Monarch Research Review

June 2019 - August 2020

Prepared by Monarch Joint Venture

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EXECUTIVE SUMMARY

In recent years, the collective body of knowledge regarding monarch butterflies (*Danaus plexippus*) has greatly expanded, driven largely by interest associated with their decline observed on overwintering grounds and their proposed listing (warranted but precluded by other priority actions) under the Endangered Species Act (ESA). Much of the literature contained in this review was considered by the US Fish and Wildlife Service (USFWS) as it developed a Species Status Assessment (SSA) for monarch butterflies, a key source of information for the ESA listing decision. Many of these resources composed a special Research Topic eBook in the Frontiers Journal Series entitled *North American Monarch Butterfly Ecology and Conservation*, and were specifically solicited to bring this latest research to the forefront prior to the ESA listing decision.

The research in this review contributes to our knowledge of monarchs both within and beyond the Americas, and covers topics of habitat quality and conservation, monarch populations and biology, as well as introspections on goal setting and community involvement in conservation. A new synopsis of global monarch distribution clarifies where monarchs have occurred in recent years, while this and other resources suggest that monarch's adaptive capacity may benefit them with regard to changing habitat conditions and availability. Within North America, physical tagging and genetic evidence speak to the interconnectedness of both the eastern and western populations.

Given drastic population declines and potential to be listed under the ESA, much of the research presented explores threats to monarchs and their habitat. Pesticides and heavy metals negatively affect monarchs at a variety of life stages. Pesticides pervade many land uses, even where not recently applied, and are likely a driving factor in the decline of monarch populations. Additionally, climate change has potential negative impacts on monarch populations both directly (e.g., reduced reproductive capacity) and indirectly (e.g., loss of overwintering habitat). Finally, we gained a more comprehensive look at the threat that predators and parasites pose to monarchs, including a newly identified suite of invertebrate predators.

Advancing our knowledge of monarch habitat is also key to understanding and conserving monarchs. This year, we learned more about the ecology of certain milkweeds, including how larval survival varies based on the age or species of milkweed on which it feeds. Habitat management recommendations range from plant species selection (e.g., based on bloom time or floral arrangement) to application of management techniques (e.g., burning as opposed to grazing). While new ways to use artificial intelligence and aerial imagery to quantify habitat are developing, we still face challenges to quantifying habitat across the United States and Mexico in a uniform way. In the meantime, research points to the most effective ways to use the Integrated Monarch Monitoring Program to characterize habitat via field surveys.

This review also contains new information on migration dynamics, including results from long-term Monarch Watch tagging datasets. We learn that the monarch migration is synced, in a way, with the angle of the sun at solar noon, and that monarchs at certain southwestern locations may migrate to either eastern or western overwintering grounds. With the aid of ever-smaller radio transmitters, we discover more about the movement of monarchs within and among patches of breeding habitat, as well as in relation to weather and the fall migration.

Since these papers have been reviewed for this summary, the USFWS has concluded that the monarch is indeed warranted for listing under the ESA but is precluded by work on high-priority species and listing actions. Thus, this research will continue to be a useful reference as the USFWS reviews the monarch's status each year until it is finally listed or it is determined that listing is no longer necessary.

METHODS & PURPOSE

To keep its partner organizations abreast of this research, the Monarch Joint Venture (MJV) has organized an annual research review. MJV staff, partners, and other contributors summarized 67 individual papers, and MJV staff compiled and condensed key takeaways herein. The set of papers summarized in this review include peer-reviewed research papers along with relevant doctoral dissertations and one article published in a non-peer-reviewed journal. We selected articles published during June 2019 and August 2020 and those that were searchable by the terms "*Danaus plexippus*" and "*Asclepias.*"

RESULTS

I. General Takeaways, Goals, & Participation

Population & Habitat Goals

Data gaps in our knowledge of monarchs outside of North America limit our ability to assess threats to the global population. Though genetically the same, eastern and western North American monarch populations face different threats and population outlooks. Milkweed stem goals may be too low, and riparian corridors could house more milkweed.

- Monarch butterflies have been documented in 74 countries or island groups since 2000. The monarchs' adaptation to novel locations indicates high adaptive capacity, within the confines of milkweed as a larval food source. More specific information is needed on monarch locations, numbers, and trends through much of their range; this would help better assess how threats may impact monarchs outside of North America (Nail et al., 2019).
- Based on current models using expert opinion to assess the influencers of eastern and western monarch populations, the population growth rate of western monarchs is unlikely to increase, even with substantial efforts. Loss of overwintering habitat had a substantial negative impact on western population growth, while additional milkweed and nectar resource availability had a modest positive impact on its growth, even under best case scenarios. With 'most-likely' conservation efforts, the eastern population probability of quasi-extinction is 40.8% within 50 years; milkweed and nectar resource availability have the most substantial positive impacts to the population in the North Central region (Voorhies et al., 2020).
- There is a higher baseline density of milkweed in urban areas than previous thought, which could mean that the existing goal of 1.8 billion stems is not adequate for restoring the eastern monarch butterfly population (Johnston et al., 2019).
- Similarly, a greater milkweed density than previously estimated was documented in midwestern conservation grasslands. The mean milkweed density across sites was 1,390 plants per hectare (median = 783) (Lukens et al., 2020).
- Restoring native habitat within 30-meter corridors along perennial and intermittent streams in the Upper Midwest could reach approximately 25% of the milkweed stem goal for the agricultural sector, and ~10% of the overall habitat goal based on Thogmartin et al. (2017). While the authors suggest that societal economic benefits of increased pollination and water quality services are greater than the farm income lost by restoring these habitat corridors, additional incentives may be needed to improve adoption of these practices (Semmens and Ancona, 2019).

