2014 Project Abstract
For the Period Ending June 30, 2017

PROJECT TITLE: Imperiled Prairie Butterfly Conservation, Research, and Breeding
PROJECT MANAGER: Dr. Erik Runquist
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2014, Chp. 226, Sec. 2, Subd. 05j1

APPROPRIATION AMOUNT: $380,000
AMOUNT SPENT: $369,464
AMOUNT REMAINING: $10,536

Overall Project Outcomes and Results

Many of Minnesota's prairie butterflies are declining. Due to ENRTF support, the Minnesota Zoo's Prairie Butterfly Conservation Program has dramatically expanded the first and only conservation rearing and breeding programs for Minnesota's imperiled prairie butterflies. We developed new rearing and breeding techniques, and increased the Zoo's conservation population of U.S. Threatened (Minnesota Endangered) Dakota skippers from 44 adults in 2014 to over 375 adults in 2017. This expansion allowed for the beginning of a multi-year reintroduction program in 2017 when 200 Zoo-reared Dakota skippers were released to reestablish a lost Minnesota population. A new augmentation program is also underway to support some of the last United States populations of the Endangered Poweshiek skipperling.

The causes of these butterfly declines are not fully understood, many factors likely contributed, and some of those threats may still exist. The ENRTF provided critical funding though to begin understanding the potential role of insecticide drift into prairies. We produced foundational data on the extent, composition, and timing of pesticides drifting into critical habitats for these protected species. The findings inform hypotheses about what may have contributed to declines of these butterflies and have spurred additional research recommendations. We are working with other agencies and parties to advance risk assessments and proper habitat management and to reduce drift exposure.

The ENRTF supported foundational Dakota skipper and Poweshiek skipperling population genetics research, filling critical knowledge gaps that inform management of these butterflies at both in the Zoo and in the wild. These studies are being published in peer-reviewed scientific literature.

We developed new outreach about butterflies, prairies, and what the public can do to help. Thanks to the ENRTF, we published two popular pamphlets in both English and Spanish, and these have been distributed free to nearly 10,000 people at the Minnesota Zoo and at other events.

Project Results Use and Dissemination

We have developed a large network of collaborators across local, state, national, and international levels. We hold frequent conference calls with several recovery and threat assessment working groups for both Poweshiek skipperling and Dakota skipper, and have attended and/or hosted several multi-day meetings and conferences for these species. We present our results to these working groups and other permitting agencies, and prepare detailed annual reports. Our results informs the actions and recommendations of the working groups. The foundational husbandry protocols we developed have also helped Winnipeg's Assiniboine Park Zoo launch a parallel and collaborative prairie butterfly

conservation rearing and breeding program. Scientific products of our ENRTF-supported work will be submitted for peer-reviewed publication.

Thanks to the programmatic expansions supported by the ENRTF, the plight of prairies and their butterflies have become much more visible and publicly known. We have presented to dozens of general public audiences (thousands of people in total), and at several University undergraduate and graduate-level courses and seminars. At least nine newspaper, radio, and television stories have been produced about the prairie butterfly conservation efforts supported by the ENRTF since 2014, including four new newspaper, radio, and television stories associated with the Dakota skipper reintroduction program in the summer of 2017.

Minnesota Zoo Facebook Live streaming event from the Hole-in-the-Mountain Prairie Preserve (https://www.facebook.com/mnzoo/videos/10155374215493788/) featuring Prairie Butterfly Conservation Program manager Dr. Erik Runquist, the Minnesota DNR's Dr. Robert Dana (project lead on this joint ENTRF for Activity 3), and staff from The Nature Conservancy and the US Fish and Wildlife Service. Viewed nearly 11,000 times, the video provided a live look at the Dakota skipper reintroduction effort, the history of the ENRTF-supported Prairie Butterfly Conservation Program, and the partnerships involved. Additional Minnesota Zoo social media and blog posts were presented throughout the summer of 2017 highlighting the reintroduction effort, our "Plant For Pollinators" campaign, and the re-introduction of the Butterfly Brew Dakota Skipper Endangered Reserve promotion through Fair State Brewing Cooperative.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2014 Work Plan

Date of Report: August 15 2017

Date of Next Status Update Report: Final Report

Date of Work Plan Approval: June 4, 2014

Project Completion Date: June 30, 2017

PROJECT TITLE: Imperiled Prairie Butterfly Conservation, Research, and Breeding Program
Part 2 (Activity 3) of the project is described in a separate work plan with an appropriation of $245,000 to the Minnesota DNR

Project Manager: Dr. Erik Runquist

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Location:

Dakota, Cottonwood, Murray, Pipestone, Lincoln, Chippewa, Big Stone, Pope, Clay, Norman, Polk, Kittson, Roseau, and potentially other counties in western and southern Minnesota with prairies.

Total ENRTF Project Budget:

ENRTF Appropriation:	**$380,000**
Amount Spent:	**$369,464**
Balance:	**$10,536**

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 05j-1

Appropriation Language:
$380,000 the second year is from the Trust Fund to the Minnesota Zoological Garden and $245,000 the second year is from the trust fund to the Commissioner of Natural Resources to prevent the extirpation and possible extinction of imperiled native Minnesota butterfly species through breeding, genetics and mortality research, inventory, monitoring, and public education. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Imperiled Prairie Butterfly Conservation, Research, and Breeding Program

II. PROJECT STATEMENT:

Prairies and their native wildlife are an important part of Minnesota's natural and cultural heritage. But with only 1% of that native prairie remaining, many prairie plant and animal species—including many species of once prevalent native butterflies—have dramatically declined. Of the butterfly species native to Minnesota prairies, 10 are of statewide conservation concern and two, the Poweshiek skipperling and the Dakota skipper, have now largely disappeared from the state and are proposed for listing under the U.S. Endangered Species Act despite being historically among the most common prairie butterflies and having their historic ranges concentrated in Minnesota. The ENTRF (Project 017-A) will allow the Minnesota Zoo will expand its conservation breeding program for butterfly species most under threat of extinction like the Poweshiek skipperling and Dakota skipper, to conduct critically needed conservation genetics studies, research potential causes of mortality associated with pesticides, and provide focused educational information on these species and efforts.

The Minnesota Zoo is collaborating with the Minnesota Department of Natural Resources (DNR) for this joint ENTRF. Classified as "Activity 3" in the joint proposal and the peer-reviewed Research Addendum, but described in a separate Work Plan, the DNR will simultaneously monitor the status of these and a number of additional targeted species on native prairie remnants across Minnesota. This joint work will provide needed information of status of not only Minnesota's native prairie butterflies, but also the greater prairie ecosystem, and steps that may be needed to further their conservation. Beyond serving as pollinators for various prairie plants and as food sources for other prairie wildlife, butterflies are sensitive "canary in the coalmine" indicators of prairie ecosystem health. The loss of prairie has significant consequences for Minnesota's water quality and wildlife interests.

III. PROJECT STATUS UPDATES:

Project Status as of November 30, 2014:

The first six months of ENTRF funding of the Prairie Butterfly Conservation Program have been successful, and we are on-track. Two prairie butterflies, the Poweshiek skipperling and Dakota skipper, were added to the U.S. Endangered Species List in October 2014 as Endangered and Threatened species, respectively. We successfully reared from egg to adulthood and then bred complete generation Dakota skippers for the first time (Activity 1), and added larvae from additional wild female lineages. We collected additional samples for population genetics research (Activity 2), collected field samples for prairie pesticides research (Activity 4), and are developing outreach content (Activity 5).

We received three sources of additional funding. The Prairie Butterfly Conservation Program received a corporate gift from Aveda that matched voluntary donations from Zoo guests during summer 2014. We had estimated $5,000 in voluntary guest donations and another $5,000 from the Aveda match in our initial LCCMR Work Plan, but donations were significantly higher than anticipated at $18,755. Aveda also raised its match to $10,000.

Following approval of the LCCMR, the U.S Fish and Wildlife Service approached the Zoo to support our prairie pesticides research (Activity 4). The Zoo and the USFWS completed a cooperative agreement and USFWS provided an extra $20,000 to support the field pesticides research that was not anticipated during our LCCMR application and Work Plan development. These three funds sources have been updated and added to Section B of the Project Budget Summary.

Project Status as of May 31, 2015:

We are continuing to advance our goals. Some aspects have been delayed by circumstances beyond our control, but we expect resolution of many of these issues by our next update. As Recovery Plans for the Dakota skipper and Poweshiek skipperling are developed by the USFWS, we remain in close contact with USFWS and all relevant parties. We currently hold 160 Dakota skipper larvae, and these will be held for additional breeding this summer (Activity 1). Construction and placement of our controlled rearing/breeding Pod has been delayed due to an extended permitting process, but we expect resolution in early summer. Limited DNA supplies have slowed

progress at the Zoo, but our genetics research collaborator has made significant advances on Activity 2 with non-ENRTF funds. We have found drift of agricultural insecticides (Activity 4) associated with soybean aphid spraying onto prairie fragments, including at potentially significant levels at Prairie Coteau SNA. We are proceeding with the publication of two educational butterfly and prairie guides (Activity 5), one of them a year earlier than planned to streamline messaging.

The majority of our non-personnel expenses during early 2015 have been covered using external sources, especially the voluntary restricted donations made by Minnesota Zoo guests in summer 2014 as well as the Cooperative Agreement with the USFWS supporting pesticides research. ENRTF support will constitute a larger proportion of our operational costs in the future as the external funds are expended.

Project Status as of November 30, 2015:

We continue to make solid progress on our goals, particularly on the critical efforts to establish the world's first and only breeding populations of US Threatened and Minnesota Endangered Dakota skippers. We remain in close partnership with the USFWS and all relevant parties. In October, the USFWS funded a three-day workshop at the Minnesota Zoo with the Conservation Breeding Specialist Group (a branch of the International Union for the Conservation of Nature) to discuss the potential future roles and forms of *ex situ* conservation programs with Dakota skippers and Poweshiek skipperlings. The workshop brought together about two dozen experts from across the ranges of these endangered butterflies. Our work was highlighted throughout the meeting, and a consensus was reached to continue and expand the Minnesota Zoo's *ex situ* program with Dakota skippers and to initiate a formal "headstarting" program with Poweshiek skipperlings in 2016. The report from the meeting is under a comment period from relevant stakeholders, and will be discussed further in future updates.

We have secured two new funding sources. First, the USFWS also provided the Minnesota Zoo with additional cooperative interagency agreement funds to support a portion of the Minnesota Zoo's needed facilities expansion to begin *ex situ* conservation with the critically endangered Poweshiek skipperling starting in 2016. Second, we were awarded a competitive grant from the Association of Zoos and Aquariums Conservation Grants Fund to provided additional facilities expansion for our work with Dakota skippers and to initiate a host plant performance study with Dakota skippers at the Minnesota Zoo.

Project Status as of June 16, 2016:

We continue to make solid progress on our goals, particularly on the critical efforts to establish the world's first and only breeding populations of US Threatened and Minnesota Endangered Dakota skippers. We remain in close partnership with the USFWS and all relevant parties. Indeed, we worked with the US Fish and Wildlife Service and other agencies to develop a "Plan for the Controlled Propagation, Augmentation, and Reintroduction of Poweshiek skipperling (*Oarisma poweshiek*)". This formal cooperative interagency plan follows the IUCN's "Guidelines for Reintroductions and Other Conservation Translocations" and lays out the specific work plan for the world's first Poweshiek skipperling augmentation by headstarting program that was recommended by experts participating in the October 2015 "Poweshiek skipperling Dakota skipper *Ex Situ* Feasibility Assessment and Planning Workshop" (Delphey et al 2016). This headstarting program will launch in the summer of 2016.

We have secured additional short-term funding: an amendment to our existing cooperative interagency funding agreement with the USFWS. This supports contracted field surveys across North Dakota to secure livestock of garita skipperling as a husbandry research surrogate for Poweshiek skipperlings, as well as two temporary part-time staff to assist with 2016 on-site husbandry operations.

Project Status as of November 30, 2016:

Our program continues to grow. The Zoo's rearing and breeding population of Dakota skippers has now grown to the size where the world's first reintroductions of this US Threatened and Minnesota Endangered species are possible. We remain in close partnership with the USFWS and all relevant parties for our programs.

In July, we secured additional short-term funding from the USFWS through another amendment to our existing cooperative interagency funding agreement to supplement temporary staffing needs on Zoo site in 2016 and 2017, and to support Zoo staff hours (including overtime pay) for work outside of Minnesota.

Project Status as of May 31, 2017:

The majority of the efforts since the last update have been focused on planning for the next phases of the Prairie Butterfly Conservation Program, particularly the initiation of the world's first Dakota skipper reintroductions using Zoo-reared individuals back to a Minnesota prairie this June. These plans are discussed in greater detail in the Zoo's ML 2016 ENRTF May 2017 update. The update for this ML 2014 update focuses on the results of husbandry operations for Dakota skippers over the 2016/2017 winter, as well as other Activities identified in this ML 2014 grant. In short though, we experienced high Dakota skipper survivorship over the winter, and the Minnesota Zoo's insurance population has grown again relative to prior years at this time. We entered spring with over 420 larvae.

We have maintained and expanded our partnerships, across federal, state, local, NGO, and academic levels. We, thanks to our research partners at New College of Florida, are making significant and foundational gains on the conservation and population genetics of Poweshiek skipperlings and Dakota skippers. We are also involved in statewide and federal pesticides research, and this ENRTF-supported work may have a significant impact on regulatory processes at the state and even federal levels. The Minnesota Zoo is also a member of Minnesota's new Interagency Pollinator Protection Team, as dedicated by the Governor's Executive Order 16-07 "Directing Steps to Reverse Pollinator Decline and Restore Pollinator Health in Minnesota".

In February, we secured additional short-term funding from the USFWS through a new cooperative interagency funding agreement to support temporary staffing needs on Zoo site in 2017 and 2018, and to install new equipment to improve microclimate control of Zoo-reared individuals.

Overall Project Outcomes and Results:

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The ENRTF supported foundational Dakota skipper and Poweshiek skipperling population genetics research, filling critical knowledge gaps that inform management of these butterflies at both the Zoo and in the wild. These studies are being published in peer-reviewed scientific literature.

We developed new outreach about butterflies, prairies, and what the public can do to help. Thanks to the ENRTF, we published two popular pamphlets in both English and Spanish, and these have been distributed free to nearly 10,000 people at the Minnesota Zoo and at other events.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Minnesota Zoo breeding conservation program for imperiled prairie butterflies

Description: The Minnesota Zoo's Prairie Butterfly Conservation Program was launched in 2012 following consultations with the U.S. Fish and Wildlife Service (USFWS) and Minnesota Department of Natural Resources (DNR) on the need to establish conservation breeding populations for endangered, threatened, and imperiled Minnesota-native prairie butterflies whose wild populations have experienced catastrophic recent declines and face the risk of global extinction. Two of these species, the Poweshiek skipperling (*Oarisma poweshiek*) and Dakota

skipper (*Hesperia dacotae*) are currently listed in Minnesota as Endangered and were proposed for federal listing as Endangered and Threatened (respectively) in October 2013. Both have disappeared from the majority of their historic ranges (90+% for Poweshiek, 50+% for Dakota) in recent decades. Dakota skippers may only remain in one Minnesota location. Poweshiek, sometimes referred to as the "Most Minnesotan Butterfly" because half of its historic range was the state, was once one of the most abundant butterflies on Minnesota's prairies, but has not been confirmed in Minnesota since 2008. It has also disappeared in North Dakota, South Dakota and Iowa between 2001 and 2008. Intensive 2013 surveys across the remaining isolated known populations in Michigan, Wisconsin, and Manitoba indicate that fewer than 500 Poweshiek skipperlings likely remained globally in 2013, making them at least three times rarer than wild giant pandas and one of the most endangered animals on earth.

The primary goal of the Minnesota Zoo's Prairie Butterfly Conservation Program is to utilize the recognized organizational capacity and experience of the Minnesota Zoo for the managed breeding of endangered species to establish large, genetically robust populations at the Zoo that can serve as an "insurance policy" against the risk of regional and global extinction of endangered species like the Poweshiek skipperling and Dakota skipper. These Zoo populations may also serve as reservoirs from which potential supplementations to wild populations and reintroductions to historic or potentially suitable sites may be drawn. These potential needs and the role of the Minnesota Zoo to achieve these goals are highlighted in the recent federal Endangered and Threatened species listing proposals for Poweshiek skipperlings and Dakota skippers (USFWS 2013). Our efforts are international, involving over a dozen partner U.S., Canadian, and tribal agencies and organizations. In consultations with our partners, we have established safeguards to ensure that our efforts protect wild population integrity.

The Minnesota Zoo constructed an outdoor butterfly breeding facility for this program in 2012 with built-in multi-level containment capabilities, but so far have lacked stable indoor space in which we can control temperature and lighting for other operations. Funding from ENTRF will allow for much needed expansion of our operations and allow us to test a variety of methodological approaches to optimize breeding success and minimize mortality. Among the remaining questions we are interested in addressing include the effects of different larval host plants on growth rates and survivorship, temperature tolerances for winter hibernation survival, and, the optimizing the conditions that provide the greatest success for mating. Our ability to perform some of these tests with the endangered species is contingent on having large, stable breeding populations, and adaptive rearing techniques may take priority over experimental arrays in the short-term to maximize survivorship. Note that the entire personnel (wage and benefits) budget for the entire program is grouped under this Activity for simplicity. In reality, both personnel supported by this ENTRF will be working on all four Minnesota Zoo Activities, but these percentages will vary proportionately within and across years.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 336,400
Amount Spent: $ 330,806
Balance: $ 5,594

Activity Completion Date:

Outcome	Completion Date	Budget
1. Purchase and outfitting of the indoor breeding chamber	May 2015	$ 52,000
2. Rearing and breeding protocols for Dakota skippers and Poweshiek skipperlings finalized	April 2017	$ 284,400

Activity Status as of November 30, 2014:

The Dakota skipper was formally listed as a Threatened Species under the U.S. Endangered Species Act on October 23, 2014. We are working with the U.S. Fish and Wildlife Service, the Minnesota Department of Natural Resources, and all other relevant regulatory agencies to ensure proper permitting and structures are in place for all current and future work.

There are no pre-existing rearing protocols for Dakota skippers, or related species. Much of the work we are doing is an evolving process as we work to maximize our rearing success. Having learned from our first summer (2013) with Dakota skipper larvae, we made modifications to our protocols this year, and these protocols are likely

to continue to evolve in the future. All protocols are available upon request in a separate Standard Operating Procedures document we are developing.

Breeding:

From wild eggs collected in the summer of 2013 by Minnesota Zoo staff, we reared 42 Dakota skippers (25 males and 17 females) through to adulthood at the Zoo. Adults were paired with unrelated individuals opportunistically as they emerged from the pupa. Mating trials consisted of pairs being kept in 9-oz cups, or larger pop-up 1 ft^3 mesh enclosures. We experimented with varying the number of males present in a mating arena and light exposure (artificial or natural). We found the greatest breeding success occurred in situations of high natural light and where males had the opportunity to encounter each other and exhibit territorial behavior before exposure to a female. We observed four confirmed copulations with three resulting in viable eggs. **This is the first time Dakota skippers have ever been successfully bred in an artificial, controlled environment, and is a critical success for the breeding program.**

Egg collection:

Using methods successfully employed in 2013, we collected additional eggs from wild Dakota skippers under permits and agreements from relevant agencies/parties to increase the genetic representation in the Zoo breeding population. We collected a total of 575 eggs from 27 wild females from four different sites in northeastern South Dakota. We also collected 39 eggs from four females from one site in Minnesota.

Rearing:

From these 614 eggs, 535 larvae hatched (87% hatch rate). This year we also hatched larvae from two pairs bred at the Minnesota Zoo**.** After being reared in sealed 150mL plastic vials with live grass (prairie dropseed) blades for the first two weeks, larvae were transferred to one of two treatment types: Tube method, or Free-range method. The Tube method consists of 9" cuttings of T12 fluorescent light protector plastic tubes with a ~1 inch plug of grass in the bottom and secured on top with white nylon mesh organza and a rubber band. The Free-range method consisted of mature grasses in 2-gallon plastic pots covered in a "tent" of white nylon mesh organza. All Dakota skipper larvae were reared on prairie dropseed.

Dakota skippers hibernate as partially grown caterpillars. At the end of 2014 growing season (mid-October), all caterpillars were transitioned to winter hibernation using standard protocols. **A total of 363 caterpillars were recovered for winter hibernation, for an overall pre-hibernation survivorship of 67.9%**. At this time last year, we had 43.7% survivorship. Using modified rearing techniques, we have therefore **increased pre-hibernation survivorship 24.2%**. Of the 425 larvae reared with the Tube method, 301 (71.0%) were recovered by the end of the growing season at the time of winter hibernation. Of the 111 larvae reared in the Free-range Method, 62 were recovered for 55.9% recovery.

All Dakota skipper caterpillars were weighed prior to being placed into individually-labeled tubes in special cups (specifications available upon request) for winter hibernation. Tube method reared larvae were on average more massive than Free-range Method larvae, but had a greater variance in size and developmental stage (instar). This may be a product of Tube-method caterpillars experiencing indoor conditions where average temperatures are higher than outside. As Dakota skippers have short adult lifespans (12-14 days), it may be crucial to our breeding efforts to keep all specimens of our population as close to the same developmental stage as possible. Furthermore, we need to make sure our *ex situ* population is developmentally in sync with wild populations if these populations are ever to be a source for potential future wild releases. Even though we had greater recovery success for Tube method reared larvae than Free-range, it will likely be advantageous to rear a larger portion of the 2015 larvae as Free-rangers. The Free-range method also requires significantly less person-hours to maintain than the more hands-on Tube method.

All Dakota Skipper larvae are now hibernating under controlled conditions that mimic what wild larvae naturally experience, either in our laboratory freezer (25°F) or outdoors in the Conservation Hoop-house. All adult females that laid viable eggs are represented in our current holdings.

Breeding Chamber:

We have met with Minnesota Zoo operations and construction staff to discuss the needs for the new breeding chamber. We are working to optimize and balance the needs for climate control, area, lighting, and containment within the available funds. This work is ongoing and will be discussed in the next update. We fully expect to have the chamber in place on schedule.

Activity Status as of May 31, 2015:

Rearing:

Dakota skipper caterpillars were brought out of hibernation in May 2015 once their host grasses had grown enough to support sustained herbivory. We found significantly higher hibernation survivorship of caterpillars that were reared outdoors under the "Free-Range" method (58/62 = 93.5%) than those reared indoors with the Tube method (102/301 = 33.9%). This may be related to two factors. First, longer exposure of Free Range individuals to outdoor variations in temperatures than those reared mostly indoors with the Tube method. Second, Tube method individuals are also necessarily handled more than Free Range individuals since the food plants (and therefore the silk shelter constructed by the caterpillar) in their tubes must be replaced 2-3 times prior to hibernation. This may pose a substantial energetic cost to those reared in tubes, at least pre-hibernation.

Breeding Chamber:

As of the time of this writing, structural designs and necessary contracts are in place with vendors to construct and place our new Breeding Pod exclusively dedicated to the Prairie Butterfly Conservation Program. However, an extended and unexpectedly complex permitting process with MMB has delayed shipment and placement of the Pod beyond the initial May completion date. We have been awaiting final approval of the necessary construction permits since late winter. We expect completion of the project in early summer. We continue to use our current office space for all operational business until the Pod is complete. The Pod will consist of a 8'x20'x'8 shipping container with water, electricity, internet, and HVAC hook-ups. It will also have an attached 8'x8'x'8 glass greenhouse. The pod+greenhouse will be placed adjacent to the Minnesota Zoo's holding pens for American Plains Bison. While not visible to the public, the proximity of *ex situ* conservation programs for the largest and smallest of Minnesota's imperiled prairie wildlife presents a unique outreach opportunity to talk about the endangered ecosystem they both depend on.

Activity Status as of November 30, 2015:

Early Summer Rearing:

Of the 160 Dakota skipper caterpillars that overwintered through to May 2015, 112 (70%) of them survived to adulthood in early July. This high percentage is comparable to our post-hibernation survivorship in 2014. A slightly higher proportion of the 2015 individuals reared with the pre-hibernation Free-Range method (44 of 58 = 75.9%) survived to adulthood than Tube-reared individuals (68 of 102 = 66.7%). Of these 112 adults were 60 males and 52 females.

Mating:

Based on lessons from our 2014 breeding efforts, we established multiple screened cages (1 ft^3 and 2 ft^3) for adults in our existing contained outdoor hoop house. We learned that mating behaviors were most pronounced when multiple males were placed in the same cage for several days before females were added. As much as possible, we placed multiple brothers or at least multiple males from the same population within the same cage so that if a mating occurred but was not observed, we could at least trace paternal grandmother origin of any resulting new larvae. In order to track parental lineages, we marked adults with unique colored Sharpie dots and recorded the identity of each butterfly. To safely mark them, we worked with Minnesota Zoo veterinary staff to develop an effective method to temporarily anesthetize (less than 30 seconds) freshly emerged adults with Isoflurane gas. This temporary anesthetization did not appear to alter behavior at any later time.

Minnesota Zoo staff and volunteers observed 15 matings, but three of these did not produce viable eggs. However, two additional unobserved matings did occur and produced viable eggs. Thus, at least 14 matings produced viable eggs in 2015. Unknown females from three cages produced additional viable eggs, but it is possible that these resulted from known matings. As in 2014, we found that mating needs to occur within the first few days of a female's life before oviposition of unfertilized eggs begins.

Egg Collection:

The Dakota skipper matings from Zoo reared individuals produced 1199 eggs. Using methods successfully employed in prior years, we collected additional eggs from wild Dakota skippers under permits and agreements from relevant agencies/parties to increase the genetic representation in the Zoo breeding population. We

collected a total of 386 eggs from 20 wild females from two sites in northeastern South Dakota. We also collected 46 eggs from five females from one site in Minnesota.

Late Summer Rearing:

While hatch rates were again high for the wild eggs we collected in South Dakota (317/386 = 82.1%) and Minnesota (43/46 = 93.5%), we had surprisingly low viability from Zoo-laid eggs. Only 280 of 1199 (23.4%) eggs hatched. The cause of this low percentage is unknown at this time. The resulting 640 neonate larvae were reared in sealed tubes (comparable to 2014) until being placed on "Free Range" potted plants. Following the poor hibernation survivorship of "Tube reared" larvae in 2014 (see previous update), we fully employed the Free Range method in 2015. After larvae were assigned to one of two treatments: as "singletons" (one caterpillar on a young prairie dropseed in a 4-inch pot) or as one of either 5 siblings or 10 siblings on an older prairie dropseed plant in a 1-gallon pot. All potted plants and associated caterpillars were wrapped in protective mesh screen, and kept outside throughout summer in our protected hoop house.

At the time of this writing, pre-hibernation survival is not known due to the extended autumn warmth. Although hibernation of Zoo-reared caterpillars will begin more than a month later than previous years, we did not artificially place caterpillars in their hibernation chambers before the advent of consistent cool, near freezing conditions so that our caterpillars remained in sync with the conditions experienced by wild caterpillars. Final pre-hibernation survivorship data will be presented in the May 2016 update.

Breeding Chamber:

Construction and permits for the new breeding chamber and attached greenhouse were completed in mid-October. These double contained, clean, and exclusively-dedicated spaces constitute a significant and critically-needed expansion of our breeding and rearing capacities. This winter, the chamber and attached greenhouse will be primarily used to house hibernating Dakota skipper caterpillars in a large new laboratory freezer that safely mimics the sub-freezing conditions caterpillars naturally experience under winter snow. Specifications of the new LCCMR-funded chamber and greenhouse are available upon request. In November, construction also began on two additional outdoor hoop houses thanks to two new non-LCCMR grants.

Activity Status as of June 16, 2016:

Late Fall and Early Winter 2015:

We entered winter with a maximum of 466 Dakota skipper larvae (see table below). The exact number was not known because some of the "Singleton" and Free-Range" caterpillars were intentionally not censused before winter to avoid the stress associated with the necessary disturbance to their shelters. These individuals, up to 170 of them, were left outdoors to hibernate, as they would do in the wild to assess overwintering survivorship in the absence of that disturbance. All larvae hibernated outdoors were well represented genetically in the *ex situ* population. The remaining individuals were censused before winter, with 296 of the possible 430 recovered from their rearing set-ups in early December. These individuals were maintained in a new freezer at -4^{o}C through winter using the Hydro-Stone cup method successfully employed previously. We had higher pre-hibernation survivorship from the smaller singleton pots (90.0%) than the multi-individual 1-gallon pots (64.0%). The recovery from the larger 1-gallon Free-Range pots is lower than hoped, but nonetheless represents an improvement for this method from 2014's pre-hibernation recovery rate (55.9%). This increase is likely due to improved plant husbandry methods and more aggressive predator removal protocols before placing larvae. We generally recovered more individuals that had been placed into their pots later in the summer, but this is to be expected given that larval mortality decreases substantially later in summer.

Winter survivorship:

Of the subset of up to 170 larvae that were hibernated outdoors, only 30% (51 of the initial possible 170) survived until early summer, with a slightly higher proportion surviving in smaller singleton pots than in the larger multi-individual 1-gallon pots. It is not determinable when the non-recovered caterpillars may have perished, either pre- or post-hibernation.

In contrast, 90.2% of the larvae that had been hibernated indoors survived winter, resulting in an overall larval survivorship (from neonate to post-hibernation) of 62.1%, despite being reared under identical conditions the previous summer. A higher percentage of larvae (96.4%, 216/224; a rise of 3% vs. the winter of 2014-2015) survived winter that been reared previously in the larger 1-gallon Free Range pots than those that had been reared

in smaller singleton pots (70.8%, 51/72) under identical conditions in the same freezer. This is inverse of overall the pattern in pre-hibernation survivorship and total survivorship between the two pot sizes. The larger plants in the 1-gallon pots may reduce pre-hibernation survivorship by posing more opportunities for small larvae to be lost and/or to experience resource competition with other larvae in the same large pot, but they may also provide more sustained resources for larvae that do survive to hibernation.

Therefore, we entered the summer of 2016 with 318 Dakota skipper larvae, nearly double the population size vs. early summer 2015. It is expected that most all of these will survive to adulthood, based on previous patterns.

		Entering Hibernation		After Hibernation		
Hibernated Indoors	**Initial Total**	**# surviving to hibernation**	**% surviving to hibernation**	**# surviving through hibernation**	**% surviving hibernation (December to May)**	**Overall % treatment recovery**
Summer reared as Free-range	350	224	64.0%	216	96.4%	61.7%
Summer Reared as Singleton	80	72	90.0%	51	70.8%	63.8%
Subtotal refrigerated	**430**	**296**	**68.8%**	**267**	**90.2%**	**62.1%**

		Entering Hibernation		After Hibernation		
Hibernated Outdoors	**Initial Total**	**# surviving to hibernation**	**% surviving to hibernation**	**# surviving through hibernation**	**% surviving through hibernation (December- June)**	**Overall % treatment recovery**
Summer Reared as Free-range	149	unknown	unknown	43	unknown	28.9%
Summer Reared as Singleton	21	unknown	unknown	8	unknown	38.1%
Subtotal Outdoors	**170**	**unknown**	**unknown**	**51**	**unknown**	**30.0%**
Grand Totals	**600**			**318**		**53.0%**

Rearing Chamber:

The LCCMR-funded climate-controlled rearing chamber and attached greenhouse was completed in December 2015, and has been successfully utilized since then for most husbandry operations.

Activity Status as of November 30, 2016:

The Minnesota Zoo continued to expand Dakota skipper husbandry operations and has now launched parallel conservation operations with Poweshiek skipperlings.

Dakota skipper Early Summer Rearing:

Of the 318 Dakota skipper larvae that survived hibernation, 228 (72%) survived to adulthood in late June and early July 2016. This percentage is comparable to our post-hibernation survivorship in 2015.

Dakota skipper Mating:

Using comparable methods to 2015, Dakota skipper adults were paired together as much as possible in multi-individual screen cages in our open-air hoop houses for breeding. Individuals were also marked as appropriate, using previously employed methods. We had 27 confirmed matings, which represents a rate comparable to 2015.

Dakota skipper Egg Collection:

The Dakota skipper matings from Zoo-reared individuals produced 693 neonate larvae. Using methods successfully employed in prior years, we also collected additional eggs from wild Dakota skippers under permits

and agreements from relevant agencies/parties to increase the genetic representation in the Zoo breeding population. We collected 313 eggs from 19 wild females from two sites in northeastern South Dakota (down from 386 eggs from 20 females in 2015), but were unable to find enough individuals at the last known viable population in Minnesota for any egg collection. All wild females were returned alive back to the sites from which they were collected within 72 hours in accordance with permitted protocols.

Dakota skipper Late Summer Rearing:

Hatch rates were lower for the eggs we collected from wild South Dakota females (181/313 = 57.8%) than in previous years (82-93%, see above) for unknown reasons. Many of the wild individuals observed, including those females temporarily held under comparable conditions for egg collections, appeared weaker than in prior years, perhaps due to drought conditions reducing nectar availability. At the Zoo, 693 of the Zoo-produced eggs hatched. The combined 874 neonate larvae were reared in sealed tubes for a few weeks until they were large enough to be reared outdoors in "Singleton" and "Free Range" potted plant setups (comparable to 2015). 189 of the best genetically represented larvae were transferred into a host plant performance study (using non-ENRTF funds) at the Minnesota Zoo to help optimize husbandry operations and potentially inform habitat management for the conservation of wild Dakota skipper populations.

At the time of this writing, Dakota skipper pre-hibernation survival is not known because extended autumn warmth has delayed our efforts to place the larvae in their hibernation setups. Final pre-hibernation survivorship data will be presented in the May 2017 update.

Poweshiek skipperlings:

In June 2016, Zoo staff initiated the newly recommended head-starting program for the critically endangered Poweshiek skipperling. Collection and captive rearing of Poweshiek skipperlings during 2016 was supported, in part, by ENRTF funds appropriated under the Minnesota Zoo's M.L. 2016 ENRTF (M.L. 2016, Chp. 186, Sec. 2, Subd. 03c1). Additional funding for the Poweshiek skipperling program was provided by the USFWS and MN Zoo. The results of this work are detailed in that project's November 2016 status update.

Manuals detailing protocols for husbandry of Dakota skippers and Poweshiek skipperlings throughout their life histories will be finalized in April, 2017. These will be living documents that will be modified as we learn more about the husbandry of these species.

Activity Status as of May 31, 2017:

Dakota skipper hibernation was successful, with increases in overall survivorship and consequent increases in the size of the Minnesota Zoo's population relative to previous springs. As noted in the overall Project Status assessment above, we have developed formal plans to reintroduce Dakota skippers reared at the Zoo back into a Minnesota prairie during the summer of 2017. This plan, along with our newer work with Poweshiek skipperling are detailed more specifically in the Minnesota Zoo's M.L. 2016 ENRTF (M.L. 2016, Chp. 186, Sec. 2, Subd. 03c1) May 2017 status update. Results from each of these efforts are being used to further refine the husbandry manuals as specified.

The majority of new expenses associated with this Activity since the last update have been for personnel, as well as some small in-state travel expenses.

Dakota Skippers, Late Fall 2016 through Winter 2016/2017:

The fall of 2016 was particularly warm and extended compared to most years. Most host grasses had senesced by the end of September, but ambient temperatures often remained above the threshold for Dakota skipper larval activity until mid-November. As such, larvae could not be fully transitioned into hibernation until early December. Once tallied though, we entered winter with a maximum of 524 larvae (446 hibernated indoors + up to 78 hibernated outdoors; see table below). The exact number was not known because (similar to 2015/2016 winter) up to "Free-Range" caterpillars were intentionally not censused before winter to avoid the stress associated with the necessary disturbance to their shelters. These individuals were left outdoors to hibernate, as they would do in the wild to assess overwintering survivorship in the absence of that disturbance. All larvae hibernated outdoors were well represented genetically in the *ex situ* population. The remaining individuals were censused before winter, with 446 of the original 648 recovered from their rearing set-ups in early December.

These individuals were maintained indoors at -4°C through winter using the Hydro-Stone cup method successfully employed previously. We had lower pre-hibernation survivorship from the smaller singleton pots (71.1%) than the multi-individual 1-gallon Free Range pots (78.4%), an inverse of the 2015/2016 winter (see June 2016 update above).

As noted in the previous update, we are performing a host-performance study (using non-ENRTF funds) to determine if Dakota skipper larvae do better on some grass species versus others. 189 of the best represented larvae were spread across seven host grasses and reared individually in Singleton pots. The treatments consisted of five native prairie grasses and two invasive grasses. More detailed reporting of this experiment is available upon request. Preliminary findings from this experiment indicate that all offered host plant species triggered larval feeding responses such that none of the grass species can be excluded as potential hosts. However, survivorship varied across grass species, with larvae surviving best to hibernation on prairie dropseed and little bluestem (see table below).

Dakota Skipper Winter survivorship:

Overall, winter survivorship was good, with 423 larvae living until emergence from hibernation in May. A total of 86.6% of the larvae that had been hibernated indoors survived winter, resulting in an overall larval survivorship (from neonate to post-hibernation) of 59.6%. Of the subset of up to 78 possible larvae that were reared during summer on large Free Range pots but hibernated outdoors, 37 were recovered in May 2017. It is important to note that for these, it is not determinable when the non-recovered caterpillars may have perished since they had intentionally not been censused since being placed on their plant in mid-summer.

A higher percentage of larvae (96.7%, 235/243; a rise of 0.3% vs. the winter of 2015-2016) survived winter that been reared previously in the larger 1-gallon Free Range pots than those that had been reared in smaller singleton pots (60.4%, 64/106) under identical conditions in the same freezer. This pattern of higher Free Range hibernation survivorship is similar to that observed the previous winter (see above).

		Entering Hibernation		After Hibernation		
Hibernated Indoors	**Initial Total**	**# surviving to hibernation**	**% surviving to hibernation**	**# surviving through hibernation**	**% surviving through hibernation**	**Overall % treatment recovery**
Summer reared as Free-range	310	243	78.4%	235	96.7%	75.8%
Summer Reared as Singleton	149	106	71.1%	64	60.4%	43.0%
Host plant study as Singleton	189	97	51.3%	87	89.7%	46.0%
Subtotal	**648**	**446**	**68.8%**	**386**	**86.6%**	**59.6%**
Hibernated Outdoors	**Initial Total**	**# surviving to hibernation**	**% surviving to hibernation**	**# surviving through hibernation**	**% surviving through hibernation**	**Overall % treatment recovery**
Summer Reared as Free-range	78	unknown	unknown	37	unknown	47.4%
Totals	**726**			**423**		**58.3%**

For those larvae in the host plant performance study, host plant did not significantly predict survivorship through winter, with all treatments surviving winter well (see table below). It is important to note though that all larvae in this study were removed from their hosts and were all hibernated indoors under identical conditions as other non-study larvae. Therefore, it is not known if larvae hibernate more successfully on certain grass species. It does not appear though that pre-hibernation host does not directly impact hibernation survivorship, and the differences in survivorship to date (with more surviving larvae fed exclusively on prairie dropseed and on little bluestem) are more explicitly tied to early larval performance (between July and August).

Host Plant Treatment	Initial	# (%) Surviving July 2016 to August 2016	# (%) Surviving July 2016 to December 2016	# (%) Surviving July 2016 to May 2017	Winter survivorship %
Prairie dropseed	27	19 (70.3%)	19 (70.3%)	17 (63.0%)	89.4%
Little bluestem	27	21 (77.7%)	18 (66.7%)	16 (59.3%)	88.9%
Porcupine grass	27	16 (59.2%)	14 (51.9%)	13 (48.1%)	92.9%
Smooth brome	27	15 (55.5%)	14 (51.9%)	12 (44.4%)	85.7%
Side-oats grama	27	12 (44.4%)	12 (44.4%)	11 (40.7%)	91.7%
Big bluestem	27	13 (48.1%)	11 (40.7%)	10 (37.0%)	90.0%
Kentucky bluegrass	27	13 (48.1%)	9 (33.3%)	8 (29.6%)	88.9%
Totals	**189**	**109 (57.6%)**	**97 (51.3%)**	**87 (46.0%)**	**89.7%**

Therefore, with more than 400 Dakota skipper larvae surviving into May 2017, and high expected survivorship through to adulthood, we remain on track for the world's first reintroduction of Dakota skippers, back to a Minnesota prairie in June 2017.

Final Report Summary:

Thanks to ENRTF support, the Prairie Butterfly Conservation Program has successfully established the world's first and only conservation breeding and reintroduction program for U.S. Threatened Dakota skippers, and made significant advances in husbandry protocols for grass skippers. We expanded our operations with exclusively dedicated clean climate-controlled husbandry spaces where all operations occur, and have composed detailed husbandry protocol manuals for Dakota skippers, Poweshiek skipperlings, and other grass skippers. Remaining funds in the appropriation associated with Activity 1 consist primarily of personnel costs that were opportunistically offset using funding from by the US Fish and Wildlife Service in 2016 to support Minnesota Zoo staff salary and travel costs for work conducted outside of Minnesota.

Dakota Skippers at the Zoo

Another successful phase of Dakota skipper husbandry was achieved during the first half of the summer of 2017. Compilation of the summer's operations will be detailed in the November 2017 update for the Minnesota Zoo Prairie Butterfly Conservation Program's M.L. 2016 ENRTF appropriation. In summary, we successfully reared about 375 Dakota skippers to adulthood by mid-July from the Zoo's existing conservation population. About 150 of these adults were retained for breeding to continue to sustain the conservation population at the Zoo into 2018. We also collected eggs from 23 more wild females from three large South Dakota populations (following protocols employed in prior years). The offspring from these Zoo breedings and wild females are currently being reared to form the 2018 adult population. The remainder of the 2017 Zoo-reared individuals were brought to The Nature Conservancy's Hole-in-the-Mountain Prairie Preserve (near Lake Benton, Lincoln Co., MN) for the first ever reintroduction of Dakota skippers back into a Minnesota prairie (see below).

Poweshiek Skipperlings at the Zoo

The U.S. Fish and Wildlife Service and the Poweshiek skipperling recovery working group recommended that the Minnesota Zoo again attempt to headstart Poweshiek skipperlings from the last best known populations in the United States in Michigan. USFWS staff provided the Minnesota Zoo with five eggs collected in July 2017. The resulting larvae are being reared at the Minnesota Zoo, and are planned to be released back to the Michigan population from which they came in June 2018 to augment the wild population.

Garita Skipperlings at the Zoo

In 2016, the Assiniboine Park Zoo (Winnipeg, Manitoba) launched a trial prairie butterfly conservation program, following the husbandry protocols developed by the Minnesota Zoo with the ENRTF support. The Assiniboine Park Zoo staff began rearing garita skipperlings from a large Manitoba population to gain familiarity with the husbandry protocols with a closely related non-endangered skipper surrogate species before initiating headstarting operations in 2017 with the only Canadian populations of the endangered Poweshiek skipperling nearby. In order to further optimize husbandry between institutions, Assiniboine Park Zoo provided the Minnesota Zoo with garita skipperling eggs following two years of unsuccessful attempts by Minnesota Zoo staff and others

to locate populations in North Dakota. These garita skipperling larvae are now being reared at the Minnesota Zoo under four different climate regimes to assess the effects of ranges of temperature and humidity on development rates and survivorship. This research will continue into 2018.

Dakota Skipper Reintroduction

Completed in April 2017, the Minnesota Zoo prepared a lengthy plan following guidelines established by the International Union for Conservation of Nature for the world's first Dakota skipper reintroduction program. With additional contributions from the Minnesota Department of Natural Resources, US Fish and Wildlife Service, and The Nature Conservancy, this report details the logistics, protocols, and justifications for the reintroduction program using Dakota skippers reared at the Minnesota Zoo. The plan is submitted along with this report. Updates about the reintroduction effort will continue to be provided to the LCCMR through semi-annual reports associated with the Minnesota Zoo's M.L. 2016 ENRTF appropriation for this work.

Approximately 200 skippers were reintroduced to the Hole-in-the-Mountain Prairie Preserve between June 21 and July 13, 2017. Individuals were brought from the Minnesota Zoo as pupae and placed at the Preserve in a protected metal screen box. Adults were released daily by Minnesota Zoo staff as they emerged from their pupae. This preserve once was home to a large population of Dakota skippers, as indicated by surveys conducted by the Minnesota Department of Natural Resources into the 2000s. For unknown reasons, the population disappeared sometime between 2008 and 2012. All other known populations of Dakota skippers had disappeared in southwest Minnesota by 2012 as well. Therefore, the reintroduction of Dakota skippers to this Preserve may represent the first known occurrence of Dakota skippers in southwest Minnesota in nearly a decade.

Minnesota Zoo staff conducted intensive surveys for the released Dakota skippers from June 21 to July 21. Fixed transects were established across the Preserve, and GPS points for all observed Dakota skippers were recorded. Individuals were not marked at the time of release, so it is not known how many unique individuals were re-observed, but it is likely that several dozen unique individuals were re-sighted at the Preserve. Most of the re-observations were within 100 meters of the fixed reintroduction point, but an independent Dakota skipper expert surveyor contracted by the Minnesota Department of Natural Resources (Activity 3 of this inter-agency ENRTF grant) observed an individual approximately 300 meters north-northeast of the reintroduction point in prime Dakota skipper habitat. In addition to accomplishing our first goal of re-sighting release adults across days, we met the year's biggest goal on the 4th of July: a female was seen laying an egg (which later hatched after being brought back to the Zoo, confirming she had mated previously) and later that day observed two separate pairs of our reintroduced Dakota skippers were observed mating near the release point. Therefore, we can be certain that at least three matings occurred at the Hole-in-the-Mountain Prairie Preserve after being reintroduced, and that there is potential to re-establish this lost population. We assume that even more breeding occurred than was observed; the Preserve is large (about 1400 acres) and usually only one Zoo staff member could be present at the Preserve every day for the month-long reintroduction and monitoring effort.

The Dakota skipper reintroduction is planned to continue through at least 2019. The reintroduction program will be evaluated yearly. Major gains were achieved, but there is no guarantee that a population of Dakota skippers can be re-established at the Hole-in-the-Mountain Prairie Preserve. There are many unknowns, but this is a groundbreaking effort and the reintroduction and the associated monitoring will provide detailed information on dispersal patterns and habitat usage. The fate of every released butterfly is not knowable, but they are being released into high quality habitat that is essentially identical to the habitat in the large northeastern South Dakota populations from which they are descended. Therefore, we do not anticipate a lack of adaptation to the conditions at the Preserve (see also the final discussion under Activity 2 below).

Overall habitat conditions at the Preserve are similar to when the skippers disappeared in the late 2000s. It is possible though that some external threat that may have contributed to the extirpation of this population is still present. While it is impossible to rule out all potential threats, particularly since the exact dates in which Dakota skippers were extirpated are not known, some of the hypothesized threats have been reduced. For example, The Nature Conservancy has agreed to manage the Preserve in a way that will promote Dakota skipper population re-establishment, and this management plan will be evaluated annually with all involved parties. These actions alleviate concerns that past habitat management operations may have inadvertently harmed the skippers. In fact, it is likely that even more high quality Dakota skipper habitat will be created at the Preserve through this management and partnership. Similarly, this ENRTF support to the Minnesota Zoo has improved our

understanding of the risks of pesticides drift from adjacent agricultural operations into skipper critical habitats (see Activity 4 below). Zoo staff have provided the data collected using ENRTF funds to the Pesticides and Fertilizer Division Management Division of the Minnesota Department of Agriculture, and helped the MDA draft a letter to pesticides applicators across the state (particularly those near known Dakota skipper populations) to raise awareness about the drift events we have observed and that these locations contain(ed) federally protected Threatened and Endangered prairie butterflies. The release point for the Dakota skipper reintroduction was also strategically placed near the center of the Preserve to reduce the possibility of any drift that may occur.

Additional Research Recommendations

While the precise mechanisms (and how they varied and interacted at local, regional, and global scales) that contributed to the declines of many prairie butterflies are not well understood (see Activity 4 below for some gains), there many more unknowns for most species and groups of pollinators. Butterflies are among the best known of all insect groups due to their high visibility and public interest. There is a basic lack of knowledge of what species of other pollinators actually exist in Minnesota, much less where those species occur, what the status of their populations are, what habitats they rely on, how they interact with each other and with their environment, and what may threaten them. Gains on these basic knowledge gaps are being made with other ENRTF-supported pollinator research programs, but much more remains. We recommend ENRTF support for 1) additional inventories of pollinators statewide, 2) assessments of the role of pollinators in the maintenance of sensitive ecosystems, 3) studies on the effects of habitat management (i.e. burning, grazing, mowing, etc.) on pollinators, 4) understanding the suitability of habitat restorations (particularly prairie) to reconnect isolated (and especially likely declining) populations of pollinators, and controlled laboratory experiments to assess the impacts of pesticides drift on prairie butterflies and other pollinators.

