

M.L. 2016 Project Abstract

For the Period Ending June 30, 2019

PROJECT TITLE: Prairie Butterfly Conservation, Research, and Breeding – Phase II

PROJECT MANAGER: Dr. Erik Runquist

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FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 03c1

APPROPRIATION AMOUNT: \$ 421,000

AMOUNT SPENT: \$ 374,874

AMOUNT REMAINING: \$ 11,126

Sound bite of Project Outcomes and Results

Many prairie butterflies are declining, and some are in danger of extinction. The Minnesota Zoo is supporting disappearing populations of Dakota skipper and Poweshiek skipperling through wild reintroductions and releases, and Zoo-based insurance populations. We are researching stressors to wild populations and the conditions needed for them to thrive.

Overall Project Outcome and Results

The Prairie Butterfly Conservation Program at the Minnesota Zoo has grown substantially during this project, and we have accomplished all Outcomes. We operate the only conservation rearing programs for two endangered butterflies in the United States. We launched the first Dakota skipper reintroduction program, rearing and releasing ~930 Dakota skippers at the Hole-in-the-Mountain Prairie Preserve near Lake Benton, and reared thousands more. We are reinforcing one of the last populations of the Critically Endangered Poweshiek skipperling, the first of its kind in the US, releasing the two adults in 2018 and 14 more in 2019. We are also improving husbandry practices with the Minnesota Threatened garita skipperling. We currently hold thousands more of these three butterflies to continue the insurance, reintroduction, reinforcement, and research programs, supported in part by a new ENRTF appropriation.

We have improved our understanding of threats to wild populations and of conditions needed to sustain and re-establish healthy populations. We have contributed to a key research need by beginning to link the quantities of pesticides found on prairie grasses that the protected skippers feed on in the wild with experimental testing of the toxicity effects of those quantities on skipper caterpillars. Significant additional research is needed in this area to fully understand the scope of the risk, particularly from pesticides that we have commonly observed in the wild but have not yet tested.

The Minnesota Zoo has received significant attention through media interviews and invited speaking engagements. We have created and established a great deal, and many agencies and organizations seek our advice as experts, but recovery of these endangered butterflies can only be fully achieved through partnerships. Successful conservation of these butterflies and the prairies they depend upon will have broad benefits to a wide range of wildlife and Minnesotans.

Project Results Use and Dissemination

Our work has garnered attention and the Minnesota Zoo has become a key agency in the international conservation programs for Poweshiek skipperling and Dakota skipper. We remain active in multiple planning processes and multi-party action teams for both species and have hosted multiple workshops and meetings, including the 2019 meeting for the International Poweshiek Skipperling Conservation Partnership. Similarly, the field component of the Skipper Identification Workshop of this joint Zoo-DNR ENRTF appropriation (Activity 4; to be discussed in greater detail in the Update provided by DNR) was held in late June 2019 at the Hole-in-the-Mountain Prairie Preserve at the site of the Dakota skipper reintroduction program as well as several other sites in southwest Minnesota.

Across the duration of this project, there have been five TV stories, two radio segments, three newspaper articles, two major blog posts, dozens of social media posts, and dozens of speaking engagements. The Dakota skipper reintroduction effort has recently been the subject of TV segments (including two live) and a newspaper story that was widely shared

across other news outlets across Minnesota and the Northern Plains, including Public TV's [Prairie Sportsman](#), [Pioneer Press](#), [KSFY-TV](#), (Sioux Falls, SD), and [KARE11-TV](#), (Minneapolis, MN). The research generated by this Project will be submitted for publication a peer-reviewed scientific journals.



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2016 Work Plan

Date of Report: August 30, 2019

Date of Next Status Update Report: Final Report

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2019

PROJECT TITLE: Prairie Butterfly Conservation, Research, and Breeding – Phase II

Part 2 (Activities 3 and 4) of the project is described in a separate work plan with an appropriation of \$329,000 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: \$421,000

Amount Spent: \$409,874

Balance: \$ 11,126

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 03c1

Appropriation Language:

\$750,000 the second year is from the trust fund. Of this amount, \$421,000 is to the Minnesota Zoological Garden and \$329,000 is to the commissioner of natural resources in collaboration with the United States Fish and Wildlife Service to continue efforts to prevent the extinction of imperiled native Minnesota butterfly species through breeding, research, field surveys, and potential reintroduction. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Prairie Butterfly Conservation, Research and Breeding - Phase 2

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. Ten of Minnesota's prairie butterflies are of statewide conservation concern and two, the Poweshiek skipperling (*Oarisma poweshiek*) and Dakota skipper (*Hesperia dacotae*), are listed as Endangered and Threatened Species under the U.S. Endangered Species Act. Both have disappeared from the majority of their historic ranges (96+% for Poweshiek, 76+% for Dakota) in recent decades. Dakota skippers may only remain in one or two Minnesota locations. The Poweshiek skipperling 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 surveys across the remaining isolated known populations in Michigan, Wisconsin, and Manitoba indicate that fewer than 500 Poweshiek skipperlings likely remain globally.