Monitoring & Community Science

Community science data contribute greatly to our understanding of butterfly populations. The Integrated Monarch Monitoring Program can be best served by increasing the number of sites monitored rather than the frequency of visits.

- Data collected by community scientists are highly valuable and have been used to develop population indices of multiple butterfly species in Canada. This approach could be extended to other parts of the monarch range. (Crewe et al., 2019)
- In a power analysis to determine the appropriate number and frequency of site visits to detect monarch population trends using the Integrated Monarch Monitoring Program (IMMP), the number of sites and years of the study affects their power more than the number of visits per year or number of subplots. To achieve the broad goals of the IMMP, monitoring over a large area might be better than highly specific monitoring of small areas (Weiser et al., 2019).
- To improve measurements of potential influences on monarch population declines (e.g., migration mortality), tag recovery search effort in Mexico should be quantified and recorded (e.g., number of individuals and time spent searching) (Fordyce et al., 2020).

Policy & Collaboration

Collaborations among stakeholders have achieved notable successes in protecting overwintering habitat in Mexico but face new challenges. Listing the monarch under the ESA may pose several complications.

- Collaboration between federal and local governmental agencies, NGOs, and academia, an increased social awareness for monarch conservation, and landowner incentives on habitat protection and restoration have contributed toward successfully curbing illegal logging in the Monarch Butterfly Biosphere Reserve (Flores-Martínez et al., 2020).
- In an opinion piece, authors propose that monarchs should not be listed under the Endangered Species Act (ESA) due to inherent difficulties in applying its restrictions and definitions, such as take and critical habitat. Listing could be a detriment to agricultural practices, private landowners, and current community education programs given that the species is so widespread and ubiquitous across North America and other parts of the world. They recommend that conservation strategies be pursued and implemented at state, regional, and international levels before invoking the regulatory protections of the ESA (Mawdsley et al., 2020).
- To garner the most support among urban residents in North America, conservation strategies should be led by not-for-profit organizations, should communicate ecological outcomes, and strive for international cooperation (Solis-Sosa et al., 2019).

II. Monarchs

Monarch Physiology

Results from several projects indicate that monarchs respond negatively to a variety of stressors, such as high numbers of visitors on the overwintering grounds or physical handling. Eastern and western populations exhibit modest phenotypic differences but are genetically similar.

- Handling of monarch larvae and pupae can cause stress, as indicated by increased heart rate. Individuals and groups involved in monarch community science or education/outreach may consider the impact that handling has on individual monarchs and adjust actions to minimize such impacts (Davis, 2020).
- Monarch caterpillars responded to low-frequency (100–900 Hz) sounds by freezing, contracting, and flicking their bodies, with the greatest sensitivity and most vigorous responses occurring between 100 and 200 Hz. Monarch larvae stopped responding after repeated exposure to sound. The authors hypothesize that hearing may aid in preventing attacks from predators or parasites and suggest that monarchs in noisy areas (near roadways, railways, or airports) may be more at risk (Taylor and Yack, 2019).
- High numbers of tourists and lower forest cover on the overwintering grounds in Mexico were correlated with higher levels of adult monarch stress, via multiple indicators, and decreased immune response (i.e., lower survival when inoculated with the novel bacteria, *Staphylococcus aureus)* (Nicoletti et al., 2020).
- Despite geographic and physical differences, eastern and western monarch populations are genetically similar. There is ongoing gene flow between the populations, and their migratory differences (distance, route) may be driven by differential gene expression induced by environmental triggers, or by alleles different from those linked to the mere presence or absence of migration. In general, North American monarchs are highly genetically diverse, suggesting they have the capacity to adapt to future environmental change (Freedman et al., 2020, Talla et al., 2020).
- Monarchs reared in the lab under fall conditions have more efficient flight and lower post-flight metabolism than those reared under summer conditions (Schroeder et al., 2020).
- Rearing commercial monarchs or those caught in the wild indoors, even with access to natural sunlight, may interfere with their ability to properly orient south, which may have implications for successful fall migration (Tenger-Trolander and Kronforst, 2020).

Migration & Movement

The presence or success of fall migratory monarchs was linked to indicators of nectar resource availability in two studies. Fall migratory monarchs have different physical conditions or abilities from non-migratory monarchs, yet some abilities may be negatively affected in indoor rearing conditions. Fall migrants are assisted by wind and warmer temperature, and larvae move regularly among milkweed stems. Adult monarch behavior is linked to plant diversity and milkweed cover, and adults exit prairies using long flight trajectories.