ACTIVITY 2: Conservation genetics research on imperiled prairie butterflies

Description: Successful conservation management of both wild and Minnesota Zoo-based populations of endangered species requires knowledge of both existing genetic variation within populations and the degree of differentiation between populations and regions of those species. To advance these needs with endangered prairie butterflies, the Minnesota Zoo has established a conservation genetics laboratory under the supervision of Program Manager Dr. Erik Runquist and formed a collaborative relationship with Dr. Emily Saarinen (Assistant Professor, University of Michigan-Dearborn/New College of Florida. Using non-ENTRF funding, Dr. Saarinen's lab extract DNA extractions for small tissue samples from the imperiled species collected under permit and then conduct "next-generation" sequencing, isolation, and identification of micro-satellite genetic markers for estimates of population-level genetic diversity. Dr. Saarinen will provide these DNA extractions to Dr. Runquist who will use ENRTF funds to 1) screen populations for the presence of *Wolbachia*, an intracellular bacterial endosymbiont that has the potential to sterilize or kill infected male butterflies when populations become infected with incompatible strains, and 2) sequence several additional known genetic markers for which evolutionary rates are better understood to estimate evolutionary divergence.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 8,000
Amount Spent: $ 7,976
Balance: $ 24

Activity Completion Date:

Outcome	Completion Date	Budget
1. Sequencing of known markers to test for population-level divergence for Poweshiek skipperlings. Screening for and strain identification of Wolbachia strains in Poweshiek skipperlings. Assessment of genetic diversity of any Zoo-bred Poweshiek skipperlings and/or Dakota skippers for ex situ breeding prescriptions.	March 2015	$ 3,500
2. Sequencing of known markers to test for population-level divergence for Dakota skippers. Screening for and strain identification of Wolbachia strains in Dakota skippers. Assessment of genetic	March 2016	$ 3,500

diversity of any Zoo-bred Poweshiek skipperlings and/or Dakota skippers for ex situ breeding prescriptions.		
3. Final sequencing and analyses for remaining individuals and species. Assessment of genetic diversity of any Zoo-bred Poweshiek skipperlings and/or Dakota skippers for *ex situ* breeding prescriptions. Preparation of results and submission to peer-reviewed scientific journals for publication.	June 2017	$ 1,000

Activity Status as of November 30, 2014:

The majority of the genetics sequencing research will begin December 2014. During summer of 2014, Minnesota Zoo staff collected small tissue samples from 59 individuals of Dakota skippers following permitted protocols. Of these, 54 were from five sites in South Dakota, and five were from one site in Minnesota. Additional samples were collected under permit by other collaborators. All of these legs are stored in 100% molecular grade ethanol and will be shipped to Dr. Saarinen's lab in December 2014 for DNA extraction and microsatellite isolation. Aliquots of these samples will then be returned to the Minnesota Zoo for sequencing as outlined in the work plan above.

Activity Status as of May 31, 2015:

Progress on this Activity has been slower than expected due to low availability of DNA samples of the two protected species, but significant gains have nonetheless been made.

Poweshiek skipperling:

The Minnesota Zoo holds small volumes of purified DNA samples from 30 Poweshiek skipperlings from one population in Michigan, with small volumes of 103 more Poweshiek skipperling DNA samples held by our collaborator, Dr. Emily Saarinen (New College of Florida). After a delay in research necessitated by a move between academic institutions, Dr. Saarinen has focused work (using non-ENRTF funds) on the identification of dozens of genetic markers from across the genomes of the Poweshiek skipperling. These markers provide quantification of the genetic diversity remaining within populations, as well as differences between populations. To date, twelve of these genetic markers have been identified, but 10-15 more are needed for statistically robust assessments of genetic diversity.

Dr. Saarinen's lab has also performed screening of all currently held Poweshiek skipperling samples for *Wolbachia* infection. All populations to date have tested positive for *Wolbachia*, with a 100% infection rate per population. The implications of this infection status are unknown, and may be entirely neutral.

Work at the Minnesota Zoo has been focused on the optimization of protocols, particularly to identify strains of *Wolbachia* known to infect Poweshiek skipperlings. Standard practices rely on the sequencing of at least five *Wolbachia* genes to identify strains, and we have positively detected three of these to date with agarose gel electrophoresis. We have also successfully amplified known Poweshiek skipperling gene sequences that are useful additional complements to work conducted by Dr. Saarinen's lab to assess potential divergence between populations. Due to the limited number of individuals and low volumes however, we have proceeded with extreme caution to not exhaust our current DNA supplies before completing the optimization process. This work has largely employed existing financial resources obtained prior to ENRTF funding. Additional laboratory tests are in progress. Poweshiek skipperlings are not currently part of the Minnesota Zoo's rearing efforts, and therefore we cannot assess genetic diversity of any *ex situ* population.

Dakota skipper:

Similar to above, the majority of the progress on this Activity has been conducted by Dr. Saarinen and her lab. At the recommendation of the US Fish and Wildlife Service, priority has been placed on Poweshiek skipperling given the much more dire global status of Poweshiek skipperling vs. the Dakota skipper. As such, fewer research advances have been made with Dakota skippers. Nonetheless, Dr. Saarinen's lab has used modern techniques to identify 10 million base pairs from the Dakota skipper genome. From this, 21,760 potentially variable genetic markers have been identified. As with Poweshiek above, the goal is to identify at least 20-25 of these to be studied across all individuals for assessments of genetic diversity and divergence. Dr. Saarinen's lab currently holds 81

Dakota skipper tissue samples, with the 59 additional samples collected by Zoo staff and partners in 2014 still awaiting DNA extraction. The Minnesota Zoo has not received any Dakota skipper purified DNA samples at this time, and Wolbachia infection status is similarly not known. We anticipate progress on Dakota skipper genetics research to proceed in late 2015. We maintain detailed records of the family (and therefore genetic) history of all Dakota skippers currently in our care.

Activity Status as of November 30, 2015:

As planned, we have not conducted any new research on Poweshiek skipperling or Dakota skipper genetics since our last update. This work will resume during the winter of 2015-2016, but will likely still be limited by the quantity of DNA available. Progress is continuing in the lab of Dr. Saarinen, using non-LCCMR funds.

Activity Status as of June 16, 2016:

No new DNA samples have been received by Zoo staff, so no new gene sequencing has occurred at the Minnesota Zoo. However, our partner Dr. Emily Saarinen (New College of Florida) has sequenced *Wolbachia* DNA from 30 Poweshiek skipperlings (mostly from Michigan). These sequences were provided to the Zoo's Dr. Erik Runquist, who performed necessary alignments and editing with previously purchased genetics software. This work is continuing, but the take-home message is that the individuals and populations screened to date appear to possess the same strain of *Wolbachia*. This reduces the likelihood that *Wolbachia* infection status will be a hindrance for potential future inter-population translocations or breeding efforts.

Activity Status as of November 30, 2016:

The majority of progress on conservation genetics research has again been conducted by our research partner, Dr. Saarinen, using non-ENRTF funds. Her lab is developing microsatellite markers for Dakota skippers and is pursuing similar analyses as described above for Poweshiek skipperling. Discussions are taking place between Zoo staff and Dr. Saarinen to plan for the data and workloads that will be needed to sufficiently address questions of population differences and diversity in these two species as identified in the work plan above. In addition, *Wolbachia* testing in remnant Dakota skipper populations will begin in January 2017. Both percentage of population infection rates and strain identification will be explored.

Although ENRTF resources were not utilized in its production, Saarinen *et al*. acknowledge the Minnesota Zoo as a key contributor in their new foundational publication on Poweshiek skipperling conservation genetics (Insect Conservation and Diversity 2016).

Activity Status as of May 31, 2017:

We have now entered into a contract with our partner Dr. Saarinen (New College of Florida) to continue to advance our understanding of Dakota skipper population genetics. Her lab has identified eight informative microsatellite markers for Dakota skippers, scored them for 176 Dakota skippers from 10 populations in Minnesota, North Dakota, South Dakota, and Manitoba, and analyzed the results. This work is detailed in an honors thesis by an undergraduate student at New College of Florida. Her lab is also performing foundational assays of *Wolbachia* infection for these individuals. *Wolbachia* protocol optimization has been more difficult than for Poweshiek skipperling and other species, but it is proceeding. Specific results for these projects are not distributable at this time, but will be once they are accepted for peer reviewed publication.

New expenses for this Activity since the last update are associated with this contracted partnership for Dr. Saarinen's efforts.

Final Report Summary:

We accomplished our goals and now possess a much clearing understanding of the genetic diversity within and differences between populations of both Poweshiek skipperlings and Dakota skippers at local, regional, and global scales. Our partnership with Dr. Emily Saarinen (New College of Florida) has been fruitful. We have learned that very little genetic diversity remains in Poweshiek skipperling populations, and that there is also very little substantive divergence between existing populations in Michigan and Manitoba. The same strain of intracellular bacteria *Wolbachia* appears to be nearly universally present in all sampled populations. Poweshiek skipperlings

have already adapted to its presence and it may not represent a conservation concern at this time. Poweshiek skipperlings are much more likely to be threatened by their current small population sizes and minimal genetic capacity to adapt to environmental changes.

Using comparable next generation sequencing and bioinformatics tools to the Poweshiek skipperling studies, Dr. Saarinen's lab completed the Dakota skipper genetics studies as contracted with a final report submitted to Minnesota Zoo staff in June 2017. This new research is not yet published in a peer-reviewed journal (in preparation for submission), so full description of the results cannot be provided at this time. Overall though, relatively high levels of genetic diversity were found in Dakota skippers at local, regional, and global scales, and the sampled populations (and clusters of adjacent populations) are not significantly divergent from each other (at least at the genetic markers analyzed). This pattern is consistent with three (potentially overlapping) explanations that 1) the existing populations are large enough to reduce genetic drift, 2) genetic diversity was historically high and populations were well connected across the once vast prairie landscape and populations have not yet been isolated long enough (only about 100 years) to diverge, and/or 3) at least some populations (particularly those in northeastern South Dakota) maintain some degree of gene flow through intermediate populations. *Wolbachia* does not appear to be common in Dakota skippers, at least in the sampled populations. Of 78 individuals sampled from across the range, only two individuals from one population in one year in northeastern South Dakota tested positive. No individuals tested positive from this population when sampled the following year, which suggests that *Wolbachia* is likely rare and not a major conservation concern for this species at this time. Additional screening for *Wolbachia* is likely warranted to see if frequencies change. The combined population genetics (relatively high local and global diversity with little substantive differentiation) and *Wolbachia* screening (largely absent everywhere) results reduce concern if populations are mixed in *ex situ* operations conducted at the Minnesota Zoo. Inter-population (and especially inter-region) breeding at the Zoo has been avoided whenever possible to date, and will still be pursued as much as possible, but these results provide some greater flexibility for breeding operations at the Zoo and potentially for possible future reintroductions to other sites.

ACTIVITY 3: ACTIVITY 3: DNR Butterfly Status Monitoring
Description: The Minnesota DNR will implement a monitoring program of prairie butterflies across Minnesota. This is described in a separate work plan with a separate appropriation to the MN DNR ($245,000).

ACTIVITY 4: Pesticides-related mortality research on surrogate prairie butterflies
Description: The historically widespread tallgrass prairies of the Upper Midwest have been dramatically reduced and fragmented, with the vast majority of the historic acreage now converted to intensive row crop agriculture. The close proximity of agricultural lands to prairie remnants that formerly or may still retain populations of threatened and endangered prairie butterflies presents the possibility that drift from agricultural pesticide applications near prairie fragments may have indirect effects on these imperiled and other prairie species (Longey and Sotherton 1997). Neonicotinoids have become one of the most important groups of agricultural and horticultural insecticides since their development in the 1990s. Their use has increased as an alternative to previously widespread applications of pyrethroid, carbamate, and organophosphate insecticides due to their lower binding potential to mammalian neural receptors and correspondingly lower human health risks. Neonicotinoids can be applied as a foliar spray, a soil treatment, and as a seed coat powder. These systemic pesticides become incorporated into plant tissues, nectar, and pollen and can persist and accumulate in soil and water for months or even years. Numerous studies have documented the negative influence of neonicotinoids on non-target invertebrates, including beneficial insects like honey bees (Pettis *et al.* 2013), aquatic macroinvertebrates (Van Dijk *et. al* 2013), and large butterflies (Krischik, in review 2014). Seed coat applications of neonicotinoids can also become airborne as dust during planting operations that can coat adjacent non-crop plants with powder that can have lethal and sub-lethal effects (Marzaro *et. al* 2011; Krupke *et. al* 2012; Tapparo *et. al* 2012).

The U.S. Environmental Protection Agency specifies the need for further data on the effects of neonicotinoids on non-target invertebrates and endangered species. A similar need for more data was also highlighted in the recent USFWS proposal to list Poweshiek skipperlings and Dakota skippers under the U.S. Endangered Species Act

(USFWS 2013), as well as at the Northern Tallgrass Prairie Lepidoptera Conservation Conference (Minnesota Zoo 2013). To begin addressing this research need, we will test for the presence of neonicotinoid residues that may be present on non-target native prairie remnants adjacent to agricultural fields. We will test insecticide residue concentrations present in grass samples and soil samples from several Minnesota prairie remnants.

This work will then inform experimental tests on the effects of varying concentrations of neonicotinoid applications on growth rates and survivorship of grass skipper butterfly caterpillars, pupae and adults. The experimental treatments will likely be three concentrations of a neonicotinoid and one control treatment with no insecticide application. The concentration of one of the three insecticide treatments will correspond with the levels of one of these neonicotinoids detected in prairie remnants. Previous studies in other U.S. states and several Canadian provinces have detected the presence of thiamethoxam, clothianidin, and (to a lesser extent) imidacloprid in prairie remnants. For these experiments, we will most likely test the effects of thiamethoxam, one of the primary neonicotinoids applied to soybean and corn production in Minnesota. Grass skippers spend the majority of their lives as caterpillars, and potential pesticide effects are expected to be greatest on caterpillars. Comparable experiments to our proposed work with Monarchs (*Danaus plexippus*) and Painted Ladies (*Vanessa cardui*) demonstrate strong effects of the neonicotinoid imidacloprid on larval survivorship but non-significant effects on the nectar feeding adults (Krischik, in review, 2014). We will perform the experimental tests using non-endangered surrogate species of related grass skippers that are similar in terms of their natural history and ecological associations to mitigate the cost of conducting these experiments with endangered species. No experiments on the effects of these pesticides on small butterflies like these skippers have been conducted to date. We plan to conduct a small-scale pilot study in 2014 to assess logistics and treatment details.

Summary Budget Information for Activity 4:

ENRTF Budget: $ 27,600
Amount Spent: $ 27,208
Balance: $ 392

Activity Completion Date:

Outcome	Completion Date	Budget
1. Begin establishment of breeding populations of surrogate species for research.	November 2014	$ 400
2. Collect plant samples from prairie remnants and submit samples for pesticide residue testing.	April 2015	$ 10,200
3. Conduct a small scale pilot study to refine protocols for controlled pesticides experiments with surrogate species.	April 2015	$ 800
4. Perform first year of controlled experiments: treat experimental plants with pesticide, track the effects on survivorship and growth on surrogate butterflies. Collect plant tissue samples from the experiments for pesticide residue analysis.	November 2015	$ 3,000
5. Collect additional plant samples from prairie remnants and perform pesticide residue testing.	April 2016	$ 10,200
6. Repeat #4 to provide replication. Analyze data and submit results for publication.	June 2017	$ 3,000

Activity Status as of November 30, 2014:

After the Minnesota Zoo was funded by the LCCMR to conduct this Activity, the Twin Cities Field Office of the USFWS (Bloomington, MN) approached the Minnesota Zoo to further collaborate on this research. A Cooperative Agreement (available upon request) between the USFWS and the Minnesota Zoo was signed July 11, 2014 and finalized in November 2014. As part of this Cooperative Agreement, the USFWS provided an additional $20,000 through the CFDA Endangered Species – Candidate Conservation Action Fund. This award (F15AC00020) will be managed by the Minnesota Zoo's Dr. Erik Runquist and used to support all of the aspects Activity 3 as outline above, especially the testing of prairie samples for agricultural insecticides. The Zoo has also established

a contract relationship with the U.S. Department of Agriculture's (USDA) National Sciences Laboratory in Gastonia, NC for the analysis of prairie samples for insecticides.

During summer 2014, we began the establishment of breeding populations of five additional common species of skippers following comparable egg collection and rearing techniques as with Dakota skippers. These surrogate species were Peck's skipper (*Polites peckius*), Tawny-edged skipper (*Polites themistocles*), Long Dash (*Polites mystic*), Least skipper (*Ancyloxypha numitor*), Hobomok skipper (*Poanes hobomok*), and European skippers (*Thymelicus lineola*). The goal of this research is to 1) document the natural history of a range of skipper species to help better inform rearing and husbandry protocols and potentially habitat management, and 2) provide a population or populations to test the effects of varying levels of pesticides on skipper larvae. Egg collection and rearing was often opportunistic, but egg collections from these common species were taken from the Minnesota Zoo and prairies in northeastern South Dakota and west-central Minnesota. As with Dakota skippers, there are no published rearing protocols for any of these five species and our work is foundational. We collected dozens to hundreds of eggs from all six species, but no European skipper eggs hatched, potentially due to low humidity. Similar to the rearing of Dakota skippers, larvae of most species were split into 9-inch tubes (with 1-inch plugs of prairie dropseed, little bluestem, or side-oats grama) or allowed to "free-range" on mature 2-gallon pots of prairie dropseed. By November 2014, we transitioned 55 Long Dash larvae (18 from tubes, 37 from free-range pots), 20 Tawny-edged skipper larvae (15 from tubes, 5 from free-range pots), and 10 Hobomok skipper larvae (all free-range pots) into winter hibernation. All of these skippers hibernate through Minnesota winters as partially grown larvae, but several of these species may produce additional generations per year in the southern portions of their ranges. We are interested in learning what may or may not trigger diapause (i.e., hibernation) in skippers, so we also are leaving some larvae of each of the five skipper species from which we obtained eggs inside and are continuing to rear them as we had during the summer without any winter hibernation. All research with the surrogate species is ongoing.

We also made progress on collecting samples from prairies for pesticide residue testing. Consulting with the USFWS and the MN DNR, we selected four prairie remnants to sample for pesticides residues. We selected two sites that currently retain extant populations of Dakota skippers and two sites where Dakota skippers and Poweshiek skippers have apparently been recently extirpated.

USFWS staff developed GIS grids using aerial photos of the sites, and classified every 10x10 m grid cell by proximity to crop fields as either "Ag Edge" (bordering agricultural field), or "Interior" (≥100 m from an agricultural or non-agricultural edge). We randomly selected 7-10 grid points for sampling within each of these two grid cell classes at all four sites. Following protocols developed in partnership with USFWS, Minnesota Zoo staff collected 4+ g of clippings of either little bluestem (*Schizachyrium scoparium*) or big bluestem (*Andropogon gerardii*) within each selected grid cell. These grass species are indicative of intact native prairies and are likely wild host plants for federally Threatened Dakota skippers and other imperiled prairie skippers. Underneath the same grasses, 25+ g of sieved soil was also collected. All samples were double-bagged in quart-sized plastic zip-loc bags, immediately placed on dry ice in the field, and then transferred to a -20°F freezer at the Minnesota Zoo for long-term storage.

Sampling occurred in mid-late August to coincide with aerial spraying of insecticides for the control of soybean aphid infestations. Prairie Coteau SNA was sampled on two consecutive days because a crop-duster plane was observed spraying insecticides over the agricultural field immediately adjacent to the northwest edge of the SNA at the conclusion of the first day of field sampling.

Site	Date	# Interior Points	# Ag Edge Points	Sampler(s)
Glacial Lakes SP	8/13/2014	10	7	Erik Runquist, Cale Nordmeyer
Felton Prairie SNA	8/19/2014	10	10	Cale Nordmeyer
Prairie Coteau SNA	8/19/2014	10	10	Erik Runquist
Prairie Coteau SNA	8/20/2014	9	10	Erik Runquist
North Enemy Swim	8/22/2014	10	9	Cale Nordmeyer

It is currently not known to what degree pesticides may or may not be present on the landscape, or which compounds may be present. It is also not known if pesticide residue concentrations from grass samples might differ from soil samples, or if residue concentrations might differ between grass species. Many more samples

were collected in August 2014 than current funding allows to be tested, so a small subset of the samples have been submitted to the USDA lab to begin initial estimates on the full range of pesticide compounds that might be present and to estimate the potential differences between grass and soil samples. The goal of these initial analyses is to optimize the most efficient use of the remaining funds dedicated for these analyses. Results from these initial analyses are pending, and will be discussed in the May 2015 update.

Activity Status as of May 31, 2015:

We submitted the grass and soil samples that were collected in August 2014 to the USDA National Sciences Laboratory in two rounds in November 2014 and April 2015. Results of the November set of samples was delayed significantly due to processing delays beyond our control at the USDA Lab and were not received until April 2015. This first set was paid for entirely through the Cooperative Agreement match funding provided by the USFWS instead of LCCMR funds. The second set of August 2014 samples submitted April 2015 will be paid for with LCCMR funds.

Only three compounds were detected in any samples tested; all are aerially-applied pesticides to control pest soybean aphids: chlorpyrifos (an organophosphate), and cyhalothrin and bifenthrin (pyrethroids). Soil samples rarely contained detectable concentrations of any of these insecticides. Full results are available upon request, but are summarized for each site below.

Prairie Coteau SNA:

Concentrations of all three compounds were significantly higher at Prairie Coteau SNA than anywhere else, both before and after the aerial spraying observed on the evening of August 19, 2014. There was a substantial rise in chlorpyrifos and cyhalothrin between the first and second day of sampling, while bifenthrin actually declined between days. Bifenthrin was also more abundant in the interior of Prairie Coteau than along the agricultural edges, inverse of chlorpyrifos and cyhalothrin.

We also found significant concentrations of all compounds in the interior of Prairie Coteau. For example, chlorpyrifos concentrations of 78 to 127 parts per billion (ppb) were found more than 0.5 miles away from an agricultural edge the day after the observed spraying. Edge chlorpyrifos concentrations ranged from 51.9 to 278 ppb. The contact dosage necessary to kill 50% (LD50) of individuals is reported to be as low as 70 ppb for both soybean aphids (Chandrasena et al 2011) and honey bees (Christensen et al 2009). There is no information on the effects of these concentrations of chlorpyrifos for butterflies. Soil samples rarely had any detectable pesticides residue concentrations (all less than 2 ppb), and only when associated grass samples had high concentrations.

Felton Prairie SNA:

We detected low levels of chlorpyrifos at Felton Prairie SNA, ranging from Not Detected to a max of 15.0 ppb. There were minimal differences (likely not statistically significant) between interior and edge samples. No other compounds were detected.

Glacial Lakes State Park:

No pesticides were detected in any sample submitted for analysis from Glacial Lakes.

North Enemy Swim, Sisseton Wahpeton Oyate (Day Co., SD):

We detected low levels of chlorpyrifos at North Enemy Swim, ranging from 1.6-5.0 ppb. There were minimal differences (likely not statistically significant) between interior and edge samples. We also detected traces of cyhalothrin in one interior sample (2.7 ppb).

Controlled Experiment

The controlled experiment to study the biological consequences of exposure to agricultural neonicotinoids is scheduled to begin in summer 2015. Final experimental design is under review, and may change following consultations from USFWS scientists participating in federal pesticides reviews. We may modify design in our initial work plan to add additional treatment concentrations. This will provide a more comprehensive view of the lifetime effects of exposures, including potential sublethal effects from long-term exposure to low concentrations. We have selected the Long Dash as the most likely species to be studied in the experiment.

Activity Status as of November 30, 2015:

We repeated sampling for pesticides residues at the same four prairie remnants as above in June 2015 and in September 2015. The two sampling periods are to assess the seasonal variability of potential pesticide presence and composition. It is important to note that each of the Point identifiers below (as well as relative to the 2014 data) are unique, and any two points with the same name do not necessarily represent the same location. GPS coordinates for all sampling points are available upon request.

June 2015 Field Samples:

We only found one pesticide in any of the June 2015 samples submitted: atrazine. Atrazine is the second most widely applied herbicide in the United States and controls broadleaf weeds around crops (primarily corn). The minimum Level Of Detection for atrazine for these analyses was 6.0 parts per billion (ppb), so "Trace" samples represent a range of 0.1-5.9 ppb. No insecticides were detected.

Prairie Coteau SNA:

Three "interior" samples and three "edge" points were sampled and analyzed, with five of the six points having paired grass and soil samples. Atrizine was detected at two of the Interior points. Trace levels were detected at one of the Edge samples and at the other Interior samples.

Atrazine Concentrations (ppb) at Prairie Coteau SNA		
Point	**Grass**	**Soil**
Edge 1	Not Detected	Not Detected
Edge 3	Trace	Not Detected
Edge 5	Not Detected	Not Detected
Interior 1	7.7	Not Analyzed
Interior 4	6.1	6.8
Interior 5	Trace	Not Detected

Felton Prairie SNA:

No detectable levels of any pesticides or their residues were found in any of the seven samples submitted for analysis from Felton Prairie.

Glacial Lakes State Park:

No detectable levels of any pesticides or their residues were found in any of the eight samples submitted for analysis from Glacial Lakes.

North Enemy Swim, Sisseton Wahpeton Oyate (Day Co., SD):

We detected atrazine on all four grass samples from North Enemy Swim. Nothing was detected in any of the paired soil samples.

Atrazine Concentrations (ppb) at North Enemy Swim		
Point	**Grass**	**Soil**
Edge 1	8.1	Not Detected
Edge 3	7.8	Not Detected
Interior 4	Trace	Not Detected
Interior 5	8.4	Not Detected

Early September 2015 Field Samples:

Due to logistical constraints, late summer sampling occurred later in 2015 than in 2014. As such, most aerial soybean aphid insecticide applications had occurred several weeks prior, and associated residue observations for those compounds were substantially lower. Nonetheless, the insecticide bifenthrin was widespread and detected in all samples analyzed, often at comparable (or higher) levels to the 2014 samples. Given that lack of any substantial or novel pesticide residues from soil samples in August 2014 or June 2015, we only collected grass samples in late 2015 for economic and logistical reasons.

Prairie Coteau SNA:

Relative to immediately before and after a known spray event in 2014, residue levels for bifenthrin and chlorpyrifos were substantially lower, and cyhalothrin was not detected in any samples.

Insecticide Concentrations (ppb) on prairie grasses at Prairie Coteau SNA			
Point	**Bifenthrin**	**Chlorpyrifos**	**Cyhalothrin**
Edge 2	12.9	3.1	Not Detected
Edge 3	20.9	4.4	Not Detected
Edge 5	9.5	Not Detected	Not Detected
Interior 3	11.7	Not Detected	Not Detected
Interior 5	8.9	2.9	Not Detected

Felton Prairie:

Low levels of bifenthrin were observed at all four points analyzed.

Insecticide Concentrations (ppb) on prairie grasses at Felton Prairie			
Point	**Bifenthrin**	**Chlorpyrifos**	**Cyhalothrin**
Edge 1	8.5	Not Detected	Not Detected
Edge 3	8.9	Not Detected	Not Detected
Interior 2	6.8	Not Detected	Not Detected
Interior 5	7.7	Not Detected	Not Detected

Glacial Lakes State Park:

While no insecticides were recorded at Glacial Lakes State Park in 2014, low levels of bifenthrin were found at all sampling points, and cyhalothrin was observed at one.

Insecticide Concentrations (ppb) on prairie grasses at Glacial Lakes St Park			
Point	**Bifenthrin**	**Chlorpyrifos**	**Cyhalothrin**
Edge 2	8.5	Not Detected	2.0
Edge 4	16.8	Not Detected	Not Detected
Interior 1	6.9	Not Detected	Not Detected
Interior 3	7.9	Not Detected	Not Detected

North Enemy Swim, Sisseton Wahpeton Oyate (Day Co., SD):

A localized insecticide application along an edge of the North Enemy Swim prairie appears to have occurred shortly before samples were collected. This is evident from the high bifenthrin level and the first observance of the pyrethroid cypermethrin in any samples to date.

Insecticide Concentrations (ppb) on prairie grasses at North Enemy Swim				
Point	**Bifenthrin**	**Chlorpyrifos**	**Cyhalothrin**	**Cypermethrin**
Edge 1	3.5	Not Detected	Not Detected	Not Detected
Edge 5	71.7	9.3	1.4	96.3
Interior 2	13.3	1.6	7.7	Not Detected
Interior 5	10.9	0.8	3.4	Not Detected

Controlled Experiment:

The controlled experiment to estimate potential lifetime effects of exposure to the common agricultural neonicotinoid clothianidin on prairie skippers was initiated in July 2015. In early July, we randomly assigned 128 little bluestem plants in 1-gallon pots to one of six treatments with 21-22 replicate plants per treatment. Plants in

Treatments 1 through 5 were watered with five solutions of clothianidin: 10 ppb, 50 ppb, 100 ppb, 500 ppb, and 1000 ppb. Treatment 6 plants were applied with a clothianidin-free water control. All plants were maintained outdoors under a plastic roof and bottom-watered with regular water as needed.

In mid-July, we placed five young Long Dash skipper (*Polites mystic*) caterpillars on each of these replicate plants. These caterpillars were collected as eggs from wild female Long Dash using previously employed egg collection protocols. These caterpillars will be tracked throughout their one-year lifespan into the summer of 2016 at several key developmental stages to assess potential lethal and sub-lethal effects of continued consumption of little bluestem that have been treated with different concentrations of clothianidin. Like Dakota skippers, Long Dash caterpillars construct shelters in their host grass. The caterpillars invest in shelter construction before feeding, and we know from prior experience that frequent censuses that disturb the caterpillars and their shelters can have a strongly negative impact on larval growth and survivorship. Therefore, we are only sampling a subset of all the larvae at each time step so that we can more accurately estimate the true effects exposure to clothianidin at each developmental stage in the absence of human disturbance. We are assessing survivorship and changes in weight at each sampling period.

At each sampling iteration, we are also collected living tissue of a subset of the plants the larvae are feeding on to estimate the concentration of clothianidin the larvae are exposed to. Preliminary data show an average 13% uptake rate (range: 0%-73%) of the applied clothianidin by the plants three weeks after treatment. This uptake rate did not appear to vary across treatment types.

Data collection and data analyses are ongoing and will be presented in future reports.

Activity Status as of June 16, 2016:

Note that the LCCMR funds available for this Activity increased since the November 2015 update. This is due to an accounting shift whereby some of the analysis costs that had previously been charged to the Zoo's LCCMR account were shifted onto the Zoo's Interagency Cooperative Funding grant from the U.S. Fish and Wildlife. Field sampling during the 2016 field season was supported by this ENRTF appropriation; field sampling for pesticide residue will continue during 2017 – 2018 with the support of a M.L. 2016 ENRTF (M.L. 2016, Chp. 186, Sec. 2, Subd. 03c1) and external funding.

Late May 2016 Field Samples:

We repeated grass and soil sampling for pesticides residues at the same four prairie remnants as above May 24-27 for the final time as part of this Activity. Crop planting had occurred at least a few weeks prior to sampling across the region. Samples will be sent for analysis and results reported in future reports.

Controlled Experiment:

On May 19, an additional six pots per clothianidin concentration treatment type were sampled. The number of surviving Long Dash skippers and their weights were recorded. Analyses are on-going, but preliminarily we did not find a statistically significant relationship between concentration and survivorship, although there is a potential inverse relationship between concentration and survivorship. Additional results will be presented in future reports.

Activity Status as of November 30, 2016:

In addition to the collection of samples in late Spring 2016, we collected another round of late summer samples in August. Results from these two seasons are described separately below. Note that despite completion of the analyses and submission of the two seasonal data sets below, the final purchase invoices for this work have not been received at the time of this writing. As such, expenditures for this research will not be presented until the May 2017 update.

May 2016 Field Samples:

We only found three pesticides in the May 2016 samples, with the widely applied herbicide atrazine being most prevalent. The minimum Level Of Detection (LOD) for atrazine for these analyses was 50.0 parts per billion (ppb), so "Trace" samples represent a range of 0.1-49.9 ppb. This is a significantly poorer resolution than in 2015 (when the LOD was 6.0 ppb) and is a change that we were not made aware of until results were received. The

insecticide clothianidin and the fungicide tebuconazole were also each detected for the first time, in only one sampling point each.

Prairie Coteau SNA:

Three "interior" samples and three "edge" points were sampled and analyzed, with four of the six points having paired grass and soil samples. Atrazine was detected at all of the Interior points. Trace levels were detected at one of the Edge samples and at all of the Interior samples.

Felton Prairie Bicentennial SNA:

Trace levels of atrazine were detected in all four grass samples (two Edge, two Interior) submitted for analysis. Nothing was detected in the soil samples.

Glacial Lakes State Park:

Unlike other sites, atrazine was not detected in any of the seven grass and soil samples from two edge and two interior sampling locations. However, a Trace sample (L.O.D. = 30 ppb) of clothianidin was found at one Edge point on the north side of the Park. This is the first and only observation of this (or any other) neonicotinoid insecticide in any of our sampling efforts to date.

North Enemy Swim, Sisseton Wahpeton Oyate (Day Co., SD):

We detected trace amounts of atrazine on two of three Edge samples and two of three Interior grass samples from North Enemy Swim. No pesticide residues were detected in any of the paired soil samples.

Late August 2016 Field Samples:

Sampling occurred at four prairies, although one new site (Hole-in-the-Mountain) was sampled instead of one previously sampled site (North Enemy Swim). The Nature Conservancy's Hole-in-the-Mountain preserve was sampled to help inform its suitability as a potential site for proposed reintroductions of Zoo-reared Dakota skippers (see project status update for a M.L. 2016 ENRTF, M.L. 2016, Chp. 186, Sec. 2, Subd. 03c1). Aerial soybean aphid insecticide applications were ongoing in southern Minnesota at the time of sampling, and the insecticide chlorpyrifos was only detected at the two southern preserves (Prairie Coteau and Hole-in-the-Mountain). No other pesticides were detected in any samples. Unlike comparable 2014 and 2015 sampling, no insecticides were detected in any samples farther north. Given that lack of any substantial pesticide residues from the paired soil samples to date, we only collected grass samples in late 2016 for economic and logistical reasons.

Prairie Coteau SNA:

Chlorpyrifos was detected at relatively low levels at four of the six points sampled. These points are spread across the SNA.

Chlorpyrifos Concentrations (ppb) on prairie grasses at Prairie Coteau SNA	
Point	**Chlorpyrifos**
Edge 1	6.8
Edge 2	Not Detected
Edge 3	6.1
Interior 1	Not Detected
Interior 2	26.7
Interior 3	8.8

Felton Prairie:

No pesticides were detected at three edge and three interior sampling points.

Glacial Lakes State Park:

No pesticides were detected at three edge and three interior sampling points.

Hole-in-the-Mountain Prairie Preserve, Lincoln Co., MN

Chlorpyrifos was detected at three of the six points sampled, at relatively low levels that are comparable to those observed at other prairies in late summer. These points are spread across the preserve.

Chlorpyrifos Concentrations (ppb) on prairie grasses at Hole-in-the-Mountain Preserve	
Point	**Chlorpyrifos**
Edge 1	Not Detected
Edge 2	9.2
Interior 2	13.5
Interior 3	5.5
Interior 4	Not Detected
Interior 5	Not Detected

Controlled Experiment:

The controlled experiment at the Zoo to estimate potential lifetime effects of exposure to the common agricultural neonicotinoid clothianidin on prairie skippers concluded in July 2016. Over a three week period in June and July 2016, the remaining 36 unsampled pots (5-7 pots per pesticide concentration treatment type) were censused for surviving adult Long Dash skippers. Due to limitations in staffing during the busiest weeks of summer, priority for Zoo personnel was necessarily given to the intense Activity #1 husbandry operations with the federally Threatened and Endangered species instead of toward this experiment. Consequently, we could not check every pot every day for surviving skippers, but we were able to recover them later. As such, we were able to record the number of individuals that had survived to adulthood on those plants since their initial placement as small caterpillars in July 2015 (the primary goal), but we were not able to record the weights of those survivors at the time of their emergence or other subtle details on other potential sublethal effects (secondary goals). Of the original 180 caterpillars placed on these 36 pots in July 2015 (5 per pot), 39 were recovered. Preliminary analyses do not suggest a statistically significant relationship between survivorship to adulthood and initial clothianidin concentration. However, interpretation of these results is limited due to confounding factors, such as relatively small sample sizes and the variable uptake of clothianidin, and additional analyses will be completed in the winter.

Our initial objective was to repeat this experiment again in 2016-2017, but we were unable to complete the replication as planned. The staffing requirements of the experiment in 2015-2016 could not be sustained in 2016-2017 due to the formal expansion of Zoo's Prairie Butterfly Conservation Program to 1) include head starting of the critically Endangered Poweshiek skipperling in 2016-2017 in partnership with the US Fish and Wildlife Service and 2) initiate the world's first re-introduction of Dakota skippers in 2017 (see Activity 1 for more information on these programs). Personnel efforts were necessarily re-directed towards these federally-listed species.

Activity Status as of May 31, 2017:

No additional field sampling has occurred since the last update, and therefore no new data is available to present. However, the remainder of the allocated funds for the Activity were fully expended since the last update to pay for analysis of those samples discussed in the previous update. Additional funds were also used to pay for these analyses from the Zoo's "Legacy Amendment" Conservation fund ($5,000) and the Zoo's ML 2016 ENRTF ($115). As noted below, this ENRTF-supported research on pesticides drift into remnant prairies and the potential link to declines in prairie butterflies was featured in a November symposium held at the University of Minnesota on behalf of the Minnesota Invasive Terrestrial Plant and Pest Center. The symposium synthesis report was published in March 2016 and contains recommendations for additional research.

Final Report Summary:

We know significantly more about the degree and prevalence of pesticides drifting into prairie remnants in Minnesota than prior to this ENRTF support. We have observed traces of insecticides at all five prairies we sampled, all of which either have or once had populations of Dakota skippers and Poweshiek skipperlings (as well as other prairie butterflies known to be in decline). We have found that the risk of drift of broad-spectrum insecticides applied against the economically damaging soybean aphid in the second half of summer is likely greater than the risk associated with dust from neonicotinoid-coated seed crops planted in the spring.

While gains have been made, substantial questions remain. With the exception of a single sample date, we do not understand the temporal or spatial origins of the insecticides that we have detected. It is not known how long these residues had been present in the prairies prior to being sampled, nor can we trace how far away they came from. We also do not understand the biological consequences of the exposures we have observed. As described previously, controlled experiments that expose caterpillars to ranges of dosages of the observed insecticides are needed to fully assess the risks. Some of this work is now being initiated under the Minnesota Zoo's M.L. 2016 ENRTF, and will be detailed in its semi-annual updates.

ACTIVITY 5: Prairie Outreach and Environmental Education at the Zoo

Description: With 1.3 million visitors annually, the Minnesota Zoo will utilize its role as Minnesota's largest environmental education center to provide educational materials about prairie butterflies, their imperiled native habitats, and actions the public can take. The Minnesota Zoo will produce at least two publications (both traditional and web-based) and graphics about Minnesota's imperiled butterflies and their prairie habitat for public education. These glossy, fold-out guides will be free to Minnesota Zoo guests at its seasonal Butterfly Garden exhibit, at other on-site displays, and at other educational outreach opportunities. These guides will also be made available online for download and incorporated into Zoo social media and other digital outreach opportunities.

Summary Budget Information for Activity 5:

ENRTF Budget: $ 8,000
Amount Spent: $ 3,474
Balance: $ 4,526

Activity Completion Date:

Outcome	Completion Date	Budget
1. Production and printing of a Prairie Butterflies Identification and Pollinator Information Guide	May 2015	$ 4,000
2. Production and printing of a Prairie Biology Guide	May 2016	$ 4,000

Activity Status as of November 30, 2014:

The winter of 2014-2015 will be the primary work time for the first deliverable associated with this Activity. We have developed some online messaging that may be incorporated into these outreach guides, and these have also been shared through the Minnesota Zoo's social media outlets. These include species guides about Dakota skippers (http://mnzoo.org/blog/animals/dakota-skipper/) and Poweshiek skipperlings (http://mnzoo.org/blog/animals/poweshiek-skipperling/), and the Minnesota Zoo's Prairie Butterfly Conservation Program (http://mnzoo.org/conservation/minnesota/saving-minnesotas-prairie-butterfly-heritage/). We also have created a "Plant for Pollinators" webpage (http://mnzoo.org/plant-pollinators/) which provides guidance on how the general public can help butterflies and pollinators in their own yards.

Activity Status as of May 31, 2015:

We have drafted content for both Guides, and are working with MNZoo's graphics staff on final edits and layouts. We decided to proceed with production and publication of both guides simultaneously so that messaging can be streamlined. We will complete and proceed with publication of these guides in early June so that they can be distributed free to Zoo guests in our Aveda Butterfly Garden starting June 2015. Both guides will credit the ENRTF.

The first guide, tentatively titled "Your Butterfly Neighbors", will provide life-sized color photos of 11 common Twin Cities butterflies, as well as photos of all butterflies currently listed by the Minnesota DNR as Endangered, Threatened, or Special Concern. It will also describe the loss of Minnesota's prairie ecosystems, the consequent declines of prairie butterflies like the Poweshiek skipperling and Dakota skipper, and the Minnesota Zoo's Prairie Butterfly Conservation Program. Finally, the guide will describe ways people can help butterflies and pollinators through wildflower gardening.

The second guide will be modeled after the Zoo's Plant for Pollinators webpage (http://mnzoo.org/plant-pollinators/). It will describe benefits native wildflowers can provide to struggling pollinators with suggestions for

the best plants for certain situations. It will also reflect some of the conservation messaging and information presented in the first guide.

Activity Status as of November 30, 2015:

We successfully produced and published two outreach pamphlets. These have been and are being distributed free to Minnesota Zoo guests at the Aveda Butterfly Garden and at volunteer tables around the Zoo. We also have provided them at various outreach events, including the Minneapolis Monarch Festival, two pollinator public policy forums, and several STEM events. The first ("Get To Know Your Butterfly Neighbors") provides life-sized color photos of some common butterflies that may be seen in the Twin Cities and across much of Minnesota, highlights the endangered, threatened, and imperiled butterflies of Minnesota's disappearing prairies, the work of the Minnesota Zoo's Prairie Butterfly Conservation Program, and what the public can do to help. We printed an initial run of 12,000 copies of this four-fold pamphlet, and have also made it available for download (http://mnzoo.org/pdfs/BG15_ButterflyPamphlet_finalWEB.pdf).

The second pamphlet ("Plant for Pollinators") is modeled after our website of similar content (http://mnzoo.org/plantforpollinators). It recommends 31 species of Minnesota-native plants (and describes the conditions they need) so that the public can help provide needed resources for pollinators. We printed an initial run of 12,000 copies of this four-fold pamphlet, and have also made it available for download (http://mnzoo.org/pdfs/BG15_PlantforPollinatorsPamphlet_finalWEB.pdf).

Activity Status as of June 16, 2016:

We have continued providing the two pamphlets free to Zoo guests at interpretive kiosks, as well as at several additional public events and forums. Several thousand copies of each pamphlet have been distributed, and several thousand more of each remain. They will be distributed again to guests in future months.

Activity Status as of November 30, 2016:

We have continued providing the two pamphlets free to Zoo guests, as well as at several additional public events and forums. A few thousand copies of each remain. Most significantly, we commissioned a Spanish translation of both pamphlets and printed 250 new copies of each. These were primarily distributed at the Minneapolis Monarch Festival in September, with remaining copies to be made available at similar events such as the Minnesota Zoo's Spanish Day.

Activity Status as of May 31, 2017:

The two pamphlets continue to be a popular resource for Zoo guests, legislators, and at a variety of public outreach events. Approximately 1,000 copies of each remain. We are working closely with Zoo staff to highlight these pamphlets around the Zoo, with the Minnesota Zoo Butterfly Garden continuing to be the primary venue for guests to discover these pamphlets. We also provide them to the public at various speaking engagements and special events. We will be producing and printing updated versions of these two pamphlets in the next month using the remaining dedicated ENRTF funds.

Final Report Summary:

All of the remaining copies of the original two pamphlets were distributed to Minnesota Zoo guests and other members of the public by late July 2017. We published updated versions of these two pamphlets in both English and Spanish in late July 2017. The content of the new pamphlets are very similar to the first versions; changes are primarily stylistic to align the pamphlets with current Zoo-wide formatting standards. They are being distributed through the Zoo's Butterfly Garden and elsewhere, and are also available online. The unspent funds remaining under the Activity 5 budget had been budgeted for the printing of these updated pamphlets, but non-ENRTF funds had to be used for due to extended production timelines. The ENRTF remains prominently credited as a funding source for their development.

V. DISSEMINATION:

Description:

The activities and results of the Minnesota Zoo's breeding and research operations will be shared with all named partners through annual reports. The outcomes of the conservation genetics and the pesticides research will be submitted for publication in independent peer-reviewed scientific journals. Findings will also be communicated through the Minnesota Zoo's marketing and education departments as much as possible, including on the Zoo's webpage (mnzoo.org), as well as presentations by the Project Manager to the public and other interested parties. Zoo staff, interns, and volunteers will also be trained to talk about the program, prairie butterflies, and the importance of prairies to the public. The produced guides described in Activity 5 will also serve as a major source of outreach and in addition to being made available free to Zoo guests, will be posted on the Zoo's webpage for download, and integrated into other outreach digital and hardcopy publications.

Activity Status as of November 30, 2014:

Reports to partners are in preparation now, with the majority of them to be completed and distributed in December 2014 and January 2015. The conservation genetics and pesticides research programs are in their formative stages, and no data or analyses are yet available for publication. In addition to the websites listed in Activity 5 above, the Minnesota Zoo's Prairie Butterfly Conservation Program was recently highlighted in a story in the November 1, 2014 Star Tribune (www.startribune.com/local/south/281186431.html). The Program and the plight of these butterflies were also highlighted during the Minnesota Zoo's summer 2014 "Big Bugs!" exhibit and in the Zoo's Aveda Butterfly Garden. Zoo staff and volunteers were trained on prairie butterfly conservation issues for communication to the general public. As noted below, the Minnesota Zoo's Prairie Butterfly Conservation Program also was the recipient of a corporate gift from Aveda that matched voluntary donations made at Zoo admissions from guests. Dr. Runquist was an invited speaker at the National Caucus of Environmental Legislators' National Issues Forum in Minneapolis, MN on August 17, 2014, where he spoke about prairie butterfly conservation and their needs.

Activity Status as of May 31, 2015:

The final report describing our 2014 work was distributed to the US Fish and Wildlife Service and other partners in late December 2014. It is available to LCCMR upon request. Now that the Poweshiek skipperling and Dakota skipper both listed species under the US Endangered Species Act, we have been involved in numerous conference calls associated with the federal Recovery Plan process for these species. The majority of our next outreach opportunities will occur in summer 2015 with several planned events. These will be outlined in the next update.

Activity Status as of November 30, 2015:

Reports to partners are in preparation now, with the majority of them to be completed and distributed in December 2015 and January 2016. The pesticides and genetics research are still in progress and not yet in a publishable state.

As discussed above, we hosted a US Fish and Wildlife Service funded workshop with the Conservation Breeding Specialist Group in October to discuss the potential role and form of *ex situ* conservation programs with Dakota skippers and Poweshiek skipperlings. This three-day workshop brought together about two dozen experts from across the ranges of Dakota skippers and Poweshiek skipperlings. The Minnesota Zoo's LCCMR-funded work was highlighted throughout the meeting. A consensus was reached to continue and expand our *ex situ* program with Dakota skippers and to expand into work with Poweshiek skipperlings in 2016. The report from the meeting is under a comment period from relevant stakeholders, and will be discussed further in future updates.