In partnership with the US Fish and Wildlife Service and the Minnesota Department of Natural Resources, the Minnesota Zoo's Prairie Butterfly Conservation Program is establishing the world's first and only conservation breeding populations for endangered, threatened, and imperiled Minnesota-native prairie butterflies. We utilize the recognized organizational capacity and experience of the Minnesota Zoo for the conservation of endangered species.

Currently largely supported by a M.L. 2014 ENTRF (M.L. 2014, Chp. 226, Sec. 2, Subd. 05j-1), this new ENTRF Project 009-A will provide the resources necessary to continue and expand the Minnesota Zoo's Prairie Butterfly Conservation Program. Based on the recommendations from an independent working group, the Minnesota Zoo has been tasked with beginning the world's first wild population supplementations and reintroductions of endangered Poweshiek skipperlings and Dakota skippers. It will also allow the Minnesota Zoo to continue to research on the risk to these endangered butterflies of potential exposure to widely applied agricultural pesticides.

The Minnesota Zoo is collaborating with the Minnesota Department of Natural Resources (DNR) for this joint ENTRF. Described in a separate Work Plan, the DNR will simultaneously monitor the status of these and a number of additional targeted butterfly species on native prairie remnants across Minnesota and will work to train new surveys to help alleviate a severe shortage of qualified observers. 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 disappearance of these historically widespread Minnesota prairie butterflies is noteworthy and troubling.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of November 30, 2016:

The Minnesota Zoo's Prairie Butterfly Conservation Program continues to expand with the support of ENTRF resources. We initiated the world's first head starting program for Poweshiek skipperlings in 2016, and we are planning to release head started individuals as part of the population supplementation efforts in 2017. A portion of the Zoo's rearing and breeding population of Dakota skippers will be released in western Minnesota during late spring and early summer, 2017, as the first phase of reintroduction efforts. We have helped Winnipeg's Assiniboine Park Zoo launch parallel *ex situ* operations with the garita skipperling (likely Poweshiek skipperling's closest living relative and a Minnesota Threatened Species) to help inform husbandry methods for Poweshiek. We remain in close partnership with the USFWS and all relevant parties for all of these 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:

Following high survivorship over the winter (see the May 2017 status update for our ML 2014 ENRTF for more details), the Minnesota 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 followed guidelines prepared by the IUCN to develop formal plans to begin reintroducing Dakota skippers to a high-quality prairie where they were historically common. This "Plan for the Controlled Propagation, Augmentation, and Reintroduction of Dakota skipper (*Hesperia dacotae*)" (Delphey, Runquist, and Nordmeyer, April 30, 2017; available upon request), details the justifications and protocols to use the Minnesota Zoo's existing Dakota skipper insurance population to attempt to reestablish a once large wild population at The Nature Conservancy's Hole-in-the-Mountain Preserve in Lincoln County, MN. In addition to the releases, planned for June 2017, we will conduct intensive monitoring of the Preserve and adjacent prairie remnants (WMAs managed by the Minnesota DNR) for Dakota skippers and other prairie butterflies. The Plan specifies that reintroductions at the Preserve should occur for at least the next three summers (through 2019), with additional intensive monitoring for two years beyond that. Critical to the reintroduction effort will be the existing partnerships that we have already established, and those partners have played an important role in the development of the reintroduction plan.

Unfortunately, the Poweshiek skipperling headstarting program has not been as successful. None of the remaining eight larvae survived winter hibernation. After extensive consultations, the US Fish and Wildlife Service recommended that the program continue as planned, with additional eggs collected this June. The installation of new federally funded equipment later this summer to provide greater manipulation of key microclimatic variables should improve our chances for success. Despite the setbacks, we believe we have learned about important but previously unknown aspects of skipperling biology. We have identified new research opportunities to advance Poweshiek skipperling husbandry, and with the additional support, we are optimistic that future efforts with Poweshiek skipperlings will be fruitful.

We continue to plan for a controlled pesticides exposure experiment, as well as for additional field sampling for pesticide residues at several prairies. Both of these activities will occur in late summer 2017.

Project Status as of November 30, 2017:

The summer of 2017 represented a major milestone in the Prairie Butterfly Conservation Program. For the first time, we began releasing Zoo-reared Dakota skippers into a Minnesota prairie where they once had been common. The Dakota skipper reintroduction effort garnered significant media attention. The Zoo's population of Dakota skippers also has continued to expand, holding more individuals than ever before. The Poweshiek skipperling headstarting program is continuing, with a limited number of new initial eggs and larvae this summer. For the first time, we are rearing garita skipperlings to our program to help optimize husbandry protocols for Poweshiek skipperlings. Equipment improvements (funded by the US Fish and Wildlife Service) resulted in no anomalously rapid larval development of Poweshiek skipperlings or garita skipperlings this summer.

Our investigations into the extent and potential biological consequences of the movement of agricultural pesticides into prairies have continued. We collected another season of sampling native grasses in late August at four Minnesota prairies, and again had them analyzed for a wide range of pesticides residues. We also initiated controlled exposure experiments in partnership with the University of Minnesota to understand the biological consequences of contact with one of those pesticides on a surrogate grass skipper species, with more detailed work planned for 2018 and beyond. This work is shared with our partners and an overview was presented at a large international scientific conference in Minneapolis in November.

