

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 ENRTF 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

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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 provide 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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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:

ENTRF 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 ft³ 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 ft³ and 2 ft³) 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°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		Overall % treatment recovery
		# surviving to hibernation	% surviving to hibernation	# surviving through hibernation	% surviving hibernation (December to May)	
Hibernated Indoors	Initial Total					
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		Overall % treatment recovery
		# surviving to hibernation	% surviving to hibernation	# surviving through hibernation	% surviving through hibernation (December- June)	
Hibernated Outdoors	Initial Total					
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).

Hibernated Indoors	Initial Total	Entering Hibernation		After Hibernation		Overall % treatment recovery
		# surviving to hibernation	% surviving to hibernation	# surviving through hibernation	% surviving through hibernation	
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 <i>Wolbachia</i> 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 <i>Wolbachia</i> strains in Dakota skippers. Assessment of genetic	March 2016	\$ 3,500

diversity of any Zoo-bred Poweshiek skipperlings and/or Dakota skippers for <i>ex situ</i> 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 <i>ex situ</i> 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 clearer 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. Atrazine 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 ENTRF-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. ENTRF 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 ENTRF 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 ENTRF Appropriation: 3.75

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

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
Zoo admissions donations from the public. <u>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.</u>	\$19,385 <u>+\$5,000</u> <u>+7,000</u>	\$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. <u>Additional Funds from the Zoo's General budget were used in late 2015 to cover construction and permitting costs.</u>	\$ 40,000 <u>+ \$65,000</u> <u>+\$40,000</u> <u>+\$5,000</u>	\$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. <u>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 ENTRF funds for the analysis of 2016 prairie pesticides residue samples.</u>
<u>MN Zoo General operating budget</u>	<u>\$17,645</u>	<u>\$17,645</u>	<u>Additional funds to cover construction costs for the new husbandry facilities.</u>
<u>US Fish and Wildlife Service CFDA Endangered Species – Candidate Conservation Action Fund Cooperative Agreement</u>	<u>\$20,000</u>	<u>\$20,000</u>	<u>To supplement all work outlined in the pesticides research in Activity 4. Funds will be available beginning November 2014.</u>

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

<div> <div>Environment and Natural Resources Trust Fund</div> <div>M.L. 2014 Project Budget</div> </div>														
<div> <div>Project Title:</div> <div>Imperiled Prairie Butterfly Conservation, Research and Breeding Program - Minnesota Zoo portion</div> </div>														
<div> <div>Legal Citation:</div> <div>M.L. 2014, Chp. 226, Sec. 2, Subd. 05j-1</div> </div>														
<div> <div>Project Manager:</div> <div>Dr. Erik Runquist</div> </div>														
<div> <div>Organization:</div> <div>Minnesota Zoo</div> </div>														
<div> <div>M.L. 2014 ENRTF Appropriation:</div> <div>\$ 380,000 to the Minnesota Zoo</div> </div>														
<div> <div>Project Length and Completion Date:</div> <div>3 years, June 30, 2017</div> </div>														
<div> <div>Date of Report:</div> <div>August 15, 2017</div> </div>														
<div> <div>ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET</div> <div> <div>Activity 1 Budget</div> <div>Amount Spent</div> <div>Activity 1 Balance</div> <div>Activity 2 Budget</div> <div>Amount Spent</div> <div>Activity 2 Balance</div> <div>Activity 4 Budget</div> <div>Amount Spent</div> <div>Activity 4 Balance</div> <div>Activity 5 Budget</div> <div>Amount Spent</div> <div>Activity 5 Balance</div> <div>TOTAL BUDGET</div> <div>TOTAL BALANCE</div> </div> </div>														
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														

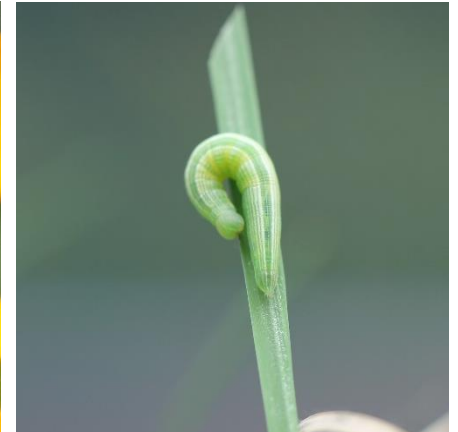
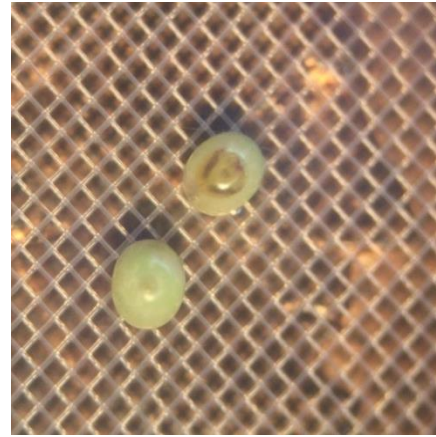