In summer, the Minnesota Zoo Foundation partnered with Fair State Brewing Cooperative to raise funds for our program with the limited edition "Dakota Skipper Endangered Reserve" beer (http://mnzoo.org/dakota-skipper-endangered-reserve/). It was sold at more than a dozen Twin Cities restaurants and helped raise awareness of the troubled butterfly and our work.

In addition to the distribution of the two pamphlets produced with ENRTF dollars, we have also worked with the Minnesota Zoo's Marketing and Public Relations Departments on social media blogs and posts. In

September, we hosted a booth at the popular Minneapolis Monarch Festival and talked with over 1000 people about prairie butterflies and the LCCMR-supported Zoo's work with them. Dr. Runquist was an invited speaker at two well-attended public pollinator forums in October, for Representatives Lillie and Hansen in October, and for Senator Dziedzic and Representatives Kahn and Loeffler. He also spoke about this program in a well-attended special symposium on the status of butterfly conservation in the US and Canada at the Annual Meeting of the Entomological Society of America in November.

Activity Status as of June 16, 2016:

The final report describing our 2015 work was distributed to the US Fish and Wildlife Service and other partners in late December 2015. It is available to LCCMR upon request. We continue to be involved in numerous conference calls and in-person meetings associated with the federal Recovery Plan process for Poweshiek skipperling and Dakota skippers. Indeed, the research conducted under Activity 4 is now being shared with the US Environmental Protection Agency as part of federal reviews of some insecticides. As discussed above, we worked with the US Fish and Wildlife Service and other agencies to develop a "Plan for the Controlled Propagation, Augmentation, and Reintroduction of Poweshiek skipperling (*Oarisma poweshiek*)". This cooperative interagency plan follows the IUCN's "Guidelines for Reintroductions and Other Conservation Translocations" and lays out the specific work plan for the Poweshiek skipperling augmentation by headstarting program that was recommended by experts participating in the October 2015 "Poweshiek skipperling Dakota skipper *Ex Situ* Feasibility Assessment and Planning Workshop" (Delphey et al 2016). The majority of our next outreach opportunities will occur in summer 2015 with several planned events. These will be outlined in the next update.

In addition to the distribution of the two pamphlets produced with ENRTF dollars, we have also worked with the Minnesota Zoo's Marketing and Public Relations Departments on social media blogs and posts. We issued a "#Plant4Pollinators Challenge".

Activity Status as of November 30, 2016:

Information and results related to the Prairie Butterfly Conservation Program's initial appropriation from ENRTF (M.L. 2014, Chp. 226, Sec. 2, Subd. 05j-1) and this project are jointly disseminated to partner organizations and the general public. Reports for partners are currently in preparation, with the majority of them to be completed and distributed in December 2016 and January 2017. We remain in close coordination with the USFWS and the Minnesota DNR about all aspects of our work. We are also beginning consultations with the US Environmental Protection Agency about the pesticides research to help inform federal review of some key insecticides.

In summer, the Minnesota Zoo Foundation again partnered with Fair State Brewing Cooperative to raise funds for our program with the limited edition re-release of the "Dakota Skipper Endangered Reserve" beer (http://mnzoo.org/dakota-skipper-endangered-reserve/). It was sold at several Twin Cities restaurants and helped raise awareness of the imperiled butterfly and our work. Publicity for the beer and the butterflies was enhanced by appearances by Dr. Runquist on KARE 11 and FOX 9 morning TV shows.

The joint work being conducted by Minnesota Zoo and the DNR program was highlighted in July in a feature-length story on Minnesota Public Radio (http://www.mprnews.org/story/2016/07/12/minnesota-prairie-butterflies-disappear-concerns, and then again in November in The Nature Conservancy's "Prairies to Pines" magazine (pdf emailed to LCCMR staff along with this update).

We completed a 'social media takeover' of MN Zoo's Facebook account in October. Cale Nordmeyer, butterfly conservation specialist at the MN Zoo, also recently filmed a segment with KARE 11 that showcased the ongoing work at the Zoo. This segment is scheduled to air in mid-December.

In November, Dr. Runquist co-chaired a workshop at the University of Minnesota that brought together individuals from academia, agencies, the agricultural sector, and conservation organizations to share information about the soybean-aphid pesticides and outline future information and research needs.

In addition to the distribution of the two pamphlets produced with ENRTF dollars, we have also worked with the Minnesota Zoo's Marketing and Public Relations Departments on social media blogs and posts. In September, we hosted a booth at the popular Minneapolis Monarch Festival and talked with over 1000 people

about prairie butterflies and the LCCMR-supported Zoo's work with them. We distributed both English and Spanish-language pamphlets at this event.

Activity Status as of May 31, 2017:

The final report describing our 2016 work was distributed to the US Fish and Wildlife Service and other partners in late January 2017. It is available upon request. We continue to be involved in numerous conference calls and in-person meetings associated with the federal Recovery Plan process for Poweshiek skipperling and Dakota skippers. In February, Program staff travelled to Michigan to participate in a multi-day Poweshiek skipperling federal recovery planning workshop, and then in April, hosted a workshop with Dakota skipper experts to develop a risk assessment model for the US Fish and Wildlife Service to help determine long-term management and recovery options for the Dakota skipper.

The synthesis report detailing the findings of the November symposium on the potential non-target effects of soybean aphid insecticides on prairie butterflies that was co-organized by Dr. Erik Runquist on behalf of the Minnesota Invasive Terrestrial Plant and Pest Center at the University of Minnesota was published March 20, 2017. It is available here: https://mitppc.dl.umn.edu/sites/g/files/pua746/f/media/mitppc_soybean.final_.pdf. The Zoo's ENRTF-supported research on Dakota skipper and Poweshiek skipperling biology and on insecticide drift that has been detailed in these status updates is centrally-featured in this report.

In April, Dr. Erik Runquist (Butterfly Conservation Biologist) was a featured speaker at the annual meetings of the Minnesota Native Plant Society (Minnesota Landscape Arboretum, Chanhassen, MN) and the Minnesota Prairie Chicken Society (Rothsay, MN).

In addition to the distribution of the two pamphlets produced with ENRTF dollars, we have continued to work with the Minnesota Zoo's Marketing and Public Relations Departments on social media blogs and posts, with more outreach planned through the spring and early summer.

Final Report Summary:

We have developed a large network of collaborators across local, state, national, and international levels. We hold frequent conference calls with several recovery and threat assessment working groups for both Poweshiek skipperling and Dakota skipper, and have attended and/or hosted several multi-day meetings and conferences for these species. We present our results to these working groups and other permitting agencies, and prepare detailed annual reports. Our results informs the actions and recommendations of the working groups. The foundational husbandry protocols we developed have also helped Winnipeg's Assiniboine Park Zoo launch a parallel and collaborative prairie butterfly conservation rearing and breeding program. Scientific products of our ENRTF-supported work will be submitted for peer-reviewed publication.

Thanks to the programmatic expansions supported by the ENRTF, the plight of prairies and their butterflies have become much more visible and publicly known. We have presented to dozens of general public audiences (thousands of people in total), and at several University undergraduate and graduate-level courses and seminars. At least nine newspaper, radio, and television stories have been produced about the prairie butterfly conservation efforts supported by the ENRTF since 2014, including four new newspaper, radio, and television stories associated with the Dakota skipper reintroduction program in the summer of 2017. New coverage since the May 2017 update includes:

- Star Tribune June 29, 2017 (front page): http://www.startribune.com/with-help-from-minnesota-researchers-imperiled-prairie-butterfly-takes-new-flight/431403283/#1
- Minnesota Public Radio, June 29, 2017: https://www.mprnews.org/story/2017/06/29/dakota-skipper-homely-butterfly-is-beautiful-sight-to-breeder
- KSFY TV (Sioux Falls), July 1, 2017: http://www.ksfy.com/content/news/Endangered-Dakota-Skipper-butterfly-reintroduced-to-the-wild-432052253.html
- Argus Leader (Sioux Falls), June 27, 2017: http://www.argusleader.com/story/news/2017/06/28/what-happened-dakota-skipper-butterfly-and-can-saved/433288001/
- Minnesota Zoo Facebook Live streaming event from the Hole-in-the-Mountain Prairie Preserve (https://www.facebook.com/mnzoo/videos/10155374215493788/) featuring Prairie Butterfly Conservation Program manager Dr. Erik Runquist, the Minnesota DNR's Dr. Robert Dana (project lead on

this joint ENTRF for Activity 3), and staff from The Nature Conservancy and the US Fish and Wildlife Service. Viewed nearly 11,000 times, the video provided a live look at the Dakota skipper reintroduction effort, the history of the ENRTF-supported Prairie Butterfly Conservation Program, and the partnerships involved. Additional Minnesota Zoo social media and blog posts were presented throughout the summer of 2017 highlighting the reintroduction effort, our "Plant For Pollinators" campaign, and the re-introduction of the #Butterfly Brew Dakota Skipper Endangered Reserve promotion through Fair State Brewing Cooperative.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	$ 273,500	1 State Program Administrator Principal at 100% FTE for 3 years; 1 Project Analyst at 25% FTE for 3 years;
Professional/Technical/Service Contracts:	$ 32,000	1 contract (RFP) pesticide residue testing; 1 contract (RFP) for DNA sequencing
Equipment/Tools/Supplies:	$ 5,600	Supplies needed to support Zoo conservation breeding operations as well as conservation genetics and pesticides research, including tables, rearing cages, butterfly nets, collecting supplies, plants, and laboratory reagents
Capital Expenditures over $5,000:	$ 52,000	Purchase and outfitting of indoor chamber for the Zoo conservation breeding program
Printing:	$ 8,000	Production of two guides on prairies and prairie butterflies and pollinators for free distribution at the Zoo
Travel Expenses in MN:	$ 3,800	Mileage, lodging, meals for travel to and between prairie sites for data collection and breeding operations
Other:	$ 5,100	Travel expenses outside of MN. Mileage, lodging, meals for travel to and between prairie sites to obtain individuals for the Zoo conservation breeding program. All known viable populations of the Minnesota-native endangered butterflies are now outside of Minnesota in Wisconsin, Michigan, North Dakota, South Dakota, and Manitoba, necessitating out of state travel to obtain founder stock.
TOTAL ENRTF BUDGET:	**$ 380,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: The Minnesota Zoo's Prairie Butterfly Conservation Program requires stable indoor space in which temperature and lighting can be controlled for breeding and rearing operations, an aspect that has been lacking to date. Funding from ENTRF will allow for required expansion of our operations to allow us to test a variety of methodological approaches to optimize breeding success and minimize mortality of these endangered species. This multi-layer containment rearing chamber will be located on Zoo grounds and will conform to USFWS and USDA guidelines. Should the Prairie Butterfly Conservation Program close, the Zoo will consult with the ENTRF on alternative arrangements or reimburse the funds.

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 3.75

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
Zoo admissions donations from the public. Remaining funds were rolled over from FY15 into FY16 and combined with an additional FY16 $5,000 and FY17 allocation from the Minnesota Zoo Foundation.	$19,385 +$5,000 +7,000	$24,385	To generally supplement all operations, including additional pesticides residue testing, genetic screening, and staff pay. Donations were solicited May 24-September 1, 2014.
Matching Gift from Aveda	$10,000	$10,000	To generally supplement all operations, including additional pesticides residue testing, genetic screening, and staff pay. Funds were available late 2014.
State			
Legacy Clean Water Arts and Cultural Heritage Fund, grant to MN Zoo for FY15, FY16, and FY17. Additional Funds from the Zoo's General budget were used in late 2015 to cover construction and permitting costs.	$ 40,000 + $65,000 +$40,000 +$5,000	$147,000 (estimated)	To support the remaining 75% of the MN Zoo's assistant worker salary and benefits. This amount has been secured for FY15 through FY17. Additional Legacy and Zoo funds were needed in summer 2015 for construction and permitting of the new butterfly breeding chamber and greenhouse. Additional Legacy funds were used in late 2016 to supplement ENRTF funds for the analysis of 2016 prairie pesticides residue samples.
MN Zoo General operating budget	$17,645	$17,645	Additional funds to cover construction costs for the new husbandry facilities.
US Fish and Wildlife Service CFDA Endangered Species – Candidate Conservation Action Fund Cooperative Agreement	$20,000	$20,000	To supplement all work outlined in the pesticides research in Activity 4. Funds will be available beginning November 2014.

US Fish and Wildlife Service CFDA Endangered Species – Candidate Conservation Action Fund Cooperative Agreement	$52,128 +$14,138 +$23,353	$60,632	To fund the Conservation Breeding Specialist Group work shop on *ex situ* conservation program feasibility/details, provided needed expansion for *ex situ* activities with Poweshiek skipperlings, surveys and husbandry with garita skipperling, and new temporary summer staff. Availability began October 2015.
Association of Zoos and Aquariums Conservation Grant Fund award	22,467	$22,467	To provided needed expansion for *ex situ* activities with Dakota skippers and to conduct a host plant performance study with Dakota skippers. Grant was received October 2015.
TOTAL OTHER FUNDS:	**341,116**	**$302,129**	

VII. PROJECT STRATEGY:

A. Project Partners:

Beyond the Minnesota Zoo and DNR partnership, we are also partnering with the numerous agencies and organizations. None will receive funding from this partnership:

- U.S. Fish and Wildlife Service: Permitting under the US Endangered Species Act; access to federal lands
- U.S. Department of Agriculture: Permitting to allow the movement of live insects between states and internationally
- Provincial government of Manitoba: Permitting under the Species at Risk Act
- Sisseton Wahpeton Oyate: Permitting under tribal endangered species provisions, access to tribal lands
- Michigan DNR: Permitting under the state's endangered species provisions; access to state lands, assistance in collections of individuals for breeding
- Wisconsin DNR: Permitting under the state's endangered species provisions; access to state lands
- The Nature Conservancy: Access to prairie preserves
- The Nature Conservancy of Canada: Access to prairie preserves
- University of Minnesota: Collaborative pesticides-associated mortality research
- University of Michigan-Dearborn & New College of Florida: Collaborative conservation genetics research, assistance in collections of individuals for breeding
- Milwaukee Public Museum: Assistance in collections of individuals for breeding

B. Project Impact and Long-term Strategy:

The Minnesota Zoo's Prairie Butterfly Conservation Program and the Minnesota DNR's survey and monitoring program are complimentary and integrative. Extensive survey efforts in Minnesota for Poweshiek skipperlings and Dakota skippers from 2006 to 2013 have pointed to a steep decline in both, to the point that the Poweshiek skipperling may be extirpated and the Dakota skipper may be close to meeting the same fate. Surveys in other states in these skippers' ranges are yielding similar results. There are troubling indications of declines in other Minnesota-native prairie species as well. This project will assist the DNR in broadening the scope of survey and monitoring efforts for prairie-dependent butterflies. The immediate benefit may be the discovery of surviving colonies of one or both of the two highest priority species. This will support the Minnesota Zoo's conservation breeding program and conservation genetics and pesticides studies. Initiation of the complementary monitoring of individual populations will provide the foundation for a higher-resolution tracking of population trends and for detection of causation.

Both the conservation breeding and wild population monitoring programs are obviously long-term commitments, and this ENTRF project will constitute only the beginning for them. We intend this project to develop monitoring and breeding protocols that will be used long-term. We will be working on strategies for funding the long-term work.

C. Spending History:

Funding Source	FY12	FY13	FY14
Legacy Clean Water Arts and Cultural Heritage Fund grant to MN Zoo to support all operations and staff of the Prairie Butterfly Conservation Program since its inception in February 2012	$ 62,000	$ 103,000	$ 107,000

VIII. ACQUISITION/RESTORATION LIST: N/A

X. VISUAL ELEMENT or MAP(S): See attached graphic of Poweshiek skipperling, Dakota skipper, and Regal Fritillary pictures.

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH ADDENDUM: See attached, for Activities 1-4.

XII. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than November 30, 2014; May 31, 2015; November 30, 2015; May 31, 2016, November 30, 2016, and May 31, 2017. A final report and associated products will be submitted between June 30 and August 15, 2017.

Environment and Natural Resources Trust Fund
M.L. 2014 Project Budget

Project Title: Imperiled Prairie Butterfly Conservation, Research and Breeding Program - Minnesota Zoo portion
Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 05j-1
Project Manager: Dr. Erik Runquist
Organization: Minnesota Zoo
M.L. 2014 ENRTF Appropriation: $ 380,000 to the Minnesota Zoo
Project Length and Completion Date: 3 years, June 30, 2017
Date of Report: August 15, 2017

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 4 Budget	Amount Spent	Activity 4 Balance	Activity 5 Budget	Amount Spent	Activity 5 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	**Zoo Conservation Breeding Program**			**Zoo Conservation Genetics Research**			**Zoo Pesticides Mortality Research**			**Zoo Prairie Butterfly and Pollinator Outreach Guides**				
Personnel (Wages and Benefits) - Overall	$273,500	$269,425	$4,075										$273,500	$4,075
Erik Runquist, Butterfly Conservation Biologist (State Program Administrator Principal @ 100% FTE; 70% salary, 30% benefits for 3 years - $237,000).														
Zoo Project Analyst worker (1 unclassified @ 25% FTE, 70% salary and 30% benefits for 3 years - $36,500) to support rearing, breeding, research and outreach operations														
Professional/Technical/Service Contracts														
TBD (competitive bid): DNA Sequencing				$6,000	$6,000	$0							$6,000	$0
TBD (competitive bid): Pesticides residue testing							$26,000	$26,000	$0				$26,000	$0
Equipment/Tools/Supplies														
Zoo conservation breeding operations: including tables, rearing cages, butterfly nets, and collecting supplies, plants	$2,000	$2,000	$0										$2,000	$0
Zoo conservation genetics research: chemicals, reagents, pipette tips				$2,000	$1,976	$24							$2,000	$24
Zoo pesticides research: chemicals, plants							$1,600	$1,208	$392				$1,600	$392
Capital Expenditures Over $5,000														
Purchase and outfitting of indoor chamber for the Zoo conservation breeding program	$52,000	$52,000	$0										$52,000	$0
Printing														
Publication of prairie and prairie butterfly guides										$8,000	$3,474	$4,526	$8,000	$4,526
Travel expenses in Minnesota														
Zoo: mileage, lodging, meals for travel to and between prairie sites for data collection and breeding operations	$3,800	$3,779	$21										$3,800	$21
Other														
Zoo Travel expenses outside of MN. Mileage, lodging, meals for travel to and between prairie sites to obtain individuals for the Zoo conservation breeding program. All known viable populations of the Minnesota-native endangered butterflies are now outside of Minnesota in Wisconsin, Michigan, North Dakota, South Dakota, and Manitoba, necessitating out of state travel to obtain founder stock.	$5,100	$3,603	$1,497										$5,100	$1,497
COLUMN TOTAL	**$336,400**	**$330,806**	**$5,594**	**$8,000**	**$7,976**	**$24**	**$27,600**	**$27,208**	**$392**	**$8,000**	**$3,474**	**$4,526**	**$380,000**	**$10,536**

Note: Activity 3 is On a separate budget sheet being managed by the DNR - Robert Dana Project Manager

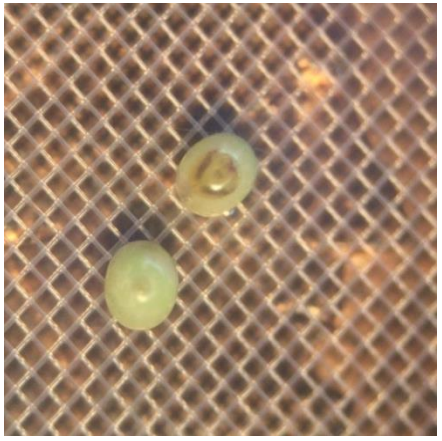
MINNESOTA ZOO

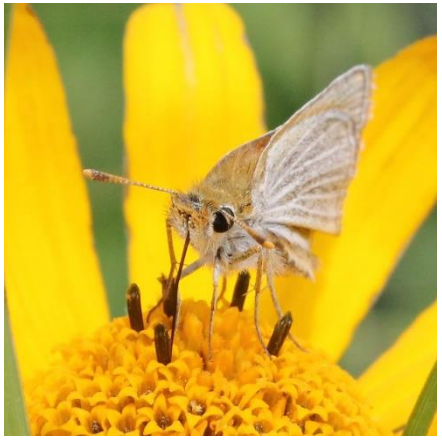
ENVIRONMENT AND NATURAL RESOURCES TRUST FUND

Thanks to ENRTF support, the Minnesota Zoo has established the first and only breeding program for the endangered Dakota skipper. This conservation population has grown from 44 in 2013 to over 375 adults in 2017.

MINNESOTA ZOO

The Minnesota Zoo is reintroducing Dakota skippers back to Minnesota's prairies for the first time. Over 200 butterflies reared at the Minnesota Zoo were released at The Nature Conservancy's Hole-in-the-Mountain Prairie Preserve in 2017. Many were re-sighted and some were observed breeding! This is the first time Dakota skippers are known to be living in southwest Minnesota since at least 2012.





MINNESOTA ZOO

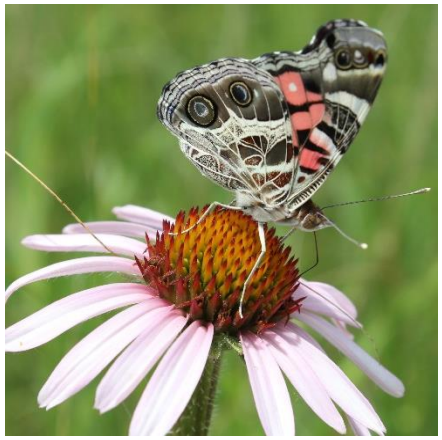
The Poweshiek skipperling was once common across Minnesota's prairies. Due to habitat loss and other threats though, it is now one of the world's most endangered animals. With ENRTF funding, the Minnesota Zoo started a rearing program to help support the last populations of this butterfly in the United States.

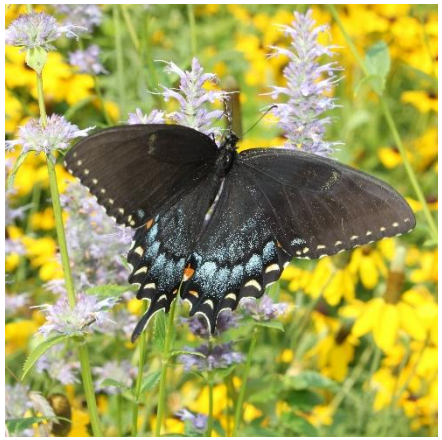
MINNESOTA ZOO

“The Chrysalis” is a new laboratory and greenhouse for the Minnesota Zoo’s Prairie Butterfly Conservation Program. Constructed with ENRTF support, it provides clean, quarantined space for the rearing of Minnesota Endangered butterflies. The ENRTF also funded personnel and supplies.

MINNESOTA ZOO

Many prairie butterfly species are declining, as shown by Minnesota Department of Natural Resources surveys supported by this joint ENRTF grant. Many factors likely contributed. The ENRTF provided the Minnesota Zoo with support for prairie butterfly surveys, prairie habitat studies, and important conservation genetics research for endangered prairie butterflies.





MINNESOTA ZOO

Bees, butterflies and other animals pollinate most wild plants, as well as much of our food. Pollinators are declining though. The Minnesota Zoo's "Plant For Pollinators" campaign provides resources for people to help. The ENRTF funded the publication of "Plant For Pollinators" and "Butterfly Neighbors" pamphlets in English and Spanish that are free to Zoo guests and at other events. More at: mnzoo.org/PlantForPollinators.

MINNESOTA ZOO

Prairie Butterfly Conservation Program Annual Report

2016

Erik Runquist
Cale Nordmeyer

I. Contents

II. Overview

Since 2012, the Minnesota Zoo's Prairie Butterfly Conservation Program has partnered with many agencies to secure a future for imperiled prairie butterflies native to the Upper Midwest. The Prairie Butterfly Conservation Program continues to grow, particularly on efforts to establish the world's first and only breeding populations of the U.S. Threatened and Minnesota Endangered Dakota skipper (*Hesperia dacotae*). This report summarizes our work in 2016 with *ex situ* breeding conservation populations of Dakota skippers, the initiation a new head-starting program for Poweshiek skipperlings, wild pesticides drift and effects studies, and public outreach.

All activities were conducted under permits and agreements issued to the Minnesota Zoo: the U.S. Fish and Wildlife Service (USFWS, TE64079B-1), U.S. Department of Agriculture (APHIS Permit #P526P-15-02728), Minnesota Department of Natural Resources (MNDNR, Special Permit #20805; State Parks #201635; and Scientific and Natural Areas (SNA) #2016-415R), Michigan Department of Natural Resources (T&E permits #2168 and #2185 and Parks permit #PRD-SU-2016-044), The Nature Conservancy (TNC; permits dated April 9, 2016), Springfield Township (Research Permit), Little Missouri State Park (dated June 15, 2016), and North Dakota National Wildlife Refuges (dated June 15, 2016). Other permissions and crucial support were generously provided by the Sisseton Wahpeton Oyate (SWO), South Dakota Game Fish and Parks, Little Missouri National Grasslands, and numerous private landowners. We are working to ensure proper permitting and structures are in place for all future work. We greatly thank all parties and hope to continue and expand these efforts in 2017 to help advance conservation of these imperiled and endangered prairie butterflies.

III. Facilities Expansion

Since the Prairie Butterfly Conservation Program began in 2012, the need for additional space has been increasing. In 2016, three new structures were built to accommodate additional lab space, the growing Dakota skipper footprint and the addition of another imperiled species. Using funds from the Environmental and Natural Resources Trust Fund, a new lab space called the 'Chrysalis' was built by modifying an empty shipping crate. This space is used exclusively for rearing skippers at the Zoo. Attached to the Chrysalis is a three-season glass greenhouse to expand efforts to propagate additional skipper host grasses.

Two new outdoor skipper rearing structures were also installed. An 18' by 26' mesh hoop-house was installed exclusively for rearing Dakota skippers using funds from the Association of Zoos and Aquariums and Disney through an award from the Conservation Grants Fund. Internally, this new structure is being referred to as the Dakota skipper Hoop-house. With the recommendation to move forward with a head-starting program for Poweshiek skipperlings, another identical hoop-house was installed using USFWS Cooperative Agreement funds (Poweshiek skipperling Hoop-house). Construction and outfitting of these hoop houses was completed in June 2016. Each of these new structures was built adjacent to the Zoo's Bison Holding area. This is a secure space with limited staff traffic and is not accessible to Zoo guests.


(Left) The Chrysalis. (Right) The Dakota skipper and Poweshiek skipperling Hoop-houses.

IV. Dakota skipper *ex situ* conservation

A. 2015/2016 Post-diapause larvae and adults

As noted in our 2015 annual report, the Minnesota Zoo's existing *ex situ* population of Dakota skipper larvae were transitioned to hibernation in early winter 2015. Dakota skipper larvae were either wintered in Diapause chambers in a freezer set to -3°C, or outdoors in their same husbandry setups under an insulated cone (see details in 2015 report). The weight and head cap width of all larvae overwintered in the freezer were measured before hibernation. Larvae left outdoors were not censused and survivorship in each setup from the time larvae were placed to the time of diapause is not known. A total of 170 larvae were placed in pots designated for outdoor hibernation. In the spring of 2016, 43 were recovered, having survived winter diapause. Of the pots censused from the freezer, there was a 68% recovery rate. If this same rate applies to those left outside, there would have been approximately 116 larvae in those setups going into diapause for an approximate winter survivorship outdoors of 37%. In contrast, 90.2% (267/296) survived overwintering in the freezer.

Collectively, 310 larvae survived winter diapause. Larvae were taken out of the freezer between 4/30/2016 and 5/8/2016. Of these larvae, 239 reached adulthood. This is a total survivorship of 40% from 2015 neonates (n=604) to adulthood. There was a post diapause survivorship of 77%, which is slightly higher than 2015 (70%) and 2014 (71%). Some of the post-diapause mortality we observed this year may have been avoidable. Specifically, a higher than expected number of young prairie dropseed plants that had been planned for rearing larvae using the tube method in spring did not survive the winter of 2015-2016, and local native plant nurseries did not have sufficient stocks to offset the loss of these grasses. For late instar larvae that had eaten down their first, or even second, prairie dropseed plug before pupating in June, it was decided to offer them prairie dropseed clippings daily from larger more mature plants to preserve the limited supplies of prairie dropseed plugs that were remaining. Though larvae were observed feeding on these clippings, circumstantial evidence suggests that larvae faired more poorly on clippings and their development was delayed. This is likely due to the propensity for clippings to dry out quickly. In the future, clippings will not be utilized to stretch larvae over until pupation if young host plants become limited. Instead, larvae should be transitioned onto another grass species. The host plant performance study (see below) should identify other suitable host alternatives.

Alternatively, larvae can be transitioned onto a larger, more mature plant, acknowledging that that plant cannot be offered to other larvae in the same season to maintain individual quarantine and parentage information.

Of 2016's 239 adult Dakota skippers produced at the Minnesota Zoo, only 85 were female. This deviation from the expected 50:50 sex ratio is likely due to the host plant shortage described above having a greater effect on females. We have previous determined that female Dakota skippers have a longer larval development phase than males (which is the source of the species' protandry). As in 2015, adult males were placed with sibling males in flight cages for mating effrots as much as possible. Mixed parentage male cages were setup in cases where males had fewer than four brothers. Unrelated females were introduced into cages opportunistically as they eclosed. The first individual of a particular sex was not marked. After that, individuals would be given a color mark unique to that particular cage. Individuals that needed to be marked were anesthetized following the protocols described in the 2015 Annual Report. Dakota skippers from South Dakota and Minnesota were never mixed in cages. As in 2015, Minnesota Zoo volunteers assisted with breeding observations. Volunteers would watch breeding cages in two hour blocks from 9:00 till 15:00 and record which skippers mated (if at all), the time copulation started and the end time. Observed start times took place any time between 10:05 and 15:00, with the average start time at 12:33. Of the observed matings where both the start time and end time were witnessed, the average mating duration was 33 min (range: 12 - 94 minutes).

There were 27 confirmed matings, 19 of which were observed. The unobserved matings were determined after females of unknown mating status had been transferred from breeding cages to oviposition chambers when they began laying eggs, and then produced viable eggs. In each of these cases, the paternal linage is not known, but is assumed to be any of the occupied males from the respective mating cage. When all breeding trials were completed, there were also viable eggs found in 12 different cages. In each of these cases, neither the maternal lineage, or the paternal linage is exactly known. For pedigree and management purposes, these individuals are assumed to be potentially descended from any of the males and any of the females that occupied that respective cage.

In one instance this year, a female mated twice. ♀ SF24B first mated with ♂EBD8-6F1.3 at 10:05 in Cage 4. Before she was removed, she was observed mating with ♂EES4-4A at 13:31. This is the first time a female Dakota skipper has been observed to have mated twice during the Prairie Butterfly Conservation Program's history.

6/25/2016, SF24B♀ and EES4-4A♂ mating in Cage 7.

B. Wild Dakota skipper surveys

In continued effort to expand the Minnesota Zoo's *ex situ* rearing and breeding population of Dakota skippers, additional egg collection efforts were carried out in 2016. Efforts were similar to 2014 and 2015, focusing on the cluster of populations on Sisseton Wahpeton Oyate lands in northeastern South Dakota and the Felton Prairie complex in west-central Minnesota. Survey efforts were initiated by the Zoo's Dr. Erik Runquist following consultation with Dennis Skadsen in South Dakota. In addition to recording information on Dakota skipper abundance, the abundance of all other observed butterfly species was also recorded, as below.

1. South Dakota, Sisseton Wahpeton Oyate, Day and Roberts Counties.

Scarlet Fawn

June 23, 2016 14:56- 18:18. Sunny, 79°F, light winds

Hesperiidae:	
Common checkered skipper, *Pyrgus communis*	1
Dakota skipper, *Hesperia dacotae*	44 (22 males, 22 females)
Peck's skipper, *Polites peckius*	2
Tawny-edged skipper, *Polites themistocles*	3
Long Dash, *Polites mystic*	10
Pieridae:	
Orange sulphur, *Colias eurytheme*	80
Clouded sulphur, *Colias philodice*	5
Nymphalidae:	
Meadow fritillary, *Boloria bellona*	3
Silver-bordered fritillary, *Boloria selene*	2
Variegated fritillary, *Euptoieta claudia*	13
Red admiral, *Vanessa atalanta*	4
American Lady, *Vanessa virginiensis*	5
Monarch, *Danaus plexippus*	1
Large wood nymph, *Cercyonis pegala*	2
Eyed brown, *Satyrodes eurydice*	3

Conditions were ideal, and the entire property was surveyed twice in a large loop. In accordance with the USFWS Recovery Permit, eight female Dakota skippers were collected for egg collections after the first 25 individuals had been observed.

Hayes Prairie

June 24, 2016 10:28-11:20. Sunny, 75-77°F, SE wind 20-30mph

Hesperiidae:	
Dakota skipper, *Hesperia dacotae*	3 (1 male, 2 females)
Pieridae:	
Orange sulphur, *Colias eurytheme*	14
Lycaenidae:	
Copper sp, *Lycaena* sp.	1
Nymphalidae:	
Variegated fritillary, *Euptoeita claudia*	1

Lady sp., *Vanessa* sp. 1

Conditions were suboptimal due to a strong wind. More Dakota skippers may have been present, but it is unlikely that enough could have been found to initiate egg collection procedures under even ideal conditions. This survey was conducted in partnership with Dennis Skadsen.

East Enemy Swim

June 24, 2016 11:34-13:45. Sunny, 80-85°F, windy

Hesperiidae:

Dakota skipper, *Hesperia dacotae*	53 (20 males, 33 females)
Tawny-edged skipper, *Polites themistocles*	4
Long Dash, *Polites mystic*	1

Pieridae:

Orange sulphur, *Colias eurytheme*	22

Lycaenidae:

Silvery blue, *Glaucopsyche lygdamus*	3

Nymphalidae:

Variegated fritillary, *Euptoieta claudia*	4
Large wood nymph, *Cercyonis pegala*	2

Despite difficult windy conditions, large numbers of Dakota skippers were observed during several large looping routes around the site. In accordance with the USFWS Recovery Permit, ten female Dakota skippers were collected for egg collections after the first 25 individuals had been observed. This survey was conducted in partnership with Dennis Skadsen.

North Owl

June 24, 2016 14:35-15:05. Sunny, 90°F, windy

Hesperiidae:

Long Dash, *Polites mystic*	4

Papilionidae:

Black swallowtail, *Papilio polyxenes*	1

Pieridae:

Orange sulphur, *Colias eurytheme*	4

Nymphalidae:

Meadow fritillary, *Boloria bellona*	1
Variegated fritillary, *Euptoeita claudia*	1
American painted lady, *Vanessa virginiensis*	5
Inornate ringlet, *Coenonympha tullia*	1

Conditions were suboptimal due to a strong wind, but it is unlikely that Dakota skipper were present due to limited purple coneflowers. This survey was conducted in partnership with Dennis Skadsen.

Scarlet Fawn

6/25/2016 12:15-3:15. Mostly cloudy, 85°F, SW 15-30 wind

The primary purpose of this trip was to attempt to collect up to two additional Dakota skipper females for egg collection. Conditions were suboptimal, but one more female was collected. A formal survey of all butterflies was not conducted, but one new species was observed: two Regal fritillaries, *Speyeria idalia* (Nymphalidae).

Scarlet Fawn

6/26/2016. 08:15-08:45. W wind 20-30. Partly sunny.

All nine female Dakota skippers that had been temporarily held for egg collection were released in the cool morning hours in accordance with permitted protocols. Without touching them directly, the females were gently transferred directly from their 50 mL tubes onto purple coneflowers. Six of the nine remained on the flowers to nectar for at least 10 minutes, and while the remaining three "flew to or perched on low herbaceous plants or grass" within 10 meters. No formal survey of other butterflies was conducted.

East Enemy Swim

6/26/2016. 09:00-09:20. W wind 20-30. Partly sunny.

All ten female Dakota skippers that had been temporarily held for egg collection were released in the cool morning hours in accordance with permitted protocols. Without touching them directly, the females were gently transferred directly from their 50 mL tubes onto purple coneflowers. Seven of the ten remained on the flowers to nectar for at least 10 minutes, and while the remaining three "flew to or perched on low herbaceous plants or grass" within 10 meters. No formal survey of other butterflies was conducted.

2. Minnesota

Felton Prairie

June 27, 2016 14:40-16:45 Partly cloudy then sunny. 67°F. Light NW Wind

Felton Prairie – Bicentennial Unit
14:40-15:55, then 16:20-16:45

Hesperiidae:
- Dakota skipper, *Hesperia dacotae* 11 (4 males, 7 females)

Pieridae:
- Orange sulphur, *Colias eurytheme* 27
- Clouded sulphur, *Colias philodice* 1

Nymphalidae:
- Regal Fritillary, *Speyeria idalia* 1
- Variegated fritillary, *Euptoeita claudia* 1
- Large wood nymph, *Cercyonis pegala* 5

Felton Prairie – Blazing Star
3:55 - 4:20

Hesperiidae:
- Tawny-edged skipper, *Polites themistocles* 1
- Long Dash, *Polites mystic* 1

Pieridae:

Orange sulphur, *Colias eurytheme*	6
Clouded sulpur, *Colias philodice*	1
Nymphalidae:	
Regal fritillary, *Speyeria idalia*	1
Meadow fritillary, *Boloria bellona*	1

The Dakota skippers were difficult to find, and largely only clustered in the northwestern portion of the Bicentennial Unit. Abundance in 2016 appeared to be lower than in prior years, perhaps due to dry conditions. Not enough Dakota skippers could be located to initiate egg collect procedures in accordance with permitted protocols. This survey was conducted in partnership with Robert Dana.

Felton Prairie

6/28/2016 10:22-14:15 Sunny 75-80^{o}F. Almost no wind

Bicentennial Unit:

Hesperiidae:	
Dakota skipper, *Hesperia dacotae*	14
Tawny-edged skipper, *Polites themistocles*	1
Long Dash, *Polites mystic*	1
Papilionidae:	
Black swallowtail. *Papilio polyxenes*	2
Pieridae:	
Orange sulphur, *Colias eurytheme*	33
Clouded sulphur, *Colias philodice*	8
Nymphalidae:	
Great spangled fritillary, *Speyeria cybele*	1
Regal fritillary, *Speyeria idalia*	1
Northern crescent, *Phyciodes tharos*	2
Red admiral, *Vanessa atalanta*	1
Large wood nymph, *Cercyonis pegala*	10

Despite ideal conditions, Dakota skippers remained difficult to find. A briefly survey along the upland ridge in the Blazing Star Unit yielded only one Dakota skipper. Again, not enough Dakota skippers could be located to initiate egg collect procedures in accordance with permitted protocols. This survey was conducted in partnership with Robert Dana.

Felton Prairie

June 29, 2016 10:30-11:15 Sunny 75^{o}F. Light winds.

Bicentennial:

Hesperiidae:	
Dakota skipper, *Hesperia dacotae*	5 (1 male, 4 females)
Papilionidae:	
Black swallowtail, *Papilio polyxenes*	1
Pieridae:	
Orange sulphur, *Colias eurytheme*	22
Clouded sulphur, *Colias philodice*	3

Nymphalidae:
Large wood nymph, *Cercyonis pegala* 7

This represented one final effort to assess the Dakota skipper flight. Encounter rates declined further from previous days, and the flight appeared to be in decline. This survey was conducted in partnership with Phil Delphey (USFWS) and Seth Stapleton (MN Zoo).

Bluestem Prairie SNA. N 1/2 SE 1/4 Sec 15 T139N R46W
June 29, 2016 12:30-13:15. Sunny 75°F. Light winds.

Hesperiidae:
Long Dash, *Polites mystic* 1
Papilionidae:
Black swallowtail, *Papilio polyxenes* 1
Pieridae:
Orange sulphur, *Colias eurytheme* 8
Nymphalidae:
Great spangled fritillary, *Speyeria cybele* 1
Meadow fritillary, *Boloria bellona* 1
Northern crescent, *Phyciodes tharos* 1
Large wood nymph, *Cercyonis pegala* 12

Efforts were focused on the portion where a female Dakota skipper had been photographed in 2015. The area surveyed appeared to be better Poweshiek skipperling habitat than the slightly more upland habitats utilized by Dakota skipper. This survey was conducted in partnership with Phil Delphey (USFWS) and Seth Stapleton (MN Zoo).

C. 2016/2017 Dakota skipper larvae

Wild Dakota skipper egg collection less successful in 2016 versus 2015. We collected 313 eggs from 19 wild females from two sites (Scarlet Fawn and East Enemy Swim) in northeastern South Dakota, but were unable to find enough individuals at the last known viable population in Minnesota at Felton Prairie for any egg collection under permitted protocols. This is down from a total 386 eggs from 20 females in 2015. All wild females were returned alive back to the sites from which they were collected within 72 hours in accordance with permitted protocols. One female, SF40, lost a leg in the oviposition cup, but was otherwise unharmed.

Table 1 – Number of eggs laid in 2016 by date for wild Dakota skippers from Scarlet Fawn prairie (SF) and East Enemy Swim (EES), Sisseton Wahpeton Oyate, Day County, South Dakota.

Female	*24 June*	*25 June*	*26 June*	*Total*
SF33	6	25		31
SF34	0	0		0
SF35	3	17		20
SF36	0	1		1

SF37	0	18		18
SF38	9	0		9
SF39	3	21		24
SF40	0	0		0
SF41			0	0
EES10		0	25	25
EES11		6	23	29
EES12		17	6	23
EES13		6	19	25
EES14		9	13	22
EES15		8	12	20
EES16		14	13	27
EES17		2	13	15
EES18		4	16	20
EES19		0	4	4

In addition to fewer wild eggs being collected in 2016, hatch rates were noticeably lower for the eggs collected from wild South Dakota females (181/313 = 57.8%) than in previous years (82-93%), for unknown reasons. Many of the wild individuals observed, especially the Scarlet Fawn females temporarily held under comparable conditions for egg collections, appeared weaker and more worn than in prior years despite efforts to again collect eggs near the peak of the flight, perhaps due to drought conditions reducing nectar availability.

At the Zoo, 693 of the Zoo-produced eggs hatched. The combined 874 neonate larvae were reared in sealed tubes for a few weeks until they were large enough to be reared outdoors in Singleton and Free-range potted plant setups. 189 of the best genetically represented larvae were transferred into a Host Plant Performance Study to help optimize husbandry operations and potentially inform habitat management for the conservation of wild Dakota skipper populations (See section D). As in 2015, Dakota skipper larvae were reared in either Singleton setups (one larva on a 4" potted plant), or in Free-range setups (3-7 larvae on a mature 1-gallon potted plant).

Table 2 – Total survivorship and measurements of the 2016-2017 generation of Dakota skippers at the time of winter diapause. 78 potential larvae were not censused to maintain.

Treatment	*Total*	*# Larvae surviving to diapause*	*% survivorship*	*Avg head cap width (mm)*	*Avg weight (g)*
Singleton	149	106	71%	1.97	0.0322
Free-range	310	243	78%	1.94	0.0297
Total	459	349	76%	1.95	0.0309
Not censused	78				

Dakota skipper Hoop-house. (Left) Exterior. (Right) Interior. This is a new hoop-house built in 2016. All Dakota skipper larvae were reared in this space after they were transitioned out of the Chrysalis (nearby lab space).

The 2016 fall season was particularly warm and extended compared to most years. Most host grasses had fully senesced by the end of September, but ambient temperatures most days were well above the threshold for Dakota skipper larvae to be active until mid-November. It is unknown what effect a longer than average autumn would have on hibernating larvae and if freezing temperatures are required by a particular time once edible food is no longer available. To explore this, and hedge our bets in case longer warm period exposure has a negative impact, we decided to stagger the time Dakota skipper larvae were transitioned in to the freezer. In previous years, we have brought all indoor over-wintering larvae within the same few days after overnight low temperatures consistently fell below freezing. Between 11/6/2016 and 11/27/2016, 100+ larvae were censused in "Waves" every 4 weeks. Each individual was only censused once, with head cap width and weight recorded. Ultimately, over the 21 day period, no significant difference was observed between census times. Had there been a negative impact on larvae, we would have expected increased mortality over time, or a decrease in weight. Neither outcome was observed

Table 3 – Results from the Dakota skipper staggered census. Wave 1 was censused on 11/6/2016, Wave 2 on 11/13/2016, Wave 3 on 11/20/2016 and Wave 4 on 11/27/2016. These results exclude a single outlier who had pupated and was 10x the weight of the next largest specimen.

Treatment	*Total censused*	*# Larvae surviving to diapause*	*% Survivorship*	*Avg head cap width (mm)*	*Avg weight (g)*
Wave 1	106	88	83%	1.95	0.0304
Wave 2	102	85	83%	1.97	0.0308
Wave 3	132	93	70%	1.93	0.0291
Wave 4	107	89	83%	1.94	0.0310

D. Hostplant performance study

Very little is known about the host-plant interactions of generalist, grass feeding insects, and this is especially true for the poorly studied Dakota skipper. Using funds received through the Association of Zoos and Aquariums' Conservation Grant Fund, we developed a study separate from our other Dakota skipper husbandry activities to 1) improve both *ex situ* husbandry protocols for the Dakota skipper and 2) inform *in situ* conservation by helping to identify adequate reintroduction sites and land management strategies for this highly imperiled prairie butterfly. To address these goals, we launched the host plant performance study in July 2016 using neonate Dakota skipper larvae. All larvae were from Zoo-bred stock. The study consisted of a no-choice experiment in which larvae were placed individually on one of seven prairie grass species (n = 27 per treatment; 189 total larvae). The grasses used were the natives: big bluestem (*Andropogon gerardii*), sideoats grama (*Bouteloua curtipendula*), little bluestem (*Schizachyrium scoparium*), prairie dropseed (*Sporobolus heterolepis*), and porcupine grass (*Stipa spartea*), as well as the invasive species smooth brome (*Bromus inermis*) and Kentucky bluegrass (*Poa pratensis*). All individuals were placed in singleton setups with their respective host plant treatment within a few hours of hatching. To standardize growing conditions and plant age, we elected to rear all larvae on grasses germinated from seed at the Minnesota Zoo in February 2016. This protocol ensured that all grasses in the study were the same age and exposed to the same light durations, temperatures, soil mix, and fertilizer regimes. Seeds were purchased from local nurseries.

Preliminary findings from this experiment indicate that all offered host plant species triggered larval feeding responses (**Error! Reference source not found.**4), such that none of the grass species can be excluded as potential hosts. However, survivorship and larval size varied among treatments. We exclusively offered prairie dropseed to larvae reared outside this experiment (see above). Neonate larvae in our study prairie dropseed treatment had a survivorship of 70.0%, which is consistent with the survivorship of larvae raised on prairie dropseed (75.3%; 517/686) outside this experiment. Consistent with our previous experiences, most Dakota skipper larval mortality was observed within the first month.

In December, all host plant study larvae were removed from their shelters to censused and transferred into Diapause chambers and stored in a -3°C freezer in the Chrysalis (Table 4). To date, there has been a total survivorship of 53% (79/182) within the hostplant study. Prairie dropseed and little bluestem have the highest survivorships at hibernation. Interestingly, the survivorship of the prairie dropseed treatment is lower than the general population of captive Dakota skippers; which have been reared on prairie dropseed. It is not clear at this time why there is a discrepancy between these Hostplant Study specimens on dropseed and the general population of Dakota skippers as a whole. Porcupine grass larvae were largest at hibernation, potentially because this grass species retained green leaves longer into autumn than the other species and larvae could continue to feed during extended autumn warmth.

Morphometric data is also being collected about the shelters Dakota skippers construct using each treatment species. Like most hesperiids, the Dakota skipper larvae stitch together grass blades to form a protective encasement within its host grass. Given the different morphologies of each host grass, we hypothesized that Dakota skipper larvae would yield variable shelter building strategies and that differences in the resulting shelters could impact survivorship of the caterpillar. At the time larvae were

censused in December, each shelter was measured for its total exterior length at the tallest point there had been silk stitching, total exterior width at the widest point, the interior tube length, the interior tube width, how much the interior structure is submerged underground, and how many independent structures were made. Analysis of this data is ongoing. We will repeat these steps post-diapause after each specimen has pupated.

An issue encountered was a fungal rust on many of the Kentucky bluegrass plants. It is not known if the rust directly affected Dakota skipper larvae, but may have been a contributing factor to Kentucky bluegrass having the lowest neonate to winter diapause survivorship of all grass species tested. It is suspected that the rust was an artifact of the mesh screens of the singleton husbandry setups reducing airflow across the plant and promoting damp conditions. Though this makes it difficult to extrapolate what larva might be experiencing if it was feeding on Kentucky bluegrass in the wild, it does inform husbandry practices. Kentucky bluegrass may not be an operationally viable host *ex situ*.

