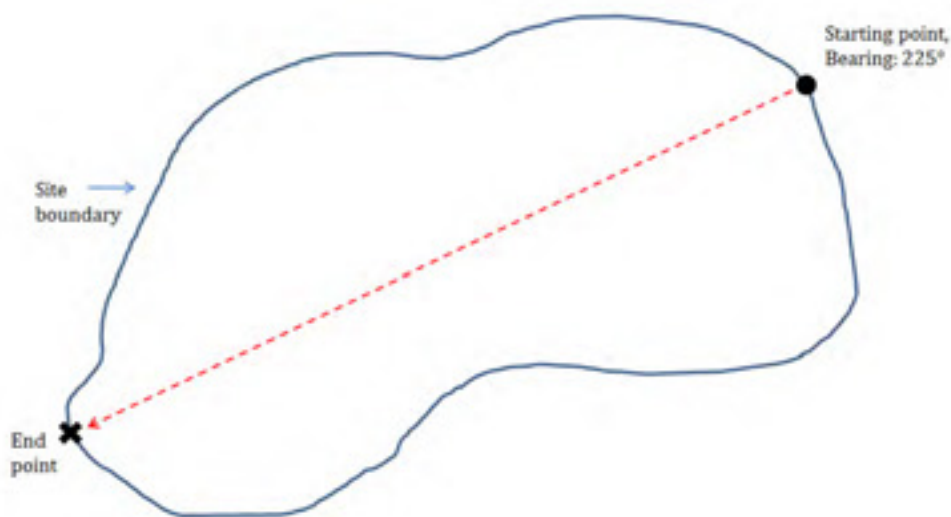


Figure 1. Map depicting route to walk across the longest portion of a grassland or prairie site for butterfly counts.

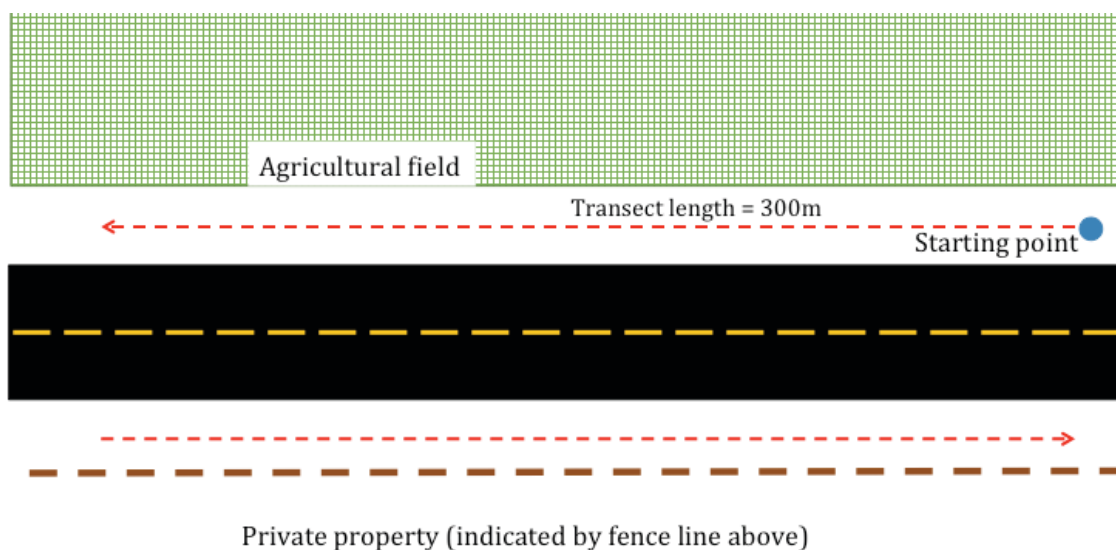


Figure 2. Map depicting route to walk (indicated by red dashed arrows) when counting butterflies along a roadside. You will walk a 300m transect on each side of the road, totaling 600m.

Conducting the Count

- 1) Walk to the starting location indicated on your sampling map provided by the monitoring coordinator.
 - a. *Using the map:* Locate the start point for the butterfly walk on your map. Use your best judgment to determine where the point is located based on landscape features pictured on the map.
 - b. *Using a mobile device:* Enter latitude and longitude coordinates into the Google Maps app or similar mapping program. To do this, open the app, enter the coordinates for the start point into the search box as they are listed on your sampling map. Walk, following the device guidance until you arrive at the point.
 - c. *Using a GPS:* Enter the coordinates for the start point as listed on your sampling map and begin walking, following the arrow and directions on your GPS until you arrive at the point
- 2) From the starting point, use a compass to find the directional bearing listed on your map and *slowly walk* in that direction across the site. Keep a pace of 30 meters per minute (or one meter every two seconds, similar to a “wedding walk” down the aisle). For roadsides, walk 300 meters parallel to the road in the center of the right-of-way area, and then repeat on the opposite side of the road. In a roadside, a 300 meter transect at this pace should take 10 minutes.
- 3) Report the location of all monarchs observed in front of or to the sides of you, including butterflies not moving. When you first observe a monarch, write an ‘A’ in the column for the distance it is first observed. This is the perpendicular distance away from the imaginary line that you are walking, and will either be 0-2.5 meters, 2.5-5 meters or “Incidental” (meaning it is outside of this sampling range). This also pertains to vertical distances, so you are essentially recording data relative to a box around the transect.
- 4) For each monarch, on the same line, record a corresponding ‘A’ based on their behavior *when first sighted*: **flying, resting, ovipositing (egg laying), mating or nectaring** (See Appendix B). If a monarch is observed nectaring, record the blooming species the monarch is nectaring from (if you know it).
- 5) If the same butterfly moves into a different distance category, on the same line, write a ‘B’ under the new distance and another ‘B’ under the column for observed behavior.

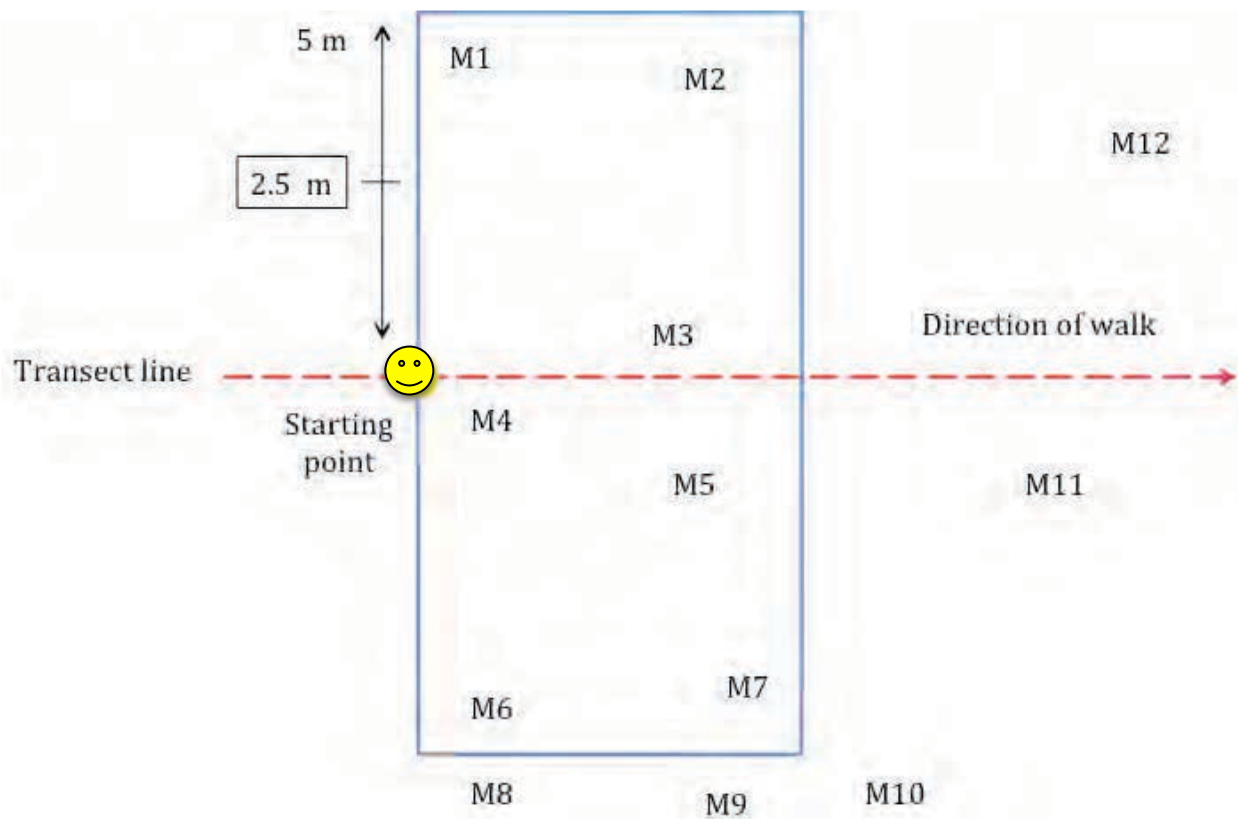


Figure 3. Monarch adult counting frame. In this example, if each “M” is a monarch, there are 7 monarchs (M1-M7) within the 5-m counting frame at this moment. M8-M12 are outside of the counting frame and are not counted. M3-M5 are within 2.5 m of the transect line. M1, M2, M6, and M7 are between 2.5-5 m from the transect line. The observer (smiley face) is moving along the transect from left to right. As the observer walks, the counting frame moves forward and some of the butterflies, such as M11 and M12, may move into the frame and be counted. The counting box extends 5 m vertically so monarchs flying within 5 m of the ground within the 5 x 5 m horizontal counting area are included in the count.

Tips for counting monarchs during the transect walk

- If you pause to record data, **pause facing the direction you will continue walking**, record your observations, and then continue the survey at a slow pace. Try to keep track of butterflies that you have already counted, and do not count them twice. If an adult monarch comes into your range and you aren’t sure whether or not you counted that butterfly before, you should count it. Do not count adults behind you.
- To estimate distances up to 5 m, we suggest practicing so you are more comfortable making these estimates in the field. Using a meter stick or tape measure, measure off 2.5 and 5 meters and practice walking while scanning that distance on either side of you. You can also take a 5-meter piece of string into the field for reference. Mark its halfway point by doubling it over and tying a knot in the middle (marking 2.5 meters). Before your count, use the string to get a sense of 2.5 and 5 m in front and to the side of you.