- Using tagging data from 1998-2015, authors suggest that the 'migration mortality hypothesis' does not explain declines in eastern monarchs because there was no disconnect between late summer and overwintering population, and because migration success was not significantly correlated with overwintering population size. Authors recommend focusing on milkweed as a primary conservation strategy. A partnership reviewer cautions, however, that the tag recovery data cannot distinguish between fall and winter mortality, weakening the case (Taylor et al., 2020).
    - A commentary in response to Taylor et al. (2020) suggests the authors do not have sufficient statistical power to conclude that monarch recovery rate has not changed over time. Fordyce et al., re-ran the data in a new model accounting for year and found that the recovery rate appears to be *increasing* over time. Authors suggest that this may be due to

increasing tag recovery effort or more monarchs dying upon reaching Mexico, but data are lacking to discern this because tag recovery effort has not been documented. Ultimately, they conclude that the debate posited by Taylor et al., (2020) (migration vs milkweed limitation) is not representative of the many factors that are likely influencing the decline and does not acknowledge important data gaps (Fordyce et al., 2020).

- Fall migration success of eastern monarchs was correlated with the level of greenness of the southern U.S (Taylor et al., 2020).
- Models containing both biotic (i.e., roosting trees and nectar plant species richness) and abiotic (i.e., climate data) factors best predict monarch distribution during fall migration. The response of monarch distribution to milkweed was only positive in one month (November); this may be due to a variable they did not account for, or to the greatest number of monarch occurrences overlapping with the greatest number of milkweed occurrences in November based on the way the monarchs are moving south over the months (Kass et al., 2020).
- Ninety percent of tagged monarchs recovered at the Monarch Butterfly Biosphere Reserve (1998-2015) were associated with sun angles at solar noon (SASN) between 46 and 57 degrees, suggesting that this is an optimal 'migration window.' Monarchs observed at a given latitude outside of these SASNs may be considered 'early' or 'late' migrants. In years with low overwintering abundance, more 'late' migrants were tagged, suggesting a relationship between timing of migration and migration completion (Taylor et al., 2019).
- Equipped with small radio transmitters, fall migratory monarchs traveled up to 143 km in a day and at 31 km per h (both wind assisted). Warmer temperatures and greater wind assistance increased the pace of migration, but there was no effect of precipitation. Sample size is small (n = 43), and the tracking devices are heavy (up to 49% of the monarch's body weight), so estimates will improve with advances in this technology (Knight et al., 2019b).
- Significant mortality occurred at two highway crossings where migrating monarchs are typically concentrated in northeastern Mexico (196,500 individuals during October 15-November 11, 2018). Efforts to mitigate this mortality include better enforced vehicle speeds during the migratory period, deflection structures to raise the height of crossing monarchs, and manipulation of habitat to lower the potential for monarchs descending to roost near key crossing points (Alvarez et al., 2019).
- Monarchs tagged on the same day in SE Arizona flew in different directions; some flew to California and while others traveled to Mexico. However, data suggest that monarchs captured earlier in the season (early/mid-September) are more likely to be recovered in California, while later-season monarchs are more likely to be recovered in Mexico. This is further evidence that the eastern and western monarch populations interact (Billings, 2020).
- Dual isotope approaches (using stable oxygen and hydrogen isotopes) may more accurately determine the natal origins of monarchs and other insects (Hobson et al., 2019).
- In grasslands in the Northern Great Plains, monarchs spent more time consuming nectar on sites with a greater diversity of plant species and lower density of milkweed. Abundance of adults and ovipositing behavior increased on sites that have *both* higher milkweed cover *and* plant species diversity (Kral-O'Brien et al., 2020).
- In a small restored prairie in Iowa, monarchs tagged with VHF transmitters made several short (<50m) steps before longer flights to exit the prairie (Fisher et al., 2020a).

- In a greenhouse study, monarch larvae regularly moved among common milkweed plants, ate from multiple plants, and always left the stem in which they were born prior to depleting that stem. Authors recommend that immature monitoring techniques include searching the ground and surrounding vegetation to avoid over estimating mortality when larvae may be moving among ramets (Fisher et al., 2020b).

Survival

Larval survival can vary based on the species or growth stage of the milkweed on which it is feeding. Immature monarch survival may be much lower than previously calculated, as least in some land use types. Monarch egg survival is reduced by certain herbicides.