(Left) Dakota skipper designation: PDS11 on prairie dropseed. This is an example of a typical shelter. Here, larvae have been exposed by peeling back two leaf blades. (Right) Dakota skipper designation: SMBR9 on smooth brome. Smooth brome shelters tended to encompass many more leaf blades, were wider, and had less symmetry than other treatment species.

Table 4 - Survivorship and development of Dakota skipper larvae by host plant treatment when larvae were transferred into their Diapause chambers for the winter.

Treatment	*Survivorship from neonate to August*	*Total % Survivorship at diapause*	*Ave Head cap width at diapause mm ± SD*	*Ave Weight at diapause g ± SD*
Prairie dropseed	70% (n=19)	73% (n=19)	1.91 ± 0.11	0.0265 ± 0.0055
Little bluestem	78% (n=21)	67% (n=18)	1.89 ± 0.08	0.0298 ± 0.0087
Porcupine grass	59% (n=16)	54% (n=14)	1.99 ± 0.16	0.0352 ± 0.0093
Smooth brome	55% (n=15)	52% (n=14)	1.85 ± 0.14	0.0292 ± 0.0201
Side-oats gramma	44% (n=12)	48% (n=12)	1.85 ± 0.11	0.0263 ± 0.0043
Big bluestem	48% (n=13)	42% (n=11)	1.80 ± 0.10	0.0121 ± 0.0035
Kentucky bluegrass	48% (n=13)	33% (n=9)	1.80 ± 0.09	0.0275 ± 0.0068
Total	57% (n=109)	53% (n=97)	1.88 ± 0.13	0.0282 ± 0.0102

V. Poweshiek skipperling *ex situ* conservation

Following the recommendations of the CBSG *ex situ* planning workshop, the Minnesota Zoo launched the world's first and only formal conservation program for the endangered Poweshiek skipperling in 2016. The program is designed to augment populations through headstarting of larvae at the Minnesota Zoo and the subsequent release of late instar larvae or pupae back at their natal sites. The formal plan for this ex situ program is detailed in the "Plan for the Controlled Propagation, Augmentation, and Reintroduction of Poweshiek Skipperling (*Oarisma poweshiek*) by Tamara Smith *et al*., June 21, 2016. That plan is available upon request.

A. Poweshiek surveys in Michigan

All Poweshiek skipperling egg collection was done by Minnesota Zoo Conservation Specialist Cale Nordmeyer. Surveys were conducted at Long Lake Fen and Brandt Rd Fen, both in Oakland County, MI. As per recovery permit TE64079B-1, up to ten females could be collected from each site, not exceeding 25% of the observed number of adults for that day (section J.2). The Long Lake Fen site is divided by what are likely two metapopulations by Long Lake itself; a western population (described as Eagle Rd.) and an eastern population (described as Eaton Rd.). During the 2015 CBSG *ex situ* planning workshop, it was discussed that Poweshieks would only be collected from the Eagle Rd. site and future releases of subsequent offspring would only be returned to the Eagle Rd. site so that the Eaton Rd. site could serve as an informative control for these activities.

The first observed Poweshiek skipperling in Michigan was reported on 6/27/2016 by Michigan Natural Features Inventory staff Clint Pogue and Mike Belitz. At the time of this first sighting, it was not known what stage the flight was in. Like many other prairie grass skippers, Poweshiek skipperling males typically eclose before females. Mr. Nordmeyer travelled to Michigan and began field surveys for gravid females on 6/29/2016. Survey durations and other butterfly species observed are listed below.

Long Lake Fen, Eaton Rd.
6/29/2016. 11:15 to 12:55pm.

Poweshiek skipperlings observed: **5**
Number of females collected: **0**

Other species observed:

Papilionidae:	
Black swallowtail, *Papilio polyxenes*	1
Pieridae:	
Cabbage white, *Pieris rapae*	2
Nymphalidae:	
Great spangled fritillary, *Speyeria cybele*	2
Baltimore checkerspot, *Euphydryas phaeton*	1
Crescent sp., *Phyciodes sp.*	2
Monarch, *Danaus plexippus*	1
Little wood-satyr, *Megisto cymela*	2

Brandt Rd. Fen

6/29/2016. 11:39 to 12:13.

Poweshiek skipperlings observed: 4
Number of females collected: 0

Other species observed:

Pieridae:	
Orange sulphur, *Colias eurytheme*	4
Cabbage white, *Pieris rapae*	2
Lycaenidae:	
Dorcas copper, *Lycaena dorcas*	12
Nymphalidae:	
Great spangled fritillary, *Speyeria cybele*	1
Crescent sp., *Phyciodes sp.*	1
Red-spotted purple, *Limenitis arthemis*	1
Eyed brown, *Satyrodes eurydice*	3

(Right) Mike Belitz and Clint Pogue at Long Lake Fen off Eaton Rd on 6/29/2016. (Left) Baltimore checkerspot at Long Lake Fen off Eaton Rd on 6/29/2016. Oakland Co., MI.

Long Lake Fen, Eagle Rd.

6/30/2016, 11:00 to 12:57

Poweshiek skipperlings observed: 17
Number of females collected: 2 (POSKA, POSKB)

Other species observed:

Hesperiidae:	
Delaware skipper, *Anatryone logan*	2
Unidentified skipper, likely *Polites mystic*	2
Papilionidae:	
Black swallowtail, *Papilio polyxenes*	3
Pieridae:	
Cabbage white*, Pieris rapae*	3
Orange sulphur, *Colias eurytheme*	3
Lycaenidae:	
Dorcas copper, *Lycaena dorcas*	9

Nymphalidae:

Great spangled fritillary*, Speyeria cybele*	3
Baltimore checkerspot, *Euphydryas phaet*on	1
Crescent sp., *Phyciodes sp.*	34
Red-spotted purple, *Limenitis arthemi*s	1
Monarch, *Danaus plexippus*	1
Little wood-satyr, *Megisto cymela*	1
Northern pearly-eye, *Enodia anthedon*	1

Long Lake Fen, Eagle Rd.
7/1/2016. 1:10 to 3:11pm.

Poweshiek skipperlings observed: 6
Number of females collected: 0

Other species observed:
Hesperiidae:

Silver-spotted skipper, *Epargyreus clarus*	1
Mulberry skipper, *Poanes massasoit*	1

Pieridae:

Orange sulphur, *Colias eurytheme*	1
Cabbage white, *Pieris rapae*	1

Lycaenidae:

Dorcas copper, *Lycaena dorcas*	9

Nymphalidae:

Great-spangled fritillary, *Speyeria cybele*	1
Meadow fritillary, *Boloria bellona*	3
Baltimore checkerspot, *Euphydryas phaeton*	2
Crescent sp., *Phyciodes sp.*	11
Monarch, *Danaus plexippus*	1


(Left) Mulberry skipper at Long Lake Fen. This was the only Mulberry skipper observed during this collecting trip, a startling contrast from the Zoo's previous visit in 2012. (Right) A male poweshiek skipperling at Long Lake Fen.

Brandt Rd. Fen
7/1/2016. 4:19 to 5:32pm.

Poweshiek skipperlings observed: 5
Number of females collected: 0

Other species observed:

Hesperiidae:	
Long Dash, *Polites mystic*	1
Papilionidae:	
Black swallowtail*, Papilio polyxenes*	1
Lycaenidae:	
Dorcas copper, *Lycaena Dorcas*	31
Nymphalidae:	
Great spangled fritillary, *Speyeria cybele*	1
Baltimore checkerspot, *Euphydryas phaeton*	2
Crescent sp., *Phyciodes sp.*	5
Northern pearly-eye, *Enodia anthedon*	3
Little wood-satyr, *Megisto cymela*	1
Eyed brown, *Satyrodes eurydice*	1

Brandt Rd. Fen
7/2/2016. 9:20 to 12:32pm.

Poweshiek skipperlings observed: 5
Number of females collected: 0

Other species observed:

Hesperiidae:	
Delaware skipper, *Anatryone logan*	1
European skipper, *Thymelicus lineola*	1
Long dash, *Polites mystic*	4
Papilionidae:	
Black swallowtail, *Papilio polyxenes*	1
Lycaenidae:	
Dorcas copper, *Lycaena dorcas*	68
Pieridae:	
Clouded sulphur, *Colias philodice*	2
Nymphalidae:	
Great spangled fritillary, *Speyeria cybele*	10
Baltimore checkerspot, *Euphydryas phaeton*	6
Crescent sp, *Phyciodes sp.*	3
Eyed brown, *Satyrodes eurydice*	4
Common wood nymph, *Cercyonis pegala*	2
Northern pearly-eye, *Enodia anthedon*	3

Long Lake Fen, Eagle Rd.
7/2/2016. 3:04 to 5:18 pm

Poweshiek skipperlings observed: 16
Number of females collected: 1 (POSKC)

Other species observed:

Pieridae:	
Sulphur sp., *Colias sp.*	1
Nymphalidae:	
Great spangled fritillary, *Speyeria cybele*	3
Crescent sp., *Phyciodes* sp.	40

Brandt Rd. Fen
7/3/2016. 12:03 to 1:43 pm

Poweshiek skipperlings observed: 8
Number of females collected: 0

Other species observed:

Hesperiidae:	
Delaware skipper, *Anatryone logan*	1
Long Dash, *Polites mystic*	1
Papilionidae:	
Black swallowtail, *Papilio polyxenes*	1
Pieridae:	
Clouded sulphur, *Colias philodice*	1
Cabbage white, *Pieris rapae*	1
Lycaenidae:	
Dorcas copper, *Lycaena dorcas*	44+
Eastern tailed blue, *Everes comyntas*	1
Nymphalidae:	
Great spangled fritillary, *Speyeria cybele*	5
Meadow fritillary, *Boloria bellona*	1
Baltimore checkerspot, *Euphydryas phaeton*	6
Crescent sp., *Phyciodes* sp.	3
Monarch, *Danaus plexippus*	1
Eyed brown, *Satyrodes eurydice*	1
Common wood nymph, *Cercyonis pegala*	3
Northern pearly-eye, *Enodia anthedon*	1

Long Lake Fen, Eagle Rd.
7/3/2016. 1:47 to 3:44pm

Poweshiek skipperlings observed: 16
Number of females collected: 3 (POSKD, POSKE, POSKF)

Other species observed:

Hesperiidae:	
Delaware skipper, *Anatryone logan*	1
Long dash, *Polites mystic*	5
Lycaenidae:	
Dorcas copper, *Lycaena dorcas*	11
Nymphalidae:	
Great spangled fritillary, *Speyeria cybele*	1
Meadow fritillary, *Boloria bellona*	1
Baltimore checkerspot, *Euphydryas phaeton*	1
Crescent sp., *Phyciodes sp.*	8
Red admiral, *Vanessa atalanta*	1
Eyed brown, *Satyrodes eurydice*	1

Brandt Rd. Fen
7/3/2016. 6:25 to 7:30pm

Poweshiek skipperlings observed: 2
Number of females collected: 0

Other species observed:

Lycaenidae:	
Dorcas copper, *Lycaena dorcas*	17
Nymphalidae:	
Crescent sp., *Phyciodes sp.*	1
Common wood nymph, *Cercyonis pegala*	1

Brandt Rd. Fen
7/4/2016. 10:18 am to 2:12pm.

Poweshiek skipperlings observed: 11
Number of females collected: 2 (POSKG, POSKH)

Other species observed:

Hesperiidae:	
Long dash, *Polites mystic*	1
Papilionidae:	
Black swallowtail, *Papilio polyxenes*	1

Pieridae:

Cabbage white, *Pieris rapae*	2

Lycaenidae:

Dorcas copper, *Lycaena dorcas*	50+

Nymphalidae:

Great spangled fritillary, *Speyeria cybele*	3
Baltimore checkerspot, *Euphydryas phaeton*	4
Monarch, *Danaus plexippus*	1
Common wood nymph, *Cercyonis pegala*	3
Little wood-satyr, *Megisto cymela*	1
Eyed brown, *Satyrodes eurydice*	1

(Left) Long Lake Fen off Eagle Rd. Area shown colloquially referred to as the 'Poweshiek City.'. (Right) Three dorcas coppers on cinquefoil. This was consistently the dominant butterfly species at Brandt Rd. Fen during the survey period. Oakland Co., MI.

(Left) European skipper at in the interior of Brandt Rd Fen. It was not uncommon to see European skipper on the road side before entering the site, but this was the first and only specimen observed in the site. (Right) A freshly eclosed common wood-nymph at Brandt Rd. Fen.


(Left) Male Poweshiek skipperling at Long Lake Fen. (Right) Collected female Poweshiek skipperling, POSKC.


(Left) Male Poweshiek skipperling at Brandt Rd. Fen. Photo taken at about 18:00. (Right) Brandt Rd. Fen. Poweshiek skipperlings were regularly seen flying later in the day than many other species. Oakland Co., MI.

B. Poweshiek egg collection

Female Poweshiek skipperlings were collected in the field by netting. Individuals were either directly transferred to a 50mL vial, or moved with a wetted Q-tip. No individuals were manipulated directly by hand. The vials were packed with a single moistened cotton makeup remover pad as a substrate and to maintain high humidity while still in the field and before setting them up back at the hotel. Vials with collected females were stored in a small cooler with chilled (not frozen) gel packs. The target temperature was between 15 and 18°C. When/if the temperature in the cooler reached 19°C, the survey was ended and all collected females would be transported to the hotel and set up in oviposition chambers.

Oviposition chambers consisted of two nested 9oz paper cups. The base of the cups each had a 2cm thick disk of Hydrostone pre-saturated with water. A small hole in the base supported grass and nectar plant cuttings. Flower cuttings (*Rudbeckia*) were collected at the site of origin and inspected for predators before placing in the oviposition chamber. An artificial nectar mix (water, organic honey, organic sugar and Brigg's amino acid spray) was also provided by soaking Q-tips in the solution and poking the Q-tips through holes in the sides of the oviposition chambers. Q-tips with nectar solution were changed daily in the mornings. All oviposition chambers were misted at least three times daily.

All collected females were held for no more than 72 hours before being returned to the same coordinates she was collected from (section J.5). Females were always returned around 06:30 (give or take 30 minutes). Females were transferred from their vial using a wetted Q-tip and always placed on blooming *Rudbeckia*.

There were no mortalities of any collected Poweshiek skipperlings. No Poweshiek skipperlings lost any legs, palps, or other body parts. The only damage that was sustained by any individuals was marginal scale loss, but nothing that would hinder flight, etc.

Eight Poweshiek skipperling (POSK) females were collected between 6/30/2016 and 7/4/2016 (see Table 1). Daily high counts were typically too low to collect more from each respective site on each given day. Females were designated with a letter (A-H) in the order they were collected. Females POSKA-POSKF were all collected within the 'Poweshiek City' at Long Lake Fen. POSKG and POSKH were both collected at Brandt Rd. Unfortunately, POSKA, POSKF, POSKG and POSKH did not produce any eggs while in captivity. It is possible that these individuals were unmated. There are no known means to definitively determine *a priori* the mating status of females. Our collection methodology assumes that females have mated before being caught. All the females that did lay eggs however, produced viable eggs. Additionally, all females that did lay eggs had done so by the end of their first 24 hours in the oviposition chambers. It is hypothesized that unmated females are less inclined to lay eggs since both POSKA and POSKF were held for the full 3-day window and never produced any eggs. Given this observation, POSKG and POSKH (the last two collected females) were released 24-hours earlier than the permit allowed. It is better to err on the side of getting these potentially un-mated females back in to wild than risk having them in captivity longer. In the future, we may want to consider a protocol where females that have not laid any eggs in their first 24 hours are always released the following morning.

Left: Female Poweshiek skipperling (POSKA) collected at Long Lake Fen off Eagle Rd. in a 50mL vial.
Right: Digital thermometer used to monitor temperature of the cooler containing any collected skippers.

Left: Oviposition chambers collected females are kept in. Right: Oviposition chambers at the hotel near a west-facing window under T5 full spectrum lighting.

Table 1 – Collected female Poweshiek skipperlings from Oakland Co., MI. 2016.

Individual	*Date collected*	*Time*	*Location*	*Coordinates*	*Eggs collected*
POSK A	6/30/2016	12:55	Long Lake Fen	42.7584687, -83.561794	0
POSK B	6/30/2016	14:36	Long Lake Fen	42.7583051, -83.5615818	25
POSK C	7/2/2016	18:06	Long Lake Fen	42.7582968, -83.5618144	28
POSK D	7/3/2016	14:58	Long Lake Fen	42.7582965, -83.5617498	10
POSKE	7/3/2016	17:02	Long Lake Fen	42.7583788, -83.5618576	33
POSKF	7/3/2016	17:07	Long Lake Fen	42.7582712, -83.5619744	0
POSKG	7/4/2016	12:34	Brandt Rd. Fen	42.8534143, -83.4682593	0
POSKH	7/4/2016	13:59	Brandt Rd. Fen	42.853042, -83.4682505	0

Table 2 – Post release female behavior

Individual	*Release date*	*Post release behavior*
POSKA	7/3/2016	Transferred on to a *Rudbeckia*. Flew quickly flew off and crawled down into the duff. Lost visual.
POSKB	7/3/2016	Transferred on to a *Rudbeckia*. Remained perched while I watched it for 15 minutes.
POSKC	7/5/2016	Transferred on to a *Rudbeckia*. Flew off about 2 minutes later onto another *Rudbeckia* head about 2 m away. Did not feed.
POSKD	7/6/2016	Transferred on to a *Rudbeckia*. Remained perched while I watched it for 15 minutes.
POSKE	7/6/2016	Transferred on to a *Rudbeckia*. Remained perched while I watched it for 15 minutes.

POSKF	7/6/2016	Flew out of the vial before it could be transferred with a Q-tip. Landed on my shoulder. It perched for 5 min before flying onto a cinquefoil, began feeding.
POSKG	7/6/2016	Transferred on to a *Rudbeckia*. Remained perched while I watched it for 15 minutes.
POSKH	7/6/2016	Transferred on to a *Rudbeckia*. Remained perched while I watched it for 15 minutes.

Non-target captures

During the Poweshiek field collecting period, four Poweshiek skipperlings were netted and placed in 50mL before being sexed as males.

Table 3 – Mis-sexed and captured male Poweshiek skipperlings

Individual	*Date collected*	*Time*	*Location*	*Coordinates*	*Post release behavior*
Male caught	6/30/2016	12:26	Long Lake Fen	42.7583599, -83.5620815	Transferred on to a *Rudbeckia*. Watched while it fed for a few minutes and then continued to perch till I left 15 min later.
Male caught	6/30/2016	13:00	Long Lake Fen	42.7584526, -83.5615161	Transferred on to a *Rudbeckia*. It chased after another male a few moments later, spiraling with the other male for a few moments.
Male caught	7/3/2016	15:28	Long Lake Fen	42.7583545, -83.5618199	Transferred on to a *Rudbeckia*. Did not feed before flying off a few seconds later. Landed ~3 meters away on a cattail.
Male caught	7/2/2016	18:14	Long Lake Fen	42.7583901, -83.5620052	Flew out of the vial before I could transfer it with a Q-tip. Lost visual.


(Left) Female POSKE post-release at Long Lake Fen. (Right) Male Poweshiek skipperling. This specimen had been initially misidentified as female and transferred from the net onto a *Rudbeckia*. Long Lake Fen, Oakland Co., MI.

C. Poweshiek skipperling larvae

The 96 Poweshiek skipperling eggs collected in from the four Michigan females were driven back to the Minnesota Zoo. Eggs were kept in 50mL vials and stored in a cooler with chilled (not frozen) gel packs. The temperature was continually monitored while driving. 96 eggs arrived at the Minnesota Zoo at 11:30pm on 7/6/2016. For internal records at the Zoo, each of the founding females were renamed. POSKB=POSK1, POSKC=POSK2, POSKD=POSK3 and POSKE=POSK4. The first head caps could be seen though eggs of POSK1 eggs on 7/8/2016 and the first two hatched on 7/9/2016.

As they hatched hatched, neonates were transferred into their own 50mL vial with prairie dropseed (*Sporobolus heterolepis*) and Pennsylvania sedge (*Carex pensylvanica*) leaf blades fed through the opening in the stopper. Pennsylvania sedge was included as a hypothesized hostplant given its presence at some mesic prairie Poweshiek populations, and reports of potential feeding on *Carex.*

Overall, 83 of the 96 eggs hatched (Table 4), a rate (86%) comparable to prior *ex situ* experience with wild Poweshiek skipperlings and Dakota skippers. All eggs and neonate larvae were initially reared indoors in the Prairie Butterfly Conservation Program office, and then transitioned outside the Poweshiek Hoop-house once the larvae matured through past earliest stages where extrinsic factors may pose the greatest risk. Indoors, vials were kept under artificial grow lights set to match daily circadian light cycles.

Table 4 – Poweshiek skipperling egg hatching success.

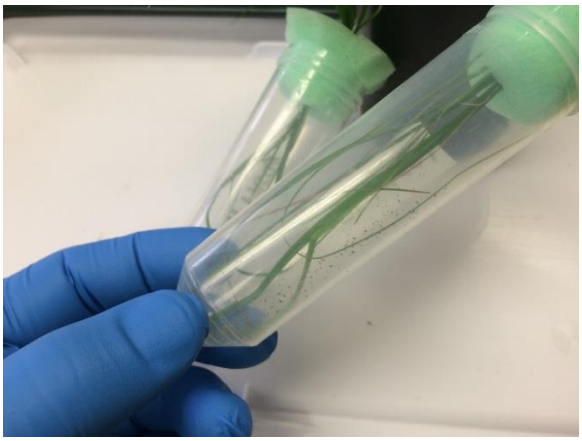
Female	*Eggs laid*	*Hatched*	*Developed but unhatched*	*Likely unfertilized*	*% Hatched*
POSK1	25	21	2	3	84%
POSK2	28	28	0	0	100%
POSK3	10	7	0	3	70%
POSK4	33	27	1	5	82%
Total	96	83	3	11	86%

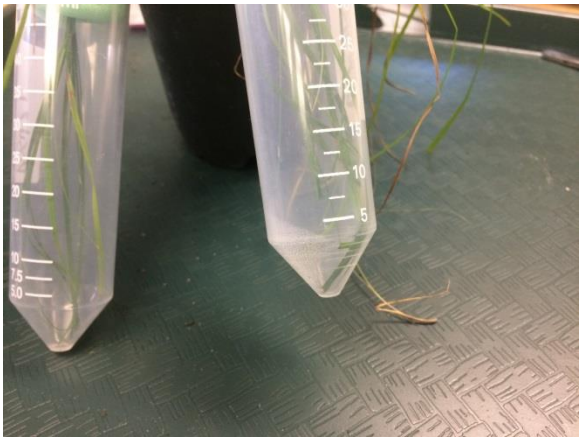
Despite these high hatch rates, there was higher than expected neonate mortality. Within the first week, 53 of the 83 larvae had died. None of these larvae showed any signs of feeding, for unknown reasons. When it was initially noticed that many of the larvae were not feeding, there was concern that the larvae may not have been exposed to enough grass leaves and were simply not coming in contact with hosts inside their tubes. In response, the density of grass blades was increased on 7/16/2016. This did not seem to help though, and 10 more larvae were later found dead despite sitting on viable prairie dropseed leaves. One additional individual died when it was accidently pinched by the vial stopper while removing some condensation in the tube.

On 8/2/2016, 31 larvae were transferred individually into singleton setups. Each 4" singleton pot was planted with both a prairie dropseed and Pennsylvania sedge. The surface of each pot was covered with a thin layer of white sand in hopes to better observe predators and frass accumulation during inspections. This addition was a slight deviation from previous singleton method protocols for Dakota skippers. Ultimately, adding the white sand made inspections significantly easier, did not seem to interfere with the plant, and would be worthwhile to implement in future years for non-ground shelter building skippers. Each pot was inspected three times by at least two different Prairie Butterfly Conservation staff members before placing a larva in the pot. Poweshiek singleton setups were kept in the office for 24 hours before being transferred to the Poweshiek Hoop-house so that the larvae could acclimate to their new setups and attach themselves to a leaf beforehand. At the time larvae were transferred, head cap widths were opportunistically measured to assess growth rates. The average head cap width was 1.28 ± 0.30mm.

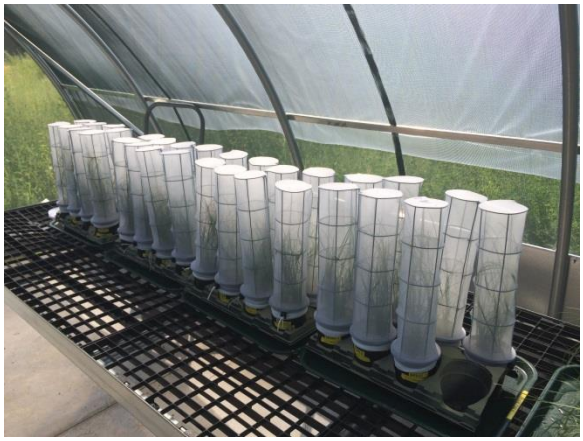
There were no new mortalities between 8/2/2016 and 8/25/2016. However, the larvae developed more quickly than expected in mid-August, with many prematurely advancing to the penultimate (5^{th}) and final (6^{th}) larval instars. Indeed, two unrelated individuals even reached pre-pupa (POSK1.17) and pupa (POSK2.01).

Poweshiek neonate larvae were first housed in doors in the lab in separate 50mL tubes. Larvae were offered leaf blades from both Pennsylvania sedge and prairie dropseed. To provide the larvae with double containment, the larval tubes were kept sealed in a pop-up mesh cage. Shown is the cage closed (Left) and open (right).





Larval vials would have to be cleaned periodically. (Left) A vial with a lot of frass accumulation. (Right) Condensation forming at the base of a tube.



(Left) Poweshiek skipperling larvae in Singlton setups after being transtioned outdoors in the Poweshiek Hoop-house. The Poweshiek hoop-house is a new hoop-house built in 2016 exclusively for Powesheik skipperlings and thier host plants. (Right) POSK1.12 on 8/21/2016. Recently molted from 3rd to 4th instar. Note: old headcap is present and the exuvea is behind the larvae.

D. Revised husbandry strategies

At the same time the rapid premature development of Poweshiek skipperling larvae occurred at the Minnesota Zoo, garita skipperling (*Oarisma garita*) larvae being reared under comparable husbandry protocols at the Assiniboine Park Zoo (Winnipeg, Manitoba) also developed rapidly. The garita skipperling is hypothesized to be the closest living relative to Poweshiek, and was identified during the conservation planning process as a key surrogate species for the optimization of husbandry protocols for Poweshiek. Both of these prairie butterflies are univoltine species with similar natural histories, and wild larvae are not expected to reach these late stages until early the following summer. Late instar larvae and pupae are not expected to survive sub-freezing hibernation, and would reach adulthood in early autumn without intervention. In contrast, hundreds of Dakota skippers reared under essentially identical conditions in the adjacent Dakota skipper Hoop-house developed normally, without any accelerated development.

Given the unexpectedly fast development of two *Oarisma* populations under nearly identical *ex situ* conditions, all Poweshiek skipperlings were moved on 8/25/2016 from their outdoor Hoop-house to a 13°C refrigerator set to slow them down while a new strategy was developed. After discussions with the USFWS and other partners, it was decided to let the Minnesota Zoo's Poweshiek population mature into adults and attempt to breed them at the Zoo. If mating was successful, any subsequent larvae would be artificially reared indoors through the autumn in attempt to synchronize adult eclosion time with that in the wild (late June 2017). Poweshiek skipperling has not been bred under *ex situ* conditions, but there have also been few well-timed opportunities for potential breeding to draw lessons from. During the 2015 *ex situ* planning workshop, population augmentation through head-starting was identified as a more appropriate strategy than developing a long-term insurance population at the Zoo because of the uncertainty of being able to get Poweshiek skipperlings to mate *ex situ*. The decision to attempt breeding was largely based on a similar occurrence at the Toledo Zoo with their population of Mitchell's satyrs. Like Poweshiek skipperlings, the Mitchell's satyr is a univoltine species with a flight time in mid-summer. In this example, some of the Toledo Zoo's Mitchell's satyrs also developed much faster than expected. Half of their population was allowed to mature that season and half were forced into winter diapause as late instar larvae. Though there was survival among the diapaused, late instar larvae, none bred the next year. In contrast, Toledo Zoo staff reported that they were successful at breeding the eclosed adults and were able to synchronize subsequent larvae adult times with the wild the next year.

Initially, it was decided to attempt to breed all 31 of remaining Poweshiek skipperlings to maximize opportunities for breeding. In order to synchronize, larvae were taken out of the refrigerator in three waves, five days apart from each other, bringing out smaller larvae first.

POSK3.2 on 9/9/2016. Final (6th) larval instar. (Left) Dorsal anterior. (Right) Showing white ventral abdominal patched also exhibited by many other grass skippers (sub-family Hesperiinae) during their final instar.

1. Hibernation of the smallest larvae

Despite the initial strategy to breed all captive poweshiek skipperlings eight of 31 larvae showed no signs of further development after they were brought back out to ambient conditions in the outdoor Poweshiek Hoop-house from the refrigerator. These eight were the smallest of the 31 larvae (each between 3rd and 4th instar), and appeared to be on the expected developmental trajectory. These eight individuals remaining were later transferred to diapause chambers and are now overwintering in a

freezer at -3°C with the hopes that they could be released as adults back at Long Lake Fen in 2017 as planned.

On 1/4/2017 the overwintering poweshiek skipperling larvae were censused. One of the eight was dead. All others had retained body moisture, but some were variable in coloration.

Table 5 – Poweshiek skipperlings overwintering as larvae. Headcap width (HC) and weight measurements were opportunistically gathered as larvae were transitioned in to overwintering diapause chambers on 10/9/2016.

Designation	*Date hatched*	*Strategy*	HC 10/9/2016 (mm)	Weight on 10/9/2016 (g)
POSK2.22	7/13/2016	winter	2.08	0.0420
POSK1.12	7/10/2016	winter	1.46	0.0194
POSK1.20	7/10/2016	winter	1.92	0.0276
POSK1.3	7/10/2016	winter	No data collected due to poor condition	
POSK1.8	7/10/2016	winter	1.90	0.0234
POSK1.9	7/10/2016	winter	2.08	0.0270
POSK4.2	7/13/2016	winter	1.02	0.0060
POSK4.26	7/14/2016	winter	1.71	0.0219
Avgerage			1.74±0.36	0.0239±0.0100

2. Adult breeding attempts

Twenty-two Poweshiek skipperling larvae were identified for use in the breeding trial. All of these had reached final instar by late August. Of these, six died as larvae and two died as pupae. A final individuak eclosed deformed (wings crumpled and proboscis unfused), and was euthanized. Ultimately,, 14 of the remaining 21 individuals reached adulthood (64%). This is a much lower than expected survivorship for late instar larvae and pupae, and may have been related to the refrigeration that had begun on 9/25/2016.

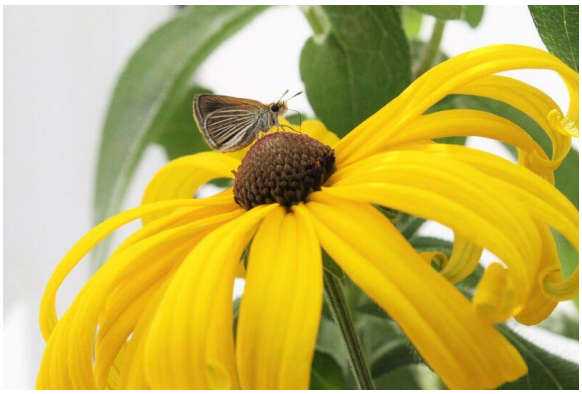
Pupae took eight to ten days to eclose. Pupae were a light green until about 48 hours before eclosure. Two days before eclosure, a bright red color could be seen under the eye caps. One day before eclosure, the pupa turned a pale yellow brown, and wings turned brown about half a day before eclosure. With the exception of one Poweshiek skipperling that pupated horizontally on the top mesh of its enclosure, they always pupated vertically, anterior up.


(Left) POSK1.4 on 9/26/2016. Eclosed 9/29/2016. Note the red yes that develop a few days before eclosing. (Right) POSK4.2 on 9/21/2016. Eclosed 9/22/2016 about 12 hours later. Eye caps have darkened and wing caps have become a light tan.

Of those that eclosed successfully, four were female and ten were male. Initially, sibling males were housed together and a single unrelated female was introduced. Due to concerns regarding handling and viability, we did not mark Poweshiek skipperling wings at first. Because we did not mark them, males in a single cage could not be distinguished from each other and therefore we were unable to collect data on individual male life expectancy.

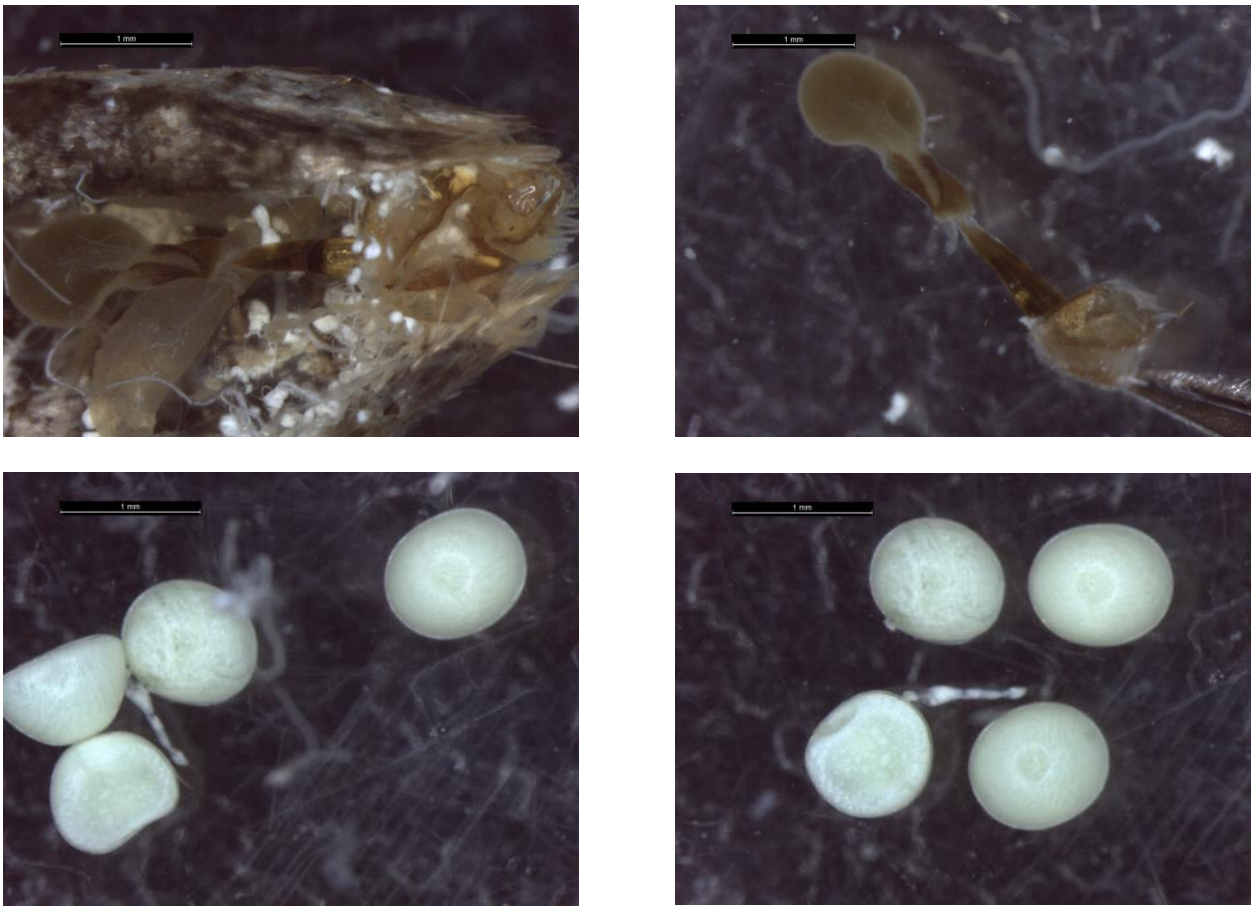
Breeding setups consisted of 12" wide x 24" tall pop-up cages filled with potted Maximillian sunflowers provided by the USFWS Twin Cities Field Office staff. Cages were setup in the Program's greenhouse to buffer from the cooler late September and early October weather. Full spectrum heat lamps and full spectrum T5 florescent lights set to an early July diurnal light cycle were used to increase lighting and heat to ultimately try to mimic conditions during a typical Poweshiek skipperling adult flight period. Adults were monitored by Zoo Conservation staff and Zoo Volunteers from 9:00 till 18:00 every day there were both male and females housed together. Staff and volunteers monitored cages for breeding and recorded feeding activities and anytime adults came in contact with each other.

Unfortunately, no Poweshiek skipperling breeding was observed. Cloudy, rainy conditions were common throughout, and adult activity was often notably depressed. One male was observed displaying stereotypical "abdominal J-ing" courtship behavior to females on 9/24 and 9/25 in different cages. Interestingly, both courtship attempts took place during the rare periods of clear skies. Though artificial lighting was provided, additional lighting may have been insufficient at simulating their needs. Full sun is likely an important stimulant for breeding, as has been demonstrated with much for the Minnesota Zoo's successful of Dakota skipper breeding program. A concern before the breeding trials was that males and females would not come in contact with each other in the cages. Watchers recorded every instance that adults physically came in contact with each other. From these observations, we can report that breeding did not take place simply because adults did not encounter one another. Every day of the breeding trials, males and females were recorded to have come in contact with each other. About half the time, contact resulted in a flight response (53%), the other half of the time resulted in no change in behavior or walking direction.



(Top) Poweshiek skipperlong breeding cages in the Chrysalis greenhouse. (Bottom) A female poweshiek skipperling (POSK1.19) (Photo credit: Dr. Tara Harris).

After the last male Poweshiek skipperling died on 9/28/2016, females were setup in their own oviposition cups to lay eggs. Though the skippers were watched continually from 9:00 am to 6:00 pm while the skippers are most active, it is possible a mating event occurred outside this time frame. Eggs were collected from the remaining females and treated as though they could have been fertilized. However, none hatched. POSK3.2 laid 14 eggs total and POSK1.19 laid 10 eggs total. Two females eclosed after the last male had died. POSK 1.4 on 9/29 and POSK 1.6 on 10/2. Neither laid any eggs before dying expiring on 10/5. To better understand potential egg load, we dissected female POSK3.2, with assistance from Dr. Snell-Rood lab at the University of Minnesota. This female was found to have 65 mature eggs with another nine developing.



Above photos are each from dissecting the abdomen of ♀ POSK3.2. She was carrying 65 fully developed eggs and nine developing eggs. Photos were taken in Dr. Emily Snell-Rood's lab at the UMN. (Above right) Internal abdominal structures showing the sclerotized genital opening, bursa, and fat bodies. (Above left) Isolated bursa. (Bottom left and right) Examples of mature eggs.

E. Concerns and future directions for Poweshiek skipperling *ex situ* strategies

Below, we highlight periods of difficulty during the Poweshiek skipperling Head Starting Program and the final attempted breeding of adults. These issues were unforeseen while developing the formal *ex situ* plan for this species. Each point will need to be addressed in order justify that future *ex situ* activities are successful (the production of a net gain of individuals to the wild population) and do not cause harm.

1) **Low numbers of skippers encountered for collections.** The Minnesota Zoo is permitted to collect eggs from up to ten females per site (Brandt Rd Fen and Long Lake Fen, Eagle Rd), with no more than 25% of the observed individual collected. During most surveys, the target threshold *and* female encounter rate could not be met due to low wild population sizes. The permitted collecting parameters were appropriate.

    - **Action: Collection/Survey Assistance.** In 2016, a single person both was responsible for surveying, collecting and carrying for collected individuals between two sites. Having additional

surveyors and collection assistance *may* help encounter rate and gauge a more accurate pupation count. Another strategy may be to have one person target Brandt while another targets Long Lake to avoid time lost traveling between the two in the same day.

- **Action: Begin surveys/collections later.** Surveys and egg collection efforts began three days after the first report of a Poweshiek at Eaton Road. The ratio of males to males in the first few days of surveys was very high. However, waiting longer before initiating egg collection efforts may yield a higher proportion of mated females, but that requires more robust pre-flight surveys than Minnesota Zoo staff can devote at this time. Note: Though we believe both the above actions are warranted, there is a low confidence of this greatly improving the number of collected females since the low numbers of Poweshiek skipperlings observed by Zoo staff were comparable to those of the Michigan Natural Features Inventory.

2) **Unexpectedly high rates of neonate mortality within about 3 days of hatching.** Despite having high hatching success of collected eggs (96 eggs collected, 83 neonates hatched), larvae either began feeding on the provided host graminoids, or did not feed at all. Susan Borkin (Milwaukee Public Museum) mentioned having similarly high levels mortality when placed on live plants in her office. It was not obvious why some individuals experience failure to thrive and do not feed.

- **Action: Provide different host graminoids.** Currently we are trying to propagate mat muhly grass, *Muhlenbergia richardsonis*. Note: *Low to medium confidence* of improving survivorship, but easy to implement if current attempts to propagate mat muhly are successful.

- **Action: Increase humidity of neonate tubes with Hydrostone.** There was a small correlation between tubes that needed to be changed out more frequently due to condensation and those larvae that fed successfully. Note: There is only *medium confidence* of this improving survivorship, and if not closely managed, could detrimentally harm neonates through mold or drowning.

- **Action: Separate individuals as eggs into setups 24h before hatching.** Note: *low confidence* of improving overall survivorship, but easy to implement.

- **Action: Provide increased airflow for neonates.** This was as suggestion by Susan Borkin during a conference call. The logistics still need to be determined, but may involve transferring neonates directly onto singleton setups rather than starting them out in tubes. There is *low confidence* of this improving neonate survivorship.

3) **Unexpected accelerated phenology.** High developmental variance and most were more developed than expected. Twenty-one of 30 larvae were 5^{th} or 6^{th} instar by the last week of August. Diapause is expected to take place at either late 3^{rd} or 4^{th} instar.

- **Action: Move outside into singleton setups earlier.** This year larvae were moved outdoors after reaching late 2^{nd} instar. Instead, it may be beneficial to place them outside earlier, such as outside in singleton setups 5 days after first feeding is observed. *Medium confidence* of improving phenology.

- **Action: Test foliar nitrogen levels in host graminoids.** High nitrogen levels in potting soil may artificially promote larval ground. A baseline of developmental rates under different soil nitrogen levels should be established with a surrogate species and compared to foliar nitrogen levels at the natal Michigan sites. This will not solve any issues for any 2017 Poweshieks, but it could have great ramifications for *ex situ* breeding of all grass feeders in future years.

- **Action: Better temperature control.** Subtle variations in temperature may have a disproportionately large effect on *Oarisma* larval development given the accelerated developments at the Minnesota Zoo and Assiniboine Park Zoo, particularly given that Dakota skippers reared using comparable methods and adjacent to the Poweshiek at the Minnesota Zoo did not develop unexpectedly. If high temperatures have an effect, cooling can be promoted through a new water bath system to cool singleton setups, periodically uncovering hoop-house film in response to high ambient temperatures, and bringing singleton setups into air-conditioned lab space during peak temperatures. *Uncertain confidence* that any of these techniques will improve phenology. If too much alteration is imposed on the larvae, developmental variance may be exaggerated.

4) **Out of sync breeding trial failure.** In response to the observed accelerated phenology, we attempted to bring 21 of our larvae to adulthood and breed them. Before breeding could be attempted, we observed high mortality as late instar larva, pupa, and had one bad emergence (n=9). Those that survived to maturity did not breed in the setups trials provided.

- **Action: eliminate, or reduce refrigeration times.** There is *medium to high confidence* that this would increase survivorship *if* future breeding were to be attempted.

- **Action: Offer different sized breeding setups**. Most breeding trails were attempted in 18" wide x 24" tall pop-up mesh cages. This configuration has been successful for Dakota skipper breeding at the Minnesota Zoo. Poweshiek may benefit from being either confined in a smaller space to increase encounter rates, or provided larger spaces to simulate more natural movement pattern. *Low confidence* of improving breeding success.

VI. Garita skipperling and North Dakota surveys

A key recommendation resulting from the *Ex Situ* Workshop was the addition of garita skipperling into the Minnesota Zoo's husbandry operations. Likely the closest living relative to Poweshiek skipperling, optimization of husbandry operations with garita skipperling could confer significant insights into Poweshiek skipperling *ex situ* rearing efforts. Garita skipperling is a small short- to mixed-grass prairie butterfly listed as a Threatened species in Minnesota (where it may be extirpated) but historically has been relatively predictable and locally common across western and central North Dakota where it is not a protected species. Therefore, the Minnesota Zoo will likely need to rely on the nearest reliable United States populations in North Dakota to obtain livestock for the recommended surrogate species husbandry program. The local nature of some garita skipperling populations, the narrow timeframe in which flights may occur, and the potentially dispersed geography of large garita skipperling populations across North Dakota also limits the ability of Minnesota Zoo staff to rapidly and

efficiently identify and collect ample numbers of gravid females, particularly given the existing husbandry demands with federally listed at the Minnesota Zoo. Attempts by Zoo staff to locate garita skipperlings at historic sites in North Dakota were not successful in 2015.

Using Cooperative Agreement funds provided by the USFWS, the Minnesota Zoo contracted with Dr. Ron Royer, Minot State University Professor Emeritus, in June 2016 to survey multiple prairie sites across western and central North Dakota to 1) help locate garita skipperling populations, 2) determine the optimum date(s) Minnesota Zoo staff should travel to those populations, and 3) potentially aid Minnesota Zoo staff in the collection of female garita skipperlings from multiple localities to maximize size and the utility of the *ex situ* garita skipperling population. Dr. Royer surveyed 25 sites across western and central North Dakota between June 17-26, 2016, focusing efforts on historic garita skipperling localities where the butterfly had once been predictably common. Despite generally good conditions during most surveys and known synchronous garita skipperling flights in adjacent southwest Manitoba, only one garita skipperling was observed by Dr. Royer (at a site where collection permissions had not been granted). The full report of the findings of the surveys, including all other butterflies observed, descriptions of sites surveyed, and comparisons to historical patterns, was successfully completed and submitted to Minnesota Zoo and USFWS staff in August 2016. This report is available upon request. Two other garita skipperling individuals were observed at one site in western North Dakota by outside surveyors. Minnesota Zoo Conservation Biologist Erik Runquist attempted to locate garita skipperlings at these and other high quality habitats recommended by Dr. Royer June 30-July 2 (see below), but was similarly unsuccessful. The disappearance of garita skipperling from a large number of historic North Dakota sites in the last 20 years is disconcerting and extends patterns of prairie butterfly diversity losses across the Upper Midwest. Dr. Royer also notes degraded habitat and decreased availability of key nectar-producing forbs at many of the historic garita sites.

In a last ditch effort to locate garita skipperling eggs, the Minnesota Zoo's Dr. Runquist surveyed several additional sites across western and central North Dakota following consultations with Dr. Royer and others. Observations of species and conditions made by Dr. Runquist are as follows:

(Left) High quality shortgrass prairies in the Badlands (McKenzie County, ND). Left: 4 miles west of Theodore National Park North Unit. Two garita skipperlings were found here several days before Minnesota Zoo staff could survey the site. Right: Atop Mile Butte, Little Missouri National Grasslands.

Badlands north of Red Wing Creek, 4 miles W of Theodore Roosevelt National Park, N Unit. 45.57°N, 103.538°W. McKenzie County, ND.

June 30, 2016. 10:30-4pm. Partly Cloudy – Sunny. 75-80 F. Light winds.