Activity 2: Counting Milkweed, Nectar Plants and Monarchs

Purpose

In this Activity, you will 1) *Find and walk transects*, 2) *Note the abundance of milkweed and nectar plant species*, and 3) *Look for monarch eggs, larvae, and adults*. The method for laying out transects differs slightly depending on whether your site is:

1. Larger than 4 acres
2. 1-4 acres
3. A roadside
4. Less than an acre

This activity will be completed at a site every three weeks throughout the growing season (May-September). Each visit will take 2-4 hours, depending on the site. The data gathered will help quantify the availability of food plants for monarchs, evaluate habitat quality and use by monarchs, and estimate monarch survival rates. **There are two levels of difficulty for this activity to account for varying ability to identify blooming plant species.** In *Level A* you will have the option to identify blooming plants to the species-level. In *Level B*, you will only note the presence or absence of blooming plants.

Habitat Attributes Measured

- Blooming nectar plants
 - *Level A: Frequency and diversity*
 - *Level B: Frequency*
- Per milkweed plant density of monarch eggs and larvae
- Milkweed density and diversity
- Temperature in shade (ambient °F)
- Abundance and behavior of adult monarchs

Equipment and Materials (* = optional)

- Paper map (provided by monitoring coordinator)
 - Datasheets, clipboard, pen/pencil
 - Subplot frame
 - Compass
 - Spooled tape measure*
 - GPS or smartphone*
 - Plant identification materials (see Appendix A)*
 - Monarch identification materials (see Appendix B)*
 - Hand lens or magnifying glass*
-

Sites Larger than 4 Acres

To sample a site, you will use a series of *transects*, or designated pathways, for collecting data. Your monitoring coordinator will determine transect placement on the site you delineated and will provide a map illustrating the transect placement (Fig. 4).

1. Navigate to the starting location of your first transect (Figure 4):
2. *Using the map:* Locate Point 1 on the map provided by the monitoring coordinator. Use your best judgment to determine where the point is located using landscape features pictured on the map.
3. *Using a smartphone:* Enter latitude and longitude coordinates into the Google Maps app or another mapping program. To do this, open the app, enter the coordinates for Point 1 as you would for a regular address, and follow the directions until you arrive at the point.
4. *Using a GPS:* Enter the coordinates for Point 1 and follow the arrow and directions on your GPS until you arrive at the point.
5. If using a measuring tape, secure the stake at the 0 meter mark of the tape at Point 1.
6. Using a compass, walk in the directional bearing indicated on your sampling map (and extend the measuring tape if you are using one).
7. Place your subplot frame at 0 meters (see Figure 5) and sample the subplot for nectar plants, milkweed, and immature monarchs using the instructions in Section II *Data recording*.
8. Place your second subplot 7 meters from the first (see Figure 5). Move along the transect placing each subplot 7 meters from the previous one.
9. Stop recording data when you reach either:
 - a. The boundary of your site
 - b. 50 subplots on one transect
10. Move to your second transect at Point 2 using the methods described in step 1 above.
11. When you reach Point 2, start your transect in the bearing indicated on your map.
12. Walk your second transect following the same instructions as steps 3-6.
13. Continue finding subsequent points and walking transects following the above instructions until you reach a total of **150** subplots sampled within the site.

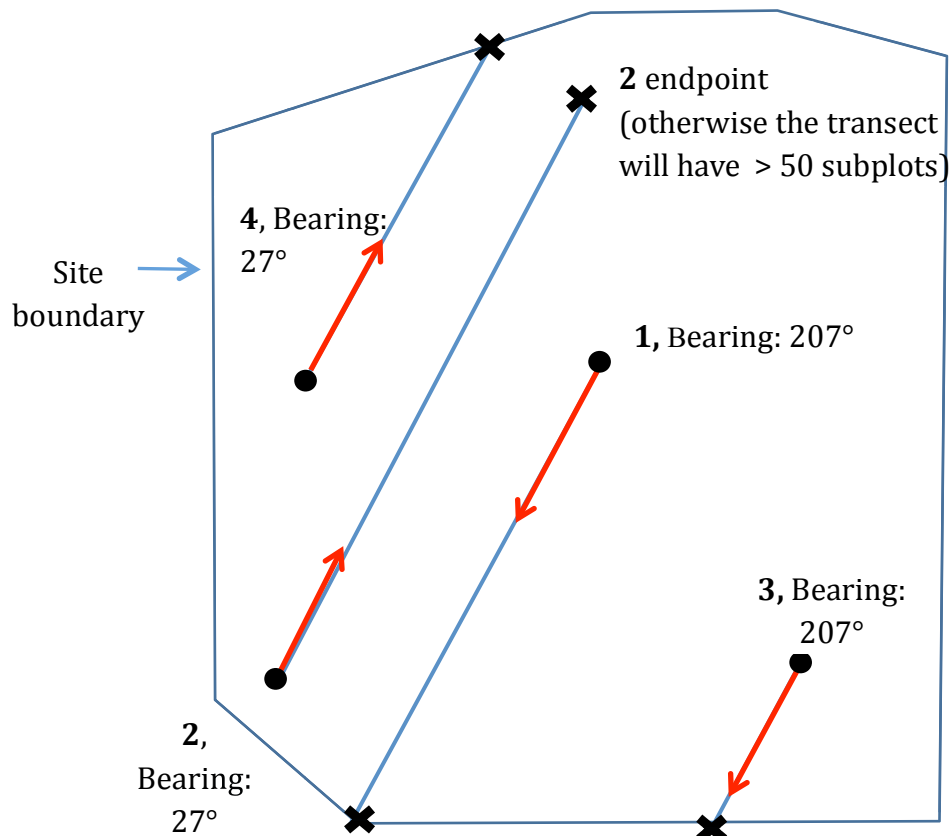


Figure 4. Transect placement within grasslands larger than 4 acre.

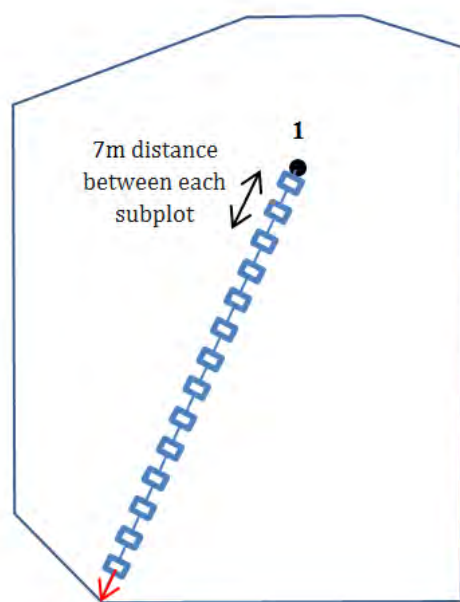


Figure 5. Subplot placement along transects.

Sites 1-4 Acres

To sample a site, you will use a series of *transects*, or designated pathways, for collecting data (Figure 7). Your monitoring coordinator will determine transect placement on the site you delineated during Activity 1 and will provide a map illustrating the transect placement.

- 1) Navigate to the starting location of your first transect:
 - a. *Using the map:* Locate the start point on the map provided by the monitoring coordinator. Use your best judgment to determine where the point is located using landscape features pictured on the map.
 - b. *Using a smartphone:* Enter latitude and longitude coordinates into the Google Maps app or another mapping program. To do this, open the app, enter the coordinates for the start point as you would for a regular address, and follow the directions until you arrive at the point.
 - c. *Using a GPS:* Enter the coordinates for the start point and follow the arrow and directions on your GPS until you arrive at the point.
- 2) Using your compass, orient yourself according to the directional bearing indicated on your sampling map. If using a measuring tape, secure the end at 0 meters and walk the tape out in the directional bearing.
- 3) Place your subplot frame at 0 meters (see Figure 5), and sample the subplot for nectar plants, milkweeds, and monarchs using the instructions in section II.
- 4) Place your second subplot 7 meters from the first (see Figure 5) and record observations as you did in the first plot. Move along the transect placing each subplot 7 meters from the previous one and recording data.
- 5) A transect is complete when you reach the boundary of the site. At this point, you should start the next transect.
- 6) The distance between transects will vary based on the size of your site (see Table 1). This allows you to sample across the entirety of your site. To find the start of your next transect, measure the appropriate distance from the end point of your last transect along the boundary (e.g. 4 meters for a 1.5 ac site). All transects should be parallel to each other.

Table 1: Distance between transects (m) for each site size category.

Site Size (acres)	Distance (meters)
1-2	4
2-3	7
3-4	11

- 7) When you reach the start of your next transect, walk the transect using the bearing indicated on the map provided by your monitoring coordinator.
- 8) Sample along your second transect like you did for Transect 1 until you reach the opposite boundary of the site.

- 9) Repeat steps 6-8 for the remaining transects, *until you've sampled 150 subplots*. If you run out of space before reaching 150 subplots, stop sampling and contact your monitoring coordinator.

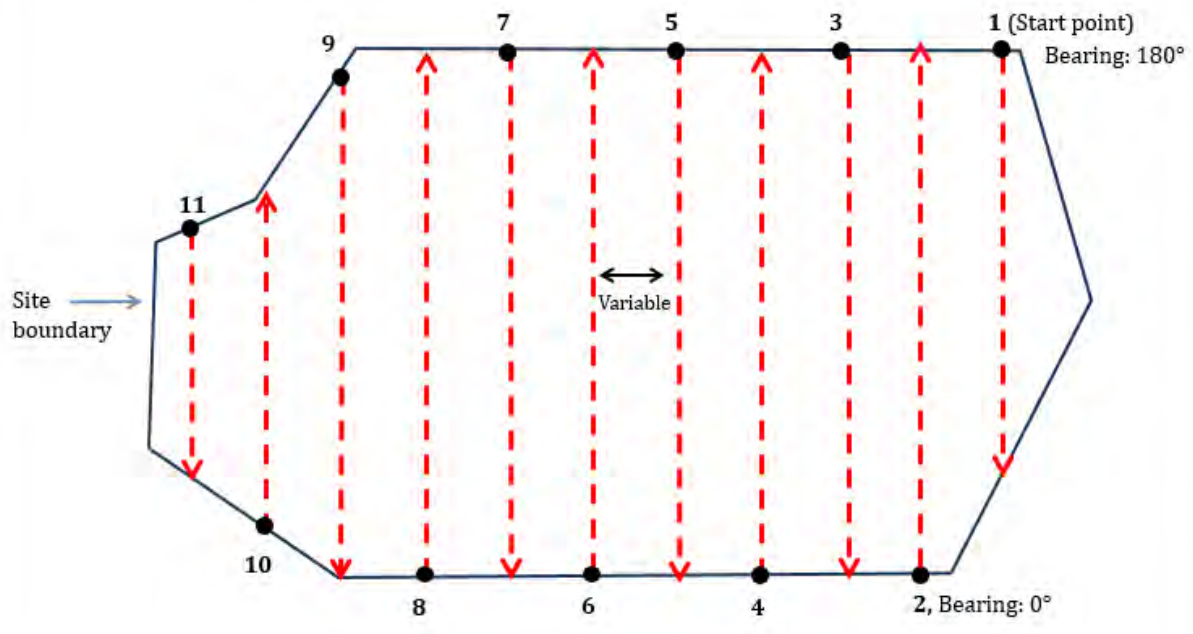


Figure 7. Transect placement in 1-4 acre grasslands. The distance between transects is variable depending on site size.