- First instar monarch survival was found to be more than twice as high on regenerating milkweed in Michigan (after mid-summer mowing) than on older stems. However, the authors caution that frequently mowing or cutting milkweeds can create ecological traps for monarchs -- initial disturbances may attract ovipositing adults, but continued mowing can kill large numbers of immature monarchs as they develop. It is important to consider the frequency of disturbance events and to further study the effects on pollinator communities as a whole (Haan and Landis, 2020).
- A recent study developed a new modeling approach to estimate the larval survival probability of arthropod species using stage-structured count data. The authors estimate an average monarch larval survival rate of 0.014 (range: 0.002 - 0.058) across rights-of-way, grasslands, and agricultural borders in Iowa, USA and Ontario, Canada. In other words, they estimate that 1.4% of monarch eggs laid survive through the 5th instar stage. This estimate is four times lower than the 0.058 survival probability previously identified in Nail et al., 2015. Estimates of overall survival (egg to adult) varied by location, habitat type, and time (year), but the highest rates were observed in agricultural edges (0.025) and lowest in natural grasslands (0.008). Authors emphasize that it is important to explore the effect of this lower survival rate on monarch modeling and that this new modeling method can be applied in many field studies (Grant et al., 2020).
- Monarch larvae in California were more successful on *A. fascicularis* than *A. speciosa*. Milkweed defensive traits change with plant age, with *A. fascicularis* defensive traits increasing much faster than *A. speciosa*. This has a significant effect on larval health (Yang et al., 2020).
- A preliminary study suggests certain herbicides, including a Round-Up glyphosate product, Bayer 2,4-D product, and 20% vinegar solution, can have direct negative impacts on the survival of monarch eggs when directly applied (Albanese, 2019). A partnership reviewer notes that in this master's thesis project, sample size was small, and the Bayer product was applied in undiluted form directly onto the butterfly eggs.

Captive Rearing

Rearing can negatively impact a variety of physical traits and orientation ability.

- Rearing commercial monarchs or those caught in the wild indoors (even with access to natural sunlight) may interfere with their ability to properly orient south, which may have implications for successful fall migration (Tenger-Trolander and Kronforst, 2020).

- Monarchs reared under late-summer conditions have lower grip strength, paler orange color, and less elongated forewings than wild-caught fall migratory monarchs. Authors hypothesize this may be due to the frequency of handling or lack of natural selection in reared monarchs (Davis et al., 2020).
- Handling of monarch larvae and pupae can cause stress, as indicated by increased heart rate. Individuals and groups involved in monarch community science or education/outreach may consider the impact that handling has on individual monarchs and adjust actions to minimize such impacts (Davis, 2020).

Population Dynamics

Integrating a variety of population model types may improve our understanding of population dynamics. The temporal scale at which monarch populations are evaluated can also impact our perception of their trends and threats. There is more evidence showing how interconnected the eastern and western populations are. Climate change and OE can limit monarch populations or their reproductive capacity.

- Grant and Bradbury (2019) provide recommendations for future modeling efforts to improve our understanding of North American monarch population dynamics.
- In a recent analysis examining the drivers of the western monarch decline, trends in western monarch abundance were more strongly associated with land use change variables (e.g., coastal development, increased pesticide use) than climate variables, though the authors acknowledge the difficulty in separating the effects of climate and land use factors. More research needs to be conducted to understand how monarch populations respond to different environmental conditions across seasons and years, and to understand the relative contribution of these factors to monarch declines (Crone et al., 2019).
- Research has come to different conclusions about the cause of monarch's decline depending on the temporal scale evaluated, in that shorter time periods suggest that the advent of genetically modified crops drives the decline, while longer time periods suggest the more general loss of small farms are the driver. In sum, there are many stressors driving the decline of monarchs and none can be addressed in a silo (Agrawal, 2019).
- Monarchs tagged on the same day can fly in different directions from SE Arizona to California or to Mexico, though data suggest that monarchs captured earlier in the season (early/mid-September) are more likely to be recovered in California, while later-season monarchs are more likely to be recovered in Mexico. This is further evidence that the eastern and western monarch populations interact (Billings, 2020).
- Climate change models that predict an increase in temperature in the early spring, combined with continued loss of breeding and wintering habitat, has the potential to limit the reproductive capacity of monarchs and their ability to recover from population lows (Crewe et al., 2020).
- A recent study suggests that the protozoan parasite, *Ophryocystis elektroscirrha* (OE) could contribute to declines of North American monarchs. The authors state that multiple routes of parasite transmission, combined with high virulence, can reduce lifetime reproductive success and reduce resident (non-migratory) monarch populations by 50%, via a reduction in lifetime reproductive success (Majewska et al., 2019).

Eastern Monarchs

Weather is an important factor in monarchs' ability to recolonize the northern portion of their breeding range. Habitat loss on overwintering grounds has played less of a role in monarch declines in the past decade, and while the population has achieved the targeted 6 hectares of overwintering occupancy recently, it is not necessarily on an upward trajectory.

- The Canadian portion of the monarch range is limited by factors acting during spring migration. Weather plays an important role in the ability to recolonize and breed in the northern portion of their summer range each year. Climate models predicting an increase in temperature in the early spring, combined with continued loss of breeding and wintering habitat, has the potential to limit the reproductive capacity of monarchs and their ability to recover from population lows (Crewe et al., 2020).
- It has been widely assumed that monarch population declines are in part attributed to due to the loss of oyamel fir forest cover in Mexico. A recent analysis shows no relationship between the loss of forest cover and recent (within the last decade) monarch population declines. Logging has been largely curtailed during the last ten years, suggesting that other threats (herbicide/insecticide use, loss of milkweed and nectar resources) are likely the main causes of monarch population declines (Flores-Martínez et al., 2019 and 2020).
- The population trajectory of eastern monarchs switched in 2014 from decreasing to "perhaps" increasing, but there is currently insufficient data to assert with statistical confidence that it is on an upward trajectory. Authors suggest that the overwintering population should average 6.85 hectares for three consecutive years to infer that the monarch population has achieved a "secure" level of 6.0 hectares (Thogmartin et al., 2020).