Hesperidae:		
	Afranius duskywing, *Erynnis afranius*	1
	Common checkered skipper, *Pyrgus communis*	2
	Ottoe skipper, *Hesperia ottoe*	5
Papilionidae:		
	Two-tailed tiger swallowtail, *Papilio multicaudatus*	1
	Anise swallowtail, *Papilio zelicaon*	1
Pieridae:		
	Clouded sulphur, *Colias philodice*	many
	Orange sulphur, *Colias eurytheme*	many
Lycaenidae:		
	Gray hairstreak, *Strymon melinus*	1
	Coral hairstreak, *Satyrium titus*	1
	Juniper hairstreak, *Callophyrs gryneus siva*	many
	Melissa blue, *Lycaeides melissa*	4
Nymphalidae		
	Regal fritillary, *Speyeria idalia*	1
	Aphrodite fritillary, *Speyeria aphrodite*	many
	Callippe fritillary, *Speyeria callippe*	3
	Sagebrush checkerspot, *Chlosyne acastus*	1
	Red admiral, *Vanessa atalanta*	1
	Wiedemeyer's admiral, *Limenitis wiedemeyerii*	1
	Least wood nymph, *Cercyonis oetus*	many
	Large wood nymph, *Cercyonis pegala*	4

This site was surveyed after an unaffiliated butterfly surveyor recorded two garita skipperlings several days prior to the Zoo's survey.

Little Missouri National Grasslands, Mile Butte. McKenzie County, ND.

7/1/2016 11:25-1:20pm. Mostly cloudy, 68°F, winds 10-20 mph

Hesperiidae:		
	Tawny-edged skipper, *Polites themistocles*	2
Papilionidae:		
	Two-tailed tiger swallowtail, *Papilio multicaudatus*	1
Pieridae:		
	Western white, *Pontia occidentalis*	1
	Orange sulphur, *Colias eurytheme*	4
	Clouded sulphur, *Colias philodice*	24
Lycaenidae:		
	Melissa blue, *Lycaeides melissa*	5
Nymphalidae:		
	Aphrodite fritillary, *Speyeria Aphrodite*	6

Weidemeyer's admiral, *Limenitis wiedemeyerii*	2
Red admiral, *Vanessa atalanta*	1
Least wood nymph, *Cercyonis oetus*	4
Large wood nymph, *Cercyonis pegala*	11

Little Missouri National Grasslands, off Binnie Pierre Rd. McKenzie County, ND.

7/1/2016 1:25-2:40pm. Sunny, 81°F

Hesperiidae:	
Uncas slipper, *Hesperia uncas*	2
Tawny-edged skipper, *Polites themistocles*	3
Papilionidae:	
Anise swallowtail, *Papilio zelicaon*	2
Pieridae:	
Western white, *Pontia occidentalis*	2
Orange sulphur, *Colias eurytheme*	2
Clouded sulphur, *Colias philodice*	11
Lycaenidae:	
Melissa blue, *Lycaeides melissa*	1
Nymphalide:	
Callippe fritillary, *Speyeria callippe*	4
Weidemeyer's admiral, *Limenitis wiedemeyerii*	1
Common wood nymph, *Cercyonis pegala*	4

Hills east of Lake Louise, Chase Lake National Wildlife Refuge. Kidder County, ND

7/2/2016. 9:55am-1:30pm Cloudy until 11:45, then sun. Calm winds.

Hesperiidae:	
Long Dash, *Polites mystic*	1 (old female)
Papilionidae:	
Black swallowtail, *Papilio polyxenes*	2
Pieridae:	
Clouded sulphur, *Colias philodice*	21
Orange sulphur, *Colias eurytheme*	22
Bronze copper, *Lycaena hyllus*	1 *
Dione copper, *Lycaena dione*	2 *
Nymphalidae:	
Great spangled fritillary, *Speyeria cybele*	4
Callippe fritillary, *Speyeria callippe*	5
Meadow fritillary, *Boloria selene*	7 *
Northern crescent, *Phyciodes tharos*	1 *
Red admiral, *Vanessa atalanta*	2
Common wood nymph, *Ceryconis pegala*	4
Eyed brown, *Satyrodes eurydice*	3 *

* Only observed in lower elevation mesic area just north of the hills.


Left: Badlands in western McKenzie County, ND, Little Missouri National Grasslands, off Binne Pierre Rd. Right: Rolling hills of the Missouri Coteau, east of Lake Louise, Chase Lake National Wildlife Refuge. Hundreds of purple coneflowers were in peak bloom, but there was very little visiting them. Lower elevations of the hills were heavily grazed.

VII. Pesticide drift and exposure research

A. Early June field samples

We only found three pesticides in May 2016 samples submitted, with the widely applied herbicide atrazine being most prevalent. The minimum Level Of Detection (LOD) for atrazine for these analyses was 50.0 parts per billion (ppb), so "Trace" samples represent a range of 0.1-49.9 ppb. This is a significantly poorer resolution than in 2015 (when the LOD was 6.0 ppb) and is a change that we were not made aware of until results were received. The insecticide clothianidin and the fungicide tebuconazole were also each detected for the first time, in only one sampling point each.

Prairie Coteau SNA:

Three "interior" samples and three "edge" points were sampled and analyzed, with four of the six points having paired grass and soil samples. Atrazine was detected at all of the Interior points. Trace levels were detected at one of the Edge samples and at all of the Interior samples.

Felton Prairie Bicentennial SNA:

Trace levels of atrazine were detected in all four grass samples (two Edge, two Interior) submitted for analysis. Nothing was detected in the soil samples

Glacial Lakes State Park:

Unlike other sites, atrazine was not detected in any of the seven grass and soil samples from two Edge and two interior points from Glacial Lakes State Park. However, a Trace sample (L.O.D. = 30 ppb) of clothianidin was found at one Edge point on the north side of the Park. This is the first and only observation of this (or any other) neonicotinoid insecticide in any of our sampling efforts to date.

North Enemy Swim, Sisseton Wahpeton Oyate (Day Co., SD):

We detected trace amounts of atrazine on two of three Edge samples and two of three Interior grass samples from North Enemy Swim. No pesticide residues were detected in any of the paired soil samples.

B. Late August field samples

Sampling occurred in mid-late August to coincide with aerial spraying of insecticides for the control of soybean aphid infestations. We collected samples of prairie grasses at four prairies during the third week of August in 2016. Three of these sites (Prairie Coteau SNA, Glacial Lakes State Park, and Felton Prairie complex) had seen comparatively sampled previously, while the fourth site (Hole-in-the-Mountain). The Nature Conservancy's Hole-in-the-Mountain preserve was sampled to help inform its suitability as a potential site for proposed reintroductions of Zoo-reared Dakota skippers. As has been done previously, we collected samples from random "Edge" (within 10 meters of a prairie-agriculture border) and "Interior" (at least 100 meters from a prairie-agricultural border) points.

Aerial soybean aphid insecticide applications were ongoing in southern Minnesota at the time of sampling, and the insecticide chlorpyrifos was only detected at the two southern preserves (Prairie Coteau and Hole-in-the-Mountain). No other pesticides were detected in any samples. Given that lack of any substantial pesticide residues from the paired soil samples in our prior research, we only collected grass samples in late 2016 for economic and logistical reasons. At both Glacial Lakes State Park and at Felton Prairie SNA, no pesticides were detected at three edge and three interior sampling points.

Table 6 - *Prairie Coteau SNA.* Chlorpyrifos was detected at four of the six points sampled, at relatively low levels. These points are spread across the SNA.

Chlorpyrifos Concentrations (ppb) on prairie grasses at Prairie Coteau SNA	
Point	**Chlorpyrifos**
Edge 1	6.8
Edge 2	Not Detected
Edge 3	6.1
Interior 1	Not Detected
Interior 2	26.7
Interior 3	8.8

Table 7 - *Hole-in-the-Mountain Prairie Preserve, Oakland Co., MN*. Chlorpyrifos was detected at three of the six points sampled, at relatively low levels that are comparable to those observed at other prairies in late summer. These points are spread across the preserve.

Chlorpyrifos Concentrations (ppb) on prairie grasses at Hole-in-the-Mountain Preserve	
Point	**Chlorpyrifos**
Edge 1	Not Detected
Edge 2	9.2
Interior 2	13.5
Interior 3	5.5
Interior 4	Not Detected
Interior 5	Not Detected

C. Pesticides exposure experiment

The controlled experiment at the Zoo to estimate potential lifetime effects of exposure to the common agricultural neonicotinoid clothianidin on prairie skippers concluded in July 2016. Over a three week period in June and July 2016, the remaining 36 unsampled pots (5-7 pots per pesticide concentration treatment type; see prior reports) were censused for surviving adult Long Dash skippers. Due to limitations in staffing during the busiest weeks of summer, priority for Zoo personnel was necessarily given to the intense *ex situ* husbandry operations with the federally Threatened and Endangered species instead of toward this experiment. Consequently, we could not check every pot every day for surviving skippers, but were able to recover them later. As such, we were able to record the number of individuals that had survived to adulthood on those plants since their initial placement as small caterpillars in July 2015 (the primary goal), but we were not able to record the weights of those survivors at the time of their emergence or other subtle details on other potential sublethal effects (secondary goals). Of the original 180 caterpillars placed on these 36 pots in July 2015 (5 per pot), 39 were recovered. Preliminary analyses do not suggest a statistically significant relationship between survivorship to adulthood and initial clothianidin application concentration. However, interpretation of these results is limited due to confounding factors, such as relatively small sample sizes and the variable uptake of clothianidin.

VIII. Outreach

In summer, the Minnesota Zoo Foundation again partnered with Fair State Brewing Cooperative to raise funds for our program with the limited edition re-release of the "Dakota Skipper Endangered Reserve" beer (http://mnzoo.org/dakota-skipper-endangered-reserve/). It was sold at several Twin Cities restaurants and helped raise awareness of the troubled butterfly and our work. Publicity for the beer and the butterflies was enhanced by appearances by Dr. Runquist on KARE 11 and FOX 9 morning TV shows.

This joint Minnesota Zoo and DNR program was highlighted in July in a feature-length story on Minnesota Public Radio (http://www.mprnews.org/story/2016/07/12/minnesota-prairie-butterflies-

disappear-concerns, and then again in November in The Nature Conservancy's "Prairies to Pines" magazine. The Zoo's work was also highlighted on TV: http://www.kare11.com/life/outdoors/new-butterfly-research-lab-at-minnesota-zoo/369545286.

We have worked with the Minnesota Zoo's Marketing and Public Relations Departments on social media blogs and posts. In September, we hosted a booth at the popular Minneapolis Monarch Festival and talked with over 1000 people about prairie butterflies and the Zoo's work with them. We have now translated these materials into Spanish.

Campaign graphic for Fair State's Dakota Skipper Endangered Reserve.

(Left) Minnesota Zoo's booth at the Minneapolis Monarch Festival on September 10th, 2016. (Right) Poweshiek skipperling coloring page activity at the Minnesota Zoo's booth.

Plan for the Controlled Propagation, Augmentation, and Reintroduction of

Dakota skipper

(*Hesperia dacotae*)

April 30th 2017

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I. Executive Summary

The Dakota skipper, *Hesperia dacotae*, has vanished from over 74% of once known occupied sites and was listed under the Endangered Species Act as Threated in 2014 (USFWS 2014). This species occurs nowhere else but remnant tall grass prairies. Suitable habitat for Dakota skippers has become largely isolated and fragmented. Natural dispersion between sites is highly unlikely without anthropomorphic intervention. For sites where Dakota skippers have become extirpated, reintroduction is the only option to restore the species to where it once was.

This document outlines a plan to reintroduce Dakota skippers from the Minnesota Zoo's Prairie Butterfly Conservation Program to The Nature Conservancy's (TNC) Hole-in-the-Mountain (HIM) Preserve (Lincoln Co., MN) beginning in 2017. The breeding program at the Minnesota Zoo began in 2013 strictly as an insurance program to preserve genetic diversity. In addition to breeding Dakota skippers at the Zoo, the *ex situ* population has been supplemented by wild-collected eggs annually from populations in northeastern South Dakota and northwest Minnesota. The Prairie Butterfly Conservation Program has successfully grown this *ex situ* Dakota skipper population each year, to the point where the insurance program can be maintained and excess progeny are being produced that can be utilized for release.

The strategy moving forward for Dakota skippers is to combine the insurance population program with reintroduction using zoo-reared pupae. These pupae represent both multi-generational Zoo lineages and newly collected specimens from extant populations the previous year (head-starting). Only specimens from northeastern South Dakota lineages will be utilized in the initial release, pending ongoing population genetics studies.

At this time, reintroduction is only being considered for sites in Minnesota at this time due to logistical, partnership, ecological, and financial benefits. The TNC HIM Preserve was chosen as the initial release location based on scoring compared to other candidate sites within the Dakota skipper's historic range in Minnesota. The Preserve is composed of six management units. The initial release will take place in the designated 'Central Unit', in a location chosen for its high quality habitat. A single release location will be used in the first year of reintroductions, to maximize the opportunity for released males and females to contact each other. The release itself will consist of placing 150-250+ pupa into a stationary closed box at the release point and releasing adults as they eclose. The expected timing of release is mid-June. A second release, one week after the initial release, will be carried out using any Dakota skippers that pupate later.

The long term goal of the project is for the TNC HIM Preserve as well as the adjacent Hole-in-the-Mountain and Altona Wildlife Management Areas (Minnesota Department of Natural Resources) to become occupied by Dakota skippers. We hope that the units will behave as a functional metapopulation. Collectively, we are referring to all of these units combined as the Greater HIM Complex.

Releases will take place within the TNC 'Central Unit' for at least three years. Each year, survey efforts will begin once the first pupae are released. Fixed transects will be established in the Central Unit and surveyed daily (as possible) to track occupancy and dispersal of adults over time, with additional regularly surveyed routes established throughout the entire Greater HIM Complex. In 2020, after three years of augmentation, releases will discontinue, followed by two years of additional surveys to track persistence. Surveys will inform future actions (e.g. if additional augmentation is needed, and where future reintroductions of Dakota skippers might take place within the Greater HIM Complex). Depending on the success of the Zoo-based rearing efforts, if there are an additional 150-250+ pupa beyond what was is needed to augment the 'Central Unit,' additional sites within the Greater HIM Complex may be identified for release. Any additional sites selected for release would follow a similar timeline with three years of continued augmentation followed by a two year evaluation period.

The following plan details these above activities and justifies the need for reintroduction planning and monitoring. Additionally, the plan addresses the need for continued communication with adjacent land owners and outreach with the adjacent city of Lake Benton. This document focuses on the reintroduction of the Dakota skipper to the Greater HIM Complex. This document does not address the long-term efforts needed for species recovery across its range, or address what will be needed for long term persistence.

II. Introduction

Controlled propagation, augmentation, and reintroduction have become important tools for the recovery of threatened and endangered organisms. In a number of cases, they form the basis for an urgent course of action to either restore or maintain existing population levels. The guiding principle of these efforts should be to minimize risks to extant populations and their habitats, and avoid harm to existing populations of non-target species. The primary purpose of augmentations or reintroductions should be to establish free-ranging, self-sustaining wild populations of the species.

U.S. Fish and Wildlife Service (Service) plans to cooperate with the Minnesota Zoo, The Nature Conservancy (TNC), and Minnesota Department of Natural Resources (DNR) to reintroduce the Dakota skipper at TNC's Hole-in-the-Mountain Preserve (HIM) in southwest Minnesota by releasing Zoo-reared larvae or pupae. This plan is intended to explain the rationale for pursuing this reintroduction, how it will be achieved to minimize risks and maximize the likelihood for success, how the project will be evaluated and, if and when appropriate, terminated.

The Dakota skipper was listed as a Threatened species under the Endangered Species Act in 2014 and has been extirpated from numerous sites within its historic range. At some of these sites, conditions, including habitat quality and land management, may be suitable to support a reintroduced population. The Minnesota Zoo's Prairie Butterfly Conservation Program has been successful at rearing the species *ex* situ to the extent that there may be enough individuals available to now attempt to reestablish a population through reintroduction.

The purpose of this plan is to provide a comprehensive risk assessment of the proposed reintroduction of Dakota skipper using zoo-reared individuals. We used IUCN guidelines (2013) to facilitate our assessment of ecological, social and economic risks, and to aid development of collection, release, and monitoring strategies.

We intend to update or amend this plan to fully evaluate long-term *ex situ* conservation activities leading to species recovery, including inter-site reintroductions and establishment of an insurance population.

III. Definitions of Terms Used in this Plan

Definitions used in this plan generally follow or are modified from the definitions provided in the International Union for the Conservation of Nature's (IUCN) Guidelines for Reintroductions and Other Conservation Translocations (2013) and the IUCN Guidelines on the Use of *Ex situ* Management for Species Conservation (2014).

Captive Rearing is defined as the careful maintenance of portions of generations, if not complete generations, of a species in a controlled environment (e.g. a zoo) to advance the conservation of the species. This includes those individuals maintained over multiple generations under these controlled conditions (e.g. the offspring of zoo-breeding in an insurance population, below) or under more short-term operations (like head-starting, below).

Ex situ is defined as conditions under which individuals are spatially restricted with respect to their natural spatial patterns or those of their progeny, are removed from many of their natural ecological processes, and are managed on some level by humans.

Head-start program is defined as a demographic manipulation that removes individuals from the wild to reduce mortality during a specific life stage and then subsequently returns those same individuals to the wild.

Insurance population program is defined as a program that maintains a viable *ex situ* population of the species to prevent predicted local, regional or global species extinction and preserve options for future conservation strategies.

Reintroduction is defined as the intentional movement and release of an organism inside its indigenous range from which it has disappeared.

Translocation is defined as the human-mediated movement of living organisms from one area, with release in another. Reintroduction is a type of translocation.

Augmentation is defined as the intentional movement and release of an organism into an existing population of conspecifics.

IV. Justification for Captive Rearing, Augmentation and Reintroduction

A. Status of the species

The Dakota skipper historically ranged in native prairie grasslands across much of the northern Great Plains, from northeast Illinois west to northern Iowa, and north through western Minnesota, eastern South Dakota, most of North Dakota, and into southern Manitoba and Saskatchewan. Populations of the Dakota skipper were distributed among patches of native grassland in the species' historical range as both isolated populations and as groups of populations that we presume interact (or at least did so historically) by dispersal - i.e., metapopulations.

However, Dakota skippers, like many other prairie butterflies, appear to have been extirpated from large portions of their historic range. Indeed, as detailed below, it has apparently disappeared from at least three-fourths of all known historically documented populations in the last few decades. It is now apparently extinct in the southern portions of its documented range in Illinois, Iowa, and southern Minnesota. Despite being present in at least 11 Minnesota counties in the early 2000s, with strong populations in three counties across four metapopulation complexes, there is now only one known remaining predictable population remaining in Minnesota. The size of this last population has also recently declined dramatically, from thousands of adults annually in the 1980s to the low hundreds annually between 2013 and 2016 (Minnesota Department of Natural Resources). The Dakota skipper was listed by the State of Minnesota in 1984 as a Threatened Species, but its status was elevated to State Endangered in 2013 in response to the numerous documented extirpations and declines. In response to concurrent range-wide declines, the U.S. Fish and Wildlife Service (hereafter, "the Service") listed the Dakota skipper federally as Threatened Species in October 2014.

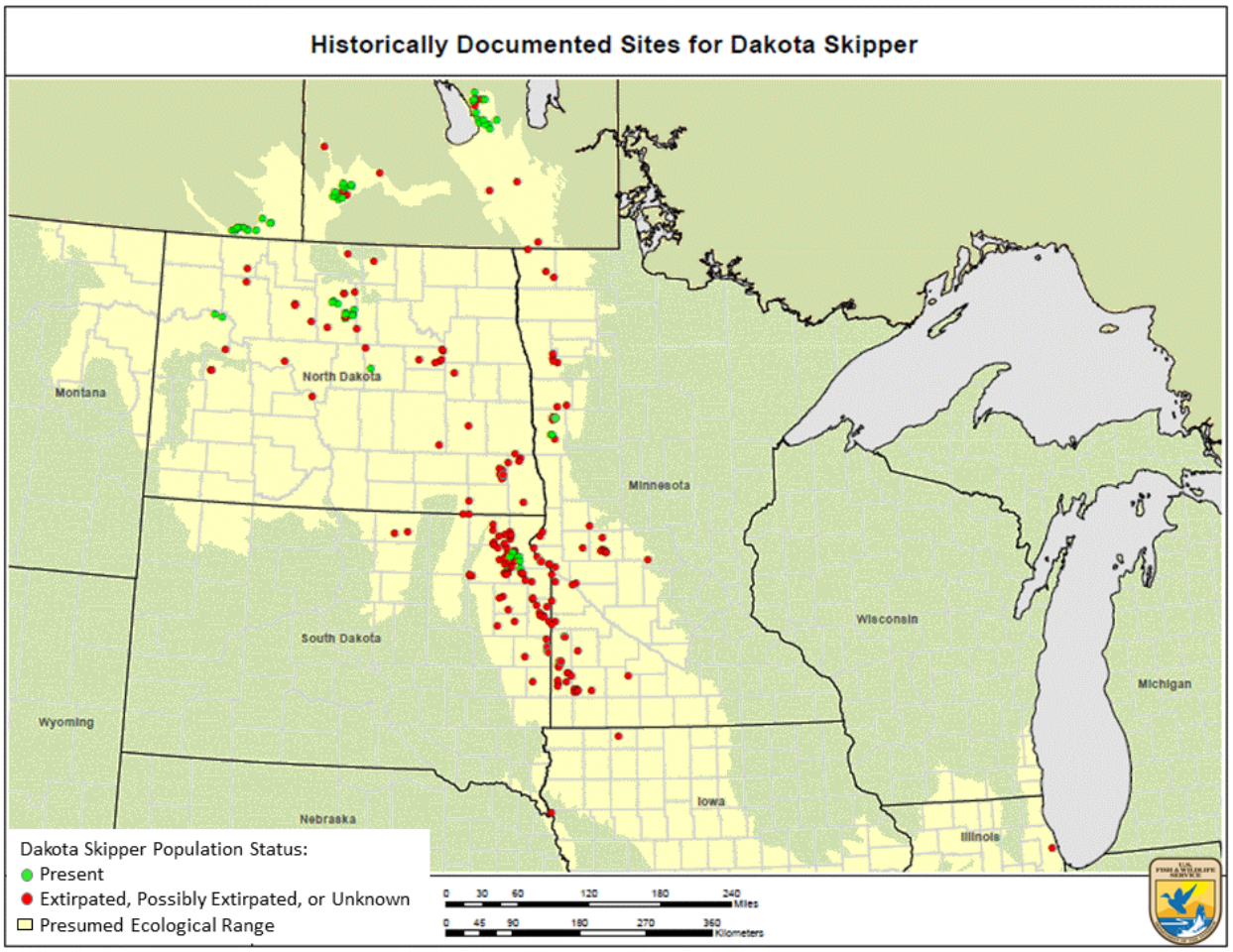

Figure 1. Presumed extant and extirpated historic populations of Dakota skipper and its inferred ecological range.

Dakota skipper populations exist as either (1) isolated populations that are too far from other populations to allow for dispersal or (2) groups of subpopulations near enough to one another to allow for dispersal. The maximum likely dispersal distance for Dakota skippers is unknown, but individuals may not typically move more than 5-8 kilometers away from core (natal) habitats (R. Westwood, pers. comm. 2016). Therefore, to group Dakota skipper survey records into putative metapopulations, the Service used the following definition, adapted from the metapopulation definition used for the Karner blue (*Lycaeides melissa samuelis*) in its recovery plan (U.S. Fish and Wildlife Service 2003): Dakota skipper metapopulations are comprised of subpopulations that occupy habitat patches that are no more than five kilometers from one another, on average, with the maximum distance separating occupied patches of no more than 8 kilometers.

To use this definition to identify metapopulations of the Dakota skipper, the Service used a survey dataset that contains geographic coordinates and other attribute data associated with over 1,900 surveys conducted in the range of the species. The geographic coordinates associated with each survey record represent 1) the approximate center point of the habitat patch surveyed; 2) the approximate center point of multiple Dakota skipper observations reported within a patch; or, 3) precise locations (points) of Dakota skipper observations.

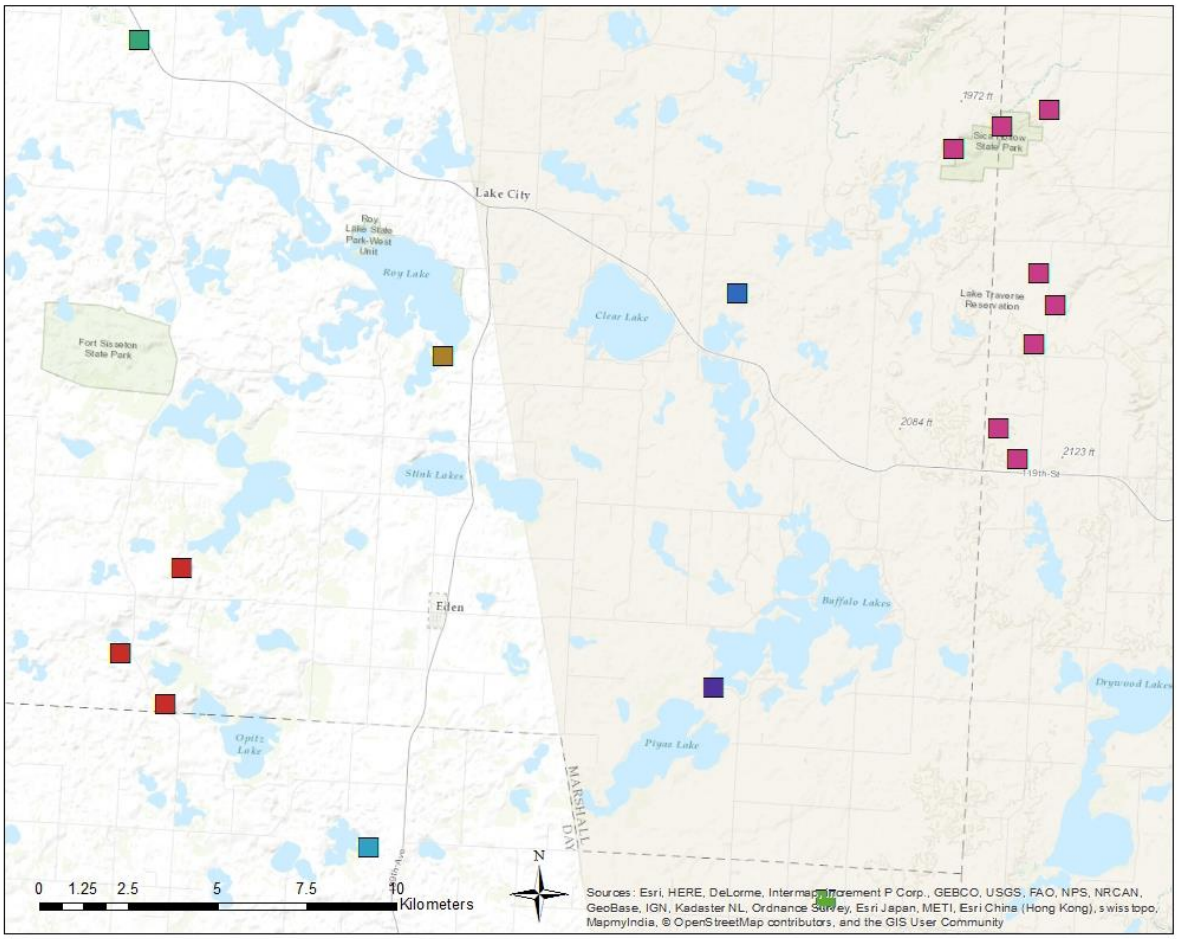

Figure 2 - An example output of the delineation of Dakota skipper populations based on the location of survey records. Squares represent approximate center points of habitat patches where the Dakota skipper was found; 2) the approximate center points of multiple Dakota skipper observations reported within a patch; or, 3) precise locations of Dakota skipper observations. Squares with the same color are grouped into putative metapopulations based on the definition described in the text; other populations are isolated.

With respect to this definition, the Service treated each survey record (point) as a distinct subpopulation (Figure 2) and reviewed mean distances among survey records to determine which should be grouped into metapopulations. For example, if the average distance among a group of survey points was greater than 5 km, outlying survey points were eliminated until the average distance among the remaining points was no more than 5 km. The resulting points represented groupings of subpopulations (metapopulations) or isolated populations. We used only extant subpopulations (survey records) to determine these groupings. Survey records that represented extirpated subpopulations were not used to determine metapopulation groupings.

This analysis indicates that there are 73 distinct Dakota skipper populations, over half of which are isolated. The mean number of subpopulations per metapopulation is 2.6, but more than half of the areas (38) consist simply of a single, isolated population (Figure 3). The viability of many populations is unclear though since the species has not been recorded since 2006 in over one-third of the 73 areas due to limited survey activity (Figure 4).

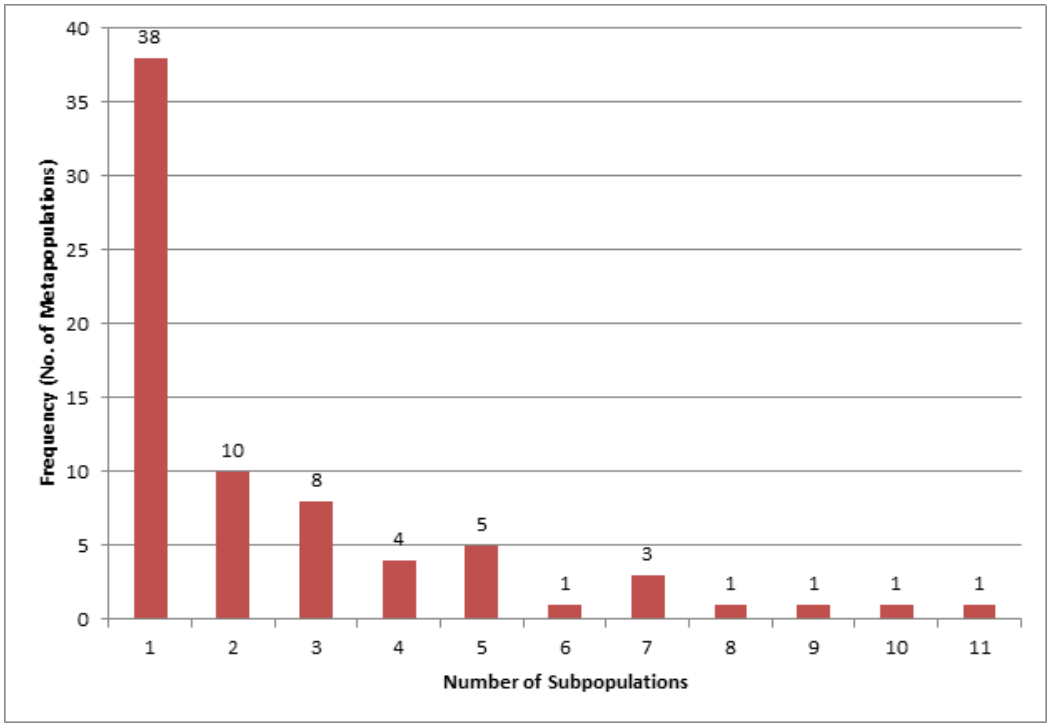

Figure 3 - The number of subpopulations that comprise metapopulations of the Dakota skipper. Over half (52%) of identified metapopulations consist simply of a single isolated population.

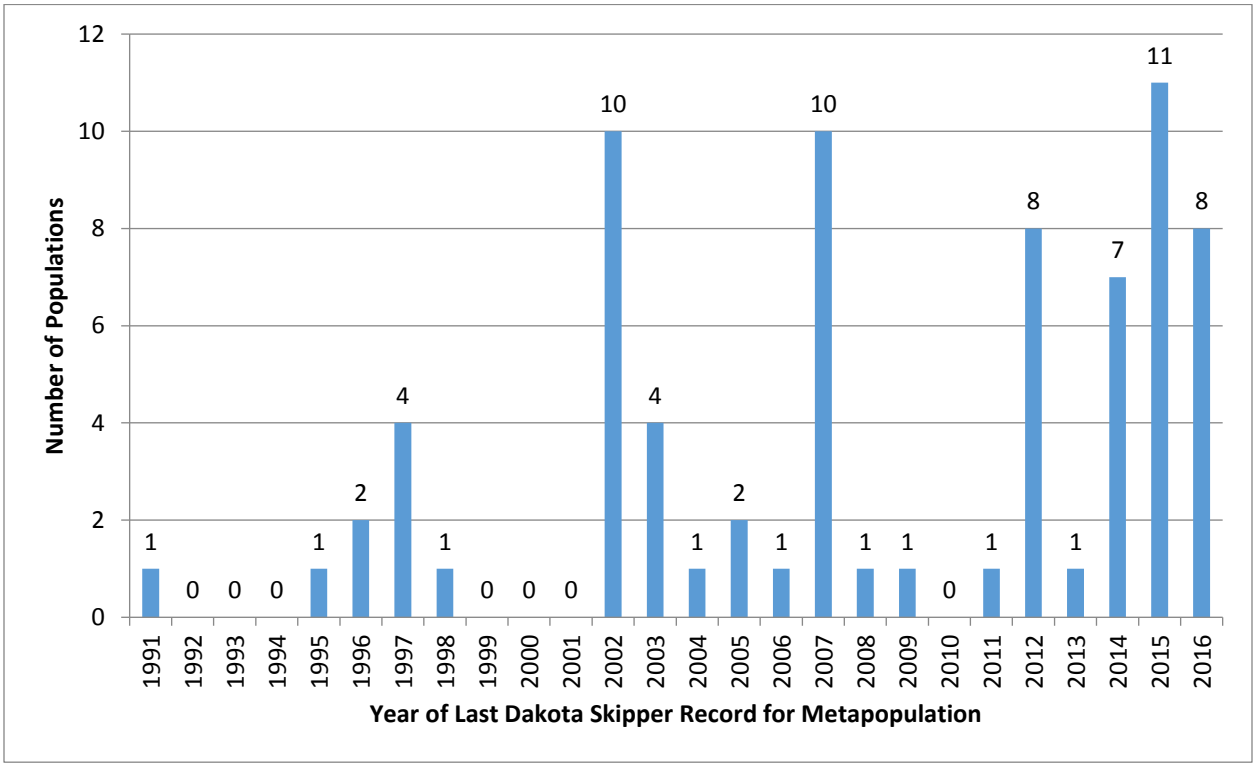

Figure 4. The last year that the Dakota skipper was recorded in the 75 extant populations (37 isolated populations and 38 putative metapopulations). Due primarily to limited survey efforts, the presence of the Dakota skipper has not been recorded since before 2006 for more than one-third of the populations.

B. Likely Population Trends

Like many butterfly species endemic to native grasslands in the Upper Midwest, the Dakota skipper has experienced dramatic declines across its historic documented range, and some remaining populations are apparently less abundant than they were historically. While it may not be at imminent risk of extinction based on the number of extant populations, the Dakota skipper is at risk of experiencing significant declines in the near future that could change the species' status dramatically. The decline of the Poweshiek skipperling (*Oarisma poweshiek*) is a case in point. The Poweshiek skipperling experienced a greater than 95% decline across its range since approximately 2000 and is now imminently imperiled with extinction. It once formerly occurred with the Dakota skipper in native prairie habitats in Iowa, Minnesota, South Dakota, and North Dakota, but recent surveys indicate that it is now apparently gone from those states and only a few hundred individuals may remain globally. The Dakota skipper itself has been extirpated from sites in Minnesota that were until recently considered strongholds for the species, including the Prairie Coteau Scientific and Natural Area and Hole-in-the-Mountain Prairie region. Numerous other populations of the species in Minnesota have evidently been extirpated and it may now exist in Minnesota in only a single metapopulation at Felton Prairie in Clay County. A single individual was found in the nearby Bluestem Prairie in 2015, but intensive efforts to find the species there in 2016 were unsuccessful.

Many of the extant Dakota skipper populations are actually of questionable status. A preliminary analysis of survey data suggests that as few as 22% of the extant metapopulations may exhibit a consistent and continuous presence that may be indicative of viability (R. Royer, Minot State University retired, pers. comm. 2016). Among metapopulations that have been surveyed during at least four years since 2000, only 22% have positive detections in greater than 90% of those years. If the threshold for positive detections is lowered to 80% of years surveyed since 2000 as a standard for 'consistent and continuous' observations, only 28% of the 73 metapopulations would still be considered to have met this preliminary standard of metapopulation health. Thus, only 8-10 metapopulations may be relatively stable globally, based on these preliminary analyses. Concerted surveys are critically needed across the range, particularly at under-studied historic localities, to better understand the actual status of Dakota skippers globally.

Metapopulations face a wide variety of threats and the continued existence of many may be subject to land management decisions that are made without regard to their effects on the Dakota skipper. Two-thirds (67%) of subpopulations are either Vulnerable or Highly Vulnerable to habitat conversion that would lead to their extirpation and about 72% are in private ownership, managed under the authority of the North Dakota Department of Trust Lands or the South Dakota Highway Department. Although Dakota skipper habitats can be conserved on these lands, the type of ongoing attention to the actions that may be needed to maintain high quality habitats for the species cannot be guaranteed as landowners and land managers make decisions based on other economic priorities.

Dakota skippers are sensitive to land management activities, but the vast majority of documented populations occur on private, county, state, and other lands not owned by the Service or federal agencies,. The listing of the Dakota skipper as a federally Threatened Species prohibits most forms of take of the species, but does not generally guarantee or require proactive measures to conserve species except by federal agencies. The long-term status of Dakota skipper populations is going to need a considerable amount of conservation effort outside of federal lands in the U.S., especially since only 17 of the 152 subpopulations in the U.S. occur on federal lands. When the Service listed the species in 2014, it included a special rule allowable under section 4(d) of the Endangered Species Act that exempted take of the Dakota skipper that occurred as part of routine livestock ranching activities on nonfederal lands. If the

status of the species changed to endangered, we would no longer be able to have such a rule in place and conservation of the species on non-federal lands could become more difficult due to decreased willingness to participate in the conservation process from private stakeholders. *Ex situ* management and reintroduction could be significant in keeping the species from becoming endangered and in maintaining our ability to recover it from its current threatened status.

C. Threats

The greatest threat to the Dakota skipper has been loss of native high quality prairie habitat. However, it remains unclear why this species has vanished from more than 76% of the last known sites in the last few decades. Hypotheses include but are not limited to: further habitat loss and degradation, isolation due to fragmentation, small population size effects (i.e. Allee Effect), pesticide drift, invasive species, over- (or under-) use of prescribed fire and other habitat management methods, climate change and extreme weather events, and perhaps diseases or novel predators or parasitoids. It is assumed that these factors vary locally and interact with each other.

Among these threats, it is unlikely that only small population sizes and isolation alone could have been enough to cause the widespread disappearance of populations within such a short period of time. For example, formerly large populations of Dakota skippers appear to have disappeared from many sites across southwest Minnesota in just a few years. Surveys by the Minnesota DNR led by Robert Dana found Dakota skippers at eight localities in Hole-in-the-Mountain region, the Prairie Coteau Scientific and Natural Area, Terrace Wildlife Management Area, and private sites in the Chanarambie Creek Valley in 2006 through 2009. Populations in the Hole-in-the-Mountain region were particularly high in these years. Surveys did not resume at these sites until 2012 however, and at which time all southwest Minnesota Dakota skipper populations appeared to have been extirpated. Focused surveys from 2012 through 2016 at sites across this region have all failed to produce detection of the species. A synchronous region-wide extirpation event within just a few years likely suggests a common causal influence that acted on all populations across a wide geographic area, such as extreme weather event(s), widespread pesticides drift, etc., or some interaction(s) thereof. This period is also just after the apparent extinction of Poweshiek skipperling from the same region.

Pursuit of reintroduction is not often recommended when known causes for extirpation have not been mitigated. Due to the number potential of causal stressors identified above and serious data gaps (i.e. survey history, etc.) regarding Dakota skipper, it is difficult to extrapolate the exact cause for each local extirpation event, let alone predict how or what to mitigate. What is apparent, given the poor dispersal ability of this species (see Section VI.A), is that once a population is extirpated it is unlikely natural recolonization will occur. Our ability to restore corridors connecting extant sites is uncertain in most situations given the long distances between sites. To expand the current known range and ensure species persistence, reintroduction activities will be needed. While reintroduction efforts are being executed, activities studying reasons for the decline by the Service and Minnesota Zoo will continue, as will long-term efforts to restore prairie landscapes. Indeed, having stable or increasing populations of Dakota skipper and other imperiled prairie butterflies is identified as a key indicator of the success of prairie restoration and prairie connectivity efforts outlined in the multi-decadal Minnesota Prairie Conservation Plan (Minnesota Prairie Plan Working Group 2011).

Among the potential threats being studied is non-target pesticide drift from adjacent agricultural operations. The Minnesota Zoo and the Service have cooperated on a drift study at five prairie remnants in Minnesota and South Dakota since 2014. Grass samples were first collected within the HIM Preserve in August 2016 and analyzed by pesticide residues. As described below, residues of some insecticides

applied against the invasive soybean aphid, *Aphis glycines*) were found. Results will be compared to baseline data collected at other sites where Dakota skippers are still present and where they have become recently extirpated. These findings and their implications are presented in Runquist and Nordmeyer (2017) and in Runquist and Heimpel (2017). This work will continue annually.

D. Contributions to the Species' Recovery

If populations of Dakota skippers can be reestablished within their historic range, the species will be closer to recovery. Important lessons can also be learned that will inform the science of prairie skipper reintroductions and population management, even if Dakota skipper reintroduction efforts described below are not immediately successful. For example, dispersal patterns of adult butterflies away from the reintroduction site(s) along fixed transects that are surveyed on a regular basis will help inform models of metapopulation connectivity parameters. Monitoring behaviors of reintroduced adults may also elucidate key habitat characteristics that promote local residency vital to long-term population viability.

If we can reestablish a viable population of the Dakota skipper at HIM, it would make a tangible contribution to the species' recovery. Recovery of the Dakota skipper will rely on the existence of at least several healthy metapopulations within each of several ecoregions. Metapopulation health will rely on several factors intrinsic to the species' population dynamics, including the density and diversity of larval food plants and nectar plants and the extent of high quality habitat. It will also rely, however, on the consistent implementation of management practices that maintain or restore these essential habitat features and that minimize short-term adverse effects to population growth. The Nature Conservancy and the Minnesota DNR Division of Wildlife each manage a significant amount of native prairie at and in the vicinity of HIM. This is likely to provide the management consistency and quality that is not guaranteed at numerous sites occupied by the species.

E. Summary of CBSG Workshop– Dakota Skipper Working Group

In 2015, the Service, in collaboration with the Minnesota Zoo, invited the IUCN/SSC's Conservation Breeding Specialist Group (CBSG) to plan and facilitate a participatory workshop process designed to use the *Ex Situ* Guidelines (IUCN/SSC 2014) as an aid to evaluate the feasibility of incorporating an *ex situ* management element into the broader conservation activities for both Poweshiek skipperling and Dakota skipper. The workshop was hosted by the Minnesota Zoo on 20-22 October, 2015 and was supported by United States Fish and Wildlife Service and the Minnesota Zoo Foundation. The workshop was facilitated overall by Dr. Philip Miller of CBSG, with his colleague Dr. Kathy Traylor-Holzer leading the participants through the application of the *Ex Situ* Guidelines to the specific conservation issues facing the two focal species. Participants in the meeting included 20 experts on species biology and management, with a few individuals with expertise on conservation of closely related species participating by conference telecommunications.

The details regarding the participants, processes, and recommendations followed during the workshop are provided in Delphey et al. (2016). Below we summarize the approach adopted by the Dakota skipper group during the workshop:

> **Reintroduction of Dakota skipper at sites within the species' historical range where it has been extirpated.** The specific objective for this management component is to establish at least one new population in the wild by 2021. Larvae for reintroduction will be produced primarily by headstarting – collecting eggs from wild females and rearing the eggs at the zoo to produce larvae

or pupae for release. Some larvae or pupae may be produced from mating of captive-reared adults at the Minnesota Zoo. This may consist largely of individuals that survive research projects (see below) and become adults at the zoo, but captive rearing and breeding to produce an F1 generation may also be used to generate a sufficient number of offspring to establish a reintroduced population.

Provision of Dakota skippers for research projects that are integral to the species' conservation. The research program would focus on gaining a better understanding of the number of larvae/pupae that must be released to reestablish a viable population of the Dakota skipper. A viable population would be one with consistent evidence of recruitment. To accomplish both the research and restoration components of the overall program would require producing at least 800 post-diapause larvae and/or pupae. Approximately 175 larvae could be used in continued larval food plant studies at the Minnesota Zoo, while an additional 30 larvae could potentially be used in a pesticide study, also conducted by the Zoo. Upon completion of those studies, all larvae produced *ex situ* would be available for population restoration unless additional research needs are identified.

Completion of a management protocol that could be used by zoos or other facilities to successfully house the Dakota skipper *ex situ*. This would likely take the form of a comprehensive husbandry manual describing the procedures and methods necessary to achieve success in management *ex situ* populations of the Dakota skipper.

This plan focuses on the first of these points and, even more specifically, on *ex situ* rearing and reintroduction of the Dakota skipper to TNC's Hole-in-the-Mountain Prairie Preserve in Lincoln County, Minnesota.

F. Reintroduction Justification

Due to the Dakota skipper's patchy extant range and fragmented available habitat, it is improbable that the species will immigrate to many of the suitable, otherwise unoccupied habitats without human aid in the foreseeable future. During the 2015 CBSG Workshop, the first recovery activity recommendation was the formation of a reintroduction program from zoo-reared individuals that are part of the Minnesota Zoo's insurance population. Population restoration via reintroduction was chosen as the conservation strategy for a number of reasons. First, reinforcement of extant populations through augmentation is not considered a worthwhile option at this time. Augmentation engenders certain risks and requires analyses that are not relevant or important for reintroductions – swamping of locally adapted genotypes, for example. If a local population appears to be experiencing rapid decline to the extent that genetic rescue may be necessary, we may initiate a reinforcement program. By only implementing a reintroduction strategy to a site where the species is extirpated, there is little risk to wild populations.

Conservation introductions are not being considered at this time for Dakota skipper recovery. Conservation introductions may take the form of ecological replacement, or assisted colonization to sites outside their indigenous range. Ecological replacement is not currently warranted for Dakota skippers, as the broader ecological impact of this species in its native prairie range is not well known and therefore a suitable substitute species cannot be identified. Assisted colonization is a risky and often controversial conservation strategy, and has a low likelihood of success. Given the poor understanding of Dakota skipper's broader ecological role, it is impossible to predict the effects Dakota skippers would have on the ecology of a site to which it was introduced to as a non-native species. Until a well-defined need is identified for conservation introductions, no such activities are being proposed at this time.

At this time, only reintroductions into remnant prairies are being considered, as opposed to restored prairies. We likely do not know enough about the species' ecological needs to identify and recreate those features within a restored site. Historically, translocations of imperiled butterflies into restored sites have had low levels of success. Theoretically, there could be an unknown element of the environment (the lack of a mutualist, etc.) that has not been incorporated into the restoration that was critical to the translocated species. Even for species that have been studied more robustly, this is the case. However, the number of remnant prairie sites apparently suitable for reintroduction are few. It is possible, if not likely, that restored prairie sites will need to be utilized for the recovery of this species at some point in the future. Establishing Dakota skippers within more sites will increase the species' overall resiliency. However, translocation to restored sites is currently outside the scope of this document.

Reintroductions to HIM will utilize Zoo-reared Dakota skippers. Acknowledging the risks of *ex situ* reared Lepidoptera (Lewis and Thomas 2001; Schultz and Dzurisin 2009), this strategy is being adopted in hopes that benefits from having a reduced impact on the extant populations will offset these risks. Many other Lepidopteran translocation and *ex situ* programs collect specimens as eggs or larvae to reduce population level impacts. Given the presumably low survivorship of early life stages, removal of relatively few eggs, or early instar larvae likely has a minimal impact on the population as a whole. Survival in the wild from egg to pupation of one rare butterfly, for example, was 3% based on a sample of 1,617 eggs (Lambert 2011, p. 110). In studies reviewed by Nail *et al.* (2015), the predicted survival rate of monarchs (*Danaus plexippus*) from egg to adult was ~4.2 to 9%, but this was conservative since the pupal stage was raised in captivity and not exposed to some important mortality factors. In the case of Dakota skippers, no technique or search image has been developed for identifying and finding eggs or larvae in the wild. The only life stage that can be located with any confidence is the adult stage. As part of the Minnesota Zoo's Dakota skipper collection protocol, adult females are collected from the field and held for no more than 72 hours. Any eggs laid in this time are integrated into the *ex situ* Zoo population. Once eggs are collected, the adult females are returned to the same GPS coordinates from which they were collected, to allow them to continue laying eggs and contribute to that local population. For more details about the Minnesota Zoo's collecting and husbandry techniques, the Hesperiid Husbandry Manual is available upon request.