Roadside Sites

Roadside sampling consists of fifteen 75-meter transects spaced 5 meters apart (8 transects on one side of the road and 7 transects on the opposite side). Each transect starts where the roadside vegetation begins (including mowed areas) and extends diagonally across the full length of the right-of-way so that it spans 75 meters from edge to edge. Along each transect you will gather data at 10 subplots spaced 7 meters apart. Transects and subplot layout are visualized in Figures 10 and 11.

- 1) Navigate to the coordinates for transect one (provided by the monitoring coordinator):
 - a. *Using a mobile device:* Enter latitude and longitude coordinates into the Google Maps app or a similar mapping program. To do this, open the app, enter the coordinates for transect one into the search box as written on the sampling map, and begin walking. Follow the directions until you arrive at the starting point.
 - b. *Using a GPS:* Enter the coordinates for transect one and begin walking, following the arrow and directions on your GPS until you arrive at the starting point.
- 2) If using a measuring tape, secure the end of the tape to the edge of the road where the vegetation begins.
- 3) Measure and record the width of the mowed vegetation, the width of the entire roadside (including mowed vegetation), and the height of the mowed vegetation on the top of Activity 2 Nectar Plants datasheet.
- 4) Flip a coin to choose a random direction in which you will run your transects (either to the left or right of the starting point). This will be consistent on the first side of the road and you will run the transects in the opposite direction when monitoring the other side.
- 5) Walk the tape in a diagonal line (based on the coin flip) through the vegetation towards the opposite edge of the roadside right-of-way (i.e. the agricultural field, fence line, etc.) (see Figure 10).
- 6) When you have laid out 75 meters of tape, adjust your 75-meter diagonal line so that one end is at the edge of vegetation adjacent to the road (your original anchor point), and the other is at the edge of the vegetation adjacent to the fence or other right-of-way boundary (see Figure 10). If there is no boundary, do not extend your transect more than 30 meters away from the road.
- 7) Place your subplot frame at 7 meters and sample the subplot for nectar plants, milkweed, and immature monarchs (see section II *Data recording*).
- 8) Place your second and subsequent subplots 7 meters from the plot before (see Figure 11).
- 9) Stop recording data when you reach the 70-meter mark (after sampling 10 subplots).
- 10) Walk in a straight line back to the road. Move 5 meters down the road. This will be the start of your second transect.

- 11) Repeat steps 2-7 until you have sampled 150 subplots (with 8 transects on one side and 7 on the other).
- When you reach the end of transect 8, collect and record the latitude and longitude coordinates for that end point. Note: If width of the roadside differs between your transects, **adjust your bearing for every transect so that it is 75 meters long, and touches both the roadside and the edge of the agricultural field or fence line.**

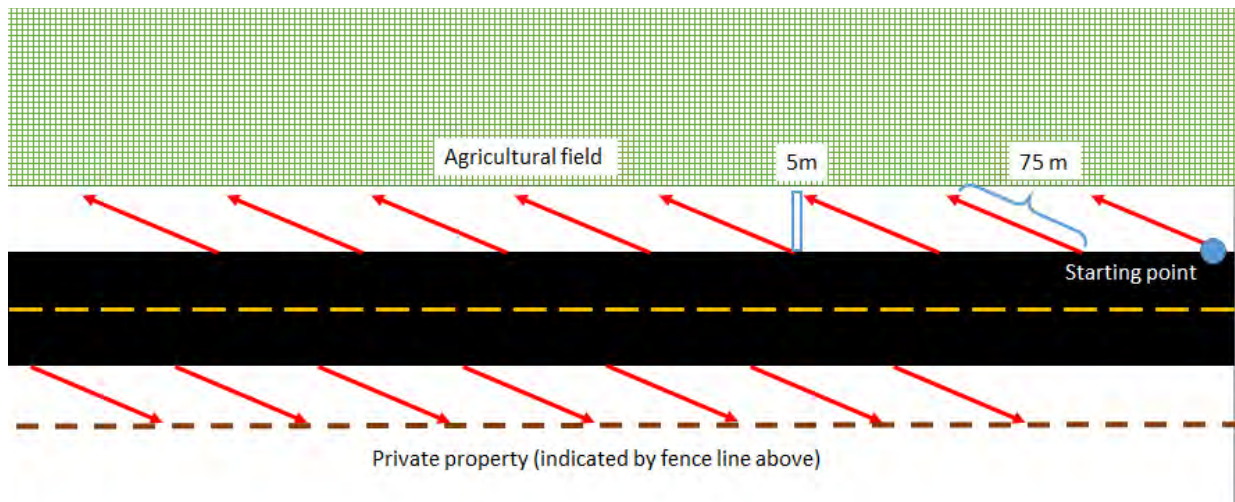


Figure 10. Transect placement in a roadside site. Transects are 75 meters long and run diagonally from the road to an edge (i.e. agricultural field or fence line shown above). The angle will differ depending on the roadside width and the direction (left or right side of the starting point) is randomly determined by flipping a coin. You will run 15 transects to sample 150 subplots: 8 on the first side of the road and 7 on the other.

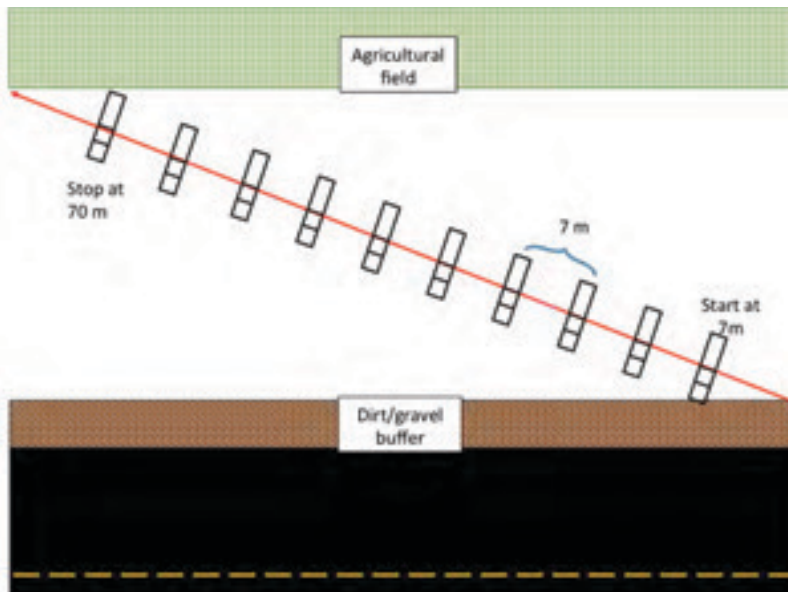


Figure 11. Subplot placement along roadside transects. The first subplot should be placed at the 7-meter mark, rather than at 0 (to avoid sampling the gravel buffer). Place each subsequent subplot 7 meters from the previous. The last subplot on each transect should be placed at 70 meters, totaling 10 subplots per transect.

Recording Data

While sampling each subplot, you will measure four attributes simultaneously:

- 1) Blooming nectar plants²
 - a. Level A: Identify the species of all blooming plants present
 - b. Level B: Record presence or absence of blooming plants
- 2) Density and diversity of milkweed
- 3) Density of monarch eggs and larvae
- 4) Adult monarch abundance and behavior

²If you are able to identify nearly all blooming plants to the species level, follow data recording instructions for **Level A**. If you will only note the presence or absence of blooming plants, follow data recording instructions for **Level B**.

Blooming nectar plants

Level A: Identify all blooming species

- 1) Place the subplot frame so that it is on the left side of the transect.
- 2) Check section A of the sampling frame (Figure 6) for blooming nectar plants (with at least one open, accessible flower) and record all the species you see on the Nectar Plants datasheet for Activity 2 Level A. You do not need to count individual plants, just record that the species is in the section.
- 3) Look at section B, and record any new blooming plant species. **Do not record a species that you found in section A.**
- 4) Flip the sampling frame to the right side of the transect to create section C.
- 5) In section C, record any new blooming plant species. **Do not record a species that you found in sections A or B.**

Level B: Presence or absence of blooming plants

- 1) Place the subplot frame so that it is on the left side of the transect (sections A and B, see Figure 6).
- 2) Check section A of the sampling frame (Figure 6) for blooming nectar plants (with at least one open, accessible flower). If there are blooming plants present, record **A** on the Nectar Plants datasheet for Activity 2 Level B. If nothing is blooming in section A, look at Section B of the sampling frame and record **B** if there are blooming plants present.
- 3) If there are no blooming plants in A or B, then flip the sampling frame to the right side of the transect to create section C. Record C if there are blooming species present. If no blooming plants were found throughout sections A, B, or C, then record zero.

Milkweed Count

- 1) Count all the milkweed plants (or stems that originate from the ground) that you find in the subplot. (You will **not** record the number of plants in each section A, B, or C, but rather, the total number per subplot or sum of A, B, and C.) If a plant has multiple stems, like *Asclepias viridis* or *A. tuberosa*, only record it if the majority of the stems are growing from soil within the subplot (see Appendix A).
- 2) Record the number of milkweed plants by species on the Activity 2 Milkweed and Monarchs datasheet. **If milkweed was blooming and therefore recorded as a nectar plant, record it a second time here.**

Monarch eggs and larvae

- 1) Check all the milkweed plants within each subplot (Figure 6) for monarch eggs and larvae. Record the number of monarch life stages that you see on the Activity 2 Milkweed and Monarchs datasheet. For more information about how to search for monarch eggs and larvae, see Appendix A.
- 2) Continue along the transect and check any milkweed plants between subplots for monarch eggs and larvae³. Monitor all milkweed plants within 1 meter (width of your sampling frame) on each side of the transect line. Record location information for these plants by noting the two subplot numbers straddling the milkweed plant observed. For example, any milkweeds checked between subplots 1 and 2 on a transect get labeled as 1.2, milkweed between subplots 64 and 65 as 64.65, and so on.

³Note: If you reach 100 milkweed plants monitored, you can stop counting and monitoring milkweed between subplots. However, **you should continue** to count and monitor milkweed within subplots.

If you see a monarch egg or larvae on a plant outside of the 1-meter swath to either side of the transect, do not record these observations. Looking at a plant just because you saw a monarch on it is not a random observation, and will skew the data you collect. However, if you see a monarch larva that is NOT on a milkweed plant, but it **is** in a subplot or an area between subplots that you are searching for milkweed, you can record it in the section for that subplot or area. Because older larvae often crawl off plants to molt or cool down, this may happen.