Project Status as of May 31, 2018:

The Prairie Butterfly Conservation Program has remained active, despite a long winter that delayed the emergence of the Threatened and Endangered species from hibernation. Winter survivorship for these larvae was

generally good and in line with expectations. Plans are in place to continue the Dakota skipper reintroduction effort back to Minnesota prairie, to continue executing the recommended Poweshiek skipperling headstarting program, and to expand both programs in 2018.

The pesticides threats assessment research has been largely static over the winter, as planned. Some data processing has occurred, but significantly more research is planned for the summer of 2018, both in terms of field sample collections and toxicity exposure testing. We have been having extensive discussions with our partners with the US Fish and Wildlife, Minnesota Department of Agriculture, Minnesota Department of Natural Resources, and University of Minnesota, and the US Environmental Protection Agency to optimize the research extent, efficiency, and applicability using the remaining ENRTF funds on hand.

Project Status as of November 30, 2018:

We remain on track to accomplish all of our stated activities and outcomes. Our butterfly rearing and wild release efforts achieved a new milestone during the summer of 2018: the first release of Zoo-reared Poweshiek skipperlings back into the wild at one of the last U.S. populations. A new, larger, generation of Poweshiek skipperlings is currently being reared at the Zoo and these individuals are scheduled to be released back into the wild again in 2019. We have also expanded our rearing efforts with garita skipperling, and were able to breed the species for the first time. We continued breaking ground with Dakota skippers through a second year of reintroductions and are maintaining the largest conservation population we have ever held at the Zoo.

The pesticides research activity also has advanced. We completed our fifth year of prairie pesticides occurrence collections, and are now preparing to publish the results in peer-reviewed scientific literature. Building from this field research, we also completed a foundational detailed experiment to assess the potential risk to wild prairie butterflies of insecticide exposure under conditions that mimic real-world exposures.

We continue to be actively involved in multi-party formal recovery planning and risk assessments for Poweshiek skipperling and Dakota skipper, at local, state, national, and international levels.

We received an additional grant from the U.S. Fish and Wildlife Service via the Great Lakes Restoration Initiative. This Interagency Cooperative Agreement is supporting much of the activities associated with the Poweshiek skipperling reinforcement program, and now offsets the out-of-state costs that had previously been partially budgeted for under this ENRTF appropriation. This grant also offsets supplies costs (such as host plants, release boxes) for the Poweshiek skipperling program, and will be effective beyond the duration of this ENRTF appropriation into 2020.

Amendment Request 11/30/2018

The addition of the Dakota skipper reintroduction program and the associated required daily monitoring by Zoo staff in southwest Minnesota over 3+ weeks each summer has raised our in-state travel costs more than originally forecast. Conversely, we have been able to offset some of our out-of-state travel costs associated with Poweshiek skipperling through another new grant from US Fish and Wildlife Service via the Great Lakes Restoration Initiative. Additionally, the winter 2019 planning meeting for the international Poweshiek skipperling working group will actually be hosted by the Minnesota Zoo, instead of being held in Michigan as previously. The 2017 Amendment Request (approved 12/18/2017) to reserve up to \$3,000 of the out-of-state travel budget for Zoo staff to participate in critical planning meetings outside of Minnesota is now no longer needed. As such, we now formally request that \$3,000 of the remaining \$4,507 currently allocated to out-of-state travel be re-allocated to in-state travel for remaining duration of this ENRTF grant. This would be utilized to support the remaining in-state field efforts, particularly in June 2019, and leave enough funding in the out-of-state travel budget to complete required operations associated with Dakota skipper work.

Amendment Approved 12/7/2018

Project Status as of May 31, 2019:

We have remained active on multiple fronts throughout the winter of 2018-2019, particularly in our interactions with and integration into US and Canadian recovery planning for Dakota skippers and Poweshiek skipperlings. The expertise that we have built thanks to support from the ENRTF has been requested by multiple

agencies, and is helping to launch possible parallel prairie butterfly conservation and pesticides exposure research opportunities. We continue to remain on-track to accomplish our objectives.

Our rearing efforts for US listed Dakota skipper and Poweshiek skipperling have remained on track. Through the winter, we have continued to maintain a record large Dakota skipper population, and early results indicate high hibernation survivorship. The majority of these individuals will be dedicated to the on-going reintroduction effort at the Hole-in-the-Mountain Prairie Preserve. We are also well-positioned to advance the Poweshiek skipperling headstarting and augmentation program with additional releases of adults back into the only known remaining US populations following similarly high overwintering survivorship.

The pesticides drift and exposure studies are also remaining on track. Following additional statistical analyses, we are preparing for submission of the five years of research that ENRTF has supported, likely in late 2019 or early 2020.