Western Monarchs

Overwintering survival and early spring breeding likely limit the western population, whose growth rate is not projected to increase even with substantial efforts.

- For the western monarch population, the most limiting part of the migratory cycle appears to be concentrated during the overwintering stage and/or in early spring. Overwintering survival was not the biggest driver of the 2017-2018 decline; rather, the density of eggs and larvae had a ten-fold decline, suggesting the drop in overwintering abundance happened prior to the 2018 breeding season, in early spring 2017 (Pelton et al., 2019).
- Based on updated models using expert opinion to assess the influencers of eastern and western monarch populations, the population growth rate of western monarchs is unlikely to increase, even with substantial efforts. Loss over overwintering habitat had a substantial negative impact on western population growth, while additional milkweed and nectar resources had a modest positive impact on its growth, even under best case scenarios. (Voorhies et al., 2020).

Threats

Pesticides, Toxins, and Heavy Metals

Monarch eggs, larvae, and adults are negatively affected by a variety of pesticides, and pesticides are prevalent throughout the landscape even where not recently applied. However, monarchs may have the ability to avoid pesticide-treated plants if presented with the option. Zinc exposure reduces larval survival.

- Pesticides are ubiquitous across many land-use types, even in places where no known pesticide application has occurred in recent years. The potential lethal and sub-lethal effects of these pesticides, especially in combinations, are still largely unknown for non-target organisms, but it is likely that monarch caterpillars are encountering biologically meaningful concentrations of pesticide contaminants in the central valley landscape of California (Halsch et al., 2020).
- Certain herbicides, including a Round-Up glyphosate product, Bayer 2,4-D product, and 20% vinegar solution, can have direct negative impacts on the survival of monarch eggs when directly applied (Albanese, 2019). A partnership reviewer notes that in this master's thesis project, sample size was small, and the Bayer product was applied in undiluted form.
- Early-instar monarchs suffered 97-100% mortality when fed milkweed leaves sprayed with the biopesticide *Bacillus thuringiensis galleriae* (strain SDS-502), designed to reduce defoliation by adult Japanese beetles. The authors discourage the use of this biopesticide in gardens with monarch host plants or those of other non-pest Lepidoptera (Redmond et al., 2019).
- Adult monarchs fed neonicotinoid-contaminated sugar water exhibited reduced life spans compared to those fed sugar water without the chemicals. Insecticide use may be contributing to the monarch population decline by reducing adult monarch longevity. Conservation strategies should therefore include limitations on imidacloprid (neonicotinoid) use, and more research should be conducted to further explore the impacts of widespread neonicotinoid-contaminated crop and wildflower nectar on monarch and pollinator populations (James, 2019).
- A study reports the transfer of the pesticide clothianidin from soil to adult monarch butterflies, through consumption of contaminated leaves. The estimated dose required to kill 50% of individuals in the tested population (LC_{50}s) (combined mortality of larvae and pupae) varied but was higher than the majority of concentrations reported in the literature for clothianidin contamination of leaves (Bargar et al., 2020).
- A study in the north-central US documents high monarch larval mortality rates in locations 0-15 meters downwind of insecticide-treated corn and soybean fields and examined the toxicity effects of commonly used insecticides on monarch larvae. Beta-cyfluthrin and chlorantraniliprole were the most toxic insecticides across all instars. Chlorpyrifos was the least toxic to first instars and thiamethoxam was the least toxic to fifth instars (Krishnan et al., 2020).
- Monarchs appear to adaptively shift their oviposition and foraging strategies to avoid contaminated host plants. Adult monarchs laid fewer eggs on milkweeds containing high levels of pesticides (concentrations commonly present in agricultural fields) and first instar larvae showed preference for pesticide-free milkweed in lab trials (Olayas-Arenas et al., 2020).
- Monarchs seem to have a low tolerance for developmental zinc exposure, a common heavy metal contaminant found along roadsides. As zinc exposure increased in the laboratory, monarch survival decreased significantly. Although not studied specifically in this research, the article points to other references that suggest that there is less pollution in vegetation along less busy roads. Strategies to minimize pollution exposure should be explored (e.g., focus roadside milkweed planting along less busy roads to minimize exposure) (Shephard et al., 2020).

Climate Change

Climate change is likely to negatively affect the eastern breeding population, is already contributing to losses on the Mexican overwintering grounds, and may be impacting where overwintering colonies form.