Always record the number of milkweed plants that you examine. ***This includes milkweeds on which you do not observe monarchs.***

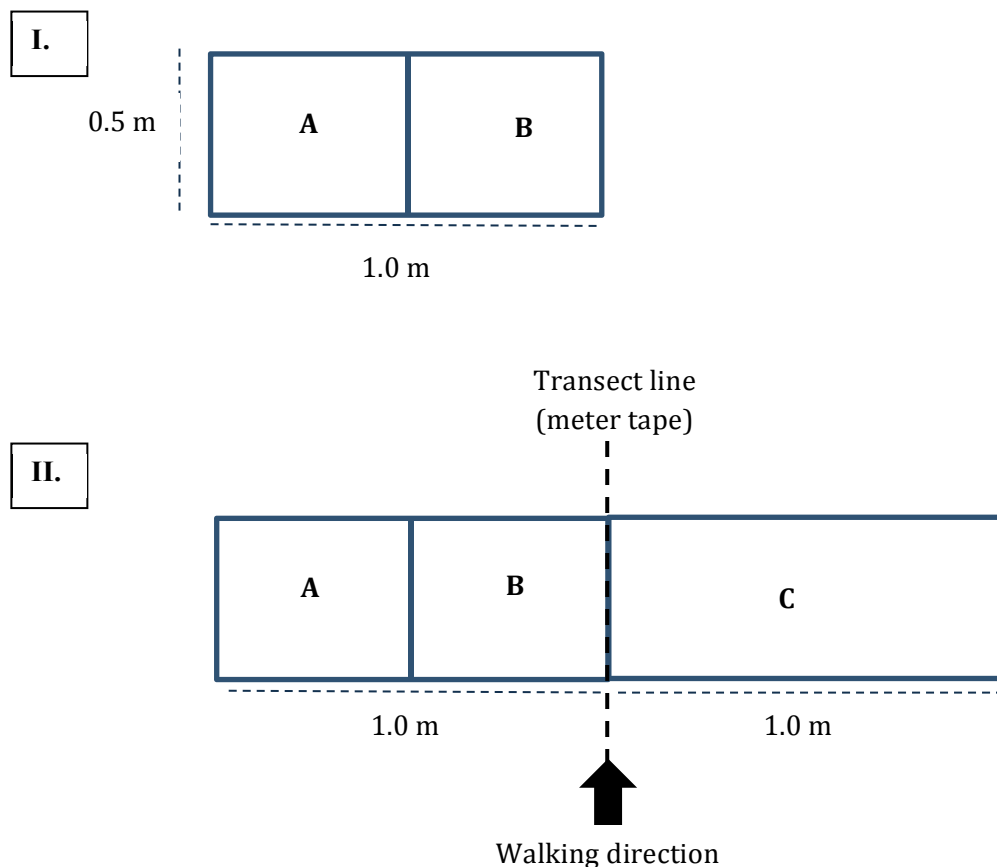


Figure 6. Measuring frame (I) and placement of subplots on transects (II). Measuring frames are 0.5 m x 1.0 m (I) and divided into two areas. Subplots are 1 m², and consist of two measuring frame placements (II). The second measuring frame placement (3rd area) is formed when the 0.5 m² frame is flipped over. When placing the measuring frame, the subdivided count should always be started to the left of the transect. The whole frame count (3rd area) should be finished to the right of the transect line.

Adult monarch abundance and behavior

If you see an adult monarch while you are monitoring milkweed and nectar plants, note its presence and behavior in the Misc. Adult Monarch Observations section at the bottom of Activity 2 Milkweed and Monarchs datasheet. Behaviors include flying, resting, ovipositing, mating, and nectaring. If nectaring, record the associated plant species they are nectaring from. See *Appendix B* for information on how to distinguish between behaviors.

Meandering Walk

After you have sampled 150 subplots, walk through your site and note any additional nectar plant and milkweed species missed by your subplots on your Site Summary Datasheet.

Summary of data recording steps (Level A):

- 1) At 0 meters, place the subplot on the left side of the transect line.
- 2) Record all blooming species present in section A.
- 3) Record all the new blooming species present in section B.
- 4) Count all the milkweed plants in sections A and B, and check them for monarch eggs and larvae.
- 5) Flip the subplot to the right side of the transect line.
- 6) Mark the new blooming species present in section C.
- 7) Count all the milkweed plants in section C, and check them for monarch eggs and larvae.
- 8) Pick up the subplot.
- 9) Between the first and second subplot, count and check all milkweed plants for monarch eggs and larvae within 1 meter of each side of the transect line.
 - a. If you reach 100 milkweed plants, you can stop doing step 9.
- 10) At every 7 meters, place the subplot on the left of the transect and begin again at step 2.

Summary of data recording steps (Level B):

- 1) At 0 meters, place the subplot on the left side of the transect line.
- 2) Record the first section (A, B, or C) in which you observe a blooming plant.
- 3) Count all the milkweed plants in sections A, B, and C and check them for monarch eggs and larvae. Record what you observe on the Milkweed Counts and Larva datasheet.
- 4) Pick up the subplot.
- 5) Between the first and second subplot, observe and record all milkweed plants for monarch eggs and larvae within 1 meter of each side of the transect line.
 - a. If you reach 100 milkweed plants, you can stop doing step 9.
- 6) At every 7 meters, place the subplot on the left of the transect and begin again at step 2.

Activity 2: Counting Milkweed, Nectar Plants and Monarchs in Small Sites (<1 acre)

Purpose

In this Activity, you will 1) *Note the presence or absence of plant species*, and 2) *Look for monarch eggs, larvae, and adults*.

This activity will be completed at a site every three weeks throughout the growing season (May-September in the Midwest). Each visit will take 30-60 minutes, depending on the site. The data gathered will help quantify the availability of food plants for monarchs, evaluate habitat quality and use by monarchs, and estimate monarch survival rates. **There are two levels of difficulty for this activity to account for varying participant ability to identify blooming plant species.** In *Level A*, you will have the option to identify blooming plants to the species level. In *Level B*, you will only note the presence or absence of blooming plants.

Habitat Characteristics Measured

- Blooming nectar plants
 - *Level A: Presence/absence and diversity*
 - *Level B: Presence/absence*
- Density of monarch eggs and larvae
- Density and diversity of milkweed
- Temperature in shade (ambient °F)
- Abundance and behavior of adult monarchs

Equipment and Materials (* = optional)

- Datasheets, clipboard, pen/pencil
 - Plant identification materials (see Appendix A)*
 - Monarch identification materials (see Appendix B)*
 - Hand lens or magnifying glass*
-

Sampling the site

For sites smaller than 1.0 acre, monitor blooming nectar plants, milkweed, and monarchs comprehensively. To do this, walk through the site using a pattern similar to what is shown in Figure 9 to reach all areas of the plot. The tightness of the curves should be adjusted as needed to ensure the site is monitored in its entirety.

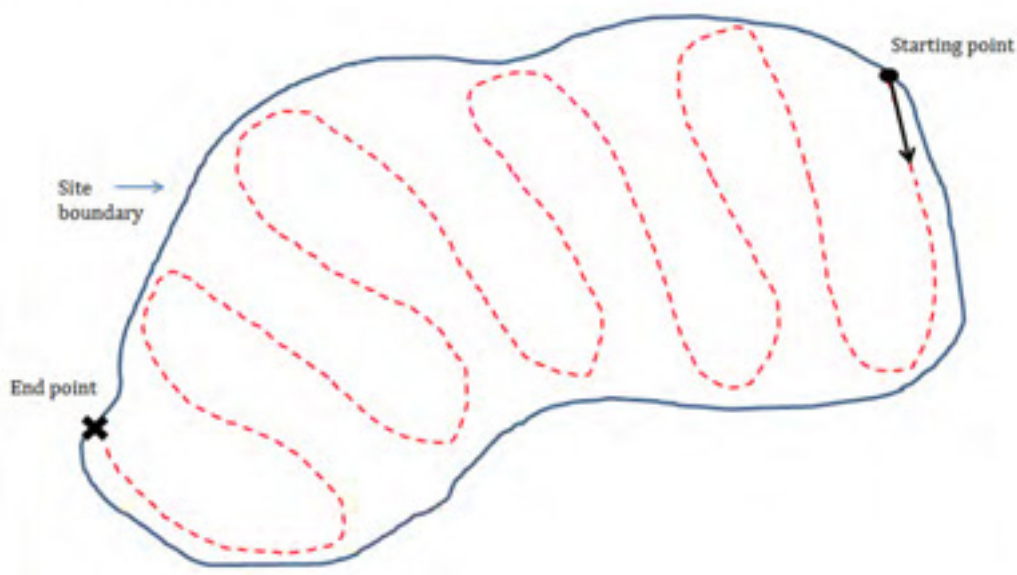


Figure 9. Monitoring path for sites smaller than 1 acre. Start from one edge and walk back and forth in order to monitor all areas of the site.

Recording data

Data for four attributes will be recorded simultaneously on Activity 2 Small Site datasheet. These are:

- 1) Blooming nectar plants⁶
 - a. *Level A: Presence/absence and diversity*
 - b. *Level B: Presence/absence*
- 2) Density and diversity of milkweed
- 3) Density of monarch eggs and larvae
- 4) Adult monarch abundance and behavior

⁶If you are able to identify blooming plants to the species level, follow data recording instructions for **Level A**. If you will only note the presence or absence of blooming plants, follow data recording instructions for **Level B**.

Level A: Identify all blooming plant species

- 1) As you walk, record all blooming flower species that you see at the site. To be considered a blooming plant there must be at least one open, accessible flower **Do not count individual plants, just record each new species you encounter. Individual blooming species should only be recorded once, even if you encounter the same blooming plant species several times.**
- 2) Count all the milkweed plants at the site and note how many species of milkweed there are, regardless if they are blooming.
- 3) Check all the milkweed plants within the site for monarch eggs and larvae. For more information about how to search for eggs and larvae, see Appendix B.

Note: If you reach 100 milkweed plants, you can stop checking them for monarchs. You should, however, continue counting and recording all milkweed plants observed at the site.

- 4) Record the presence of milkweed, blooming nectar plants, and monarchs on the Activity 2 Small Sites datasheet.

Note: If you encounter more than 1000 milkweed plants at your site, contact your monitoring coordinator for assistance devising a different sampling method.

Level B: Presence or absence of blooming plants

- 1) Record whether or not there are blooming plants at your site (Yes/No). To be considered a blooming plant there must be at least one open, accessible flower.
- 2) Count all the milkweed plants at the site, and note how many of each species there are.
- 3) Check all the milkweed plants within the site for monarch eggs and larvae. For more information about how to search for eggs and larvae, see Appendix B.