Overall Project Outcomes and Results:

The Prairie Butterfly Conservation Program at the Minnesota Zoo has grown substantially during this project, and we have accomplished all Outcomes. We operate the only conservation rearing programs for two endangered butterflies in the United States. We launched the first Dakota skipper reintroduction program, rearing and releasing ~930 Dakota skippers at the Hole-in-the-Mountain Prairie Preserve near Lake Benton, and reared thousands more. We are reinforcing one of the last populations of the Critically Endangered Poweshiek skipperling, the first of its kind in the US, releasing the two adults in 2018 and 14 more in 2019. We are also improving husbandry practices with the Minnesota Threatened garita skipperling. We currently hold thousands more of these three butterflies to continue the insurance, reintroduction, reinforcement, and research programs, supported in part by a new ENRTF appropriation.

We have improved our understanding of threats to wild populations and of conditions needed to sustain and re-establish healthy populations. We have contributed to a key research need by beginning to link the quantities of pesticides found on prairie grasses that the protected skippers feed on in the wild with experimental testing of the toxicity effects of those quantities on skipper caterpillars. Significant additional research is needed in this area to fully understand the scope of the risk, particularly from pesticides that we have commonly observed in the wild but have not yet tested.

The Minnesota Zoo has received significant attention through media interviews and invited speaking engagements. We have created and established a great deal, and many agencies and organizations seek our advice as experts, but recovery of these endangered butterflies can only be fully achieved through partnerships. Successful conservation of these butterflies and the prairies they depend upon will have broad benefits to a wide range of wildlife and Minnesotans.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Breeding and potential reintroduction of endangered butterflies

Description:

In October 2015, the US Fish and Wildlife Service commissioned the Conservation Breeding Specialist Group (a branch of the International Union for the Conservation of Nature) to conduct an *ex situ* assessment and conservation planning workshop for the U.S.-listed Poweshiek skipperling and Dakota skipper. The workshop brought together experts from across the global ranges of these Minnesota endangered butterflies. Workshop attendees reached a consensus to continue and expand the Minnesota Zoo's *ex situ* programs with these species. Attendees recommended the initiation of the world's first wild reintroduction program of Dakota skippers using individuals that are reared and/or "headstarted" at the Minnesota Zoo. This reintroduction program will likely be fully implemented in the summer of 2017, but smaller efforts may begin in 2016 depending on site selection and availability of individuals. It was also recommended that the Minnesota Zoo initiate a new formal headstarting program with critically endangered Poweshiek skipperlings beginning in 2016 to help support the last relatively reliable populations in the United States in Michigan and reduce the risk of global extinction.

Continued funding from ENTRF will provide personnel and material support for expansion of our operations to conduct these recommended *ex situ* actions. It will also allow us to advance research on 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 hostplants 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 populations, and adaptive rearing techniques may take priority over experimental arrays in the short-term to maximize survivorship. We will also use non-endangered surrogate species closely related to the listed species to explore many of these questions. Note that the entire personnel (wage and benefits) budget for the entire program is grouped under this Activity for simplicity. In reality, personnel supported by this ENTRF will be working on some or all Minnesota Zoo Activities, but these percentages will vary proportionately within and across years.

Summary Budget Information for Activity 1:

ENTRF Budget: \$ 386,000
Amount Spent: \$ 374,874
Balance: \$ 11,126

Outcome	Completion Date
1. Collect first set of Poweshiek skipperling larvae and head start them at the Zoo; collect additional Dakota skipper larvae as needed and rear them at the Zoo	July 2016
2. Conduct first set of population supplementations of Poweshiek skipperlings	June 2017
3. Conduct first summer of reintroductions of Dakota skippers	June 2017
4. Collect second set of Poweshiek skipperling larvae and head start them at the Zoo; collect additional Dakota skipper larvae as needed and rear them at the Zoo	July 2017
5. Conduct second set of population supplementations of Poweshiek skipperlings	June 2018
6. Conduct second summer of reintroductions of Dakota skippers	June 2018
7. Collect third set of Poweshiek skipperling larvae and head start them at the Zoo; collect additional Dakota skipper larvae as needed and rear them at the Zoo	July 2018
8. Conduct third set of population supplementations of Poweshiek skipperlings	June 2019
9. Conduct third summer of reintroductions of Dakota skippers	June 2019

Project Status as of November 30, 2016:

Poweshiek skipperling head starting

Minnesota Zoo staff initiated egg collection for the new recommended head starting program for the critically Endangered Poweshiek skipperling in summer, 2016. Adhering to strict protocols, we collected 96 eggs from four females from one site in Michigan. Four additional temporarily-held females did not lay eggs. All wild females were returned alive back to the sites from which they were collected within 72 hours in accordance with permitted protocols.

The egg hatch rate was consistent with prior experiences and our goals ($83/96 = 86.5\%$), but neonate larval survivorship was lower than expected despite deliberate care, with 31 surviving past the first few days. Of those that survived, there was no further mortality until late-August. About two thirds of the larvae grew more rapidly than expected, with the majority reaching the final caterpillar stage (instar) by late-August; two even reached pupation. With just one generation per year, Poweshiek skipperlings are not expected to reach pupation until the following June. The reason for this accelerated development is not understood, but suggests that Poweshiek skipperling larvae may be particularly sensitive to small variations in temperature and other unknown conditions that we did not fully replicate. Similar unexpectedly rapid development was also observed at the Assiniboine Park Zoo (Winnipeg, Manitoba) with their new parallel butterfly conservation efforts with the closely related Garita skipperling using identical rearing methods.