- Climate change, and in particular warming spring temperatures, will likely have a negative effect on the eastern population of monarch butterflies (Crewe et al., 2020).
- Climate is currently the major driver of forest loss and is expected to continue to have future impacts on overwintering habitat degradation as climate change progresses. Forest cover losses from climate-related events (wind and rainstorms) have increased since 2012 at the Monarch Butterfly Biosphere Reserve (Flores-Martínez et al., 2019).
- Researchers used modeling based on climate change and reports by the National Commission on Protected Areas of Mexico to locate two new colonies of overwintering monarchs in the Sierra Nevada, both in mature oyamel fir forests. This could signify that monarchs are already seeking out new overwintering grounds and could impact how overwintering habitat is prioritized for conservation in the face of climate change (Perez-Miranda et al., 2020).

Habitat Loss and Degradation

Climate change and avocado production are substantial drivers of forest cover loss in and around the overwintering grounds in Mexico. Habitat loss of overwintering grounds on the west coast is a large driver of western monarch population decline. Urban and roadway habitat pose risks to monarchs that may be mitigated through structural changes.

- Overall forest cover loss in Mexico's Monarch Butterfly Biosphere Reserve has declined since 2012, largely due to the decrease in illegal logging. During this time, however, losses from climate-related events (wind and rainstorms) have increased. Climate is currently the major driver of forest loss and is expected to continue to have future impacts on overwintering habitat degradation as climate change progresses (Flores-Martínez et al., 2019).
- An estimated 20% of forest loss in Michoacán is associated with the expansion of avocado orchards. This includes areas of global significance for the conservation of biodiversity, i.e., Key Biological Areas per the International Union for Conservation of Nature (IUCN) criteria. The author recommends using existing data on the farm-level origin of avocados to increase supply chain transparency and give consumers the ability to reward sustainable avocado production, ultimately reducing the impacts of avocado production on Michoacán forest loss (Cho, 2020).
- In a recent analysis examining the drivers of the western monarch decline, trends in western monarch abundance were more strongly associated with land use change variables (e.g., coastal development, increased pesticide use) than climate variables, though the authors acknowledge the difficulty in separating the effects of climate and land use factors. They state that habitat conservation and restoration will be an effective first step in recovering the population, but that more research be conducted to understand how monarch populations respond to different environmental conditions across seasons and years, and to understand the relative contribution of these factors to monarch declines (Crone et al., 2019).
- While urban habitats have the potential to support milkweed habitat, it is important to consider its overall contribution to monarch conservation goals. Urban environments may be more predisposed to invasive species, heightened fragmentation and habitat loss, and intensive application rates of

pesticides and other chemicals. Paying close attention to fitness and reproductive success may be necessary to more closely approximate the role of urban environments in monarch conservation (Baker and Potter, 2020).

Predators and Parasites

Increased milkweed toxicity may present some level of protection against certain parasites. Monarchs experience very high egg mortality in grasslands, primarily nocturnal predation, and an extremely diverse group of predators. Higher levels of cardenolides can provide some level of protection against parasites. OE has the potential to reduce resident monarch populations by 50%.

- Monarch larvae suffer significant predation by the invasive paper wasp, *Polistes dominula,* in urban gardens. *Polistes* wasps were observed in a majority of the urban gardens studied in Kentucky and monarch larvae in urban settings sustained significantly more predation by *Polistes* wasps than monarch larvae in rural settings (Baker and Potter, 2020).
- New research documented 36 previously unreported monarch predators, including 4 new orders (Orthoptera, Dermaptera, Lepidoptera and Opiliones) and 11 taxa (Acrididae, Gryllidae, Tettigoniidae, Forficulidae, Anthocoridae, Geocoridae, Lygaeidae, Miridae, Nabidae, Erebidae and Opilliones). Several herbivores readily consumed immature monarchs, both in a targeted fashion or incidentally as a result of herbivory (Hermann et al., 2020).
- In midwestern grasslands, a large proportion (up to 74%) of monarch egg predation occurs at night. Some predators, a few of which have never before been recorded predating monarchs, include European earwigs (*Forficula auricularia*), tree crickets (*Oecanthus* sp.), lacewing larvae (Neuroptera), plant bugs (Miridae), small milkweed bugs (*Lygaeus kalmii*), ground beetles (*Calleida* sp.), and spined soldier bugs (*Podisus maculiventris*) (Myers et al., 2020).
- Egg mortality can reach 90% within the first 72 hours in perennial grasslands, while only 10–30% in corn fields. Weekly monarch egg surveys may be too infrequent to distinguish oviposition habitat preferences from losses due to egg predation (Myers et al., 2019).
- Monarchs containing more cardenolides (from consuming milkweeds with higher toxicity) are less likely to be attacked by the parasitic wasp *Pteromalus cassotis.* However, if attacked, the higher cardenolide levels do not increase monarch survival. *P. cassotis* brood size and wasp survival was lower in monarchs with high levels of cardenolides (Stenoien et al., 2019).
- Researchers describe three transmission routes (adult transfer, environmental transmission, and adult to offspring (vertical) transfer) that produce and sustain high OE infection prevalence in monarch populations. They estimate that these transmission routes, combined with high parasite virulence, can reduce resident (non-migratory) host populations by 50%, via a reduction in lifetime reproductive success (Majewska et al., 2019).
- Researchers documented the first report of an OE-like parasite outside of the *Danaus* genus (found in four Lepidoptera species including *Helicoverpa armigera, H. assulta*, *H. punctigera, and Parthenos sylvia*). Genetic analyses indicate high similarity between this parasite and OE. When testing infection across species, monarchs were successfully infected with the OE-like parasite (Gao et al., 2020).