Note: If you reach 100 milkweed plants, you can stop checking them for monarchs. You should, however, continue counting and recording all milkweed plants observed at the site.

- 4) Record the presence of milkweed, blooming nectar plants, and monarchs on the Activity 2 Small Sites datasheet.

Note: If you encounter more than 1000 milkweed stems at your site, contact your monitoring coordinator for assistance devising a different sampling method.

Adult monarch abundance and behavior

If you see an adult monarch while you are monitoring milkweed and nectar plants, note its presence and behavior in the lower portion of the Activity 2 Small Site datasheet. Behaviors include: flying, resting, ovipositing, mating, and nectaring. If nectaring, record the associated plant species they are nectaring from. See *Appendix B* for information on how to distinguish between behaviors.

Activity 3: Tracking Parasitism and Monarch Survival

Overview

Monarchs often die of causes that are difficult to diagnose, or they may be parasitized by fly or wasp parasitoids that kill them (Monarch Larva Monitoring Project, 2016). This activity will help to estimate monarch larva and pupa survival in addition to the prevalence of some monarch natural enemies, including parasites (especially the protozoan parasite *Ophryocystis elektroscirrha* or *Oe*) and parasitoids (wasps and flies that lay their eggs inside immature monarchs). If you choose to participate in the *Oe* study, you'll need to contact project coordinators at the University of Georgia to obtain a free sampling kit (see instructions below, Project Monarch Health, 2015).

The activity takes 2-5 hours a week depending on the number of late-instar larvae collected. Only do this activity if you have the time and facilities to raise monarchs collected from your monitoring sites.

Collect any 4th or 5th instars each week during your site surveys while conducting any of the other activities. You may collect larvae from your monitoring site or other locations (if you collect them from locations other than assigned monitoring sites, keep track of when and where you collected them and note all relevant information on Datasheet 4).

Rear larvae indoors and record whether they survive to adulthood, and, if not, what caused their death (including if the cause of death is unknown). If you choose to test butterflies for the OE parasite, do this before releasing them. Preserve the adult parasitoids (and the pupal cases) of flies that emerge from the monarchs and mail them to the University of Minnesota for identification.

Equipment and Supplies

Rearing monarchs

- Containers in which to put larvae (see 'Detailed Instructions', Step 3 below)
- Disinfectant (20% bleach-water solution)
- Markers or tape for labeling cages
- Markers
- Paper towels or filter paper
- Screen-sized mesh, cloth, or other porous material for containers (they need air flow)
- Milkweed to feed caterpillars (collected locally)
- Cooler for collecting larvae on hot days

Collecting parasitoid flies

- Monarch Larva Monitoring Project label (Figure 5)
- Small containers (e.g. pill bottles)
- Tissues

Testing for Oe

- Oe testing kit requested from Project Monarch Health (see end of protocol)
- Disposable gloves
- Disinfectant (20% bleach-water solution in spray bottle)

Detailed Instructions for Rearing Larvae to Track Survival

- 1) Collecting 4th and 5th instars will minimize the amount of time required to care for them. Caring for larvae takes time (~30-60 minutes per day depending on the number you collect), so only collect as many as you can care for.
- 2) If it is over 85°F on a monitoring day, avoid collecting monarch eggs or larvae, or bring a cooler for them. Even if it is cooler, plastic or glass containers act as small greenhouses, so containers with monarchs should be kept out of the sun, in a cooler, cloth bag, or other opaque carrier. As you collect the monarchs, ensure that you collect the milkweed leaf on which you find them, and enough additional milkweed to last the rest of the day. If the caterpillar is on a stem, gently remove it from the plant and give it a leaf from the plant on which you found it. Occasionally caterpillars are located on vegetation near a milkweed plant, in which case you can collect the caterpillar and leaves from the nearest milkweed.
- 3) Keep individual larvae in a jar, quart-sized deli container, or another container. When they are smaller, they can be in smaller containers (Figure 13). Containers should be easy to open; have screen or cloth coverings, or holes for airflow; and be large enough for the adult to expand its wings when it emerges. Keep it out of the sun or other hot places. Keep only one larva in each container to combat disease spread, and to allow accurate tracking of individual larvae.



Figure 13. Example of rearing set-up (Photo courtesy of Ilse Gebhard).

- 4) Clean jars daily. Empty out the caterpillar frass (excrement) and old milkweed. Wash your container before using it for another caterpillar using a 20% bleach-water solution.

5) 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 milkweed bag.

6) 4th and 5th instars will likely pupate within a week. They'll crawl to the top of their cage and form a pre-pupal "J" before shedding their skin for the last time (Figure 14). Be careful to not jostle the container while larvae are pupating. If the pupa becomes detached, carefully tie dental floss or thread around the stalk of the pupa (cremaster) and tape it to the top of the cage.



Figure 14. Pupation sequence (Photos courtesy of Siah St. Clair).

7) The pupa stage lasts 9-14 days. Pupae turn darker the day before butterflies emerge, and look black on the day they emerge (Figure 15). At this point, the wings are visible. The butterflies usually emerge in the morning. Their wings will be soft and flexible when they emerge, but they'll be ready to fly in ~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, after recording information on Datasheet 4, and, if possible, testing it for *Oe* (see below).

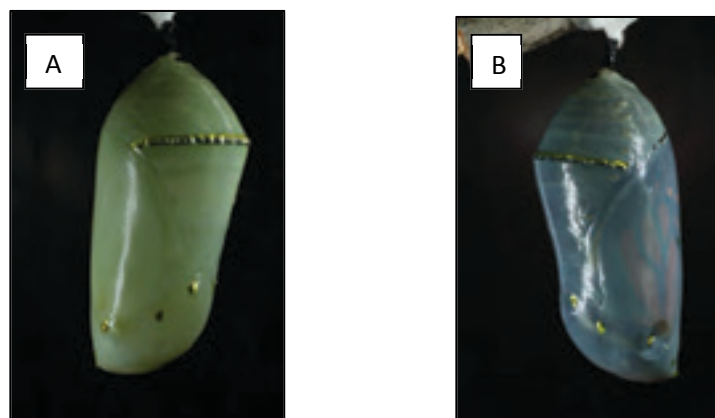


Figure 15. Monarch pupae (A) early stage chrysalis/pupa and (B) mature monarch chrysalis/pupa about to eclose. Note that wings are visible through the chrysalis wall (Photos courtesy of Siah St. Clair).

8) Larvae that have been parasitized by flies will often not pupate successfully, but will hang limply and die, although some flies will emerge from the pupa. Fly maggots come out of the host on a silk-like thread (Figure 16A), 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.



Figure 16. Tachinid fly parasitoids of monarchs. (A) Monarch pupae with silk-like thread from tachinid fly parasitoids (Photo courtesy of Sonia Altizer). (B) Three tachinid fly larvae (maggots) from parasitized caterpillar (Photo courtesy of Jaap de Roode). (C) Soon after emerging, the flies pupate, turning reddish-brown (Photo courtesy of Sonia Altizer). (D) Adult tachinid fly (Photo courtesy of UMN Monarch Lab).

9) If monarch larvae die, wait a few days to see if parasitoids emerge from them. Dispose of the monarch larvae carefully (washing your hands after handling them and disposing of them in a different room from where living larvae are kept). Search the dried milkweed leaves for fly pupae.

10) Please send adult parasitoid flies or wasps that come from monarchs you rear to the Monarch Larva Monitoring Project (MLMP). After the **adult flies** emerge from their pupae (7-10 days after they emerge from the monarch), put the flies or wasps **and** pupa cases in small containers (e.g. pill bottles, small boxes, Ziploc storage containers), with tissue to prevent them bouncing around. Do not use cotton balls, as the cotton filaments become attached to parts of the fly that are used for identification purposes. If no adult flies or wasps emerge, please send the pupae (with the same information on the label) because we may be able to identify the fly genus or species from the puparium. **Each container should hold all of the parasitoids that emerged from ONE monarch.** Put them in a freezer until you have several or until the end of the season. Either label each container with the information noted on the container label below (Figure 17), or number the containers and write the information on a separate table sent with the containers. Contact info@monarchlab.org to notify us when a package of flies should be expected. Send the specimens to:

Monarch Larva Monitoring Project
University of Minnesota
Dept of FWCB
2003 Upper Buford Circle, 135 Skok Hall
St. Paul MN 55108

Date monarch was collected:

Stage of monarch at collection:

Milkweed species on which monarch was collected:

Location of collection:

of flies and date emerged from monarch*:

Other notes:

**NOT date adult fly emerged from fly pupal case*

Figure 17. Label for container.

Instructions for Testing Monarch Adults for Oe (optional)

- 1) Obtain a sampling kit from [Project Monarch Health](mailto:monarchhealth@gmail.com). To request a free kit, email: monarchhealth@gmail.com. You will send the sampling card plus a copy of your datasheet to Monarch Health (see address below).
- 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 *Oe*, wear gloves to prevent contamination and change them frequently (after checking every adult monarch). 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 (Figure 18A). 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 against the abdomen of the monarch. Press down so that it wraps around and sticks to the sides of the abdomen (Figure 18B).
- 5) Gently peel the tape off and stick it to the index card (Figure 18C). You will remove scales in the process, but it will not harm the monarch. Label the tape sample with a number that corresponds to Activity 4 datasheet entry (Figure 18D).
- 6) Sanitize your working surface with 20% bleach-water 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 data, send a copy of Datasheet 4 and the index card to:

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

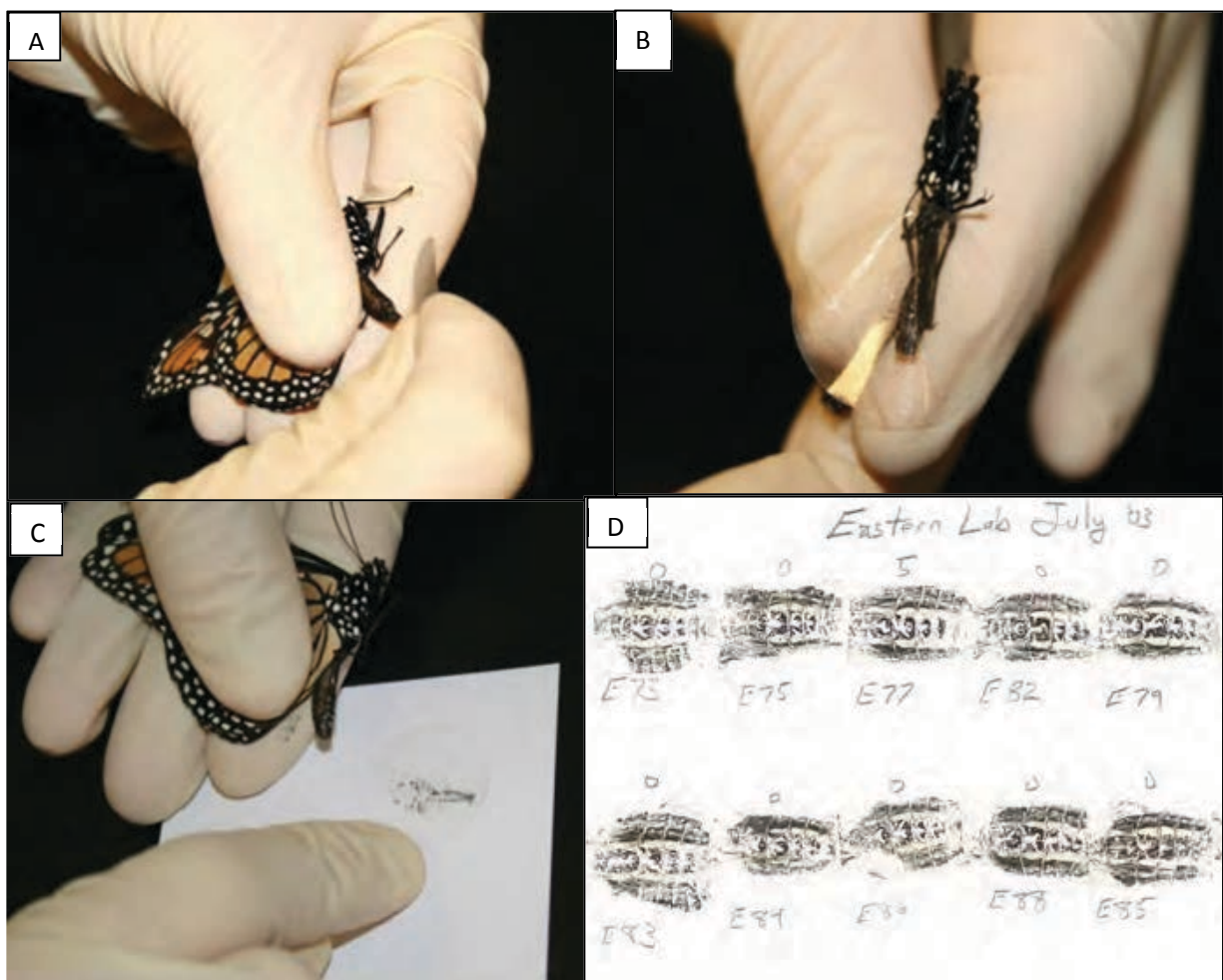


Figure 18. Sampling adult monarch for Oe. (A) Hold the monarch gently with gloved hands (B) Wrap tape around the abdomen (C) Gently peel the tape off and stick it to the Oe index card (D) Example of labeled Oe index card.

Sampling Checklist Sheet

STEP 1:

- ☐ Write the start time on your Site Summary Datasheet
- ☐ Describe the site (or if you have already sampled at this site just note new disturbances)

STEP 2:

- ☐ Navigate to the start of your butterfly walk
- ☐ Use the bearing provided to walk across your site (or along the road), noting monarchs on the Activity 1 Datasheet

STEP 3:

Materials

- ☐ Paper map (provided by monitoring coordinator)
- ☐ Datasheets, clipboard, pen/pencil
- ☐ Sampling frame
- ☐ Compass
- ☐ Rope or spooled tape measure *
- ☐ GPS or smartphone *
- ☐ Plant ID materials (see App. A) *
- ☐ Monarch ID materials (see App. B) *
- ☐ Hand lens or magnifying glass *
- ☐ Safety gear * (for roadside sites)

Sites Larger than 4 Acres

- ☐ Use your phone/GPS or the surrounding landscape to find your start point
- ☐ Use the bearing provided to run the transect tape until you reach the site boundary or you run out of tape
- ☐ Place the sampling frame every 7-m, recording nectar plants, milkweed, and monarchs (also record milkweed and monarchs between subplots)
- ☐ Move to your next point when you reach 50 subplots or the site boundary

Sites 1-4 Acres

- ☐ Use your phone/GPS or the surrounding landscape to find your start point
- ☐ Use the bearing provided to run the transect tape until you reach the site boundary or you run out of tape
- ☐ Place the sampling frame every 7-m, recording nectar plants, milkweed, and monarchs (also record milkweed and monarchs between subplots)
- ☐ At the end of the transect, measure either 4, 7 or 11 meters along the boundary and start your next transect

Roadsides

- ☐ Use your phone/GPS to or the surrounding landscape to find your start point
- ☐ Measure the width of the roadside and the width/height of mowed vegetation on the first transects on each side of the road
- ☐ Run a 75-m transect diagonally across roadside
- ☐ Place the frame every 7-m, recording nectar plants, milkweed, and monarchs (also record milkweed and monarchs between subplots)
- ☐ At the end of the transect, shift over 5-m and run another 75-m transect
- ☐ Run 8 transects on one side of the road and 7 on the other

STEP 4:

- ☐ Meandering walk: Walk through your site and note any additional nectar plant and milkweed species missed by your subplots
- ☐ Note the end time on your site summary sheet

Appendix A: Milkweed and Plant Identification

Counting Milkweed Plants



Figure 19. *A. syriaca* (common milkweed) plants. Above-ground stalks (ramets) could be from different or the same plant. Count each individual stalk as an individual plant unless there is no soil separating stalks (*Photo courtesy of Denise Gibbs*).



Figure 20. *Asclepias tuberosa* (butterfly weed) plant. If the stems originate from the same central place (similar to the example above), count each bush of stems as one plant (*Photo courtesy of Chip Taylor*).

Native Milkweed Species Commonly Found in the Midwest

Asclepias syriaca, Common milkweed

Key Characters:

- Broad leaves, sometimes with reddish vein
- Flowers pink and fragrant
- Stout stem and hairy leaves



Photos courtesy of Wendy Caldwell

Asclepias incarnata, Swamp milkweed

Key Characters:

- Leaves typically hairless and leaf margins toothless
- Leaves opposite and tapered to a point
- Flowers pink and fragrant



Photos courtesy of Wendy Caldwell

Asclepias tuberosa, Butterfly weed

Key Characters

- Flat topped clusters of orange flowers
- Does not have milky sap
- Leaves are stiff, pointed and sessile (have no leaf stalk)
- Flowers have no noticeable scent



Photo courtesy of Chip Taylor



Photos courtesy of Wendy Caldwell

Asclepias verticillata, Whorled milkweed

Key Characters:

- Long skinny leaves whorled in clusters of 3-6



Photos courtesy of Wendy Caldwell

Asclepias sullivantii, Sullivant's milkweed

Key Characters:

- Leaves smooth and lacking stalks
- Leaves oblong or egg-shaped and heart-shaped at the base
- Side veins do not extend to the edge of the leaf



Photo courtesy of Bill Johnson



Photo courtesy of Tim Osborne

Asclepias speciosa, Showy milkweed

Key Characters:

- Leaves with broad base and pointed tip
- Leaf underside hairy
- Red mid-vein



Photos courtesy of Wendy Caldwell



Asclepias exaltata, Poke milkweed

Key Characters:

- Leaves large, broad and tapered to a point
- Leaf stems $\frac{1}{4}$ to an inch long
- Flowers droopy with the horns sticking out of the hoods
- Leaf attachment opposite



Photos courtesy of Janet Allen

Appendix B: Monarch egg, larvae, and adult identification

Eggs and Caterpillars

Remember that monarch eggs and caterpillars can be hard to find!

- Look carefully at all parts of the plant, including the bottoms of the leaves, the area within the small leaves at the top of the plant, and buds and flowers.
- Keep an eye out for caterpillar clues, such as chew marks on the leaves.
- Handle the plants carefully to avoid knocking any larvae off of them.
- Use the pictures of the eggs and 5 larval instars below.

Monarch butterfly eggs and larvae (caterpillars) are found on species of milkweed plants (*Asclepias* spp.). Monarch caterpillars go through 5 stages of development called instars (illustrated below).



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 ridges. (*Photo courtesy of Michelle Solensky*)



Monarch egg (left) and sap bubble from milkweed (right). (*Photo courtesy of Anurag Agrawal*)



Live monarch egg about to hatch. Monarch eggs will develop a dark spot when close to hatching. (*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)*



1st instar feeding damage. This circular feeding pattern is an indication that a monarch first instar was on the plant. Note that striping appears after the caterpillar begins to eat. *(Photo courtesy of Tom Collins)*



1st instar consuming eggshell. First instars are dull greenish-grey when they first emerge from the egg, and have a dark black head. They have tiny front and back tentacles that are difficult to see with the naked eye. *(Photo courtesy of Mary Holland)*



2nd 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).*



3rd instar. This third instar monarch has just molted. As monarch larvae develop, they increase in size and their stripes become more distinct. The front tentacles extend to the tip of the head but not past it (*Photo courtesy of Monarch Lab*).



5th instar. Older larvae have bright yellow, black and white striping and the front tentacles are about 1 cm. long (*Photo courtesy of Richard Hicks*).



4th instar. The front tentacles now extend beyond the tip of the head. Internal changes, including the development of reproductive structures, begin to occur in late instar monarchs (*Photo courtesy of Monarch Lab*).



Monarch instars. The entire larval stage in monarchs lasts from 9-14 days under normal temperatures. The speed of monarch development is temperature dependent (*Photo courtesy of Monarch Lab*).