To remain within the scope of the goals of the Poweshiek skipperling head starting effort while maximizing the chances for success, the nine smallest and most appropriately sized larvae were artificially transitioned into hibernation for release next summer back to their natal site (see below), while those largest individuals that had

surpassed viable hibernation size were allowed to develop directly through to adulthood in early Autumn. After consultation with USFWS and other partners, we attempted to breed these adults, but due to cool cloudy weather and an incomplete understanding of the required conditions, there was no successful mating.

Dakota skipper rearing

Collection and captive rearing of Dakota skippers during 2016 was primarily supported by ENRTF funds appropriated under the Minnesota Zoo's M.L. 2014 ENRTF (M.L. 2014, Chp. 226, Sec. 2, Subd. 05j-1). The results of this work are detailed in that project's November, 2016 status update.

Poweshiek skipperling population supplementation

The Minnesota Zoo has engaged with numerous partners including the Minnesota DNR, the USFWS field offices in the Twin Cities and East Lansing, Michigan, and the Michigan Natural Features Inventory to plan for the release of Poweshiek skipperling larvae. Although once considered a common species of Minnesota's prairies, Poweshiek skipperlings have been extirpated from Minnesota and are now known to occur in only three areas worldwide. Sites in Michigan support the most robust of these isolated, remnant populations. To consider reintroduction of Poweshiek skipperlings in Minnesota, we first need to stabilize the existing populations and use our experiences to inform husbandry efforts and potential reintroduction programs. We secured non-ENRTF funds to support this foundational out-of-state work for 2016 as well as future years. In late spring, 2017, remaining individuals will be transferred as larvae or pupae to the Michigan site. We have evaluated release options and tentatively selected a conservative approach that will safeguard the larvae and pupae from potential predators and parasites, but will require regular and frequent maintenance to release any individuals that have emerged. MN Zoo staff will be participating in meetings in Michigan in late January and early February to continue the planning process for the 2017 field season, including confirming plans for population supplementation and egg collection from wild butterflies.

Dakota skipper reintroduction

In collaboration with the USFWS, the Minnesota DNR, and the Nature Conservancy, we have identified a probable site in western Minnesota for reintroduction of Dakota skippers. The vetting process for selection of a reintroduction site has included multiple visits by MN Zoo staff to evaluate management plans with site managers and to assess overall site suitability, particularly the presence and abundance of purple coneflower, a primary nectar plant for Dakota skippers, and the presence of pesticide residue (see Activity 2 below). Larvae and pupae currently maintained at the Zoo will be selected for reintroduction based on their genetic contributions to ongoing captive rearing and reintroduction efforts. These individuals will be transferred to the site during late spring, 2017. We are partnering with the USFWS to compile a Dakota skipper reintroduction plan to outline the framework for the reintroduction process and subsequent monitoring efforts in 2017 and beyond. We will also continue to work with site managers this winter to ensure that management activities (e.g., prescribed burns) remain conducive to a successful reintroduction program in future years.

Project Status as of May 31, 2017:

We have made progress planning and preparing for the 2017 season. While efforts with Poweshiek skipperling have not gone as planned, we remain on track for a new Dakota skipper reintroduction program. New non-payroll expenses since the last update primarily consist of new host plants and associated new supplies, particularly for the Dakota skipper reintroduction program.

Poweshiek skipperling head starting

As noted in the previous update, most of the Poweshiek skipperling larvae in our care experienced unexpected extremely rapid growth in late summer, with most individuals sprinting past the presumed hibernation age. The largest individuals (5th and 6th instars) were allowed to develop through to adulthood in early fall, but breeding attempts were unsuccessful. We then entered the 2016/2017 winter with eight remaining larvae. These were the smallest larvae remaining (3rd and 4th instars), and are thought to have been at the appropriate developmental stage for successful hibernation. These larvae were transitioned into hibernation

using our previously developed methods that have proven successful for Dakota skippers and other skippers. Following our established hibernation protocol, larvae were maintained in secure open-air facilities outdoors until host plants senesced and overnight temperatures consistently approached freezing, which in 2016 did not occur until late November. The larvae were then collected from their host plants, weighed, and placed into individual plastic 5 mL microcentrifuge tubes that were then placed into a lidded plastic cup containing a layer of moistened Hydro-Stone plaster (to increase humidity). This cup was then placed into an indoor specialized refrigerator/freezer that was incrementally decreased in temperature over several weeks to a hold temperature of -3°C to mimic the stable conditions wild larvae typically experience under a healthy snowpack.

In spring, we transitioned the remaining Poweshiek skipperling larvae out of hibernation, first by gradually raising the temperature in the refrigerator/freezer from -3°C to +2°C over a two week period. The larvae were censused and weighed before placement onto a new netted potted host plant for continued growth outside. Unfortunately, none of the remaining Poweshiek skipperling larvae survived hibernation and/or the full transition to spring. This unusually high mortality is unprecedented for our program, and its cause is not known at this time. This protocol works well for other grass skippers that we have reared, as well as for the handful of Poweshiek skipperlings the Minnesota Zoo has reared in the past. We suspect that the larvae were weak going into hibernation, and that the difficulties experienced in late summer and fall are the ultimate cause, and not the hibernation protocols.