An alternative approach to this reintroduction strategy would be to translocate gravid adult females from other *in situ* populations. This strategy was not adopted largely due to the above explanation of trying to mitigate negative impacts on removing specimens from the source populations. Removal of a fraction of a female's reproductive load is likely of much less impact than removing the female herself. Additionally, translocating adults may have added complications. Adult skippers often do not travel well and are prone to damaging themselves. By utilizing *ex situ*-reared specimens, we are able to translocate pupae, with little risk of injury.

At this time, reintroduction is only being considered for sites in Minnesota due to logistical, partnership, ecological, and financial benefits. The TNC HIM Preserve was chosen as the initial release location based on its ranking compared to other candidate sites within the Dakota skipper's historic range in Minnesota (see Selecting Release Sites, below). The long term goal of the project is for the TNC HIM Preserve as well as the adjacent Hole-in-the-Mountain and Altona Wildlife Management Areas (Minnesota Department of Natural Resources) to become occupied by Dakota skippers. We hope that the units will behave as a functional metapopulation. Collectively, we are referring to all of these units combined as the Greater HIM Complex.

V. Goals, Objectives and Actions

We will use the following definitions for this plan (IUCN 2014:5):

A **Goal** is a statement of the intended result in terms of conservation benefit;

Objectives give clear and specific details for how the goal will be realized; and,

Actions are statements of what should be done to meet the objectives.

Goal

The goal of this plan is to ensure continuity of a self-sustaining population of Dakota skipper at Hole-in-the-Mountain Prairie in Lincoln County, Minnesota. A self-sustaining population would be one in which the species can be reliably detected at the reintroduction site for at least five years after the cessation of any release of individuals produced partly or entirely *ex situ*.

Objectives

The following are objectives - and underlying actions - of this plan. Note that for the specific actions addressed by this plan, parts or all of objectives 1-3 were completed before or during plan development.

1. Select one or more sites where it would be warranted to attempt the reintroduction of the Dakota skipper.

1.1. Consult with species experts and review survey data to identify candidate sites.

1.2. Evaluate candidate sites according to the criteria described in the section, Reintroduction Site Selection.

1.3. Secure landowner permission to carry out the reintroduction. Ensure that owners of the reintroduction site are apprised of the contents of the Dakota Skipper Propagation and Reintroduction Plan.

2. Identify Dakota skipper populations that are healthy enough to sustain the removal of a limited number of eggs for captive rearing and that are ecologically similar to the reintroduction site.

2.1. Consult with species experts and review survey data to determine which extant populations are likely to have populations sufficiently robust to allow for egg collection based on the protocols.

2.2. Ensure that any potential collection sites are in the same Ecological Sections as the reintroduction site as described by Bailey *et al*. (1995).

2.3. Ensure that all necessary approvals and permits are secured to allow for egg collection and transport to Minnesota Zoo.

3. Carry out egg collection at identified collection sites and rear eggs to pupal stage for release according to the best practices identified in the Minnesota Zoo's Hesperiid Husbandry Manual.

4. Develop site-specific release plans before releases occur.

4.1. Site plans for reintroductions will consist of the following, at a minimum: (1) a map that delineates the limits of the site and the extent of Dakota skipper habitat at the site; (2) the results of any previous surveys for Dakota skipper conducted at the site; (3) a description of the individuals, organization or entities who will carry out release activities, manage habitats at the

site, and carry out surveys; (4) a description of previous and planned survey and habitat assessment methods; (5) a description of the land ownership and statements of approval or authorization from the landowner(s) for the area where releases will occur; (6) the precise methods of release, including a) the life-stage - or life-stages - at which immature Dakota skippers will be released; b) any structures to be used and how they will be used; c) the number and identity of personnel who will carry out each task; d) methods to be used to transport the animals to the release site; e) the methods that will be used to determine the time of release; f) the information that will be collected as each release is carried out, including fates of released individuals, timing of eclosion, etc.; g) the location within the reintroduction site where releases will occur; and, h) contingency plans for each aspect of the release, as appropriate.

5. Ensure that funding, facilities, and personnel are in place to carry out planned activities.

5.1. By the end of each calendar year, identify gaps in facilities, funding, and personnel needed to complete the planned activities for the following year.

5.2 No later than April 1 of each calendar year, or as deadlines dictate, apply for funding as needed to fill forecast gaps in funding, facilities, and personnel while this plan remains in effect, to carry out the actions described in Section VII. Monitoring and continuing management are secured and in place to support those actions for the succeeding twelve months.

5.3. No later than April 1 of each calendar year while this plan remains in effect, ensure that personnel have been identified to fulfill all of the needed roles and responsibilities as described in Section IX. Monitoring and continuing management, as needed for the succeeding twelve months.

6. Ensure that planned activities are communicated to all stakeholders before any egg collection or releases.

6.1. Develop an outreach plan to inform stakeholders about planned activities.

6.2. No later than April 1 of each calendar year while this plan remains in effect, implement the recommended activities of the outreach plan to ensure that all identified stakeholders have been informed of the release plans.

7. Determine success or failure of captive rearing and releases and whether to continue reinforcement.

7.1. Monitor release sites to determine whether Dakota skipper adults are present and where they occur at release sites.

7.1.1. Develop and implement a monitoring plan that includes the following: (1) a description of the survey methods to be used; (2) a description of the qualifications of persons who will conduct surveys; (3) a map and description of the area(s) to be surveyed; (4) a description of the timing, frequency, and number of surveys to be conducted during each flight period; and, (5) a description of the manner in which results will be reported.

7.1.2. Evaluate adult survey data collected after releases to determine the abundance and distribution of the Dakota skipper.

7.2. If five years of releases have failed to establish the species at the reintroduction site, determine whether releases should continue. Continue releases until it is determined that additional releases are unlikely to maintain or further improve population trends. This determination may be made based on the trends in relative abundance – or another appropriate

metric – of the Dakota skipper at the reintroduction site(s); the extent of suitable habitat that the species occupies at the site; and/or, the likely trends in the quality and extent of habitat at the site. If suitable habitat remains unoccupied at the site or if management actions are likely to lead to significant improvements or expansion of suitable habitat at the site, continued releases may be prudent.

7.3. Annually evaluate survival from egg to release while in captivity to determine whether it is likely to exceed survival in the wild. Survival rates from egg to pupa or late-instar larva that are less than 3% may not exceed survival rates in the wild. If after three years of *ex situ* management, survival rates during the egg to release stage do not exceed 3-5%, the program will cease pending further review.

VI. Basic Biological Knowledge

A. Dispersal

Dakota skippers are not known to disperse widely; the species was evaluated among 291 butterfly species in Canada as having relatively low mobility. Experts estimated Dakota skipper to have a mean mobility of 3.5 (standard deviation = 0.7) on a scale of 0 (sedentary) to 10 (highly mobile) (Burke et al. 2011, p. 2279; Fitzsimmons 2012, pers. comm.). Dakota skippers may be incapable of moving more than 8 kilometers (km) (5 miles (mi)) between patches of prairie habitat separated by structurally similar habitats, but typical movements may be less than one km (Cochrane and Delphey 2002, p. 6, R. Westwood, University of Winnipeg, pers. comm., 1 Sep 2016). Royer and Marrone (1992a, p. 25) concluded that Dakota skippers are not inclined to disperse, although they did not describe individual ranges or dispersal distances. McCabe (1979, p. 9; 1981, p. 186) found that concentrated activity areas for Dakota skippers shift annually in response to local nectar sources and disturbance.

In a mark–recapture study, average adult movements of Dakota skipper were less than 300 meters (m) (984 feet (ft)) over 3–7 days; marked adults crossed less than 200 m (656 ft) of unsuitable habitat between two prairie patches and moved along ridges more frequently than across valleys (Dana 1991, pp. 38–40). Dana (1997, p. 5) later observed reduced movement rates across a small valley dominated by exotic grasses compared with movements in adjacent widespread prairie habitat. Roads and crop fields were suspected to be impediments for movement among prairie patches along two sites of the main valley (Dana 1997, p. 5), although movements beyond the study area were beyond the scope of the 1997 mark-recapture study (Dana pers. comm. 2013). Skadsen (1999, p. 2) reported possible movement of Dakota skippers in 1998 from a known population at least 800 m (2625 ft) away to a site with an unusually heavy growth of purple coneflower; he had not found Dakota skippers in three previous years when coneflower production was sparse. The two sites were connected by native vegetation of varying quality, interspersed by a few asphalt and gravel roads (Skadsen 2001, pers. comm.).

In summary, the best information we have suggests that dispersal of Dakota skipper is limited due in part to its short adult life span and single annual flight. The precise relationship between natural repopulation and the distance to the nearest extant population is unknown, but the species' extirpation from a site may be permanent unless it is close enough to a population that generates enough emigrants to repopulate the site or is the subject of a human-mediated reintroduction. Even sites greater than one km from another populated site likely face a reduced chance of recolonization.

B. Dakota Skipper Habitat Descriptions

Core habitat patches are areas that contain the vegetation and physical features that provide nectar, sites for oviposition, larval food, and shelter required by Dakota skipper during its life cycle. Dakota skipper occurs in two general core habitat types. 'Type A' core habitats consist of low wet-mesic prairie with little topographic relief that occurs on near-shore glacial lake deposits (Royer et al. 2008, p. 14-16). The second core habitat type, referred to as 'Type B' by Royer *et al.* (2008, p. 14), occurs primarily on rolling terrain over gravelly glacial moraine deposits and is dominated by little bluestem (*Schizachyrium scoparium*), needle or porcupine grasses (*Hesperostipa spp.*), sideoats grama (*Bouteloua curtipendula*), and prairie dropseed (*Sporobolus heterolepis*). Typically, 'Type B' habitats have generally sustained larger populations of Dakota skippers than 'Type A' habitats.

'Type A' Habitats

In the United States, Dakota skipper occurs in two general habitat types. The first is a low wet-mesic prairie with little topographic relief that occurs on near-shore glacial lake deposits – Royer et al. (2008, p. 14-16) (Figure 5). In the United States, 'Type A' Dakota skipper habitat occurs primarily in North Dakota, but it may also comprise a small amount of the species' habitat in northeastern South Dakota. 'Type A' habitat may be flooded in some years, but has "sufficient relief to provide segments of non-inundated habitat during the spring larval growth period within any single season" (Royer et al. 2008, p. 15; Royer et al. 2014, p. v). 'Type A' habitats are also found in the "Interlake" region of Manitoba, between Lakes Winnipeg and Manitoba.

Figure 5 - 'Type A' Dakota skipper habitat in McHenry County, North Dakota (Royer et al. 2014). Note the abundant mountain deathcamas (white flowers) and the scattered prairie lilies (orange flowers). A male Dakota skipper perched on a prairie lily in 'Type A' (USFWS photo).

'Type B' Habitats

The second Dakota skipper habitat type, referred to as 'Type B' by Royer *et al.* (2008, p. 14), occurs primarily on rolling terrain over gravelly glacial moraine deposits and is dominated by big bluestem, little bluestem, and needle or porcupine grasses (6). As in 'Type A' habitats, bluebell bellflower and prairie lily are present in 'Type B' habitats, but 'Type B' habitats support more extensive stands of narrow-leaved purple coneflower, upright prairie coneflower (*Ratibida columnifera*), and common gaillardia (blanketflower; *Gaillardia aristata*) (Royer et al. 2014, p. 1-2). Each of these flowers is a documented

nectar source for the Dakota skipper in 'Type B' habitats (McCabe 1981; Dana 1991). Little bluestem and porcupine grass (*Hesperostipa spartea*) are the predominant grass species in South Dakota 'Type B' habitats, but side oats grama, needle-and-thread grass (*H. comata*), and prairie dropseed are also typical (Skadsen 2006, p. 1-2). In a variant of 'Type B' habitats found in western North Dakota (Figure 6), western wheatgrass (*Pascopyrum smithii*) is also typical (Royer et al. 2014, p. 1).

Dakota skipper 'Type B' habitats typically support a high diversity and abundance of native forbs, including purple coneflower, purple prairie clover (*Dalea purpurea*), white prairie clover (*D. candida*), yellow sundrops (*Calylophus serrulatus*), prairie groundsel (*Packera plattensis*), groundplum milkvetch (*Astragalus crassicarpus*), eastern pasqueflower (*Pulsatilla patens*), old man's whiskers (prairie smoke, *Geum triflorum*), western silver aster (Symphyotrichum sericeum), dotted blazing star (*Liatris punctata*), tall blazing star (*L. aspera*), meadow zizia (heartleaf golden alexanders; *Zizia aptera*), blanket flower (Gaillardia sp.), prairie sagewort (*Artemisia frigida*), and leadplant (*Amorpha canescens*) (Skadsen 2006, p. 1-2). Prairie milkvetch (*Astragalus laxmannii* Jacq. var. *robustior*) also occurs in 'Type B' habitats in Minnesota (Dana 1997, p. 8).

In the rolling terrain of river valleys and the Missouri Coteau of North Dakota, on the western edge of the species' known range, Dakota skippers inhabit a variant of 'Type B' habitats. These habitats typically contain an association of little bluestem, big bluestem, and needlegrasses that is often invaded by Kentucky bluegrass (*Poa pratensis*) (Royer and Marrone 1992, p. 22). These prairies, also typically contain prairie lily, bluebell bellflower, coneflowers, and other asters as nectar sources; in some areas, mountain deathcamas also occurs (Royer and Marrone 1992, p. 22). 'Type B' habitats also occur in far southwest Manitoba and southeast Saskatchewan.

Figure 6 - 'Type B' Dakota skipper habitats in southwest Minnesota (upper left), northeast South Dakota (upper right), and western North Dakota (bottom). The site at upper left is The Nature Conservancy's Hole-in-the-Mountain Prairie Preserve, the reintroduction site addressed in this plan. Photos from USFWS and Royer et al. (2014).

C. Habitat Needs by Life Stage

Adults

"Regular access to nectar is clearly important" for adult Dakota skippers, most critically as a source of water and secondarily as a source of carbohydrates to support survival and reproduction (Dana 1991, p. 47). Adult Dakota skippers nectar on flowers "regularly throughout the day" and do not obtain water from mud, pond margins, etc. as do other skippers (Dana 1991, pp. 21; 48).

In Minnesota, Dana (1991, p. 50) found that almost all nectaring occurred in dry-mesic habitat. Dakota skippers relied mostly on four plant species that have "concealed" nectar that is available only to species with a "slender trophic apparatus" (e.g., proboscis) that is 5 mm or longer (Dana 1991, p. 48; Table 1). North of the range of the purple coneflower in Minnesota at Lake Bronson State Park, Dakota skippers used oval-leaf milkweed (*Asclepias ovalifolia*) and prairie milkvetch (*Astragalus adsurgens*) for nectar.

Table 1 - Four species used most as nectar sources by Dakota skippers in Minnesota 'Type B' habitats (Dana 1991). Number of nectaring observations by Dana: V = very common (many hundreds, not enumerated); C = common (about 35 visits); F = frequent (11-25); 0 = occasional (1-10); R = rare (2-4).

Species	Males	Females
Narrow-leaved purple coneflower (*Echinacea angustifolia*)	V	V
Prairie milkvetch (*Astragalus laxmannii* Jacq. var. *robustior*)	C	F
Hoary vervain (*Verbena stricta*)	O	F
Purple locoweed (*Oxytropis lambertii* Pursh var. *lambertii*)	F	R

The "standing crop" of nectar in the species used commonly by the Dakota skipper may be greater than in species that may be used by a wide range of nectar feeders (Dana 1991, p. 48). When favored species of nectar are unavailable, Dakota skippers may switch to less favored species that may produce less nectar or are accessible to a large number of other insects (Dana 1991, p. 48).

Dakota skippers use vegetation that rises above the grass canopy for reproduction and for unobstructed flight. In Minnesota, males typically perch on *Echinacea angustifolia* flowers 0.3-0.5 meters "above the grass canopy" and chase butterflies from perches, in pursuit of potential mates (Dana 1991, p. 21). The need for unobstructed flight from perches and larval habits (see below) may explain why Dakota skipper habitat is comprised primarily of mid-height grasses, such as little bluestem (*Schizachyrium scoparium*) and prairie dropseed (*Sporobolus heterolepis*), and why it is generally absent from grassland dominated by taller species, such as big bluestem (*Andropogon gerardii*).

Eggs

Habitat patches must be capable of supporting oviposition and must not be subject to intensive herbivory while unhatched eggs are present. Dana (1991, p. 50) found no evidence that oviposition occurred outside of the species' dry-mesic habitat in Minnesota. Females oviposited on plants "in the grass stratum" with little or no selectivity among plant species (Dana 1991, p. 14; 47). This lack of selectivity may be an adaptation to the ubiquity of the native grass species that function as larval food plants in high quality Dakota skipper habitat (see Larvae and Pupae, below). In Minnesota sites inhabited by Dakota skipper, Ottoe skipper (*H. ottoe*) larvae emerged from eggs laid on purple coneflower and dropped from the flowers into underlying grasses soon after hatching (Dana 1981, p. 77). The Minnesota Zoo has observed similar non-specific oviposition deposition in female Dakota skippers in open air mesh cages under *ex situ* conditions. Dakota skippers may behave similarly after hatching, although they may not oviposit frequently on purple coneflower (Dana 1991, p. 17). Removal of vegetation before oviposition or before hatching (e.g., by herbivory) may reduce or eliminate oviposition sites or destroy eggs (e.g., Lambert 2011, p. 97).

Larvae and Pupae

Larvae construct a series of shelters as they grow that are built from with plant material at or near the soil-surface interface and they pupate in similar shelters (Dana 1991, p. 16). Larvae require ready access to non-senescent tissue of food plants to develop through at least four instars before entering diapause (Dana 1991, p. 46). The bunchgrasses, little bluestem, prairie dropseed, and sideoats grama, provide Dakota skipper larvae with a dense cluster of erect blades in close proximity to "an abundance" of edible leaf tissue (Dana 1991, p. 46). The shelter-building habit of Dakota skipper larvae may render the native grasses big bluestem and Indiangrass (*Sorghastrum nutans*) unsuitable as larval hosts after spring and early summer due to the distance that would have to be travelled between shelters and palatable tissue and hairiness of stems that may hinder travel of the larvae along the stem, respectively. The Minnesota Zoo is currently studying the performance of Dakota skippers reared on seven potential host grasses (prairie dropseed, little bluestem, side-oats grama, big bluestem, porcupine grass, Kentucky bluegrass, and smooth brome) through larval no-choice experiments. This experiment will continue through 2017.

D. Climate

IUCN (2013:15) recommends that climate requirements of the focal species should be understood and matched to current and/or future climate at the destination site. This will be important for potential reintroductions for the species broadly throughout its historical range. Currently, this document only addresses the reintroduction to a single site. It is important to note that HIM is further south than any other known extant Dakota skipper site. This plan makes the assumption that climate change is not a contributing factor in the decline Dakota skippers. Climate change remains a poorly analyzed variable in Dakota skipper population trends and warrants further attention. In the next version of this document we will do the following, as adapted from IUCN (2013:15):

- Assess key climate parameters in the Dakota skipper's current and historical ranges, as appropriate, to estimate the breadth of climatic conditions potentially suitable for the species;
- Use the resulting bio-climate envelope in models of predicted climate change to assess how the Dakota skipper might respond to scenarios of future climate; Supplement the climate change modeling with a study of other factors that might determine habitat suitability and distribution, such as the presence of essential species and habitats, disease etc.; and,
- Determine whether the climate is predicted to remain suitable for the Dakota skipper for long enough to achieve the desired outcome for the species, in light of the uncertainties inherent in climate projections. Variables that will need to be addressed will include, but are not limited to, include: air temperature, microsite temperature and humidity in the duff layer and winter snowpack.

VII. Feasibility and Design

A. Selected Founder Populations and Genetic Considerations

The apparent extinction of Dakota skippers from all documented populations in southern Minnesota (including the Hole-in-the-Mountain complex) necessitates that any reintroduction efforts there must rely on populations from outside this historic metapopulation. Ideally, reintroduction programs should derive source individuals from populations as close geographically and ecologically to the extinct populations. Mixing contributions from multiple disparate source populations may result in reduction of fitness if genetically-mediated phenotypes are drawn away from locally-selected optima. Conversely, hybrid vigor may be produced if beneficial genetic variation that had been lost through genetic drift in small populations is reintroduced through deliberate mixing of lineages.

To date, the only published research on Dakota skipper population genetics and phylogeography is Britton and Glasford (2002). The researchers assayed allelic diversity at 21 isozyme loci from 278 individuals across nine populations from Minnesota, South Dakota, and Manitoba. Included in this study are populations that would likely serve as source ("Enemy Swim Lake", South Dakota) and destination (Hole-in-the-Mountain, Minnesota; now apparently extinct) for the reintroduction program discussed here. The Felton Prairie complex, which is perhaps the now only remaining viable Dakota skipper population in Minnesota and is also represented in the Minnesota Zoo's *ex situ* rearing and breeding program, was also included in the study. Low levels of genetic differentiation were observed globally, though there were statistically significant relationships of pairwise genetic isolation by distance between populations both globally and between the Minnesota and South Dakota populations. This pattern of increasing genetic differentiation with increasing distance between populations is consistent with expectations for species with historically large and continuous ranges (like prairie endemics). Due to modern habitat loss and the consequent interruption of historic gene flow between populations, genetic drift is likely the primary generator of modern differentiation between extant populations. The two Manitoba populations were moderately divergent from the seven studied U.S. populations, and some additional lower divergence was found between the southern Minnesota and South Dakota populations. Populations were also found to have low heterozygosity, suggesting inbreeding.

While the isozyme electrophoresis methods employed by Britton and Glasford were sound for their time, interpretation of these results requires some caution. Technological advances in molecular genetics in the years following publication of the study have dramatically improved analytical power,

particularly through "next generation" genomic sequencing. Modern DNA-based techniques provide significantly greater resolution than isozyme-based techniques. Isozymes are also more likely to be under selection than the presumably neutral genome-wide nucleotide polymorphisms revealed through modern DNA sequencing techniques. Therefore, patterns emerging from these two data sources can vary from each other. New research utilizing these modern next generation methods on Dakota skipper population genetic and phylogeographic are now underway, led by Dr. Emily Saarinen of the New College of Florida. Important to this work will be the incorporation of additional populations from across the range, including for the first time, populations from the western edge of the range in North Dakota.

Until this deeper understanding of the extant (and ideally historical) genetic diversity and divergence within and across populations can be developed (as well as other husbandry considerations), it is recommended that Dakota skipper reintroduction efforts to southwest Minnesota's Hole-in-the-Mountain utilize the nearest known viable extant populations, in northeastern South Dakota. These South Dakota populations are found on tribal Sisseton Wahpeton Oyate lands and also constitute the large majority of the Minnesota Zoo's existing rearing and breeding programs, and are also derived from ecologically comparable habitats to that of the Hole-in-the-Mountain Preserve.

B. Animal welfare

The welfare of all individuals (handled or otherwise) is of paramount concern. The imperiled state of all known remaining Dakota skipper populations requires that significant care be undertaken. Any handling of individuals must be done for deliberate reasons by trained personnel under explicit authorizations. As much as possible, field censuses of Dakota skipper populations should be conducted by visual counts. Specific protocols for the recommended operation are discussed below in Section VII.B of this document and can be found in more depth in the Minnesota Zoo's Hesperiid Husbandry Manual (available upon request).

C. Disease and parasite considerations

Larvae reared by the Minnesota Zoo and other potential institutions are done so under controlled conditions that are designed to reduce the risk of disease and parasite transmission. The full history of the Minnesota Zoo's rearing methods is outlined in annual reports. To date, no known diseases or parasites have been recorded in any of the Minnesota Zoo's rearing efforts with any skipper species. Larvae are either reared individually or at low densities amongst siblings on potted host grasses that have been enclosed in a fine mesh cage. Clean conditions are maintained, and Dakota skipper operations are also quarantined from parallel husbandry operations with Poweshiek skipperling, Garita skipperlings (*Oarisma garita*), and other grass skippers through the establishment of multiple hoop houses exclusively dedicated to each species. All equipment used to handle Dakota skippers (forceps, paintbrushes, etc.) are disinfected with 20% bleach solution, or ethanol between specimens. No equipment is shared across species of butterflies. Protocols are also established so that, within a given day, Zoo staff care for Dakota skippers before other less endangered butterfly species to further minimize the risk of transmitting disease to threatened species.

Dakota skipper collection and rearing protocols mitigate the risk of unintentional introduction of parasitoids when individuals are released. At the Minnesota Zoo, all individuals are double contained at all times. Larvae and adults are housed inside fine nylon mesh cages that exclude parasitoids such as Tachinid flies, and Braconid and other small wasps. Eggs are obtained either through breeding at the Minnesota Zoo or from wild females temporarily held under controlled settings. These measures

eliminate potential parasitoid encounter. In the event that a zoo-reared Dakota skipper was found, or suspected to be parasitized, it would be excluded from any potential release and quarantined from other Dakota skippers.

D. Social feasibility

For the Dakota skipper reintroduction program to be successful, it must be socially feasible and take into account the priorities and agendas of various stakeholders. For this plan, we will use the following definition for stakeholder - one who is involved in or affected by a course of action.

The known or potential stakeholders that we have identified thus far are:

- U.S. Fish and Wildlife Service
- Minnesota Zoo
- Sisseton-Wahpeton Oyate Tribe
- South Dakota Department of Game, Fish, and Parks
- Minnesota Department of Natural Resources, Division of Wildlife
- Minnesota Department of Natural Resources, Division of Ecological and Water Resources
- The Nature Conservancy
- City of Lake Benton, Minnesota
- Lincoln County Parks
- Minnesota Department of Transportation
- Lincoln County Highway Department
- Lincoln-Pipestone Rural Water
- DM&E Railroad (owner of electrical transmission line) and, private landowners immediately adjacent the reintroduction site

We will ensure that each of these stakeholders has at least a general understanding of our plans and has access the plan's details and associated documents (e.g., permits). In addition, we will seek their comments on our plan and ask them to tell us of any concerns that they might have related to the plan. We will attempt to resolve any concerns, as feasible.

A local awareness campaign has already begun in the city of Lake Benton. Minnesota Zoo staff have been reaching out directly to local land managers that manage land immediately boarding HIM. Zoo staff have begun sharing reintroduction plans with Lake Benton city council members. The Minnesota Zoo is also planning multiple public presentations at the Lake Benton Public Library in 2017. The intent of these outreach activities is predominantly to generate local awareness about the plight of the Dakota skipper and inspire people to care. It is a goal to dispel/ mitigate any local misconceptions about the Dakota skipper listing and make ourselves available for questions. The purpose of this campaign is not to have local land mangers change their agricultural practices to better accommodate Dakota skippers. Though some agricultural practices may be harmful for Dakota skippers, such as unintentional pesticide drift (see Section IV.C), sufficient data is lacking to recommend changes land management procedures in

agricultural spaces. At this time the Dakota skipper working group is pursuing a 'knowing is caring' approach.

According to IUCN guidelines, successful translocations must not only contribute to conservation of the intended species, but any gain from this conservation must be balanced against potential "collateral harm to other species, ecosystems or human interests" (IUCN 2013). Potential risks that may occur (biological, financial, socio-economic, and others) are discussed further in the Risk Assessment section of this propagation plan. However, at this time, we do not anticipate any negative consequences of *ex situ* management that would outweigh the conservation benefits for Dakota skipper.

E. Regulatory compliance

Federal Endangered Species Act Compliance

The Endangered Species Act, 1973, as amended (16USC 1531 et seq.) (ESA) makes it unlawful to take (includes harm, harass, pursue, hunt, shoot, wound, kill, trap, capture, or collect any wildlife within the United States), possess, ship, deliver, carry, transport, sell, or receive unlawfully taken wildlife. These prohibitions apply to live or dead animals, their progeny and parts or products derived from them. Some activities are allowed in accordance with permit provisions. For protected species, like the Dakota skipper, permits may be issued for scientific research, enhancement of propagation or survival, and taking that is incidental to an otherwise lawful activity.

The Minnesota Zoo holds a Section 10(a)(1)(A) Threatened and Endangered Species Recovery Permit (#TE64079B-1, expires on 12/31/2017) from the Service authorizing the handling and collection of a limited number of eggs from a limited number of temporarily held female Dakota skippers. We anticipate that this permit will be renewed and amended, as necessary to ensure that the goal of this plan is achieved.

On May 2, 2016, the U.S. Fish and Wildlife Service's (Service) finalized a revised Intra-Service biological opinion (BO) on the effects of issuing section 10(a)(1)(A) scientific research permits to personnel conducting surveys for the Dakota skipper in Iowa, Minnesota, North Dakota, and South Dakota and for captive rearing efforts for the species. The biological opinion was prepared in accordance with section 7 of the ESA and replaces the original opinion for the subject actions, which was completed on June 16, 2015. The Service will update and revise this biological opinion, as needed, to ensure that the issuance of section 10(a)(1)(A) permits is carried out in compliance with ESA section 7.

NEPA Compliance

The National Environmental Policy Act (NEPA) was enacted to facilitate national policies protecting the environment and ecological processes and provide means to carry out these policies. The captive rearing of the Dakota skipper is completely covered by a categorical exclusion contained in 516 DM 6, Appendix 1 (Federal Register 1997 62:2375-2382). The applicable categorical exclusion applies to the reintroduction or supplementation (e.g., stocking) of native, formerly native, or established species into suitable habitat within their historic or established range, where no or negligible environmental disturbances are anticipated. This categorical exclusion precludes the need to prepare an environmental assessment for this project.

USDA/APHIS Compliance

The Minnesota Zoo holds a required Interstate Movement Permit from the U.S. Department of Agriculture Animal and Plant Health Inspection Service for "Live Plant Pests, Noxious Weeds, and Soil". All live phytophagous insects are classified as potential "plant pests" by the USDA, and intentional movement across of them between states is regulated, regardless of their Service or State listing status. This permit (P526P-15-02728) was issued June 11, 2015 and is valid through June 4, 2018. An additional USDA permit (P526P-17-01270; issued April 4, 2017, expired April 4, 2020) allows for the release of zoo-reared individuals originating from South Dakota to be released in Minnesota. Individuals derived from within state boundaries do not require additional USDA permitting. In the future, founders may be acquired from North Dakota or Manitoba, but an amendment to existing permitting would be required before releases of those individuals outside of their founder jurisdictions could be conducted.

State Compliance

The Minnesota Department of Natural Resources issued the Minnesota Zoo a state permit in 2016 authorizing the collection and holding of Dakota skipper at their facility. This permit will need to be renewed and updated to allow for the Dakota skipper reintroduction program. All landowner permissions will be obtained prior to accessing lands. Additional permits from other agencies will also be secured as necessary.

F. Resource availability

The Minnesota Zoo has established personnel and infrastructure capacity to perform the captive rearing and breeding program. A dedicated rearing chamber (termed "The Chrysalis") and an attached greenhouse were constructed in fall 2015. These double-contained, clean, climate-controlled facilities are exclusively dedicated to the Prairie Butterfly Conservation Program. In addition to work tables, the chamber also houses a large laboratory hibernation freezer that safely mimics the sub-freezing conditions that wild hibernating caterpillars naturally experience under winter snow.

The Minnesota Zoo maintains three open air hoop houses with built-in containment capabilities for this program. Each of the three hoop houses is dedicated exclusively to a given species: 1) Dakota skippers and their host plants, 2) Poweshiek skipperlings and their host plants, and 3) Other surrogate skipper species (like Long Dash, *Polites mystic*) and their host plants. These lockable hoop houses are solely designated outdoor rearing and husbandry space for the Prairie Butterfly Conservation Program. The footprints of the hoop houses are wrapped in 3-foot high sheet metal to prevent access from mice and other small vertebrates that may eat plants and/or butterflies. The upper portions are wrapped in durable fine-mesh outdoor screening that excludes insect predators and parasitoids. Each hoop house contains work tables and a waterline, and is partially wrapped with clear plastic for protection from rain/hail. The screen for these hoop houses is not snow-bearing, so it is peeled back in late autumn prior to the first snow. All caterpillars kept in this facility are transferred to double containment hibernation containers prior to screen removal.

The Minnesota Zoo's Prairie Butterfly Conservation Program has two full-time personnel. Dr. Erik Runquist (Butterfly Conservation Biologist) is the Program manager, and Cale Nordmeyer (Butterfly Conservation Specialist) coordinates and conducts much of the husbandry operations. The Zoo plans to hire additional seasonal staffing to assist in routine husbandry operations as necessary during the summer of 2017 and beyond as funding allows.

G. Disaster preparedness

Equipment failures, human error, and other potential catastrophic events have the potential to cause the loss of some or all of the individuals being held or maintained in captivity. The maintenance of Dakota skipper at more than one facility would reduce the potential extent of adverse effects that could occur as a result of a catastrophic event at any single facility. At present though, no other facilities are prepared to maintain *ex-situ* populations of Dakota skipper. Limiting the number of facilities reduces the potential for human error and other issues which may result from the involvement of unqualified personnel. The Minnesota Zoo staff is trained and experienced in the care and handling of skippers. As discussed above, the Zoo maintains separate and dedicated facilities for Dakota skipper to prevent the spread of disease. Skippers are always maintained under at least double containment conditions in all facilities to prevent individuals from escaping confinement and to exclude enemies. All facilities where skippers may be held have been equipped with remote temperature and humidity monitors that automatically notify Zoo staff via cellular networks when conditions vary outside of predetermined tolerance thresholds so that appropriate remediation operations can be promptly undertaken. The Minnesota Zoo also possesses backup generators that may be used in the event of a power outage, but under most circumstances, a power outage represents minimal threat since the majority of larval rearing operations are likely to occur outdoors. Unauthorized disturbance of animals at these facilities is prevented by restricted and secured access.

In accordance with institutional and U.S .Fish and Wildlife Service policies, the Minnesota Zoo's Prairie Butterfly Conservation Program has developed a disaster preparedness plan (available upon request). It details critical activities that would take place and which staff would be responsible for those activities in the event of fire, high winds, severe weather, reduced workforce (e.g. pandemic or other personnel crises), and power outages. These plans are broken down by which portion of the Dakota skipper lifecycle may be affected when the emergencies might occur (two "Active Periods" = May through October, and "Diapause Period" = late October through April).

VIII. Risk Assessment

A. Risk to source populations

Removal of natural parental stock may result in an increased risk of extinction by reducing the abundance of wild individuals and reducing genetic variability within naturally occurring populations. To minimize this risk, protocols are in place to prevent over-collection at a site. As outlined in the Service Recovery Permit issued to the Minnesota Zoo, Dakota skipper adult females may be temporarily held for egg collection only after a minimum of 25 individuals are observed at a site within a 24-hour period during the yearly flight. This 25-individual minimum threshold was subjectively established in early permits issued to the Minnesota Zoo in 2013, and was designed to allow eggs to be collected only from relatively large populations. It is an estimated number based on survey data, in that at least 25 individuals can be regularly encountered within a day at the best and apparently most stable populations of Dakota skippers. Even if this threshold is met, however, only 10 females may then be used for egg collection from any single site. Each female may be held, using approved protocols, for up to 72 hours and those females must be cared for by trained staff. These parameters establish a minimum floor that excludes smaller and potentially less stable populations, and are meant to ensure that source populations are robust enough to not be substantively harmed by the removal of a limited number of eggs. When eggs from wild gravid females

are removed to controlled enemy-free *ex situ* conditions, eggs may be reared to larval or pupal stage at significantly higher survivorship rates than those expected in the wild.

The health of the temporarily held wild females is absolutely central to egg collection protocols. Female grass skippers (especially Dakota skippers) that have been returned to the habitats in which they were found within 48-72 hours of their removal have only very rarely shown signs of ill effects using current methods (Runquist, pers. comm.; Runquist 2012, Runquist 2013, Runquist and Nordmeyer 2014, Runquist and Nordmeyer 2015). Females are to be released under calm, cool weather conditions in the earlier morning and placed directly from their temporary holding vials onto a preferred nectar source (especially *Echinacea*, Table 1). This maximizes the likelihood that the females will resume normal behavior and "stay" in their original, and presumably preferred, habitat.

We expect egg removal from source sites to be minimal relative to the number of eggs that will be laid by females in the respective wild populations. The number of eggs removed from any single wild population of Dakota skipper may approach 300, but is likely to be less than 15% of all eggs that would be laid in the population. Female Dakota skippers may lay about 15-20 eggs per day (Runquist and Nordmeyer 2014, Runquist and Nordmeyer 2015) and maximum potential fecundity is "probably between 180 and 250 eggs" (Dana 1991). Female Dakota skippers held for a maximum of 72 hours may lay about 30 eggs for use in captive rearing – or about 16% of each female's potential fecundity if we assume she might be able to produce 180 eggs under ideal conditions. As stated earlier, no females may be captured for egg collection unless at least 25 individuals are found within a 24-hour period. We assume that at least twelve females will be observed before any are captured if sex ratios are approximately 1:1. If we assume that ten females held for egg collection (as permitted), and that these ten are different from the 12 seen during the initial survey, there may be at least 22 females present at any site where egg collection takes place. If we conservatively assume an expected fecundity of 90 eggs per female– approximately half of the species' assumed potential fecundity of 180 eggs – due to early death and other factors, total fecundity of these 22 females may be 1,980 eggs. If 300 eggs of these are removed for captive rearing (from the 10 held females), it would reduce total oviposition in the wild by about 15%. It is actually likely to be less than 15% because affected populations are likely to include more than 22 females and the number of females captured is likely to be lower than the maximum of ten that would be permitted – in 2014, for example, an average of six female Dakota skippers were captured for egg collection at five sites. If we alter the assumption that the ten females held for egg collection were different from the 12 observed during the initial survey and instead assume that all ten of the females held for egg collection were members of the initial 12 observed during the survey, then the total egg production of the population would be lowered to 1080 (12 females x 90 eggs/female), with a consequent change in the percentage of the population's eggs collected rising to 27.8% (300/1080). Regardless of the total population size though, based on an estimated 3% survival from egg to adult in the wild (see below), each population would be reduced by about nine adults as a result of the permitted collection of 300 eggs.

Survival of *ex situ* reared Dakota skipper has been measured across four distinct stages: 1) neonate to larval diapause; 2) larvae during winter diapause; 3) larvae from end of diapause to pupation; and, 4) pupal stage to adult. In 2015, 82% of the 432 eggs collected in the wild developed and hatched. (Runquist and Nordmeyer 2015, p. 6). Combining wild-collected neonate caterpillars and those bred at the Zoo in 2015 (n=604), 40% eclosed as adults in 2016 (Table 2).

Table 2- Survival of Dakota skippers during successive life stages at Minnesota Zoo, 2015-2016.

Stage	Estimated Survival	Basis for Estimated Survival (Year/Period)	Cumulative Survival from Egg to End of Stage
Neonate to Larval Diapause	67%	2015	67%
Larval Diapause	77%	2015-2016	51%
Diapause to Pupation	80%	2016	41%
Pupation to adult	96%	2016	40%

Based on these results with the Dakota skipper, about 60% of the Dakota skippers that are collected as eggs may die before reaching adulthood. This level of mortality is likely much less than what Dakota skippers would likely experience in the wild. A wide variety of factors may kill butterflies during each life stage, including drowning or physical damage due to flooding; predation; ungulate herbivory; and, parasitoids (Benrey and Denno 1997; Borkin 2000; Severns *et al.* 2006, p. 368; Lambert 2011). Survival in the wild from egg to pupation of one rare butterfly, for example, was 3% based on a sample of 1,617 eggs (Lambert 2011, p. 110). Studies reviewed by Nail *et al.* (2015) predicted survival rate of monarchs (*Danaus plexippus*) from egg to adult was about 4.2 to 9%, but this is likely an overestimation of survivorship to adulthood since the pupal stage was raised in captivity and not exposed to some important mortality factors.

For the foreseeable future, we anticipate that no more than 900 Dakota skipper eggs will be collected in any single year; that is, 300 eggs/site from up to three sites per year. If the above mortality rates found for the Dakota skipper at the Minnesota Zoo occur, this would result in the production of about 360 Dakota skipper adults (but note, actual release is likely to occur during the pupal stage). In contrast, if we assume a 3% survival rate from egg to adult in the wild, only 27 Dakota skipper adults would be expected from these same eggs if left in the wild. Thus, if survivorship observed in other butterflies is transmissible to Dakota skippers, we anticipate our collection protocol to be minimally invasive while still having significantly higher survivorship than those in the wild.

Effects of Netting Wild Adults for *Ex Situ Operations*

The act of hand-netting adults for egg collection incurs risks of sub-lethal, or lethal injuries (USFWS 2016). In 2015, 42 Dakota skippers were captured and no injuries were reported. The health of collected adults will continue be reported as outlined in USFWS permits.

It is unclear whether netting and releasing Dakota skippers could have significant adverse effects on post-release behavior and survival. In 2015 we began to collect information on behavior of Dakota skippers immediately after release. Data from 2015 is not conclusive, but do not suggest any significant effects to behavior of Dakota skippers related to netting and release (Table 3). It is assumed that post-release dispersal into atypical habitat (like into “Tall Shrubs or Trees”, below) or over long distances that prevent identification of perching substrate (“Flew Away” or “Unknown”, below) is detrimental in that individuals may land in areas that are not conducive to the health of the individual or the population. We

will continue to compile data on the initial post-release behavior of any Dakota skippers netted and released.

Table 3 - Post release behavior of Dakota skippers captured and released in 2015. Surveyors were asked to report post-release behavior as: Flew to and perched on herbaceous vegetation, low shrubs, or to out-of-sight location in herbaceous vegetation.

Source	Number Netted & Released	Post-Release Behavior					Comments
		Flew to Perch in Herbaceous Vegetation or Low Shrubs	Flew Into Tall Shrubs or Trees	Flew Away	Did Not Disperse	Un-known	
Stegeman (2016)	1	1	0	0	0	0	
Selby (2016)	13	2	0	11	0	0	Two that flew away first perched on the net and/or a finger.
E. Runquist, pers. comm. 2016	20	7	0	0	13	0	All individuals were released gently from vials directly onto *Echinacea* in the cool morning hours. Thirteen stayed directly on the *Echinacea* flower to nectar and did not fly away. The remaining 7 flew a short distance (≈10 m) into grass or to another *Echinacea*.
C. Nordmeyer, Minnesota Zoo, pers. comm. 2016	7	0	0	2	3	1	All individuals were released gently from their tubes directly onto *Echinacea*. Three stayed on the *Echinacea* flowers; two flew away to an unknown location; one died before release that was "quite old" when captured, with faded ragged wings and a skinny abdomen. She did not lay any eggs in captivity and likely died of old age, not due to any causes directly related to handling.

B. Ecological Risk

Ecological concerns associated with the Dakota skipper reintroduction program can be partitioned between 1) the source populations and 2) the site of reintroductions. First, as discussed above, removal of eggs from the source populations may diminish the health of the source populations if conducted in an unsustainable manner. The actual removal of the eggs (and therefore ultimately adults) from the source environment however is not thought to be substantially disruptive to general ecological process in the source environment. We will minimize the risk of diminished survival and reproduction by ensuring that state-of-the-art methods will be used throughout the *ex situ* process by the Minnesota Zoo.

Second, the ecological risk of reintroducing Dakota skippers into sites at which they are no longer present are also minimal. Dakota skippers presumably occupied the reintroduction site(s) for significant periods of time before their recent extirpation. Furthermore, stochastic events (such as large wildfires or

extreme weather events) likely extirpated Dakota skippers from historically occupied sites like the Greater HIM complex in the past, but the region could be re-colonized by dispersal from connected populations. The modern fragmentation of the suitable habitat precludes natural recolonization of the Greater HIM complex, and reintroduction is likely the only mechanism by which Dakota skippers will return to the area. The sites of origin and the reintroduction site also must necessarily be ecologically similar to each other for reintroduction efforts to be successful. Therefore, the likelihood that the individuals released or their progeny will have deleterious or undesirable effects on other species or ecosystem functions is likely discountable. However, reintroduction efforts may require some alterations to habitat management plans to promote the conditions Dakota skippers need, and those conditions may not necessarily be optimal for other species presently or potentially occupying the same habitat.

Bailey's Provinces reflect similarities in "vegetational macrofeatures" and climatic characteristics (Bailey 1995, p. 2). The vegetational macrofeatures used to distinguish the Provinces inhabited by the Dakota Skipper include the relative amounts of short-, mid-, and tallgrass prairie; and, the amount and relative dominance of forest and grassland. Climatic differences among provinces that are likely to be related to adaptations in the Dakota skipper include the degree of aridity; frequency of drought; and, variation in temperatures. Having similar ecological features with the sites of origin is likely to minimize the risk that survival and reproduction of released Dakota skippers will be lower due to their poor adaptation to the characteristics of the reintroduction site. The gg source and reintroduction sites are both in the same Bailey's Province (Figure 7).

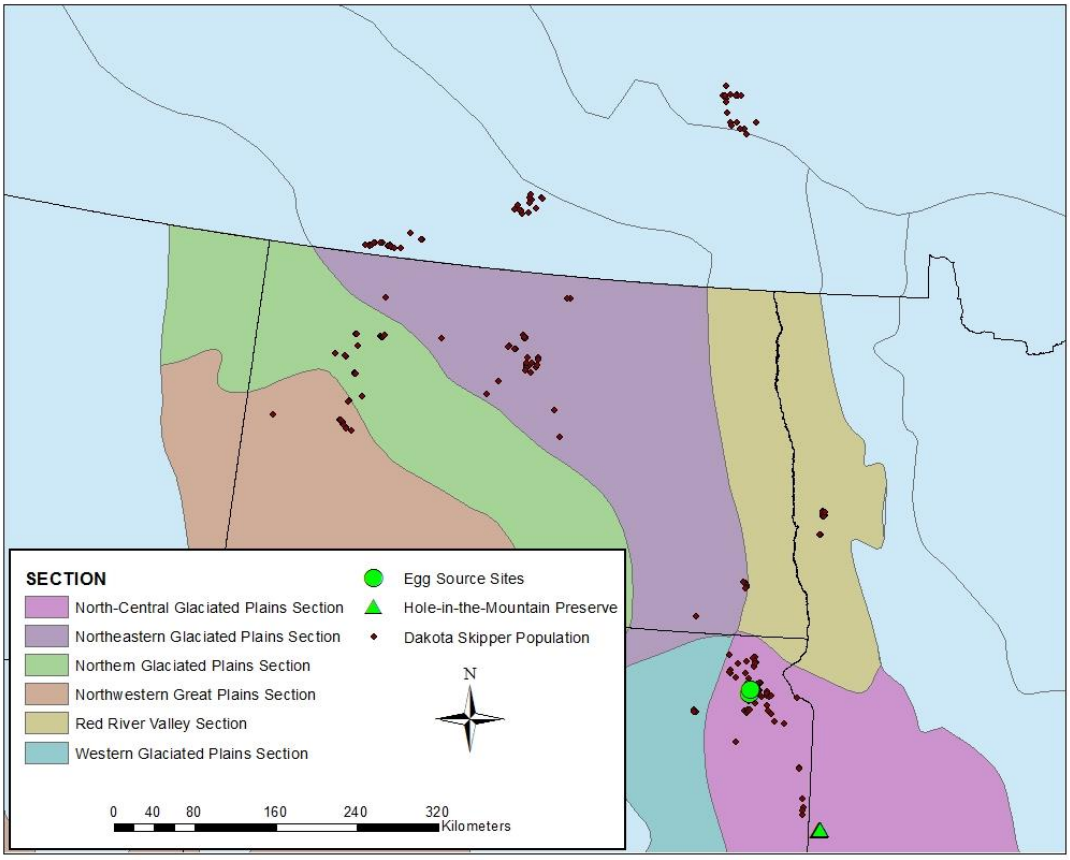

Figure 7 - The locations of the egg source sites (circle) and the reintroduction site (triangle), relative to Bailey's ecological Sections (Bailey 1995). The egg source sites and the reintroduction site are each in Bailey's North Central Glaciated Plains Section.

At an even finer level of resolution in the classification hierarchy, the source sites and reintroduction site may be separated into two subsections - the Outer Coteau des Prairie (northeast South Dakota) and the Northwest Iowa Plains (Hole-in-the-Mountain). Subsections are delineated primarily with the use of soil and surficial geology criteria (McNabb et al. 2007, p. 1). Primary nectar flowers and native grasses that skippers may use as larval foods appear similar between sites. Purple coneflower is the primary nectar species at both sites, where habitats fit the description of 'Type B' Dakota skipper habitat (Royer et al. 2008).