Adult Monarchs

Male monarchs 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*)

Monarch adult behavior:

Flying	Individual butterflies are in flight
Resting	Alighted on a plant with no sign of mating, ovipositing, or nectaring
Ovipositing	A female adult monarch arching the abdomen and depositing eggs (on milkweed plants)
Mating	Male and female adults are observed copulating
Nectaring	Adults are alighted on a plant and actively extending the proboscis into a flower to obtain nectar

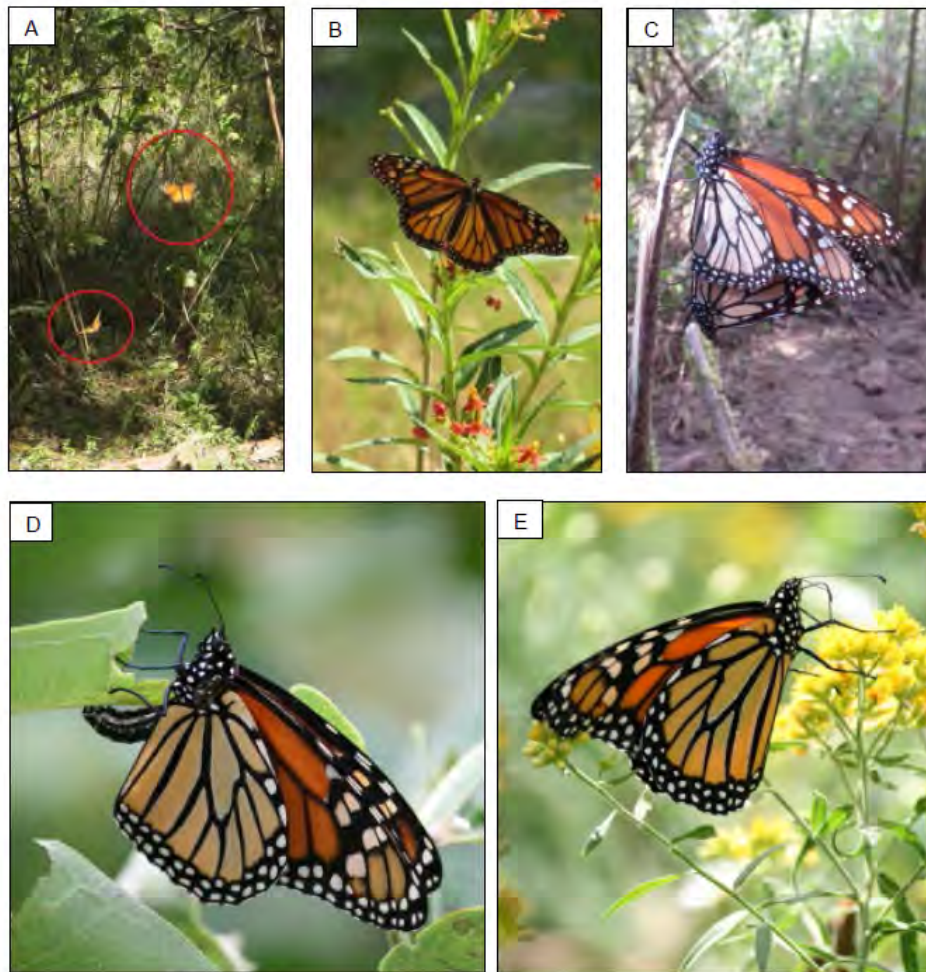


Figure 21. Monarch behavior: (A) Two monarchs flying, (circled in red; photo courtesy of Holly Holt), (B) Resting (photo courtesy of Chuck Patterson), (C) Mating (photo courtesy of Holly Holt), (D) Ovipositing (photo courtesy of Candy Sarikonda), and (E) Nectaring (note that monarch's proboscis is extended into flower; photo courtesy of Candy Sarikonda).

Monarch Butterfly Mimics

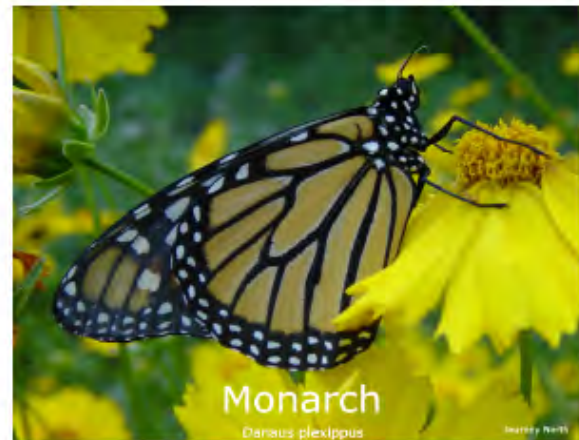
Watch out for mimics! There are a few butterfly species that look very similar to monarchs in the larval, pupal, and/or adult stages.

Viceroy, *Limenitis archippus*



Viceroy adults look very similar to monarch adults. They have the same color and similar wing patterns. To differentiate between them, look at the lower wing. Viceroy has a horizontal line across the bottom of their hindwings (as shown above). Viceroy flight patterns also tend to be faster and more erratic than monarchs (*Photos courtesy of Candy Sarikonda and Journey North*).

Queen, *Danaus gilippus*



Both queen and monarch caterpillars feed on milkweed. You can tell adults apart by looking at the forewings. Queen adults (left) have white spots scattered on the upper forewing (as shown above) (Photos courtesy of Journey North).



Queen larvae have three sets of tentacles. Monarchs only have two (front and back, no middle set) (Photo courtesy of Hayley Schroeder).

Black swallowtail, *Papilio polyxenes*



Black swallowtail larvae have yellow spots and lack the white stripes of monarch larvae. They eat plants in the carrot family (parsley, dill, carrots, etc.).

Appendix C: Using a GPS Unit (designed for Garmin products and PC computers)

If you have access to a GPS unit, we encourage you to use it to delineate site boundaries. These instructions cover how to walk with the GPS in the field to record spatial information about your site, and download and send this information to your monitoring coordinator. **We recommend practicing these steps near home before going to your field site.**

How to Walk with the GPS

A few things to note before beginning your walk with the GPS:

- The GPS will track its movement wherever it goes, so **make sure that you only walk along the outside boundary of the site.**
- The GPS collects data continuously, so if you stand at the same location for a long time, it will collect multiple points at that point. **Try to move at a constant, steady pace.**
- Keep the GPS held out in front of you so you know where it is recording. Remember, it only tracks its own location, not the location of your body.

Delineating Site Boundaries

Directions are for Garmin etrex 10, but most Garmin products are similar

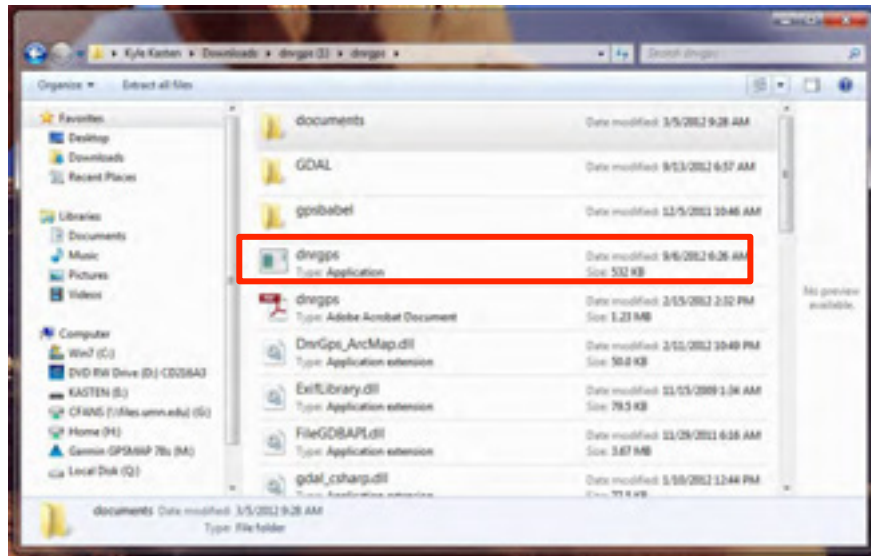
- 1) Proceed to a starting point somewhere on your site's boundary.
- 2) Press and hold the LIGHT button to turn on the device.
- 3) Ensure that it has acquired a satellite signal by checking the map. It should show where you are. You can zoom in or out using the (Λ) or (v) keys
- 4) Press the MENU key twice to display the Main Menu screen.
- 5) Highlight the "Track Manager" option using the toggle.
- 6) Press the ENTER (press the toggle straight down) to display the Tracks screen.
- 7) Press the ENTER on "Current Track".
- 8) Toggle to "Clear Current Track" and press ENTER. Then select "Yes"
- 9) Toggle down to "View Map" and press ENTER.
- 10) Begin walking around your site (see *How to Walk with the GPS* directions above).
You can zoom in or out to view your track.
- 11) Finish at the same point you started.
- 12) Press MENU, and return to the "Track Manager" page.
- 13) Press ENTER on "Current track", then toggle up to "Save Track" and press ENTER.
- 14) Delete the default name, and enter a name that you will remember later. When the name is entered, hit "Done" at the bottom of the screen.
- 15) The GPS will ask you if you want to "Clear the current track and associated trip data". This just means that it will stop recording your movement. Select "Yes" to save your track.
- 16) You can view your track by selecting it from the menu in "Track Manager".
- 17) Turn off the GPS by pressing and holding the LIGHT button.

Downloading GPS data (only compatible with PC)

These directions describe one free program for downloading GPS information. If you are more comfortable with a different program, please feel free to use it.

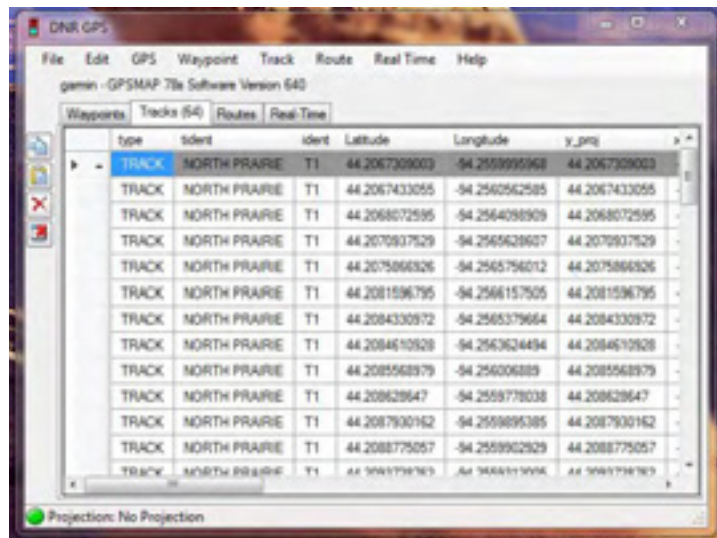
1. Download and install DNR GPS

- 1) Search “DNR GPS” on your web browser, and select the link to the Minnesota DNR. Alternatively type, in the following web address:
<http://www.dnr.state.mn.us/mis/gis/DNRGPS/DNRGPS.html>
- 2) Scroll down until you find “DNRGPS 6.0.0.15”, and click on the link to download the zip file.
- 3) Open the zip file, and run the dnrgps Application.