Hurdles were expected given the dearth of natural history information and prior rearing efforts with Poweshiek skipperling, but the concurrent rapid development of closely related Garita skipperling larvae at Winnipeg's Assiniboine Park Zoo using comparable methods and the simultaneous lack of rapid development of Dakota skipper larvae at the Minnesota Zoo indicates that the two skipperlings present similar rearing challenges that may be addressed together.

Poweshiek skipperling next steps

The unexpected and unfortunate loss of all Poweshiek skipperling larvae at the Zoo from late summer 2016 through spring 2017 precludes the plan that some individuals would be returned back to their natal site to supplement the wild population this year. This eventuality is obviously not the desired outcome, but was discussed as a possible outcome in the formal cooperative interagency "Plan for the Controlled Propagation, Augmentation, and Reintroduction of Poweshiek Skipperling (*Oarisma poweshiek*)" that was developed in June 2016 (available upon request). Detailed discussions have been held with all of our key Poweshiek skipperling conservation partners (U.S. Fish and Wildlife Service, Michigan DNR, Minnesota DNR, Assiniboine Park Zoo, etc.) to determine the proper course of action given the outcomes of the headstarting effort to date and the tenuous state of all known global Poweshiek skipperling populations. Two of Minnesota Zoo's Prairie Butterfly Conservation Program staff members, Dr. Erik Runquist (Butterfly Conservation Biologist and Program coordinator) and Cale Nordmeyer (Butterfly Conservation Specialist) participated in a January 2017 Poweshiek skipperling conservation planning meeting in Michigan (using non-ENRTF funds) to discuss next steps and identify needs for 2017. Minnesota Zoo staff provided a detail review of the results, the lessons learned from each stage of the effort to date, possible actions to improve outcomes, and identified areas for further research. Following the Michigan planning meeting and several conference calls, the US Fish and Wildlife Service and our other partners determined that the Poweshiek skipperling headstarting program should continue as originally planned in 2017/2018, with new eggs collected from the same site or sites in Michigan in 2017.

A leading hypothesis for the accelerated development of larvae in late 2016 is that temperatures in the outdoor hoop house at the Minnesota Zoo where the larvae were reared were warmer than the conditions wild larvae typically experience in the Michigan prairie fens they originated from. To improve chances for success in 2017, the US Fish and Wildlife Service has now entered into a new interagency cooperative agreement with the Minnesota Zoo to provide additional funding to improve rearing facilities that better control microclimatic conditions. The new USFWS-provided funds will support the construction of a "water mat" system to cool potted host plants containing Poweshiek skipperling larvae to mimic the prairie fen environment, as well as the purchase and installation of two sophisticated "growth chambers" in which light, temperature, and humidity levels can be subtly manipulated and programmed as needed. Field researchers with the Michigan Natural Features Inventory will also regularly provide the Minnesota Zoo with temperature and humidity data from locations in the Michigan

prairie fens where wild Poweshiek skipperling larvae are expected to live, against which rearing conditions at the Minnesota Zoo can be compared and adjusted as needed. The USFWS funding will also provide additional support to fully fund the remaining portions of two seasonal staff members that will work at the Zoo on husbandry operations.

Given the existing personnel limitations in the Prairie Butterfly Conservation Program, growing demands for the Dakota skipper reintroduction program, and the strong overlap in the timing of the adult flights of Poweshiek skipperling and Dakota skippers, we will be training non-Zoo staff in June to collect new Poweshiek skipperling eggs for us on our behalf. As USFWS employees, these individuals would be fully authorized to conduct the work but not need use ENRTF funds to do so.

In addition to receiving field temperature and humidity data from the Michigan prairie fens where Poweshiek skipperlings remain, we are also working closely with the Assiniboine Park Zoo. As noted in the previous status update, Assiniboine Park Zoo launched a parallel prairie butterfly conservation program in 2016, beginning with garita skipperling (likely the closest living relative to Poweshiek skipperling). It has been a key recommendation and goal that the Minnesota Zoo add garita skipperling to our current program, but attempts to obtain them from North Dakota (where they were historically common) have not been successful in 2015 or 2016 despite major survey efforts. The Assiniboine Park Zoo will continue *ex situ* operations with garita skipperling in 2017, and they have agreed to collect some garita skipperling eggs on our behalf. Siblings will be split between the two zoos, providing a unique and rare opportunity for larvae to be reared using comparable methods in two locations to test for site specific differences that may alter developmental and survivorship patterns. We have secured the necessary permits to conduct this work.