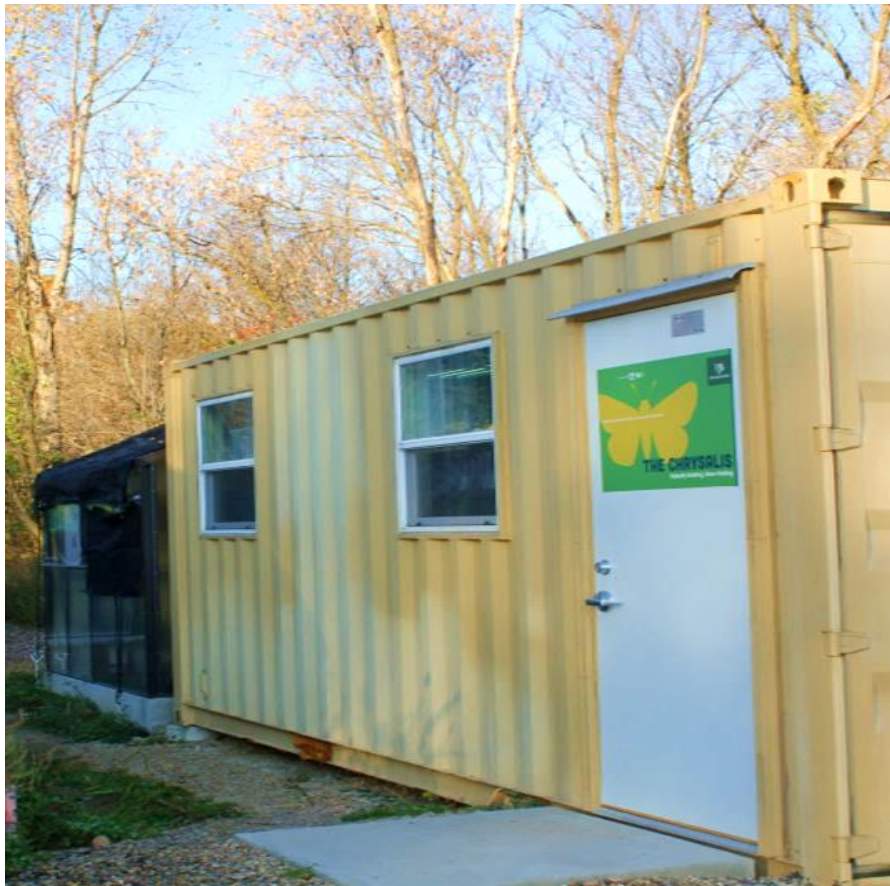
Thanks to ENRTF support, the Minnesota Zoo has established the first and only breeding program for the endangered Dakota skipper. This conservation population has grown from 44 in 2013 to over 375 adults in 2017.



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.



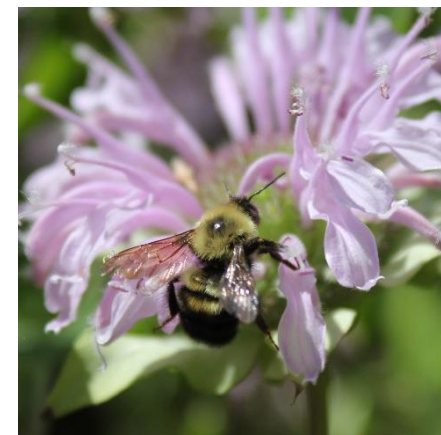
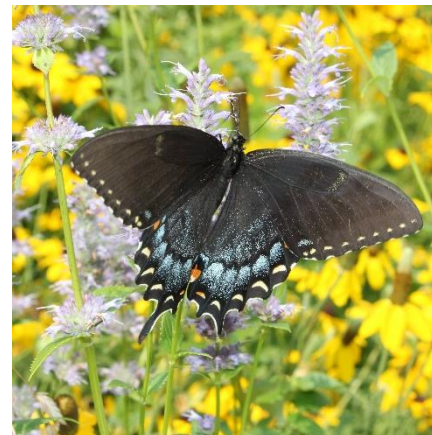
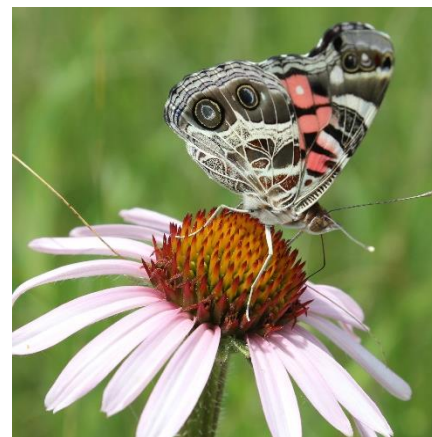
The Poweshiek skipperling was once common across Minnesota's prairies. Due to habitat loss and other threats though, it is now one of the world's most endangered animals. With ENRTF funding, the Minnesota Zoo started a rearing program to help support the last populations of this butterfly in the United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



“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.



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.



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.