The Nature Conservancy owns and manages the reintroduction site and is an integral partner in the development of plans to carry out the proposed reintroduction. TNC has already provided key information to identify the portions of their Hole-in-the-Mountain Preserve where a primary nectar source, purple coneflower, is especially abundant. Moreover, TNC has prioritized the specific area where reintroduction is likely to occur for a burn in 2017 that would occur before the release and that is likely to further enhance its ability to support Dakota skippers. Prescribed burns are known to promote coneflower blooming (a known concentrator of Dakota skipper adults) and to reduce invasive plants.

TNC has indicated a willingness to cooperate on efforts to minimize stressors to the Dakota skipper. Fire management is likely to be a key issue. Partitioning the habitat into smaller areas so that only relatively small sections of habitat occupied by the skippers are burned at any one time, and rotating burn treatments across multiple years will likely be essential to ensure that the population is able to grow in spite of mortality in the burned areas. That is, that the level of mortality is offset by movement of Dakota skippers back into the burned area.

Another possible stressor to both source and reintroduced populations of the butterfly is the potential for drift of pesticides from nearby agricultural operations. Pesticides were detected in samples collected by the Minnesota Zoo at HIM in August 2016. This sampling occurred during the known aerial application season of insecticides to control soybean aphids, a severe invasive pest on adjacent agricultural fields. Grass and soil samples were collected from six points within the HIM preserve. Relatively low levels of the organophosphate insecticide, chlorpyrifos, were found at three of them (5.5, 9.2, and 13.5 parts per billion). These points were not clustered geographically within the preserve. No other insecticides were detected in any of the samples. This pattern of low levels of broadly scattered chlorpyrifos detection is comparable to concurrent observations at nearby Prairie Coteau SNA and other prairies farther north in August 2016 and also in prior years. Two pyrethroid insecticides, bifenthrin and lambda-cyhalothrin, that are also commonly applied against soybean aphids, have also been found in within federally designated Dakota skipper critical habitat in these surveys. Additional details are summarized in Runquist and Heimpel (2017). It is not known whether these levels - found on grass species used as larval food by the Dakota skipper - would harm the species. This uncertainty has resulted in recommendations to conduct insecticides exposure experiments with grass skipper larvae of common species to estimate the consequences of these exposures to wild skippers (Delphey *et al*. 2016, Runquist and Heimpel 2017). Agricultural land is nearby and the presence of the pesticides suggests the potential for drift to affect reintroduced Dakota skippers and any wild progeny. We may seek avenues to reduce the potential for drift by working cooperatively with nearby agricultural landowners. Minnesota Zoo staff will continue sampling and conducting pesticide residue analysis at the release site in both the early and late summer for the foreseeable future, with this data informing reintroduction plans and operations as appropriate. The initial release location within the Central Unit of HIM (Figure) was selected partly on the basis of it being distant from agricultural edges and the presence of ridges that may act to shield specimen from pesticide drift on the prevailing winds.

C. Disease risk

The motivation for the reintroduction program for Dakota skipper is to expand the global population of the species by reestablishing extirpated populations. Many factors have likely contributed to those extirpations, and those factors are expected to vary and interact differently at local levels. Habitat loss and fragmentation is likely the primary driver of population loss, but other intrinsic factors such as disease could have contributed. Viral, bacterial, or fungal diseases and parasites and parasitoids that are specific to Dakota skippers are not known. This lack of understanding of Lepidopteran pathogens is one of the key reasons it was decided to conduct releases at a known extirpated site. The risk of disease infection of Zoo-reared individuals cannot be fully precluded, but it can be minimized by through strict clean protocols and quarantine procedures. Any individual that appears to be in sub-optimal health will be removed from the release program and potentially euthanized if deemed necessary. Disease outbreaks in ex situ Lepidopteran programs often take the form of mass population mortality. In the event of unexplained mass population mortality of zoo-reared larvae the situation would be assessed and any releases would likely be forgone for that generation.

Beyond diseases associated with viral, bacterial, and fungal infections that may be mitigated by clean practices, infection from *Wolbachia* is a concern. It has been recommended that all Lepidopteran *ex-situ* programs consider *Wolbachia* (Hamm *et al.* 2014). This maternally-inherited intracellular bacteria is a common symbiont across insects (Hilgenboecker *et al.* 2008), including many imperiled Lepidoptera (Hamm *et al.* 2014). It can have significant impacts on population demographics if it is introduced to uninfected populations or if infected populations become infected with novel strains. These impacts can include killing of males, sterilization of males via cytoplasmic incompatibilities, feminization of males, parthenogenesis, or other effects (Werren *et al*. 2008).

As noted earlier, population genetics studies are underway by Dr. Emily Saarinen (New College of Florida) and Minnesota Zoo staff, and this work includes assays for the presence of *Wolbachia* in Dakota skippers from several key populations. If found to be present, a pattern of uniform infection within and across populations is expected for this formerly wide-ranging species. Such a pattern generally results from a historic infection that has stabilized and no longer presents a demographic risk to the population(s) (Hilgenboeker *et al*. 2008, Nice *et al.* 2009). *Wolbachia* infection status should not by itself be a constraint to translocate individuals or their genes between populations. For example, translocation is not considered problematic if all populations are uniformly infected with the same strain of *Wolbachia*. Ultimately, any *Wolbachia*-induced phenotypes in their host butterflies cannot be known without detailed experimental crosses. Until *Wolbachia* strain identification is completed with Dakota skipper egg collection sites, geographically distant populations will treated as though they are incompatible. In the initial 2017 planned release at HIM, only specimens representing crosses or recently collected individuals from metapopulations in northeastern South Dakota will be released, to avoid these risks. If multiple *Wolbachia* strains are later identified across sites, rigorous, multiyear crosses would need to be conducted in the lab to determine if negative effects of those crosses exist. *Wolbachia* is also only one of several other microbes that are known to alter reproductive dynamics in arthropods (Duron *et al.* 2008). The status of these organisms in Dakota skippers is unknown, and protocols to isolate and identify them are not well established as they are with *Wolbachia*. Given the isolation of HIM from other known Dakota skipper sites, there is reduced risk of reintroduced Dakota skippers having a negative effect on known extant populations if *Wolbachia* infection status or strains were to differ.

D. Associated invasion risk

With any reintroduction, care should be taken to ensure that potentially invasive species are not accidentally released, along with the focal species. The small number of Dakota skipper larvae or pupae that may be released requires that only small containers holding each individual be brought into the field. Any live plant material associated with releases would be only be native to the site, so that even if some were to be accidentally introduced to a site it would already be a constituent species of the site.

E. Gene escape and population genetic consequences

"Gene escape" refers to the potentially negative consequences of mixing historically isolated populations, or of hybridization with closely related species or subspecies. There are other grass skipper species that would be sympatric and synchronic with Dakota skippers at HIM. Long Dash (*Polites mystic*) and tawny-edged skipper (*P. themistocles*) are likely two of the closest living relatives of Dakota skipper that still occur at the site and have overlapping flight periods. The congeneric Ottoe skipper (*Hesperia ottoe*), once occurred at HIM but is now likely extirpated as well. There is no evidence that Dakota skippers can hybridize with any other skipper species. It should also be noted that Dakota skippers only became extirpated from the site relatively recently. Reintroducing Dakota skippers back to HIM is expected to have no negative genetic impacts on related species.

Other population genetic consequences, however, may be relevant to this planned Dakota skipper reintroduction. A central consideration of any *ex situ* program in which partial or complete generations are maintained under artificial conditions is the selection of phenotypes that are maladaptive under *in situ* conditions. This can have significantly negative consequences for any augmentation or translocation program by reducing the viability of the destination populations (e.g. Nichols and Pullin 2000; Crone *et al*. 2007). Captivity may also reduce the apparency of the accumulation of these negative effects, and these effects compound the longer individuals and generations are reared under captive conditions (Joron and Brakefield 2003). Rearing practices at the Minnesota Zoo have been developed to mitigate artificial selection of captive Dakota skipper stock. Larvae are reared *ex situ* using methods that mimic "wild" conditions to the greatest extent as is possible (i.e. outside under regular temperature ranges, on potted host grasses), while still protecting them from most dangers. Once releases begin, no lineage of Dakota skippers will be kept in captivity for more than three generations as adopted from the Oregon Zoo's Taylor's Checkerspot protocol (Lewis, 2014 pers. comm.). Releases will comprise both Zoo-bred individuals and head-started individuals (collected as eggs the year before). Zoo-reared adults are measured (under anesthesia, while being individual marked for breeding cages) and their morphometrics are compared to wild-collected museum specimens (wing length and width). If wing aspect ratio, or other physical characteristics appear distorted among a particular lineage at the Zoo, those lines would be barred from release.

F. Socio-economic risks

The reintroduction of Dakota skippers back to suitable habitat is unlikely to have any direct effects on people and livelihoods, as there are no currently known direct negative impacts. However, there may be some perceived danger of increased regulations by releasing a threatened species back into the wild, particularly if we begin reintroductions to sites where the Dakota skipper is currently extirpated. Dakota skippers do not persist outside of high quality native prairie habitat, and are not expected to disperse into suboptimal habitat. For example, Dakota skippers would not inhabit cropground or non-native hayfields (e.g., alfalfa fields) and a special rule exempts any take of the Dakota skipper that would occur as part of

routine livestock ranching activities. The current reintroduction would directly affect only a single landowner - The Nature Conservancy – which has expressed support for the species' conservation on the site. Additionally, we do not anticipate any indirect harm (e.g., actions that could threaten ecosystem services) through our work at these Dakota skipper sites. The only indirect ecological effect that we anticipate is some light trampling of habitat while capturing and releasing Dakota skippers. Care will be used to minimize damage by training all individuals conducting field work.

In addition to direct and indirect effects of the reintroduction itself, there may be some persons who disagree with the conservation benefits of removing Dakota skippers from source sites for *ex situ* management. The current source site landowners have been part of ongoing discussions regarding the *ex situ* programs and have so far been supportive of conservation efforts. Landowners of areas that may be considered in future years will be engaged early in future discussions. In order to ensure continued support and to address any future concerns of interested parties, we will put into place the communication and outreach plans highlighted in the "Social feasibility" section of this document. These efforts will also improve our understanding of public perceptions, and provide a venue to address concerns (see Section X.F).

G. Financial risks

The Minnesota Zoo's Prairie Butterfly Conservation Program is currently exclusively supported by a collection of short-term grants from various federal, state, nonprofit, and private sources that provide all of the Program's routine staff payroll and operational costs. The primary funding source has been Minnesota's Environment and Natural Resources Trust Fund (ENRTF), as recommended by the Legislative-Citizen Commission on Minnesota Resources. Key support has been also provided through grants to the Zoo from the Minnesota's Legacy Amendment Arts and Cultural Heritage Fund, and through interagency Cooperative Agreement Grants with the U.S. Fish and Wildlife Service. The Minnesota Zoo provides office and facilities space, and covers administrative, maintenance, and utilities costs. This relative independence from the Zoo's general operating budget provides a buffer against fluctuations in the Zoo's budget, but also provides uncertainty in the long-term. The grants currently held have varying expiration dates between 2017 and 2019. Institutionally, the Minnesota Zoo administration is supportive of the Program as it matches the Zoo's mission "to connect people, animals, and the natural world to save wildlife". Perhaps the biggest hurdle to the long term expansion of the Prairie Butterfly Conservation Program is sufficiently trained full-time staffing, particularly during the high-intensity summer months when husbandry operations at the Minnesota Zoo are simultaneous with the flights of Dakota skippers, Poweshiek skipperlings, and other species of interest. The ENRTF, Service, and Legacy funds have and will continue (at least in the short-term) to pay for summer seasonal staff.

The U.S. Fish and Wildlife Service's Twin Cities Ecological Services Field Office (TCFO)[1] allocated funds that are used to support salaries of Service personnel to plan and coordinate captive rearing activities; ensure regulatory compliance with provisions of the Endangered Species Act and with Service policies; and, to assist with field activities, as needed. If the use of TCFO-allocated funds to support staff time on this project no longer occurs, it would be difficult to carry out the program in a manner necessary to meet project objectives. The amount of funds allocated to TCFO may vary from year to year and their use is at the discretion of the TCFO project leader. Therefore, a significant reduction in funds allocated to TCFO or a change in priorities of the project leader could have a significant adverse effect on the likelihood of project success. Neither of these, however, seems likely at this time.

[1] Recently renamed the Minnesota/Wisconsin Ecological Services Field Office.

A critical component of the plan is routine and standardized quantitative surveys for adult Dakota skippers at the reintroduction site (section X.A), both the summer(s) of release(s), and in the years following cessation of any reintroductions. Evaluation of program success depends on rigorously collected data over multiple years. Unfortunately, there are few trained personnel with the necessary skills to accurately identify Dakota skippers in the wild. This personnel deficit poses an annual risk that surveyors cannot be secured during the flight. Contracted surveys for Dakota skippers are often costly, particularly given the current competition for the same surveyors from fossil fuel companies in North Dakota. Agencies like the Service, Minnesota DNR, and the Minnesota Zoo that are charged with successful implementation and monitoring of the Dakota reintroduction program almost exclusively bear this financial and personnel burden.

H. Risks of inaction

Given the poor dispersal ability of Dakota skippers (Dana, 1991), emigration to an extirpated site from any of the extant sites is improbable. Even if all anthropomorphic causes for a site's extirpation have been mediated, local natural disasters still risk the remaining small populations. If no action is taken, we risk the continued loss of Dakota skipper metapopulations with no ability to reestablish the species in suitable habitats. In short, we would be foregoing the benefits of the proposed program that are described in the section, Reintroduction Justification, above. There are also risks of waiting too long to attempt *ex situ* operations. If wild populations fall to critically low levels, as is currently the case with Poweshiek skipperling for example, there may be too few individuals remaining to launch effective *ex situ* conservation programs. Acting now with Dakota skippers will help buffer that risk, and will also build institutional knowledge of how *ex situ* and reintroduction programs should be conducted.

IX. Release and Implementation

A. Selecting Collection Sites

As stated above, Dakota skipper populations on Sisseton Wahpeton Oyate lands in northeast South Dakota are best suited for reintroductions to the Hole-in-the-Mountain Preserve. They are the current source of the majority of the Minnesota Zoo's current Dakota skipper *ex situ* insurance population, are from ecologically similar habitats, and are most likely least genetically divergent from the now extinct populations in the Hole-in-the-Mountain region. Collection protocols have been set in place to be as minimally invasive as possible. Only sites that appear to have robust Dakota skipper populations have been targeted for egg collection to date, and this is reflected in the permitted egg collection protocols (see section VII.A above.). Ongoing genetic analysis will help identify genetically distinct populations. In the future, these distinct populations may warrant higher priority for collection site selection. Collection sites are also determined based on landowner permissions and other project logistics.

B. Collections and *Ex situ* Rearing

The collection of eggs from wild Dakota skipper females and the consequent rearing of progeny at the Minnesota Zoo (sometimes for a generation or more) until reintroduction will be performed in accordance with all permitted protocols and in a manner that prioritizes the welfare of each individual. Full protocols are discussed in the Minnesota Zoo's Hesperiid Husbandry Manual and in prior annual reports to the USFWS (available upon request).

C. Selecting Release Sites

Site identification

Only Minnesota sites are being considered for reintroduction at this time. Although the source populations for this reintroduction effort are from within the Lake Traverse Reservation in northeastern South Dakota, reintroductions back into extirpated locations in Minnesota are prioritized, for several reasons. First, Dakota skippers formerly occurred in dozens of prairies across Minnesota, but only one predictable population is currently known statewide. Re-establishing populations in Minnesota will advance recovery of the species by expanding the range of extant populations to buffer against losses in regionally clustered populations and metapopulations that are impacted by common phenomena. For example, a local drought in northeast South Dakota may negatively impact the entire cluster of Dakota skipper populations in that region, but may not occur in Minnesota.

Second, prairies in Minnesota are prioritized at this time because they have generally have better recent survey history, which has produced a better understanding of the current status of Dakota skipper occupancy. Outside of Minnesota, the best surveyed areas are in northeastern South Dakota, but the prevalence of extant subpopulations in this area reduce conservation needs and potential gains. Indeed, a central consideration is the potential for extant Dakota skippers at or within dispersal distance of a site. If there is not a history of multiple negative surveys at a site and at adjacent suitable habitat, it remains possible that Dakota skippers are not actually extirpated from that site and/or from adjacent suitable habitat. Sites where Dakota skipper extirpation is highly likely are much stronger candidates for reintroduction, at least in the near term, so that the gene pools of any extant are not altered by the introduction of non-natal genotypes (assuming doing so would be detrimental, see Sections VII.A. and VIII.C.).

Third, sites where Dakota skippers were once common but have apparently been extirpated from in North Dakota and Iowa are located in different ecoregions than where Dakota skippers in the Minnesota Zoo's current *ex situ* population have been derived from. These individuals may be less adapted to conditions in other ecoregions (as well as the 'Type A' vs. 'Type B' habitats discussed earlier).

Finally, logistical and financial operations are situated in Minnesota. The majority of the Minnesota Zoo's Prairie Butterfly Conservation Program operational budget is funded by grants from the State of Minnesota, and those resources need to be expended in Minnesota. Minnesota also possesses a strong network of partners at federal, state, and local levels that are prepared to support this reintroduction effort, and these have not been as well established in other states.

We drafted seven criteria and a scoring system to rank candidate reintroduction sites in Minnesota that could begin as early as 2017. All of these sites historically had Dakota skippers (Appendix A). These criteria are designed to incorporate biological needs and social necessities, and include the following:

1. The status of the Dakota skipper at the site and in suitable habitat within two kilometers of the site
2. The extent of landowner approval for the reintroduction effort
3. The potential for public controversy from adjacent landowners
4. The extent of unplowed prairie
5. The quality of habitat
6. Assurance of appropriate habitat management at the site
7. Distance from row crop agriculture

Based on these metrics, The Nature Conservancy's Hole-in-the-Mountain Preserve in southwest Minnesota ranks most highly (Table 4). Sites like Glacial Lakes State Park, Chippewa Prairie, and Bluestem Prairie where Dakota skippers may still be present, either within those sites or nearby, are weaker candidates for releases until concerted surveys indicate that Dakota skippers are likely extinct in those areas. A significant additional positive to performing the first reintroductions in the TNC Hole-in-the-Mountain Preserve is the possibility for colonization of bordering suitable habitats managed by the Minnesota DNR: the Hole-in-the-Mountain Wildlife Management Area (WMA) and the Altona WMA. Both of these WMAs had historically significant Dakota skipper populations, and the colonization of these WMAs by reintroduced individuals could eventually re-establish a functional metapopulation in the Greater Hole-in-the-Mountain Complex.

Table 4 - Rankings of six candidate prairie remnants for Dakota skipper reintroduction. Scoring schema are described in Appendix A. Higher scores indicate higher suitability for reintroduction efforts. Scoring was completed by Erik Runquist (Minnesota Zoo) and Robert Dana (Minnesota DNR).

	Hole in the Mountain TNC	Glacial Lakes State Park	Prairie Coteau SNA	Tympanuchus WMA	Chippewa Prairie TNC	Bluestem Prairie SNA
Dakota skipper status within 2km	1	-1	1	1	-1	-1
Habitat extent	1	2	0	0	2	0
Habitat quality	2	1	2	1	1	1
Landowner approval	1	0	-1	0	0	-1
Potential for public controversy	0	0	0	0	0	0
Assurance of appropriate management	1	0	-1	-1	-1	-1
Distance from row-crop ag	-1	0	-1	-1	-1	-1
Totals:	**5**	**2**	**0**	**0**	**0**	**-3**

Release site selection within the TNC Hole-in-the-Mountain Preserve

The Nature Conservancy's Hole-in-the-Mountain preserve is divided into multiple management units with varying sections of habitat quality and historic Dakota skipper occupancy (Cross Unit, Triangle Unit, Central Unit, East Unit, South Unit and Southwest Unit). Additionally, adjacent to the HIM TNC owned land are two DNR Wildlife Management Areas, Hole-in-the-Mountain WMA and Altona WMA. It is the goal of this program that all high quality habitat patches within the above described locations are eventually occupied by Dakota skippers as a functional metapopulation. Given the current number of

Dakota skipper larvae at the Minnesota Zoo (Section IX.E.), only a single release location within TNC's HIM preserve will initially be utilized.

In order to facilitate contact between eclosing adult Dakota skippers, all pupa releases will be in a condensed central area (< 50m^2). Initial releases within the HIM preserve will take place in the TNC-designated Central Unit (Figure). This release area was chosen based on its high habitat quality designation, density of purple coneflowers and proximity to other high quality patches. Other criteria for this location included its proximate distance from the nearest agricultural borders. The topography of the site may act as a barrier from pesticide drift, as well as cater to Dakota skipper's propensity for 'hill topping.' Initially, a location in the northwestern border had been identified. It was believed that the forest edge may also act a barrier from pesticide drift while simultaneously corralling adults together to facilitate 'stay time.' The northwestern portion was later rejected as the initial reintroduction location due to at least one known past spraying for periodic population outbreaks of forest tent caterpillars (*Malacosoma disstria*) in the mid-2000s. The chosen location is also removed from planned 2017 controlled burns, but close enough to expect released individuals to possibly disperse into them and benefit from the burn effects.

Future releases will also aim to be in small condensed areas, but the location within the Central Unit may change. Survey efforts will inform where these releases take place and may shift to locations with greater adult skipper abundance. Releases into the other TNC units and adjacent WMAs will be evaluated annually. Criteria will be based on post-release dispersal data, habitat quality and captive breeding success.

D. Release strategy

Life stage – Pupa

Generally, only Dakota skipper pupae will be released during reintroduction efforts. Releasing adults involves more risk due to their fragile bodies and short reproductive lives, particularly given the long 3+ hour drive from the Minnesota Zoo to the HIM. Additionally, critical timing of life history events may be missed by waiting to transport and conduct releases after individuals become adults. Evidence from zoo-reared individuals at the Minnesota Zoo suggest that there is a short critical period for females to mate after eclosing. The likelihood of a female accepting male advances has shown to decreases over time and females typically begin laying eggs after 3 days whether they've mated or not. Typically, females become unreceptive to male mating advances once egg laying begins. It is unknown if this same behavior is also exhibited *in situ*, but barring more field data, raises a serious concerns as to risk any delay in getting eclosed skippers in to the field.

Releasing pupae allows for 1) the Minnesota Zoo to maintain individuals under safe *ex situ* conditions for as long as possible to maximize survivorship, 2) transportation to the release site with negligible stress to the individual, 3) placement of individuals into the best possible locations in the reintroduction habitat for those individuals (adults may disperse erratically if released in a stressful manner), 4) the determination of the sex of every individual released, and 5) the phenological and circadian timing adjustment of individuals to key biological and microclimatic triggers at the reintroduction site for optimal adult skipper emergence. Releasing larvae would generally be avoided due to 1) an incomplete understanding of larval host plants, and 2) increased *in situ* risks to the survivorship of the individual due to exposure to predators and parasitoids and/or extreme weather events.

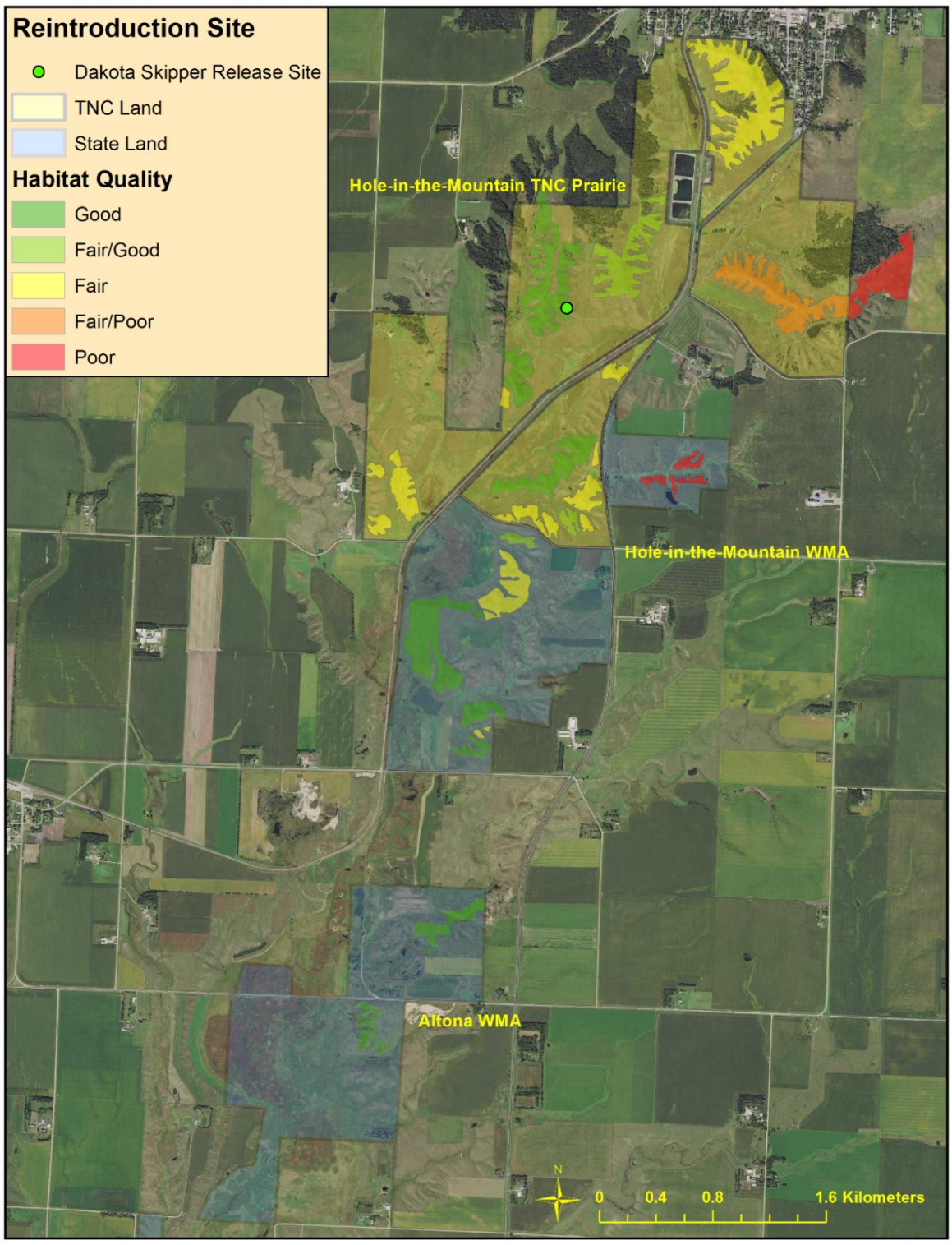

Figure 8– The greater Hole-in-the-Mountain Complex in Lincoln and Pipestone Counties, Minnesota, with habitat quality assessments from Dana (2015, unpublished) overlaid by TNC Preserve and Wildlife Management Areas (WMA) boundaries. The identified initial release site within the 'Central Unit' of the TNC Preserve is indicated by a green dot.

Release Technique

We have identified four different potential release techniques (Table 5). Each technique has pros and cons and the decisions regarding which technique to implement will be largely contingent on available survey staff. Currently, the Dakota skipper working group has identified utilizing a "closed box" which should offer the greatest amount of security to pupa brought to HIM. For the closed box technique, pupa will be housed in a sealed closed box in the center of the release site (Figure 8). The box will checked multiple times per day to release eclosed adults beginning no later than 8:00 am. A concern with the closed box is that the box itself may be an attractant and utilized as a perch; artificially attracting or concentrating local birds. The box itself would exclude predation of pupae, or newly eclosed adults prior to releasing them. However, there is a high probability that released Dakota skippers will concentrate around the release box. It is not our goal to eliminate all the potential predatory threats, but we do want to maximize success of the released individuals. After the release box is installed, but before the first skippers eclose, we will evaluate whether the box is being heavily utilized as a perch and decide if some kind of deterrent (i.e. scarecrow) is warranted.

Annual reports will include descriptions of the implemented release methods used and any logistical modifications (Section X.G). If release strategies change, the relevant sites plans will also be revised.

Table 5 - Potential Release Strategies

Release technique	Description	Pros	Cons
Closed box	Pupae are kept in a hopper-esque box on a post that is stationed at the release site. The hopper-esque box would offer full protection from predators/parasitoids and be shielded from rain. The sides are constructed with fine mesh to allow airflow, but prevent escape of the butterflies.	-Offers full protection until release -Allows us to obtain complete census data	-Requires producing a specialized box. -Requires daily (+) monitoring. - Could become a perch for prairie birds
Open box	Pupae are kept in a hopper-esque box on a post that is stationed at the release site. The hopper-esque box would offer some protection from ground predators and shielded from rain. The sides are constructed with wire mesh with gauge so that adult butterflies can escape once they have eclosed (~1").	-Requires less monitoring than a closed box -Offers limited protection -Allows us to census exuviae	-Requires producing a specialized box -Would not completely shield pupa from predators -Could become a perch for prairie birds
Direct release with open barrier	Pupae are placed directly into the base of host bunchgrass. The grass itself would be surrounded by mesh cylinder of Lumite screen that is open on top.	-Offers more protection from predators or extreme weather than direct release -Requires no additional personnel post-release -More 'natural' condition than either box strategy	-Only mitigates predation by ground predators like mice. Could allow avian predators to form a search image. -May not allow us to accurately census exuviae if they degrade
Direct release	Pupae are placed directly into the base of host bunchgrass.	-Requires no additional hardware or personnel post-release -May provide the most 'natural' condition experienced	-Offers no protection for released pupa -Least opportunity to accurately census exuviae

E. Disposition of excess progeny

Only a portion of the Minnesota Zoo's standing *ex situ* Dakota skipper population would be used for the reintroduction each year. Roughly 150 individuals will be retained each year as part of the Minnesota Zoo's breeding stock. Any and all remaining individuals could be used for release, unless otherwise determined by the working group. As described in Section IX.E, no individuals will be retained at the Minnesota Zoo's breeding colony for more than three generations. These individuals would be prioritized for release, unless those individuals display atypical characteristics (morphological, behavioral, etc.) that may be maladaptive in wild populations, which case they would be phased out of the Zoo-breeding and reintroduction programs.

Other individuals would be excluded from the Zoo-rearing program and the reintroduction program if they were suspected to be carrying a disease or parasite/parasitoid. Any such individuals would be either destroyed to prevent pathogen transfer or preserved for additional study, in accordance with protocols outlined in the Minnesota Zoo's Section 10(A)(1)(a) permit.

We anticipate some pupation time variance within the Zoo's population. Some of this variance may be due to individual genetic differences and/or developmental responses to different husbandry techniques. On average, within past Minnesota Zoo-reared Dakota skippers, females pupate 3-4 days later than males. This phenomenon was also observed by Dana (1991). To ensure the released population is diverse and captures both males and females, at least two separate release events to HIM will take place one week apart. All individuals will be confidently sexed prior to release through examination of pupal abdominal morphology under a dissecting microscope using established protocols.

We will strive to represent at least 20 distinct lineages during each year of releases, with no one lineage representing > 10% of the released population. Both multi-generational Zoo-bred lineages and newly collected individuals will released. The longest Zoo-bred lineages at the Minnesota Zoo will be prioritized for release. The purpose of this is to mitigate the possibility of artificial selection while in Zoo care.

Release Size

No minimum number of individuals for release at a single site has been identified. Persistence at the release site is unlikely if there are too few individuals to come in contact with each other and mate, or the released population is not genetically diverse enough. Currently, a target of 150-250+ individuals for the initial release would be ideal and within the scope of the Minnesota Zoo's breeding program. This metric is derived from the successful reintroduction of the large blue, *Maculinea arion*, into the UK (Thomas 2009).

X. Monitoring and continuing management

A. Demographic performance

Each reintroduction site should be monitored yearly during the adult flight period following reintroduction. Several methods for monitoring rare butterflies exist, but biological and financial tradeoffs exist between methods (Haddad et al 2008). For example, mark-recapture methods are powerful, providing detailed information on both population size and demographic parameters, but mark-recapture may be harmful to rare butterflies (particularly small, muscular species like skippers) if not done with extreme care. Mark-recapture is also significantly more time-intensive to conduct than Pollard-Yates

transects. The occurrence and distribution of adult Dakota skippers are the primary parameters of interest for the Dakota skipper reintroduction. Therefore, transect-based methods will be employed, at least initially. Distance sampling (Brown and Boyce 1998, Buckland *et al*. 2000) would allow for estimates of population density and detection probability. For example, models developed by Zonneveld *et al.* (2003) can describe peak counts as low as five individuals. INsect Count Analyzer (INCA), is a freely available user-friendly software program analyze transect counts using the Zonneveld model (Bruggeman and Zonneveld 2002; Longcore *et al.* 2003).

Observers will visit extant sites in the weeks leading up to the predicted adult flight to determine the start of the flight period. This will involve surveys starting approximately in mid- to late June through mid-July, depending on initial flight date.

Adult Monitoring Study Design:

The proposed 2017 release site(s) is located at the southern end of the northwestern-most ridge of the Central Unit of TNC's HIM Preserve (Figure 8). We delineated 'available habitat' in the Central Unit based on uplands approximated from satellite imagery (Google Earth) and cross-checked with Robert Dana's habitat characterizations. We buffered these upland regions by 25 m (upslope) to eliminate areas of less suitable habitat near the bottomlands.

We hypothesized that Dakota skippers have a very low dispersal ability (rarely more than a few hundred meters). Thus, to more efficiently allocate sampling effort, we stratified the Central Unit into high- and low-density sampling zones based on proximity to the release site. The high-density zone encompasses available habitat within ~250 m of the release site, and the low-density strata includes all remaining available habitat in the sampling habitat.

In the Central Unit, most sampling for Dakota skippers will be conducted along fixed transects oriented in a general east-west direction (Figure 9). Transects in the high- and low-density strata are systematically spaced at 75 m and 150 m intervals, respectively. In Figure 9, the upland transects that had been estimated through aerial imagery are color-coded based on likely habitat quality an incorporation historic land use patterns (e.g. tilling) In addition, we will sample from a single, ad hoc transect along the primary ridgeline of each delineated upland. These complementary transects will improve our understanding of dispersal and inform future monitoring efforts. East-west transects in the Central Unit total ~5.0 km, and ridge transects total ~1.5 km. The exact positions of each transect will not be determined until ground-truthing can be completed in late spring 2017 to maximize sampling efficiency.

Similar delineation will be made of available habitat in several peripheral sites (the Cross Unit, South Unit, and West Unit in TNC's HIM property and the southern unit of the HIM WMA). Although we do not anticipate that Dakota skippers released in the Central Unit will disperse to these sites, these surveys will serve to provide baseline data to inform future monitoring efforts and assess current species richness. These transects may be less formal, potentially consisting of semi-fixed meandering routes.

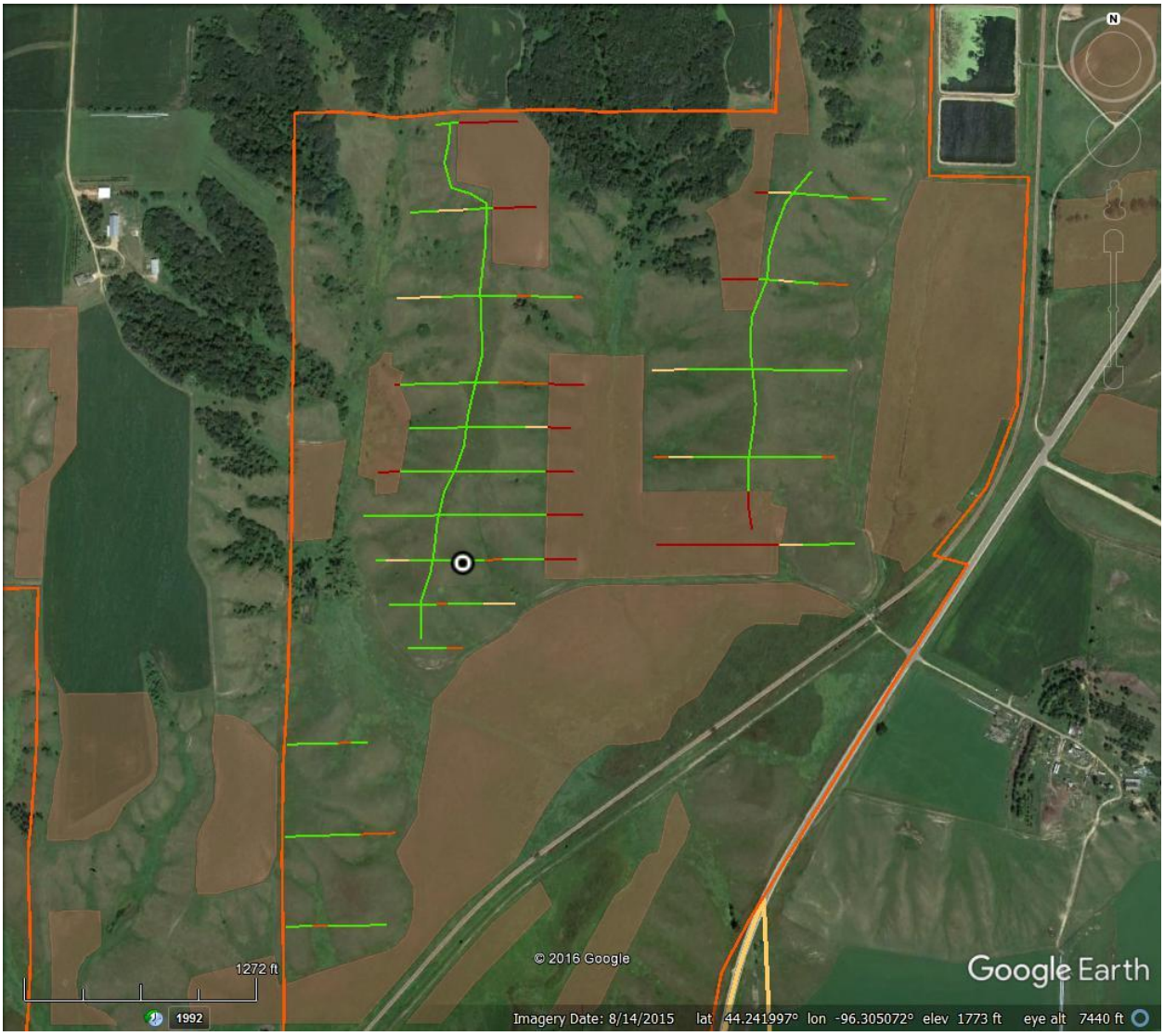

Figure 9 – Monitoring transects in the Central Unit of the TNC Hole-in-the-Mountain Preserve. Transects are coded by habitat quality (green = good; yellow = poor; orange = non-habitat; red = formerly cultivated land). Formerly cultivated land under restoration is indicated with brown polygons, and is presumed to not be suitable habitat. Habitat quality assessments and cultivation history is provided by Robert Dana (unpublished). The approximate location of the proposed release site in indicated by a white and black dot. All transect locations are approximate and will be refined in 2017 prior to the reintroduction.

Monitoring of adult abundance and dispersal will commence on the first day after pupae are established on site and continue for two to three weeks after the second wave of pupae are established to encompass as much of the flight as possible. Surveys will be conducted daily in the Central Unit during hours of peak butterfly activity. Sampling at the peripheral sites identified above will be completed on a rotational basis, such that one peripheral site is visited per day. This schedule will ensure that peripheral sites are revisited once every four days (conditions permitting), while enabling us to focus our efforts in the Central Unit release site. However, we anticipate that sampling will actually occur less frequently than planned due to inclement weather.

Surveyors will sample from transects at a slow, consistent pace. All Dakota skippers sighted in front of the observer will be recorded while on-transect, unless the observer is confident that an individual was sighted previously. Surveyors may deviate from transects to verify identity and to GPS observed Dakota skipper locations. Surveyors must be able to rapidly identify Dakota skippers visually from up to 10 - 15 meters. The ability to differentiate Dakota skippers from sympatric skippers is critical. Surveyors need to be physically fit and able to spend long hours hiking in hilly, uneven terrain in potentially difficult weather conditions for multiple consecutive days.

After at least five years of stable or increasing Dakota skipper relative abundance or abundance at a particular location, we will suspend augmentations at HIM and consider the population to be contingently self-sustaining. Annual monitoring to track trends will continue for at least five more years. If annual monitoring indicates that the species is not establishing self-sustaining populations at HIM, we will review the potential causes and consider the resumption of augmentations. Short of marking all individuals that are released each year or only releasing mated females (both of which would require a shift in methods to release adults and not pupae), there is no definitive means of identifying whether or not an adult Dakota skipper observed at HIM is the product of *in situ* breeding from individuals reintroduced the prior year or if it was an individual released that year. Presumably though, individuals that are observed in areas where they were not reintroduced to (at least separated by large swaths of unsuitable habitat, or the persistence of individuals in areas where reintroductions had occurred but had been ceased, would be indications of successful reintroduction efforts. The intensive transect monitoring scheme that has been developed will help determine dispersal patterns away from the reintroduction point, and therefore produce a landscape model of where individuals may disperse into in future years. Assays of genomic relatedness of both released and wild adults could also conceivably be conducted, but personnel and financial shortfalls, a lack of known informative genetic markers, and increased risk of severe harm to individuals preclude this technique.

B. Behavioral monitoring

While monitoring the behavior of reintroduced animals is important, its value depends on comparative data from other natural populations (IUCN/SSC 2013). This comparison would be difficult, given the present lack of data. Every effort will be made to assess adult behaviors at extant sites. This may include recording numbers of adults nectaring, perching, flying, chasing, ovipositing, and mating (e.g., Monfils and Cuthrell, 2014). This data set can be used as baseline information to help determine what set of behavioral characteristics should be represented in the reintroduced population and if there are any impacts to source populations from the egg collection efforts. However, since many of these behaviors are weather and micro-climate influenced, departures from the “norm” will need to be interpreted carefully.

C. Ecological monitoring

We currently do not foresee any negative ecological impacts due to the implementation of this plan. After the initial reintroduction in 2017, we plan annual releases under this plan. The intended demographic targets for augmentations are not expected to increase the abundance or densities of local populations of the species past its historical levels.

D. Genetic monitoring

In addition to the population genetics research described above (Sections VI.A., VII.C., and VII.E.), and in the release strategy (Section VIII.D.), detailed lineage data of the Minnesota Zoo's entire population (and therefore all individuals ultimately selected for the reintroduction program), will be closely maintained. Explicit genetic comparisons between individuals in the Zoo's population are not currently possible until microsatellite or other next generation genetic data is extracted from representative individuals in the Zoo's population. As such, lineages at the Zoo are ultimately tied back to the wild founder female(s) from which eggs were first collected from (which can range from one to three generations removed). Each of these wild maternal lineages are presumed to be distinct from each other. As stated previously, morphometric characters of Zoo-reared adults will be compared to historic museum specimens to test for deviations away from wild type characters that may indicate potentially maladaptive effects of rearing under controlled conditions.

E. Health and mortality monitoring

Individualistic and quarantined rearing of immature Dakota skippers provides the opportunity to monitor the health of every individual under *ex situ* care. However, short lifespans of Dakota skipper adults, and the inability to find eggs, larvae, and pupae under natural conditions prevents monitoring of the health status wild populations. Diseases, predators, and/or parasitoids that may be specific to Dakota skippers are also completely unknown, and therefore also cannot be directly monitored. Year-to-year fluctuations in population sizes may or may not be attributable to diseases or other factors that mediate population health. Weather may be a more direct influence on population sizes.

F. Socio-economic monitoring

To better understand the socio-economic impacts and perceptions of our work with Dakota skippers, we will maintain conversations with stakeholders on these subjects throughout the timeline of this plan. We will not have time to conduct a thorough survey before reintroductions begin in 2017, but we will instead conduct socio-economic and financial impact discussions after the first round of Dakota skipper releases in 2017. This will not prevent us from soliciting and responding to concerns and questions raised by stakeholders during our initial outreach attempts. As we continue to implement Dakota skipper releases at HIM (and similarly at other potential reintroduction locations), we may also ask stakeholders if their opinions towards Dakota skipper conservation have changed since *ex situ* work and outreach efforts began.

If there appears to be a sharp decline in support from landowners and other interested parties, or if the results show severe negative perception of our conservation efforts, outreach will be focused on specific issues of concern and targeted communication will be had with relevant stakeholders. If perceptions continue to decline or remain negative, we will discuss this with the appropriate agencies and individuals and determine possible reasons for these negative perceptions and potential ways to address concerns. Possible solutions may include but are not limited to modifying and increasing outreach, hosting meetings for interested stakeholders to share and discuss their concerns, and modifying the propagation plan to take into account stakeholder concerns.

G. Continuing Management

As stated in IUCN (2013, p. 21), some translocations require management over many years and monitoring results provide the basis for either continuing or changing management regimes and the justification for any change in translocation objectives or time schedules. We have already stated above in the Goals, Objectives, and Actions Section of this document how we plan to assess the effects of reintroduction and will not repeat that in detail here.

Much of the year-to-year analysis and reevaluation of the program will be expressed in updates to this plan. The team will review and revise the plan annually. Release plans will be drafted for any site where reintroduction will take place. Included in annual release plan revisions, as needed, will be an update to maps and survey results; detailed plans for the upcoming year, including any updates or revisions proposed for the upcoming year to survey, egg collection, transport, rearing, or release techniques; any changes in personnel; and, any modifications to contingency plans.

In addition to release plan revisions and updates, each year the team will draft an annual report of activities. The Minnesota Zoo may include the majority of the relevant information in its own annual reports, but the team will prepare a supplementary report, if needed. Annual reports will be completed by March 1st and will include detailed descriptions of the egg collection, transport, and rearing activities conducted over the previous year; the precise locations where each activity was carried out; the proportion of collected eggs that are released at later life stages; and, a description of the extent and quality of Dakota skipper habitat at reinforcement or reintroduction sites. The results of Dakota skipper adult surveys, including the locations where the species was recorded during surveys, will also be summarized and appended to the annual report. The report will include a description of the extent of habitat that the species occupies at the site and the trends in the quality and extent of the habitat. This information will be essential for determining whether reinforcement or reintroduction activities should proceed at a site and whether habitat management activities are needed.

Information that will be critical for determining whether – and when – to cease reinforcement at any individual site or generally will include adult survey data collected in a manner that will allow us to estimate relative abundance - or to assess another appropriate metric for population dynamics - and the proportion of collected eggs that survive to release. These are also addressed in the Goals, Objectives, and Actions section of this plan, above.

Activities associated with captive rearing of the Dakota skipper require authorization by the U.S. Fish and Wildlife Service under section 10(a)(1)(A) of the ESA. In addition, the Service's issuance of each permit requires that it conduct an internal consultation under section 7(a)(2) of the Act to ensure permitted activities will not reduce appreciably the likelihood of the species' survival and recovery in the wild. These regulatory requirements will provide an additional means by which the activities conducted under this plan are assessed and will take into account any significant new information.

H. Habitat management

The first and only site under consideration for reintroduction currently is TNC's Hole-in-the-Mountain Preserve, which will be subject to habitat management, as specified in an endangered species recovery permit that USFWS issued to TNC in March 2016. The permit allows TNC to carry out habitat management with a primary intent of conserving Dakota skippers on several preserves, including HIM.

The TNC permit application contains details regarding its proposed approach to habitat management, including how adverse impacts to the Dakota skipper will be minimized. To subdivide

potential Dakota skipper subpopulation areas, TNC has divided Hole-in-the-Mountain into 6 "sub-sites," 5 of which occur on TNC property, based on input from Dr. Robert Dana, Minnesota Biological Survey (TNC 2016). TNC proposes to plan separately prescribed fire and monitoring activities within each of these sub-sites following the general parameters described below.

TNC's prescribed burns will be conducted in the spring, fall, or a combination. Burns will be conducted in such a way as to minimize impact to Dakota skipper populations and individuals. TNC's proposed prescribed fire treatments take a conservative approach by following best practices identified in the literature and guidelines to the full extent possible, while also taking into account site-specific constraints and logistical challenges inherent to prescribed fire management. Using the best information available for each site, including historical occurrence records, recent surveys, Dakota skipper habitat suitability assessments, and expert knowledge, TNC will identify the areas where the larvae are most likely to be present.