Dakota skipper rearing

Collection and captive rearing of Dakota skippers from the winter of 2016/2017 was primarily supported by ENRTF funds appropriated under the Minnesota Zoo's M.L. 2014 ENRTF (M.L. 2014, Chp. 226, Sec. 2, Subd. 05j-1). The results of this work are detailed in that project's May 2017 status update. In short, over 400 Dakota skipper larvae survived winter hibernation at the Minnesota Zoo, with high survivorship anticipated through to adulthood. We estimate that about 150 of these individuals are needed to maintain the Zoo's existing insurance population (with the planned infusion of more eggs from wild females in 2017), with the remainder likely available for reintroduction back into a Minnesota prairie this June. All individuals are being reared under established protocols at the Minnesota Zoo. Detailed ancestry records are maintained for all individuals.

Dakota skipper reintroduction

We have prepared formal plans to reintroduce Dakota skippers to The Nature Conservancy's Hole-in-the-Mountain Preserve, in Lincoln County MN. The "Plan for the Controlled Propagation, Augmentation, and Reintroduction of Dakota skipper (*Hesperia dacotae*)" (Delphey, Runquist, and Nordmeyer, April 30, 2017; available upon request), follows a format recommended by the IUCN to weigh the pros and cons of the actions, identify means to remediate difficulties, specify methods for the operation, and to consider non-biological components for programmatic success, including socio-economic considerations and long-term sustainability hurdles. It details the justifications and protocols to use the Minnesota Zoo's existing Dakota skipper insurance population to attempt to reestablish a once large wild population at The Nature Conservancy's Hole-in-the-Mountain Preserve. The reintroduction is planned for the release of Zoo-reared pupae (likely 150+) from a protected box placed in prime Dakota skipper habitat in the Preserve. We will perform frequent transect-based monitoring across the Preserve to census adults that emerge, and hopefully determine patterns of adult movements and key habitat characteristics that drive recolonization and population persistence. The reintroductions are planned to occur over multiple years at the Hole-in-the-Mountain Preserve. We will also conduct surveys at adjacent TNC Hole-in-the-Mountain parcels and at the adjacent Hole-in-the-Mountain and Altona WMAs to understand the broader context of butterfly diversity in the region, and to perhaps locate individual adult Dakota skippers straying far away from the reintroduction point. The Minnesota DNR (through Robert Dana) will likely contribute to these reintroduction monitoring efforts.

Project Status as of November 30, 2017:

Our program reached new milestones, and made significant advances in programmatic research efficiencies during the summer of 2017. The biggest achievement was the initiation of the world's first Dakota skipper reintroduction program. We also continued Poweshiek skipperling headstarting and surrogate species husbandry research. As noted above, the US Fish and Wildlife Service provided funding to improve control over microclimatic conditions that may be important for the proper development rates of Poweshiek skipperling larvae. These were installed in June 2017.

Poweshiek Skipperling

The Poweshiek skipperling recovery working group and the U.S Fish and Wildlife Service requested that the Minnesota Zoo move forward with the headstarting program, as planned. Personnel from the US Fish and Wildlife Service collected five eggs from three wild Poweshiek skipperling females in Michigan in early July 2017 and provided them to the Minnesota Zoo. All eggs hatched, but three of the larvae died at an early age despite being provided healthy conditions, an expected pattern in r-selected species like Poweshiek skipperlings and many other grass skippers. The remaining two larvae were reared in their dedicated open air hoop house on the "cool" side of the new water mat (typically 1-3°C cooler than ambient). The larvae grew at an appropriate rate, and were transitioned into their hibernation chambers as normal in early November 2017. The plan is to release these individuals back to the Michigan population from which they came in June 2018 to augment the wild population.

Amendment Request:

Out-of-State travel funding is currently itemized to support travel for only the collection of eggs of Poweshiek skipperlings and Dakota skippers and to conduct wild supplementations and/or reintroductions of those species outside of Minnesota. All of these activities occur in the spring and summer, and we still intend to utilize these funds primarily for this purpose. Since the approval of this grant however, the Poweshiek Skipperling Conservation Working Group (an international collaborative of federal, state, provincial, and local agency representatives, research scientists, and zoos) has now formed and holds annual multi-day in-person winter planning meetings. Formal work plans and research priorities central to the success of our program are developed at these meetings, and the Minnesota Zoo needs to be in attendance. This winter, the 3-day meeting will be held in Michigan in February 2018, adjacent to the last reliable populations of Poweshiek skipperlings in the US. We hereby request an amendment to this out-of-state travel budget for a portion to be allocated to travel to these meetings.

We have under-utilized the out-of-state travel budget in this ENRTF in 2017, thanks to a FY 2017 overlap in out-of-state travel funding for our M.L. 2014 ENRTF as well as some support from new interagency cooperative research grants awarded to the Minnesota Zoo by the US Fish and Wildlife Service after the approval of the current ENRTF grant. We request that up to \$3,000 of the currently remaining \$6,563 be eligible for travel to these critical planning meetings in the winters of 2017/2018 and 2018/2019. No other sources of funding are available to support travel to these meetings. Amendment Approved by LCCMR 12/18/2017.