Non-native Plants

- In the fall, tropical milkweed in the southern US triggers reproductive activity in monarchs, more so than native milkweed species in the same setting. Larvae fed tropical milkweed are more likely to be

reproductively active, and wild females display greater egg development when exposed to tropical milkweed (Majewska and Altizer, 2019).

- Conservation organizations and educators should continue to discourage the planting of non-native species like tropical milkweed (*Asclepias curassavica*) due to its negative impacts on migratory behavior, parasite transmission, and overall population health (Majewska et al., 2019).

III. Habitat

Milkweed Ecology

Age affects defensive traits of some milkweed species. Asclepias tuberosa can be propagated from cuttings.

- Older *Asclepias fascicularis* and *A. speciosa* plants have greater defensive traits (i.e., latex exudation and trichome density) than younger ones, in a comparison of 4-, 6-, and 12-week-old plants grown in greenhouse conditions (Yang et al., 2020).
- *A. tuberosa* can be successfully propagated from cuttings; success is higher if cuttings are from mature plants, as opposed to juvenile, and taken between dormancy and flowering initiation. Neither the hormone potassium salt nor indole-3-butyric acid or 1-naphthaleneacetic acid improve cutting survival or root number or length (Lewis et al., 2020).

Habitat Establishment and Management

Breeding Habitat

Planting habitat is critical to increase the likelihood that monarchs are able to withstand threats associated with climate change. In certain geographies, habitat with more early season forbs, flowers with radial patterns, specific milkweed species, and burning treatments may be preferred by or benefit monarchs. Improvements can be made to CRP lands in the Southern Great Plains to better benefit pollinators. Mowing has a variety of positive impacts on monarch abundance and survival but can create an ecological trap if overapplied. Mowing has mixed effects on milkweed stem density.

- Conservation practitioners should ensure adequate breeding, migratory, and overwintering habitat for monarch butterflies so that they can withstand the threats associated with climate change (Crewe et al., 2019).
- Early season nectar resources were less frequent and diverse than mid- or late-season resources on conservation grasslands. Thus, more early-season forb species should be included in habitat projects in the upper Midwest, as monarchs arriving to the Midwest after an energy-intensive migration period need nectar sources (Lukens et al., 2020).
- In Midwest conservation plantings, *Asclepias incarnata* and *A. tuberosa* had higher densities than when not planted, suggesting that these are the most cost-effective milkweed species to seed in this region. *A. syriaca* was almost always observed at sites in which it was planted and at all sites in which it was not planted, suggesting it is an excellent colonizer species and may not need to be seeded (Lukens et al., 2020).

- Arizona milkweed (*A. angustifolia*) is recommended over pineneedle milkweed (*A. linaria*) for monarch habitat conservation projects in the Southwest within its natural range based on greater oviposition preference in experimental and wild conditions and larval performance. Both species are commercially available (Pegram and Melkonoff, 2019).
- A recent genetic and germination analysis of wild populations and commercial seed sources for two priority species of milkweed suggests that seed sourcing guidelines should be set at the species-level rather than genus. The authors suggest that swamp milkweed (*A. incarnata)* should be sourced more conservatively than common milkweed *(A. syriaca)*, as there was more variation among local sources of swamp than common (Finch, 2019).
- Seed mixes that include flower species with radial symmetry patterns may be most attractive to monarch butterflies (Esmaile and Rodrigues, 2020)
- Habitat alteration in southeastern Arizona has potential to impact both eastern and western monarch populations, given that monarchs tagged on the same day in SE Arizona were found to migrate to both California and Mexico (Billings, 2020).
- Fire might be a better monarch habitat management tool than grazing in remnant prairies in Minnesota. Burned sites had a higher abundance of monarch butterflies than grazed sites (but there was no impact of management type on milkweed or flowering plant frequency). However, it is unclear what the mechanism for that difference is, or if the findings can be applied to a larger geographic or temporal scale (Leone et al., 2019).
- Researchers documented a lower abundance and species richness of Hymenopteran pollinators (bees, wasps, flies) in Southern Plains Region CRP habitat compared to cropland and native grassland. Future CRP enrollment in this region should prioritize CP42 Pollinator Habitat practices (the incorporation of native grasses and forbs) to maximize their potential for providing quality pollinator foraging and nesting habitat (Begosh et al., 2020).
- While first instar monarch survival was found to be more than twice as high on regenerating milkweed in Michigan (after mid-summer mowing) than on older stems, frequently mowing grasslands can create ecological traps for monarchs. Initial disturbances may attract ovipositing adults, but continued mowing can kill large numbers of immature monarchs as they develop. It is important to consider the frequency of disturbance events and to further study the effects on pollinator communities as a whole (Haan and Landis, 2020).
- At a 43°N latitude (New York, southern Wisconsin, Wyoming), mowing roadsides in the second and third weeks of July resulted in higher egg abundance per milkweed plant compared to unmowed controls or several other mowing treatments. Overall, mowed plots had higher egg abundance than unmowed controls (Knight et al., 2019a). Note that this study did not find mowing to extend the egg-laying season, though other studies have found this. A partnership reviewer considers that the greater number of eggs in the mowed plots may result from shifting laying preference from nearby older milkweeds to the cut milkweed, potentially pulling monarchs from the safety of a nearby prairie to the more dangerous roadside right-of-way.
- Roadside programs that mow three or four times annually do not appear to have serious negative consequences for the long-term viability of green milkweed (*A. viridis*) populations in the southern Great Plains. Mowing frequency was not found to alter the age of green milkweed populations in roadside and grassland habitats, though 5–11-year-old milkweed plants that are mowed more