2. Download data from the GPS

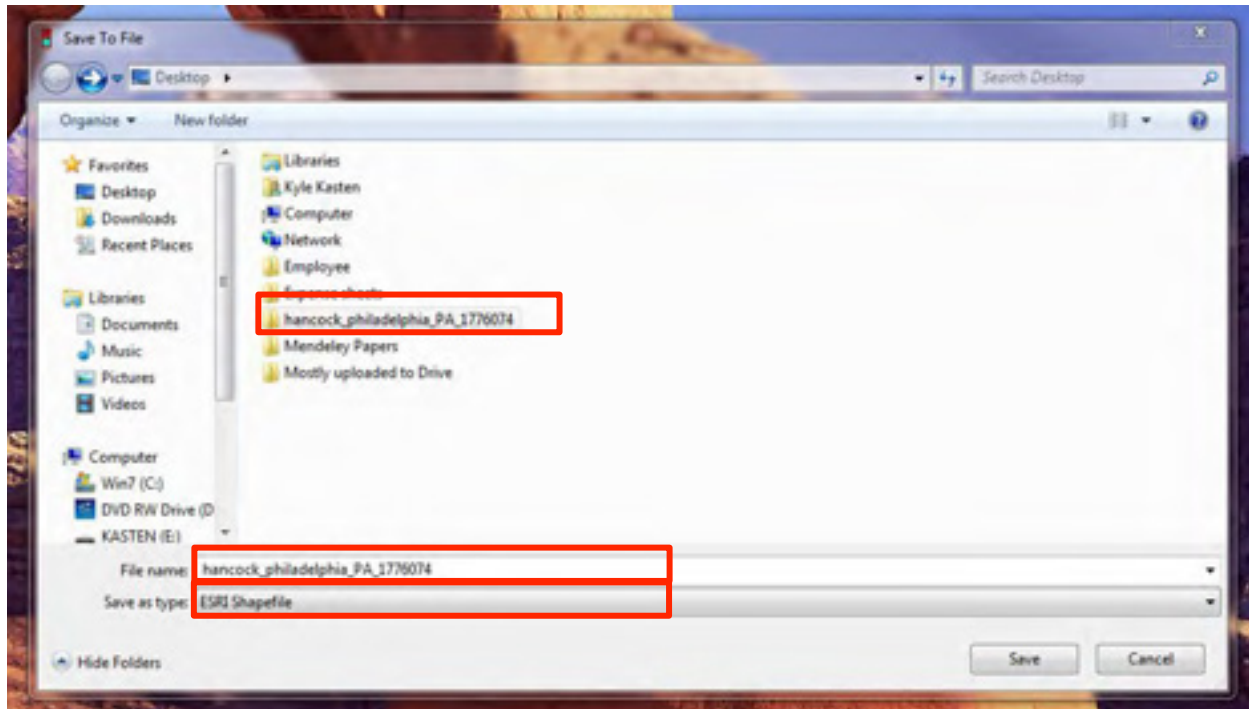
- 1) Connect the GPS to your computer using the cable it came with.
- 2) To download only tracks (aka Polygons), select the track menu along the top bar, then click Download (Track => Download).
- 3) Select the file (whatever you named it in the GPS) and click OK. Your track data should now appear in the table like this:



type	ident	ident	Latitude	Longitude	y_prio
TRACK	NORTH PRAIRIE	T1	44.2067309003	-94.2559995968	44.2067309003
TRACK	NORTH PRAIRIE	T1	44.2067433055	-94.2560562585	44.2067433055
TRACK	NORTH PRAIRIE	T1	44.2068072595	-94.2564098909	44.2068072595
TRACK	NORTH PRAIRIE	T1	44.2070937529	-94.2565628607	44.2070937529
TRACK	NORTH PRAIRIE	T1	44.2075866326	-94.2565756012	44.2075866326
TRACK	NORTH PRAIRIE	T1	44.2081596795	-94.2566157505	44.2081596795
TRACK	NORTH PRAIRIE	T1	44.2084330972	-94.2565379664	44.2084330972
TRACK	NORTH PRAIRIE	T1	44.2084610928	-94.2563624494	44.2084610928
TRACK	NORTH PRAIRIE	T1	44.2085568979	-94.256006889	44.2085568979
TRACK	NORTH PRAIRIE	T1	44.208628647	-94.2559778038	44.208628647
TRACK	NORTH PRAIRIE	T1	44.2087930162	-94.2559895385	44.2087930162
TRACK	NORTH PRAIRIE	T1	44.2088775057	-94.2559902929	44.2088775057
TRACK	NORTH PRAIRIE	T1	44.2089778165	-94.2560110004	44.2089778165

3. *Save data to a file*

- 1) Create a folder on your desktop using the naming system lastname_city_state_yyyy.mm.dd. This will be empty for now, but you will save files to it later. For example: hancock_philadelphia_PA_1776.07.04
- 2) Back in DNRGPS, go to **File => Save To => File**.
- 3) Change your “save to” location to the folder you just created on your desktop.
- 4) Use dropdown menu to change **Save as type** to “ESRI Shapefile”.
- 5) Change the **File name** using the same system (lastname_city_state_yyyy.mm.dd).
- 6) Click Save. At the pop-up box, select Shape Type as “Polygon” and click OK.



4. *Sending data to your monitoring coordinator*

- 1) Files can be sent like a normal email attachment, but first, they need to be compressed.
- 2) Address your email as you would normally, and click attach files.
- 3) Navigate to the folder on your desktop named “ hancock_philadelphia_PA_1776.07.04 ” and right-click on the folder.
- 4) In the right-click menu, find **7-Zip=>Add to hancock_philadelphia_PA_17760704.zip**. Click OK.
- 5) The new “.zip” file is now compressed enough to send. Find it in your files, attach it in your email, and send it to your monitoring coordinator!

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Site Summary Datasheet			Date:	Observers:		
Site:			Start Time:	End Time:	Temperature in the Shade:	
Before Sampling						
Disturbances: check up to 5 recent (< 1 year) disturbances observed on >10% of the plot that may affect the structure and composition of the vegetaion.			Site Condition			
No Disturbance		Grazed				
Pesticide Treatment		Contruction (road, trail, or structure)				
Herbicide Treatment		Prescribed burn/wildfire		Surrounding Landscape		
Fertilizer		Wind event/blow down				
Plowed/disked		Other:				
Flooded						
After Sampling (Only if participating in Activity 2)						
Meandering Walk: In your final walk through your site note any blooming nectar plants and milkweed species that were missed by your transects. Level B participants just note additional milkweed species.				Misc. Adult Monarch Totals (Add totals from the bottom of the milkweed datasheets)		
Blooming Nectar Plants		Milkweed		Behavior	Total	
				Flying		
				Resting		
				Ovipositing		
				Mating		
				Nectaring		
				Unknown		
				Nectar Plant I.D.		

Activity 1 Butterfly Walk				Start Time:				Site:		Date:	
				End Time:				Observer:			
Notes:											
Directions: Walk at a pace of 30 meters per minutes. For each monarch you see, write an 'A' in the box for the distance it is first observed and the behavior it is engaging in. If the same butterfly moves into a different distance category, write a 'B' in the new distance and behavior category.											
	Distance (m)			Behavior							
Monarch	0-2.5	2.5-5	Incidental	Flying	Resting	Ovipositing	Mating	Nectaring	Unknown	Nectar Plants	
<i>Example:</i>	<i>B</i>	<i>A</i>		<i>A</i>				<i>B</i>		<i>Purple Coneflower</i>	
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
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16											
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18											
19											
20											

MCSP Integrated Monitoring Strategy Data Sheets
Version: 06.01.2017

MCSP Integrated Monitoring Strategy Data Sheets
Version: 06.01.2017

Activity 2 Nectar Plants Datasheet (Level A), Sites >1 acre																Date:		page <u>1</u> of <u>3</u>								
Monitoring site:																Observer:										
When you encounter a blooming nectar plant, write the species or common name and then record the first section of the sampling frame (A, B, or C) for every subplot you find it in.																										
Subplots		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Nectar Plant Species	Example		A			A	C				B				B									A		
Subplots		26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Nectar Plant Species																										

Activity 2 Nectar Plants (Level A)							Date:				Notes:										page <u>2</u> of <u>3</u>					
Subplots	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	
Nectar Plant Species																										
Subplots	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	
Nectar Plant Species																										

Activity 2 Nectaring Plants (Level A)							Date:				Notes:									page <u>3</u> of <u>3</u>						
Subplots	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	
Nectar Plant Species																										
Subplots	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	
Nectar Plant Species																										

Activity 2 Nectar Plants (Level B), Sites >1 acre																		Date:							
Site:																		Observer:							
Notes:																									
In each of the 150 subplots listed below record the first section of your sampling frame (A, B, or C) in which blooming nectar plants were observed. If no blooming plants are present in that subplot write a 0.																									
Subplot #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Section																									
Subplot #	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Section																									
Subplot #	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
Section																									
Subplot #	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Section																									
Subplot #	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125
Section																									
Subplot #	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
Section																									

Activity 2 Nectar Plants Datasheet (Level A), Roadsides																Date:			page <u>1</u> of <u>3</u>						
Site:																Observer:									
End point Latitude, Longitude:																			Height of mowed vegetation:						
Roadside width:		Side 1				Side 2				Mowed width:		Side 1				Side 2									
Subplots	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Mowed (Y/N)																									
Species Acronym	Example			C			C			B			A										A		
Subplots	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Mowed (Y/N)																									
Species Acronym																									

Activity 2 Nectar Plants (Level A)						Date:				Notes:								page <u>2</u> of <u>3</u>							
Subplots	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
Mowed (Y/N)																									
Species Acronym																									
Subplots	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Mowed (Y/N)																									
Species Acronym																									

Activity 2 Nectar Plants (Level A)						Date:				Notes:										page <u>2</u> of <u>3</u>									
Subplots	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125				
Mowed (Y/N)																													
Species Acronym																													
Subplots	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150				
Mowed (Y/N)																													
Species Acronym																													

Activity 2 Nectar Plants Datasheet (Level B), Roadsides																		Date:							
Site:																		Observer:							
End point Latitude, Longitude:																						Height of mowed vegetation:			
Roadside width:		Side 1				Side 2				Mowed width:		Side 1				Side 2									
In each of the 150 subplots listed below record the first section of your sampling frame (A, B, or C) in which blooming nectar plants were observed. If no blooming plants are present in that subplot write a 0.																									
Subplot #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Mowed (Y/N)																									
Section																									
Subplot #	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Mowed (Y/N)																									
Section																									
Subplot #	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
Mowed (Y/N)																									
Section																									
Subplot #	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Mowed (Y/N)																									
Section																									
Subplot #	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125
Mowed (Y/N)																									
Section																									
Subplot #	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
Mowed (Y/N)																									
Section																									

Activity 2 Small Site Datasheet: Nectar Plants, Milkweed, and Monarchs				Date:		Observer:			Level (Circle one) A or B		
				Site:							
Blooming Nectar Plants				Milkweed/Egg and Larvae Counts							
Level A: Nectar Plant Species (List each species observed in the boxes below)				Milkweed Species	No. of plants	Eggs	1st	2nd	3rd	4th	5th
Level B: Were nectar plants observed (Circle)? YES/NO											
Misc. Adult Monarch Observations (tally for each behavior observed)	Flying	Resting	Ovipositing	Mating	Nectaring	Unknown	Nectar Plants (if any observed nectaring)				