TNC will minimize the possibility of fire-related injury or death to individuals within a given burn unit to the extent possible. For example, prescriptions may be designed specifically to encourage patchiness of the burn. Burn supervisors may also choose to reduce fuel loads prior to the burn. From the perspective of emerging Dakota skipper larvae, early spring and fall are the most desirable times for burns, to avoid the timeframe when larvae are above the litter layer (Dana 1991; USFWS Conservation Guidelines 2015). Seasonality of the burn will be factored into burn plans accordingly, weighing desired outcomes for Dakota skipper habitat and reducing the risk of injury or death to individuals. For example, a fall burn may be required to increase the availability of nectar plants the following spring, or a late spring burn may be needed if cool-season, non-native grasses are degrading Dakota skipper habitat. TNC will take an adaptive approach to burning with prescriptions that are informed by Dakota skipper and habitat monitoring conducted as part of this research. There is significant topographic relief at the site that precludes some other management techniques, such as haying

The prescribed burn framework for this project emphasizes spatiotemporal factors that are compatible with sustaining and recovery of Dakota skipper populations. The identified breeding habitat will be divided as well as possible into units for burning—striving for at least three, and ideally four burn units, of which only one would be burned in any given year (USFWS Conservation Guidelines 2015). The minimum fire return interval for each burn unit as described above will be 4-to-6 years (Panzer 1988; Panzer 2002; USFWS Conservation Guidelines 2015, Robert Dana, pers. comm. December 8, 2015). Care will be taken to reduce the impacts of prescribed burns on Dakota skipper populations. TNC will coordinate with adjacent ownerships that have documented occurrences of Dakota skipper and/or mapped likely breeding habitat (e.g., Bluestem and Blazing Star) to ensure that these spatiotemporal parameters are maintained consistently over time.

TNC will coordinate with the US Fish and Wildlife Service as they make plans for annual prescribed fire on the 6 sites identified in this proposal, offering an opportunity for review of these plans. For context, annual burn plans may be submitted with relevant spatial information about management history, in particular prescribed fire—conducted in the year preceding the study period and cumulative histories over the course of the project.

XI. Exit strategy

The monitoring discussed in the previous sections will help managers assess whether the objectives are being met and can be used to determine when to end the program under seven potential scenarios:

Scenario 1 - Recovery of the species has been achieved. A recovery plan for the species will require development of numerical targets to measure recovery and inform any decision to down-list or remove the species from the Threatened species list. Developing such targets is outside the scope of this plan and is in progress by the Service. This plan has largely addressed the means by which reestablishment of extirpated Dakota skipper populations might be achieved, and the risks that might put at risk such efforts. As the objectives of this plan are achieved, additional objectives and actions may be considered to further the conservation of the species. It is possible that additional *ex situ* actions, in combination with other *in situ* actions (*e.g.*, habitat management) will need to be successful for the species to be considered fully recovered.

Scenario 2- Objectives of the plan are achieved and the species has established self-sustaining, stable or growing and genetically viable populations in the Greater HIM complex such that the ex situ programs are no longer necessary in that area, but recovery of the species is needed elsewhere. Resources could be shifted from recovery efforts at HIM to other regions.

Scenario 3- Objectives of the plan cannot be achieved due to lack of observations of Dakota skippers near the release site and on the fixed monitoring routes throughout the Greater HIM Complex, despite repeated releases of hundreds of individuals per year over at least a five years. This would indicate that releases are not successful (for many potential reasons), and that Dakota skippers are not re-establishing in the Complex despite concerted efforts.

Scenario 4 – Failed Zoo-based rearing program – Survivorship of Zoo-reared eggs to release stage is at zero or nearly so (<3%) for three consecutive years, or source populations reach such low numbers (for two or more years) that collection of adult females for eggs is no longer feasible. Annual evaluations of the program will help assess the options under this scenario.

Scenario 5- Habitat becomes no longer suitable. Releases should halt of habitat quality in the Complex becomes no longer suitable for Dakota skippers, or there are increased extrinsic threats in the greater landscape that increase risks to skipper safetly beyond an acceptable threshold (e.g. dangerous pesticides drift, etc). Current estimations of risk and agreements of habitat management are discussed above in VIII.B and X.H.

Scenario 6- Objectives of the plan can no longer be achieved due to the total loss of capacity (e.g., funding) to continue the program in the foreseeable future. The financial assurances and risks are discussed in Section VI.G of this document.

Scenario 7 - Objectives of the plan cannot be achieved due to species extinction.

Under each identified scenario, the Service will engage with relevant partners to discuss the exit strategy, before a final course of action is taken.

XII. Dissemination of Information

Annual reports described in the section, Continuing Management, above, will be distributed and made available to ensure that all stakeholders and any other interested parties may stay abreast of progress and activities implemented under this plan.

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XIV. Appendix A- Criteria for Reintroduction and Founder Site Selection

The following are the criteria and associated scoring system to weigh candidate sites against each other for Dakota skipper reintroduction suitability. These factors attempt to weigh biological factors with social feasibility. The most appropriate answer that applies to the Hole-in-the-Mountain TNC preserve for each criteria is indicated in bold type. The scores of six candidate sites are provided in the main text.

1) Status of the Dakota skipper at the site and in suitable habitat within two kilometers of the site:

a. The site and all suitable habitat patches within two kilometers of the site have had at least three consecutive negative survey years. This indicates that Dakota skippers are likely extirpated from the site and adjacent areas. 1 point

b. The site and all suitable habitat patches within two kilometers of the site have had less than three consecutive negative survey years. This indicates that Dakota skippers may still be present at the site or in adjacent areas. -1 point

The intent of this factor is to ensure that the Dakota skipper is not present at the potential reintroduction site or at sites from which it could emigrate readily. If it were present, we would not release the species at the site. Instead, we would focus on whatever might contribute to the health of the population.

2) Extent of unplowed prairie

a. The extent of unplowed prairie at the site exceeds 140 ha. 2 points

b. The extent of unplowed prairie at the site exceeds 140 ha, but it is fragmented. 1 points.

b. The area of unplowed prairie at the site is between 30 and 139 ha. 0 point

c. The extent of unplowed prairie at the site is less than 30 ha. -1 point

Swengel and Swengel (1999) found that relative abundance of Dakota skippers was highest in prairies that exceeded 140 ha in extent and was intermediate in prairies in their "medium" (30-130 ha) size category. Robert Dana, Minnesota Department of Natural Resources, has conducted Dakota skipper research and monitoring at HIM for thirty years or more and in 2015 qualitatively assessed the Dakota skipper habitat conditions there (Dana, 1991; Dana 1997; Figure). There are over 140 ha of unplowed prairie in areas where the Dakota skipper has been previously recorded at HIM and there is additional prairie without previous records for the species immediately adjacent.

3) Habitat quality

a. The methods described in Ahlering and Narem (2015) – or methods substantively similar – were used to assess habitat quality at the site and overall habitat quality was ranked as A or A-. 2 points

b. The methods described in Ahlering and Narem (2015) – or methods substantively similar – were used to assess habitat quality at the site and overall habitat quality was ranked as B. 1 point

c. The methods described in Ahlering and Narem (2015) – or methods substantively similar – were used to assess habitat quality at the site and overall habitat quality was ranked as B-. 0 point

d. The methods described in Ahlering and Narem (2015) – or methods substantively similar – were used to assess habitat quality at the site and overall habitat quality was ranked as C or C-. -2 points

Our ranking of habitat quality is currently based on a combination of qualitative assessments provided by Robert Dana (Minnesota DNR) and quantitative data collected by the Minnesota Zoo. The qualitative assessments by Dana follow the Condition Ranking Guidelines for Upland Prairie Systems (Minnesota DNR 2014) that incorporate estimations of the prevalence and extent of native plant diversity associated with high quality Dakota skipper habitat, the extent of non-native plant invasion, topography (Dakota skippers are generally concentrated along higher ridgelines in 'Type B' habitats), and management history (e.g. some areas may have been degraded by overgrazing). These rankings were also influenced by historic abundance records for Dakota skippers observed by Dana and others (i.e. some locations historically had higher concentrations of Dakota skippers than others, and therefore were considered better quality). The quantitative data on the prevalence of narrow-leaved purple coneflower collected by the Minnesota Zoo (see below) also contributed to the overall assessments of Dakota skipper habitat quality across sites. The ranking is not strictly transferable to the letter ranking scheme devised by Ahlering and Narem (2015). If we convert Dana's rankings to a letter ranking and arrive at an overall score for habitat quality by weighing habitat scoring based on the relative proportion of the total HIM area, the ranking would be somewhere between B and A- (Table 6).

Table 6 – Extent and relative quality of Dakota skipper habitat at Hole-in-the-Mountain in areas that contain historical records for the Dakota skipper, as determined by Robert Dana, Minnesota Department of Natural Resources in 2015., and the resulting combined scoring of the HIM Preserve using the Ahlering and Narem method.

Habitat Quality (Dana 2015, unpublished)	Habitat Quality Letter Ranking	Habitat Quality Numeric (1-6)	Hectares	Proportion of Site	Weighted Score
Poor	C	1.2	16	0.10	0.116788321
Fair/Poor	B-	2.4	19	0.11	0.268613139
Fair	B	3.6	56	0.34	1.217518248
Fair/Good	A-	4.8	17	0.10	0.502189781
Good	A-	6	58	0.35	2.087591241
Total/Overall Score			166		**4.19270073**

As stated previously, narrow-leaved purple coneflower (*Echinacea angustifolia*) is the primary nectar source for Dakota skippers in the 'Type B' habitats of northeast South Dakota and southern/central Minnesota, and adults are almost exclusively found congregating on the flowers in areas of high density purple coneflower (Table 1). Therefore, coneflower density is a key characteristic of habitat quality that must be assessed across candidate reintroduction sites. To advance this indexing, the Minnesota Zoo's Cale Nordmeyer and Seth Stapleton conducted density surveys of flowering purple coneflowers at three historic Dakota skipper locations in August 2016 to inform the Dakota skipper reintroduction site selection. Strip transect sampling of all 2016 flowering purple coneflowers within an approximately 10

meter half width (and assumed detection probability of 1.0 within that strip) was conducted at TNC's Hole-in-the-Mountain preserve (Central Unit), Glacial Lakes State Park (Pope County, MN), and Felton Bicentennial Prairie (Clay Co, MN). Like Hole-in-the-Mountain, Dakota skippers are believed to be extirpated from Glacial Lakes State Park (despite historically large populations). The Felton Bicentennial Prairie is thought to be the only viable Dakota skipper population remaining in Minnesota. Surveys were only performed in the northwest quadrant of Felton Bicentennial because this is the area of the property with the highest Dakota skipper densities. Glacial Lakes State Park possesses extensive native prairie acreage, contained historically large Dakota skipper populations, and appears to be relatively well buffered from pesticides drift (Minnesota Zoo and USFWS unpublished data).

These surveys found that although coneflower densities at Hole-in-the-Mountain are somewhat more variable than at Felton Bicentennial, the best coneflower sites surveyed at Hole-in-the-Mountain were comparable with those at Felton Bicentennial where Dakota skippers maintain an apparently viable population. The densities were markedly lower Glacial Lakes State Park, at least in the areas sampled. It should be noted though that coneflower density surveys did not occur in the properties directly east of the Park where Dakota skippers historically had the highest densities, due to lack of landowner permissions (Table 7, Figure 10Figure 11Figure 12). Additional predictors of habitat utilization should be identified through additional research.

Table 7 - Summary statistics of purple coneflower densities along transects at three historic Dakota skipper populations in Minnesota, August 2016. Minnesota Zoo data.

	Dakota skipper status	Transects (n)	Mean (plants/10 m)	SD	Range
Felton Bicentennial	Present	4	8.96	50.4	5.50 - 16.89
Hole-in-the-Mountain	Presumed Extirpated	8	7.35	50.7	0.7 - 16.11
Glacial Lakes	Presumed Extirpated	6	0.13	0.08	0.02 - 0.28

Figure 10- Densities of purple coneflowers along transects at Felton Bicentennial prairie in the quadrant where most Dakota skippers occur, August 2016. Small, dark red dots indicate 1 – 4 plants at the point (within the strip width). Large, bright yellow dots indicate to >60 flowering plants per point. Different shades of red, orange and yellow and different sized dots follow along this color / size continuum. White border is the Felton Bicentennial boundary.

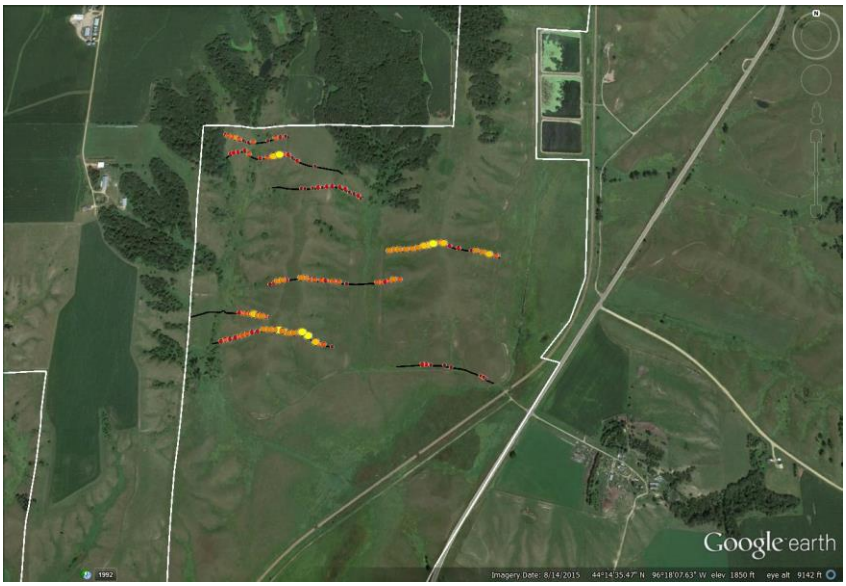

Figure 11 - Densities of purple coneflowers along transects in the central unit of TNC's Hole-in-the-Mountain Preserve, August 2016. Small, dark red dots indicate 1 – 4 plants at the point (within the strip width). Large, bright yellow dots indicate to >60 flowering plants per point. Different shades of red, orange and yellow and different sized dots follow along this color / size continuum. White border is the Preserve boundary.

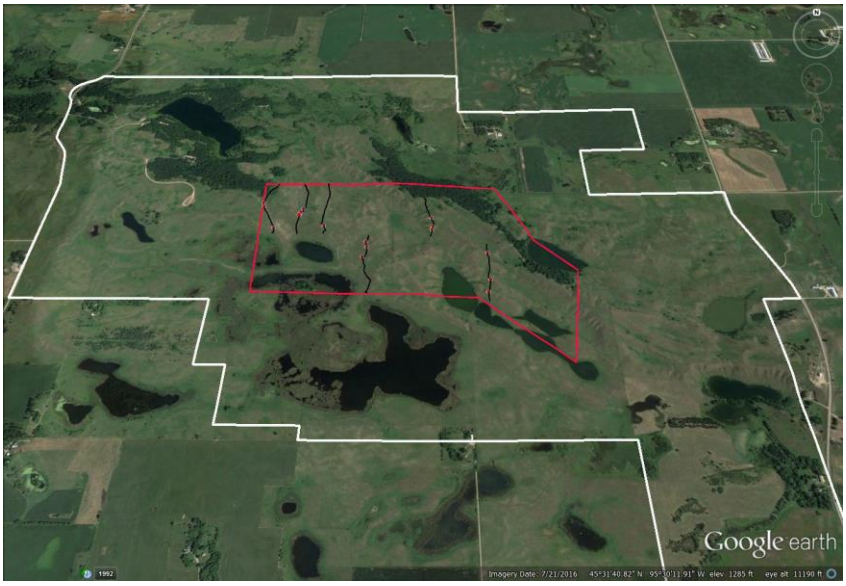

Figure 12 - Densities of purple coneflowers along transects at Glacial Lakes State Park, August 2016. Small, dark red dots indicate 1 – 4 plants at the point (within the strip width). No points had more than 8 observed coneflowers, unlike at the sites above. White border is the State Park boundary. Red border is the area of highest quality habitat in the center of the State Park where random transects were placed.

4) Land manager approval:

If more than one land manager owns land within the site, points will be weighed according to the proportion of land owned.

a. The land managers (s) has agreed in writing to the reintroduction of the Dakota skipper on their property 2 point

b. The land manager(s) has agreed verbally to the reintroduction of the Dakota skipper on their property. 1 point

c. The land manger has expressed informal interest in the possibility of the reintroduction of the Dakota skipper on their property. 0 points.

c. The land manager's position on the reintroduction of the species is unknown. -1 point

d. The land manager has expressed opposition to the species' reintroduction. - 2 points

Additional conversations with other land managers are warranted, particularly to develop habitat management plans than would increase the pool of candidate sites for future reintroductions beyond those planned in the near team, as well as to raise the suitability scores of those sites already considered.

5) Potential for public controversy

a. There is no reason to think that the reintroduction will be controversial with adjacent landowners whose lands contain potentially suitable habitat or the state conservation agency. Each landowner with potentially suitable habitat and the state conservation agency has been polled on the question and has expressed no opposition to the reintroduction. 1 point

b. There is no significant reason to think that the reintroduction will be controversial with adjacent landowners or the state conservation agency, but some of their opinions remain unknown. 0 point

c. One or more adjacent landowners or the state conservation agency has expressed opposition to the reintroduction. -1 point

Minnesota Zoo staff met with landowners adjacent to the HIM Preserve in March 2017, and also presented the proposed work to the Lake Benton City Council. Reactions ranged from neutral to positive, so we do not expect significant initial controversy with the proposed reintroduction. Our consideration of this criterion for HIM brings to mind a few faults with the way the criteria approach the potential for public controversy. First, we should consider the potential for controversy among potentially affected stakeholders, which may include persons or entities with a basis for interest other than land ownership. Second, there will probably be few situations when reintroduction of a threatened or endangered species will not be controversial with at least one stakeholder.

For the proposed reintroduction of the Dakota skipper to HIM, it is reasonable to assume that at least one of the stakeholders listed in Section F. (Social feasibility) above, may have reservation and concerns. In the end, we do not expect opposition to be significant for the following reasons:

1. We will reach out directly to each stakeholder to explain our plans and their potential implications and will attempt to address each of their concerns and questions promptly. Initial conversation with landowners near the Hole-in-the-Mountain reintroduction site have been beneficial;
2. For issues raised by stakeholders that may be complex, may not be addressed quickly, or both, we will work to resolve them with minimal delay;
3. The Dakota skipper has specific habitat requirements and is only likely to inhabit high-quality, unplowed native prairie; and,
4. The Service may exercise its authority to permit activities that would otherwise be prohibited as take under section 9 of the Endangered Species Act; and,
5. A special rule published concurrent with the species' listing as threatened in 2014 allows take of the Dakota skipper that occurs as a result of routine livestock operations on non-federal lands.

We anticipate that the most significant concerns may be raised by persons involved with farming of agricultural row crops near HIM. In the section, Ecological Risk, above, we describe briefly the potential threat that pesticide drift may pose to the Dakota skipper at HIM. When we address this threat, we will deal, directly or indirectly, with private landowners near HIM. We are uncertain how receptive the landowners will be on this topic, but may engage with University of Minnesota Extension for assistance.

From the other landowners, we expect interest, but not significant opposition. We do not expect opposition because we expect the Dakota skippers to inhabit only high-quality native prairie; we are committed to dealing with stakeholders openly and cooperatively; and, we expect that we will be able to identify acceptable solutions to any potential conflicts that arise with the identified stakeholders.

6) Assurance of appropriate management

If more than one landowner owns land that contains unplowed prairie within the site, points will be weighed according to the proportion of land owned.

a. The landowner(s) has agreed in writing to implement land management practices in a manner conducive to the conservation of Dakota skippers for at least ten years on the species' habitat at the site. 2 points

b. The landowner(s) has agreed in writing to implement land management practices in a manner conducive to the conservation of Dakota skippers for at least five years on the species' habitat at the site. 1 point

c. The landowner(s) has agreed verbally to implement land management practices in a manner conducive to the conservation of Dakota skippers for the foreseeable future on the species' habitat at the site. 0 point

d. The landowner has not agreed verbally or in writing to implement land management practices in a manner conducive to the conservation of Dakota skippers on the species' habitat at the site. -1 point

e. The landowner has expressed opposition to the species' reintroduction and has not agreed verbally or in writing to implement land management practices in a manner conducive to the conservation of Dakota skippers on the species' habitat at the site. - 2 points

At this stage, TNC has expressed support for the reintroduction and is cooperating with the Service and Minnesota Zoo to select the site where reintroduced Dakota skippers may be most likely to thrive, based on high relative density of purple coneflower; is planning to burn the reintroduction area to further enhance habitat quality; and, has expressed informally its implicit support to reestablish the species on its property at HIM. In addition, TNC has proposed a Dakota skipper habitat management study that would last at least five years at HIM and at other sites where the species may be present in Minnesota, North Dakota and South Dakota. They have applied to the Service for an endangered species permit that would cover any take of the Dakota skipper that could occur as a result of their habitat management in these areas. Prescribed fire intended to enhance habitat for the Dakota skipper, for example, could result in take of the species. TNC has proposed to manage lands where the Dakota skipper may be present, including HIM after its release, in a manner that is presumed to maximize the likelihood that the populations present will persist. TNC's permit application is evidence that it will engage with the Service to cooperatively manage their lands in a way that will conserve Dakota skipper populations.

7) Distance to row-crop agriculture

a. There is no row-crop agriculture within two miles of the site. 2 points

b. There is no row-crop agriculture within one mile of the site. 1 point

c. There is row-crop agriculture within 0.5-1 mile of the site. 0 point

d. There is row-crop agriculture within one-half mile of the site. -1 point

This ranking factor is an index to the threat posed potentially by drift of pesticides from row-crop areas. This potential threat is recognized above, in the section, Ecological Risk. A preliminary assessment of pesticide residues at HIM carried out by Minnesota Zoo in 2016, showed that there is some pesticide drift into HIM (Section VII.B.). Similar, if not higher levels have been observed at other candidate sites considered for reintroduction in this plan (Prairie Coteau and Glacial Lakes State Parks).

XV. Acknowledgements

Numerous partners have contributed to the development of this plan. Useful comments on early versions of this draft were provided by Dr. Ron Royer (Minot State University Emeritus), Dr. Richard Westwood (University of Winnipeg), Marisa Ahlering (The Nature Conservancy), Dr. Tara Harris (Minnesota Zoo), Gerald Selby (Ecological and GIS Services, Indianola, IA), and Robert Dana (Minnesota DNR). Comments and advice from other Dakota skipper and prairie butterfly researchers has been vital. We owe our understanding of the state of Dakota skippers to decades of concerted research.

The Minnesota Zoo's Prairie Butterfly Conservation Program has been primarily funded since 2013 by grants from Minnesota's Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizens Commission on Minnesota Resources, Interagency Cooperative Agreement Grants from the U.S. Fish and Wildlife Service, Minnesota's Legacy Amendment Arts and Cultural Heritage Fund, the Minnesota Zoo Foundation, and the Association of Zoos and Aquariums Conservation Grants Fund via the Disney Conservation Fund. Permits and permissions for Dakota skipper *ex situ* operations have been granted to the Minnesota Zoo from the U.S. Fish and Wildlife Service, the Minnesota Department of Natural Resources, the Sisseton Wahpeton Oyate of the Lake Traverse Reservation, The Nature Conservancy, and the U.S. Department of Agriculture's Animal and Plant Health Inspection Service.

COMMON BACKYARD Butterflies

There are roughly 146 species of butterflies regularly occurring in Minnesota. These are some of the most regular visitors to backyard gardens and parks in the Twin Cities and beyond.

MOURNING CLOAK

MONARCH

CABBAGE WHITE

EASTERN TIGER SWALLOWTAIL

BLACK SWALLOWTAIL

EASTERN TAILED-BLUE

SILVER-SPOTTED SKIPPER

PEARL CRESCENT

ORANGE SULPHUR

RED ADMIRAL

Minnesota Butterflies THREATENED AND ENDANGERED

MN ENDANGERED:

Persius duskywing
Erynnis persius persius

Ottoe skipper
Hesperia ottoe

Dakota skipper*
Hesperia dacotae

Assiniboia skipper
Hesperia assiniboia

Uncas skipper
Hesperia uncas

*Karner blue
Lycaeides samuelis

Poweshiek skipperling*
Oarisma poweshiek

Uhler's arctic
Oeneis uhleri varuna

MN THREATENED:

Garita skipperling
Oarisma garita

MN SPECIAL CONCERN:

Arogos skipper
Atrytone arogos iowa

Disa alpine
Erebia mancinus

Leonard's skipper
Hesperia leonardus

Nabokov's blue
Lycaeides idas nabokovi`

Grizzled skipper
Pyrgus centaureae freija

Regal fritillary
Speyeria idalia

*** U.S. Threatened/Endangered**

Photo credit: *Andrew Warren, butterfliesofamerica.org*

MINNESOTA ZOO SAVING PRAIRIE BUTTERFLIES

Prairie butterflies and other pollinators are struggling. Minnesota Zoo scientists are working with many partners to save Minnesota's endangered prairie butterflies. We are breeding butterflies at the Zoo and studying reasons for their decline in the wild.

MALE FEMALE

DAKOTA SKIPPER

Hesperia dacotae
Habitat: Tallgrass and Mixed prairie
Status: U.S. Threatened, MN Endangered
Host plant: Native grasses

The Dakota skipper is an adorable prairie specialist butterfly. Males are orange-yellow and females are latte colored. This species has vanished from more than 75 percent of its former range. The Minnesota Zoo is breeding this species to create "insurance populations" to prevent their extinction. The Zoo has also begun reintroducing Dakota skippers to prairies they have disappeared from.

Learn more about the Minnesota Zoo's work to save butterflies at **mnzoo.org/savebutterflies**

WHAT CAN YOU DO TO ATTRACT BUTTERFLIES AND PROTECT POLLINATORS IN YOUR GARDEN?

- Plant wildflowers native to your region. Check out a big list of Minnesota-native pollinator favorites at **mnzoo.org/plantforpollinators**
- Choose your plants so that there are different flowers blooming from spring to fall.
- Avoid pesticides and avoid purchasing plants that have been treated with pesticides. Ask your garden store for pesticide-free plants.
- Choose plants of different heights.
- Choose plants that provide food for butterfly caterpillars. For example, monarch caterpillars can only eat milkweed.
- Get out and look for butterflies and your other insect neighbors!

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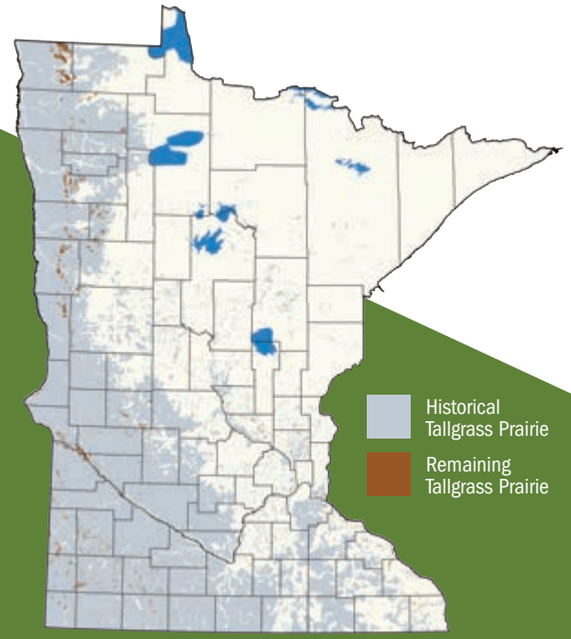
MINNESOTA ZOO

GET TO KNOW YOUR Butterfly Neighbors

Minnesota's LOST PRAIRIE

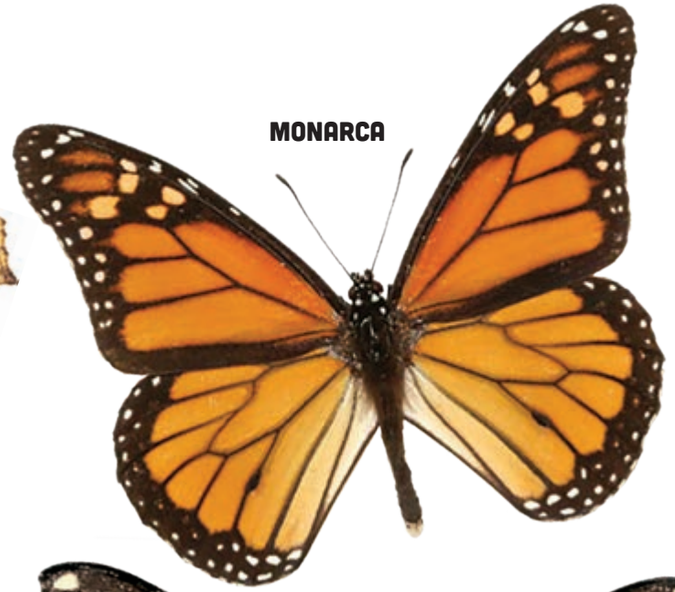


The tallgrass prairie once covered about 33 percent of Minnesota and it shaped our history. Today only one percent of it remains. Many animals and plants that need prairie have declined or vanished. Habitat loss is the major cause, but some species declined rapidly for other unknown reasons in recent years. Butterflies are "canary in the coalmine" indicators of prairie health because of their sensitivity to changes in their habitats.

Mariposas
COMUNES DE JARDÍN

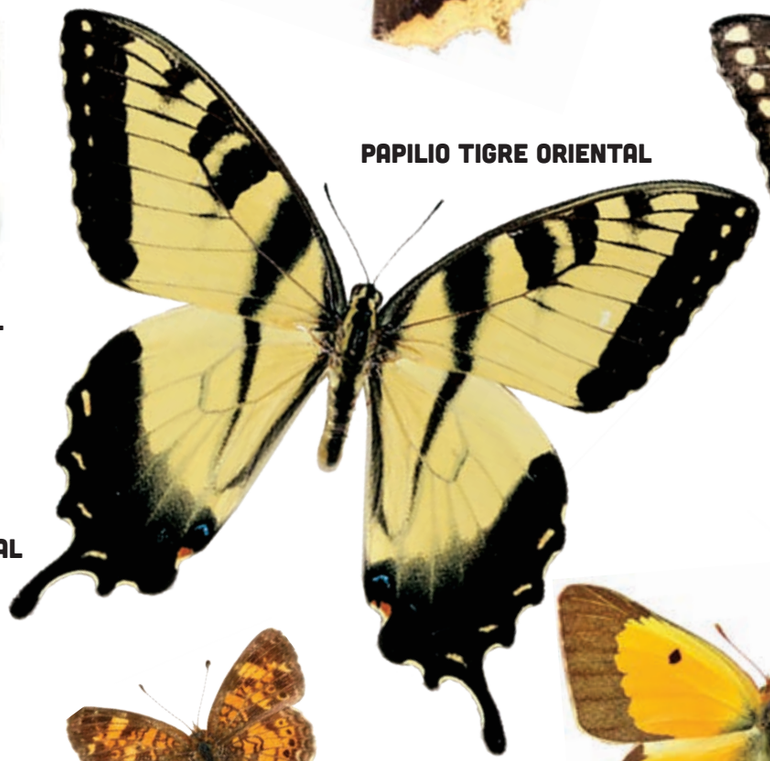
En Minnesota hay cerca de 146 especies de mariposas presentes habitualmente. Estas son algunas de las visitantes más comunes de los jardines y parques de las Ciudades Gemelas (Twin Cities) y el resto del estado.

CAPA DE LUTO



MONARCA

BLANQUITA DE LA COL



PAPILIO TIGRE ORIENTAL

PAPILIO NEGRA

AZUL DE COLA ORIENTAL

HESPÉRIDO DE MANCHAS PLATEADAS

CRECIENTE PERLADA

AZUFRE NARANJA

ALMIRANTE ROJO

Mariposas de Minnesota
EN PELIGRO DE EXTINCIÓN Y AMENAZADAS

ESPECIES DE MN EN PELIGRO DE EXTINCIÓN:

Hespérido Persius
Erynnis persius persius

Hespérido Ottoe
Hesperia ottoe

Hespérido Dakota*
Hesperia dacotae

Hespérido Assiniboia
Hesperia assiniboia

Hespérido Uncas
Hesperia uncas

*Azul Karner
Lycaeides samuelis

Hespérido Poweshiek*
Oarisma poweshiek

Ártica de Uhler
Oeneis uhleri varuna

ESPECIES DE MN AMENAZADAS:

Hespérido Garita
Oarisma garita

ESPECIES DE MN DE PREOCUPACIÓN ESPECIAL:

Hespérido Arogos
Atrytone arogos iowa

Alpino Mancinus
Erebia mancinus

Hespérido de Leonard
Hesperia leonardus

Azul de Nabokov
Lycaeides idas nabokovi

Hespérido canoso
Pyrgus centaureae freija

Fritillaria real
Speyeria idalia

*** Especies de EE. UU. amenazadas/en peligro de extinción**

Fotografías: *Andrew Warren, butterfliesofamerica.org*

ZOOLÓGICO DE MINNESOTA: SALVAMOS A LAS MARIPOSAS DE LA PRADERA

Las mariposas y otros polinizadores de la pradera están luchando por sobrevivir. Los científicos del Zoológico de Minnesota están trabajando con varios colaboradores para salvar a las mariposas de las praderas de Minnesota que se encuentran en peligro de extinción. Estamos criando mariposas en el zoológico y estudiando las razones de su disminución en la naturaleza.

MACHO HEMBRA

HESPÉRIDO DAKOTA

Hesperia dacotae
Hábitat: praderas de hierbas altas y mixtas
Condición: especie amenazada en EE. UU.; en peligro de extinción en MN
Planta huésped: hierbas autóctonas

El hespérido Dakota es una mariposa de pradera muy admirada por los especialistas. Los machos son de color naranja amarillento y las hembras son de color café con leche. Esta especie ha desaparecido de más del 75 % de su hábitat antiguo. El Zoológico de Minnesota está criando esta especie para crear "colonias de protección" a fin de evitar su extinción. Asimismo, el Zoológico ha empezado a reintroducir a los hespéridos Dakota en las praderas de las que habían desaparecido.

Obtenga más información sobre el trabajo que realiza el Zoológico de Minnesota para salvar a las mariposas en **mnzoo.org/savebutterflies**

¿QUÉ PUEDE HACER PARA ATRAER A LAS MARIPOSAS Y PROTEGER A LOS POLINIZADORES EN SU JARDÍN?

- Plante flores silvestres autóctonas de su región. Consulte una extensa lista de plantas favoritas de los polinizadores autóctonos de Minnesota en **mnzoo.org/plantforpollinators**
- Escoja sus plantas de manera que florezcan flores diferentes de primavera a otoño.
- Evite usar pesticidas y evite comprar plantas que hayan sido tratadas con pesticidas. Pida plantas libres de pesticidas en su vivero favorito.
- Escoja plantas de diferentes alturas.
- Escoja plantas que proporcionen alimento para las larvas de mariposa. Por ejemplo, las larvas de la mariposa monarca solo pueden comer algodoncillo.
- ¡Salga y mire las mariposas y otros insectos vecinos!

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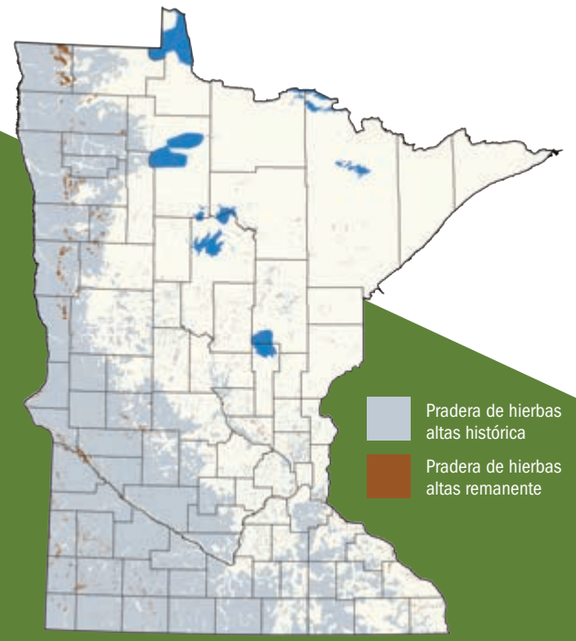
CONOZCA A SUS

mariposas vecinas

Las praderas perdidas DE MINNESOTA



Antiguamente, la pradera de hierbas altas cubría aproximadamente el 33 % de Minnesota y dio forma a nuestra historia. En la actualidad, solo queda un 1 %. Muchos animales y plantas que necesitaban la pradera han disminuido o desaparecido. La pérdida del hábitat es la causa principal, pero en los últimos años algunas especies han disminuido rápidamente por otros motivos desconocidos. Las mariposas son el "conejillo de indias" que sirve como indicador de la salud de la pradera debido a su sensibilidad a los cambios que se producen en su hábitat.

MINNESOTA NATIVE
SPRING FLOWERS:

American pasqueflower
(1/2–1ft tall)
Anemone patens

Wild columbine
(1–3ft tall)
Aquilegia canadensis

Marsh marigold
(1ft tall)
Caltha palustris

Wild geranium
(1–3ft tall)
Geranium maculatum

Virginia waterleaf
(1–2ft tall)
Hydrophyllum virginianum

Prairie smoke
(1ft tall)
Geum triflorum

Wild lupine
(1–2ft tall)
Lupinus perennis

MINNESOTA NATIVE
MID-SUMMER FLOWERS:

Fragrant hyssop
(2–4ft tall)
Agastache foeniculum

Butterfly weed
(1–3ft tall)
Asclepias tuberosa

Common milkweed
(2–4ft tall)
Asclepias syriaca

Yellow coneflower
(3–5ft tall)
Ratibida pinnata

Purple prairie clover
(1–3ft tall)
Dalea purpurea

Rattlesnake master
(3–5ft tall)
Eryngium yuccifolium

Wild bergamot
(2–5ft tall)
Monarda fistulosa

MINNESOTA NATIVE
LATE-SUMMER-FALL FLOWERS:

Spotted joe pye weed
(4–10ft tall)
Eutrochium maculatum

Bottle gentian
(1–2ft tall)
Gentiana andrewsii

Maximilian sunflower
(4–8ft tall)
Helianthus maximiliani

Rough blazing star
(2–3ft tall)
Liatris aspera

Dwarf blazing star
(1–2ft tall)
Liatris cylindracea

Goldenrod
(3–4ft tall)
Solidago sp.

Smooth blue aster
(1–3ft tall)
Symphyotrichum laevis

WHY PLANT NATIVE PLANTS?

- Native plants are adapted to our climate, so they require very little care once they are established.
- Many native plants grow long root systems. This makes them useful for erosion control and makes many resistant to drought.
- Many cultivars and hybrid plant species produce very little, if any, nectar or pollen for pollinators. They have been bred by people for extra petals or novel colors, not their benefit to pollinators.
- Native plants are beautiful!

You can see many of these native wildflowers (and more!) in the prairie demonstration space around the Tiger and Toucan parking lots at the Minnesota Zoo!

Check out mnzoo.org/plantforpollinators for more pollinator favorites!

3. Meadow Blazing Star

Liatris ligulistylis (3–5 feet tall)
Blooms Late Summer to Early Fall
This is the preferred plant for monarch butterflies in late August and September as they load up on energy before migrating to Mexico for the winter.

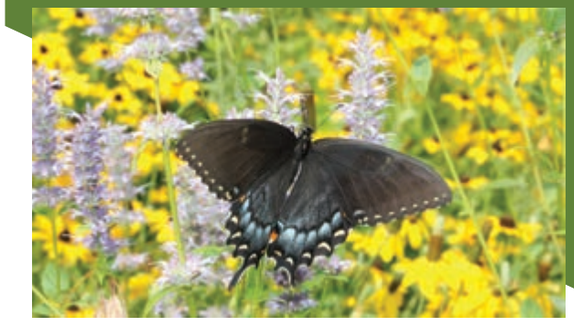
4. Black-eyed Susan

Rudbeckia hirta (1–3 feet tall)
Blooms June through September
Black-eyed susan is an iconic plant and an important food source for many butterflies and bees. They can thrive in a variety of soil types.

5. New England Aster

Symphyotrichum novae-angliae (1–4 feet)
Blooms Autumn
Bright purple flowers make New England asters one of the most striking plants of fall. It is loved by almost all pollinators and is a host plant for crescent butterflies.

TIPS FOR ATTRACTING POLLINATORS TO YOUR GARDEN



- Plant wildflowers native to your region.
- Choose a variety of plants that bloom from spring to fall.
- Avoid pesticides and avoid purchasing plants that have been treated with pesticides. Ask your garden store for pesticide-free plants.
- Choose plants of different heights.
- Choose plants that provide food for butterfly caterpillars. For example, monarch caterpillars can only eat milkweed.

For more resources about gardening for pollinators and to learn more about Minnesota Zoo's work to save butterflies visit mnzoo.org/savebutterflies

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PLANT FOR POLLINATORS

Bees, butterflies and other animals pollinate most wild plants, as well as at least a third of our food. Pollinators have been declining due to habitat loss and the lack of nectar and pollen resources.

Minnesota Zoo scientists are working with many partners to save Minnesota's endangered prairie butterflies. We are breeding butterflies at the Zoo and studying reasons for their decline in the wild.

You can make a difference in your own garden. No matter how large, pollinator gardens with native plants can have a huge impact!

TOP FIVE BEST CHOICE NATIVE PLANTS

- Full Sun
- Partial Sun
- Shade
- Wet
- Moderate
- Dry

1. Rose (swamp) Milkweed

Asclepias incarnata (2–4 feet tall)
Blooms mid-summer
They thrive in a variety of soil types, particularly moister soils and are a caterpillar host for Monarchs. The clusters of pink flowers will attract many pollinator species.

2. Purple Coneflower

Echinacea sp. (3–4 feet tall)
Blooms mid-summer
Purple coneflowers are rich nectar and pollen sources for pollinators. Narrow-leaf purple coneflower (*E. angustifolia*) is native to western Minnesota prairies. The most commonly sold species is purple coneflower (*E. purpurea*), but it is not actually native to Minnesota.

AUTÓCTONAS DE MINNESOTA
FLORES DE PRIMAVERA:

Pulsatilla americana
(15-30 cm de altura)
Anemone patens

Aguileña silvestre
(30-90 cm de altura)
Aquilegia canadensis

Caléndula acuática
(30 cm de altura)
Caltha palustris

Geranio silvestre
(30-90 cm de altura)
Geranium maculatum

Hoja de agua de Virginia
(30-60 cm de altura)
Hydrophyllum virginianum

Humo de pradera
(30 cm de altura)
Geum triflorum

Lupino silvestre
(30-60 cm de altura)
Lupinus perennis

AUTÓCTONAS DE MINNESOTA
FLORES DE MEDIADOS DE VERANO:

Hisopo de anís
(60-120 cm de altura)
Agastache foeniculum

Hierba de las mariposas
(30-90 cm de altura)
Asclepias tuberosa

Algodoncillo común
(60-120 cm de altura)
Asclepias syriaca

Flor cónica amarilla
(90-150 cm de altura)
Ratibida pinnata

Trébol de pradera violeta
(30-90 cm de altura)
Dalea purpurea

Maestra de serpientes de cascabel
(90-150 cm de altura)
Eryngium yuccifolium

Bergamota silvestre
(60-150 cm de altura)
Monarda fistulosa

AUTÓCTONAS DE MINNESOTA
FLORES DE FINAL DE VERANO/OTOÑO:

Maleza de Juan Pye
(1.2-3 m de altura)
Eutrochium maculatum

Genciana cerrada
(30-60 cm de altura)
Gentiana andrewsii

Girasol Maximiliano
(120-240 cm de altura)
Helianthus maximiliani

Estrella ardiente áspera
(60-90 cm de altura)
Liatris aspera

Estrella ardiente enana
(30-60 cm de altura)
Liatris cylindracea

Vara de San José
(90-120 cm de altura)
Solidago speciosa

Margarita azul
(30-90 cm de altura)
Symphyotrichum laevis

¿POR QUÉ PLANTAR FLORES AUTÓCTONAS?

- Las plantas autóctonas están acostumbradas a nuestro clima, así que requieren muy pocos cuidados una vez plantadas.
- Muchas plantas autóctonas desarrollan redes de raíces largas. Esto las convierte en plantas muy útiles para el control de la erosión y las hace resistentes a la sequía.
- Muchas especies de plantas híbridas y cultivadas producen muy poco néctar o polen para los polinizadores, si es que lo producen. Han sido cultivadas por la gente por su gran cantidad de pétalos o por sus colores originales y no por los beneficios que aportan a los polinizadores.
- ¡Las flores autóctonas son bellas!

Puede ver varias de estas flores silvestres autóctonas (¡y muchas más!) en el espacio de demostración de praderas que rodea los estacionamientos Tiger (Tigre) y Toucan (Tucán) del Zoológico de Minnesota.

¡Visite mnzoo.org/plantforpollinators para ver otras plantas favoritas de los polinizadores!

3. Estrella ardiente de la pradera

Liatris ligulistylis (90-150 cm de altura)
Florece entre el final del verano y el principio del otoño
Esta es la planta preferida por las mariposas monarca a fines de agosto y septiembre, cuando adquieren una gran cantidad de energía antes de emigrar a México para pasar el invierno.

4. Rudbeckia hirta

Rudbeckia hirta (30-90 cm de alto)
Florece de junio a septiembre
La Rudbeckia hirta es una planta icónica y una fuente importante de alimento para muchas mariposas y abejas. Pueden crecer bien en una variedad de tipos de suelo.

5. Margarita de Nueva Inglaterra

Symphyotrichum novae-angliae (30-120 cm)
Florece en otoño
Sus brillantes flores moradas hacen que la margarita de Nueva Inglaterra sea una de las plantas más espectaculares del otoño. Les encanta a la mayoría de los polinizadores y es una planta huésped para las mariposas del género creciente.

CONSEJOS PARA ATRAER A LOS POLINIZADORES A SU JARDÍN

- Plante flores silvestres autóctonas de su región.
- Escoja una variedad de plantas que florezcan de primavera a otoño.
- Evite usar pesticidas y evite comprar plantas que hayan sido tratadas con pesticidas. Pida plantas libres de pesticidas en su vivero favorito.
- Escoja plantas de diferentes alturas.
- Escoja plantas que proporcionen alimento para las larvas de mariposa. Por ejemplo, las larvas de la mariposa monarca solo pueden comer algodoncillo.

Para ver más fuentes de información sobre jardinería para polinizadores y más detalles sobre el trabajo que está realizando el Zoológico de Minnesota para salvar a las mariposas, visite mnzoo.org/savebutterflies

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PLANTE PARA LOS POLINIZADORES

Las abejas, las mariposas y otros animales polinizan la mayoría de las plantas silvestres, así como al menos un tercio de nuestros alimentos. Debido a la pérdida de sus hábitats y la falta de fuentes de néctar y polen, los polinizadores se encuentran en una fase de declive.

Los científicos del Zoológico de Minnesota están trabajando con varios colaboradores para salvar a las mariposas de las praderas de Minnesota que se encuentran en peligro de extinción. Estamos criando mariposas en el zoológico y estudiando las razones de su disminución en la naturaleza.

Usted puede marcar la diferencia en su propio jardín. ¡Independientemente de su tamaño, los jardines con plantas autóctonas para polinizadores pueden tener un gran impacto!

LAS MEJORES CINCO PLANTAS AUTÓCTONAS

Pleno sol
Sol parcial
Sombra
Húmedo
Moderado
Seco

1. Algodoncillo rosa (de pantano)

Asclepias incarnata (60-120 cm de altura)
Florece a mediados de verano
Crecen bien en una variedad de tipos de suelo, especialmente en suelos más húmedos, y son una planta huésped para las larvas de las mariposas monarca. Las inflorescencias plurifloras de color rosa atraen a muchas especies de polinizadores.

2. Equinácea morada

Echinacea speciosa (90-120 cm de altura)
Florece a mediados de verano
Las equináceas moradas son una rica fuente de néctar y polen para los polinizadores. La equinácea morada de hoja estrecha (*E. angustifolia*) es una planta autóctona de las praderas del oeste de Minnesota. La especie que se vende más habitualmente es la equinácea morada (*E. purpurea*), pero esta no es autóctona de Minnesota en realidad.