frequently (roadside habitats) had fewer overall stems and flowering stems than plants of the same age in less frequently disturbed grassland sites (Dee and Baum, 2019).

- In Iowa, milkweed density and milkweed species richness are higher on remnant prairies than on roadside or non-roadside prairie plantings. Milkweed density was positively correlated with higher soil pH, greater site age, and lower bulk density soil. Rural roadside sites outperformed non-roadside conservation plantings with regard to milkweed stem density (Kaul and Wilsey, 2019).

Urban Gardens and Landscapes

Urban garden habitat for monarchs can be improved by including a variety of native nectar sources, multiple milkweed stems, and grouping milkweed plants near garden edges. Risks associated with paper wasp predation may be mitigated by preventing this predator from inhabiting wooden structures.

- In an urban garden experiment, monarchs laid more eggs on milkweed plants in mixed-species plots, as opposed to a stand of primarily milkweed monoculture. Larval survival was similar in both plot types, despite that there was higher natural enemy richness, wasp, and predatory bug abundance in the mixed-species plots (Nestle et al., 2020).
- Monarch gardens and habitats should include multiple milkweed plants to support larval development since larvae regularly move among milkweed plants (Fisher et al., 2020b).
- Monarch eggs and larvae were more abundant in gardens with milkweed planted around the perimeter compared with those where milkweed was surrounded or intermixed with other nectar plants and grasses (Baker and Potter, 2020).
- Metropolitan areas provide important habitat opportunities and should be included prominently in monarch conservation strategies. Existing milkweed densities in urban areas are higher than previously published and they have the potential to contribute a sizeable portion of the milkweed needed to support the eastern population of monarch butterflies (Johnston et al., 2019).
- Monarch larvae suffer significant predation by the invasive paper wasp, *Polistes dominula,* in urban gardens. *Polistes* wasps were observed in a majority of the urban gardens studied in Kentucky and monarch larvae in urban settings sustained significantly more predation by *Polistes* wasps than monarch larvae in rural settings. Authors recommend checking wooden structures (pollinator houses, bluebird boxes, etc.) regularly for paper wasps (Baker and Potter, 2020).

Overwintering Habitat

Some California overwintering sites may be improved with wind protection. Overwintering habitat in Mexico has seen increased losses due to climate change and decreased loss due to illegal logging.

- Monarchs' occupancy at a Californian overwintering site is the best indicator of how suitable the conditions of that site are. Areas exposed to disruptive winds (>= 2m/sec) may need wind protection (Leong et al., 2020).
- Overall forest cover loss in Mexico's Monarch Butterfly Biosphere Reserve has declined since 2012, largely due to the decrease in illegal logging. Losses from climate-related events increased during this time and are expected to continue to have impacts on overwintering habitat degradation as climate change progresses (Flores-Martínez et al., 2019).

Habitat Quantification

Milkweed may best be quantified in mid-July in the Midwest, and new technologies are being developed to generate stem counts. New information helped locate new overwintering colonies, but there are still limitations to using land cover data to map habitat.

- Using vehicle-mounted cameras to survey pollinator habitat on road rights-of-way provides a promising technology and could significantly increase the efficiency of documenting progress toward meeting milkweed conservation targets (Ozcan et al., 2020).
- In a recent study on midwestern conservation grasslands, milkweed densities peaked during mid-July, suggesting that monitoring efforts intending to capture peak milkweed density for similar sites in this bioregion should occur during mid-summer (Lukens et al., 2020).
- Models based on climate change and reports by the National Commission on Protected Areas of Mexico helped locate two new colonies of overwintering monarchs in the Sierra Nevada, both in mature oyamel fir forests. This could signify that monarchs are already seeking out new overwintering grounds and could impact how overwintering habitat is prioritized for conservation in the face of climate change (Perez-Miranda et al., 2020).
- A cohesive analysis of monarch migratory habitat across the US and Mexico is not currently possible in large part because land use/land cover datasets that span the border have too coarse of resolution to meaningfully identify pollinator habitat. Newer datasets, including cloud-based imagery (e.g., Google Earth) that is frequently updated, have the greatest potential for assessing pollinator habitat and its changes. If using LiDAR or similarly coarse imagery, monarch migratory habitat is best mapped at local and regional scales (Moreno-Sanchez et al., 2019).

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For additional literature on monarch butterflies, visit the MonarchNet Research Library.

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