Garita Skipperling

As noted in our May 2017 status update, Poweshiek skipperling larvae (at Minnesota Zoo) and garita skipperling larvae (at Winnipeg's Assiniboine Park Zoo) simultaneously developed anomalously fast during the summer of 2016. To help optimize skipperling husbandry methods and test for sensitivities to high temperatures, Assiniboine Park Zoo staff collected garita skipperling eggs from a reliable population in Manitoba, and then provided half of these eggs to the Minnesota Zoo (under a permit from the US Department of Agriculture). This allowed for the simultaneous rearing of sibling garita skipperlings at both zoos to compare protocols.

The garita skipperling larvae hatched well from their eggs at the Minnesota Zoo (133/174, 76.4%). After being initially reared indoors in 50 mL plastic vials with live grasses (as usual), the Minnesota Zoo's garita skipperling larvae were transferred into 4" pots of prairie dropseed (our typical "singleton" method), and then were split into four treatment groups. Two of the four groups were reared in the open-air hoop house on the wet mat discussed above, with one group exposed to ambient temperatures and the other group exposed to water chilled air (the same conditions as the Poweshiek skipperlings, discussed above). The two remaining groups were

separately reared indoors in the two new controlled-climate growth chambers. One of these chambers was programmed to cycle daily through the average temperatures and humidities observed at 10 hours over the previous week in the ambient open-air hoop house (where the first group of garita skipperling were). The second chamber was programmed to cycle similarly to the first chamber, but at 80% of the ambient open-air hoop house temperatures. The daily light cycles in both chambers were also programmed to match sunrise and sunsets for that week. Survivorship was good, and 57 garita skipperling larvae were transitioned into hibernation at the Minnesota Zoo in early November 2017.

No anomalously rapid development of Poweshiek or garita skipperling larvae was observed in 2017, at either Zoo. Full analysis of larval size data is pending, but preliminary results suggest little differences in skipperling growth rates between the four temperature treatment groups. Unlike 2016 however, August 2017 was cooler than average in the Twin Cities, with no high temperatures above 90°F. While the exact cause of the 2016 rapid development is not yet known, the lack of high outdoor temperatures may indeed be related.

Dakota skipper rearing and breeding at the Minnesota Zoo

Dakota skipper rearing at the Minnesota Zoo again proceeded successfully during the summer of 2017, with a total of 305 individuals reaching adulthood, including 196 reintroduced back in the wild – see below). This represents a continued increase in the total population of the conservation population relative to prior years. Of the 109 adults retained at the Minnesota Zoo, 12 mating events were confirmed. This percentage of breeding adults is similar to prior years, and we are interested in ways to increase this percentage in future years. From this Zoo-based breeding, 530 new larvae were produced. Minnesota Zoo staff also collected an additional 384 larvae from 25 females from three populations in northeastern South Dakota, following protocols previously employed. A single egg was also collected from an observed oviposition following the reintroduction program at the Hole-in-the-Mountain Prairie Preserve (see below), and this egg hatched. Thus, 915 new larvae were added to the program in the summer of 2017 – a new high for our program. These 915 larvae were transitioned to three rearing set-ups (“singleton” pots with prairie dropseed: 35 larvae; larger “free-range” pots of multiple larvae with prairie dropseed: 661 larvae; and a repeat of the 2016 host plant performance study on seven different prairie grass species: 219 larvae). Larvae were reared in these set-ups through the summer in the dedicated open-air Dakota Skipper Hoop House at the Zoo. Transitioning of these larvae into their secure winter hibernation setups began in November 2017, and is still in progress at the time of this writing. Full results can be presented in future reports, but recovery rates of larvae from mid-July to hibernation appear promising and consistent with prior years.

Dakota skipper reintroductions

For the first time, Dakota skippers have been reintroduced back into the wild, to a Minnesota prairie where they were once common. In late June and early July 2017, Dakota skippers from the Minnesota Zoo’s population were brought as pupae to the Hole-in-the-Mountain Prairie Preserve (near Lake Benton, Lincoln Co., MN) and released daily as individuals reached adulthood. Over a three week period, at least 196 adults were released at the Preserve. Zoo staff conducted intensive surveys during and after the release, recording the precise locations of all Dakota skippers seen at the Preserve. Dakota skippers disappeared from the Hole-in-the-Mountain Preserve (and all of southwest Minnesota) sometime between 2009 and 2012, so all individuals sighted were the direct result of the reintroduction. A total of 111 Dakota skippers were sighted at the Preserve by mid-July. Most individuals were seen in close proximity to the release point in a large patch of purple coneflowers (their preferred nectar source), but three individuals were observed more than 100 meters from the release point. This location observational data is foundational and will help inform estimates of dispersal distances for Dakota skippers and habitat management for Dakota skippers. The re-sighting of released skippers was a milestone, but the biggest success was the confirmation of breeding in the wild by those skippers. A total of five matings were observed, with two more breeding events plausible. Thus, for the first time in at least 5 years, Dakota skippers are reproducing in the wild in southwest Minnesota. Reintroductions are planned to continue at this Preserve for at least two more years to help re-establish this lost population.

Project Status as of May 31, 2018:

Much of the recent activity since the last update has centered on data processing and planning for the 2018 season. New ENRTF expenses since the last update consist primarily of payroll, along with new host plants and associated supplies, and some out-of-state travel (authorized an amendment in the previous update). In February, two Minnesota Zoo staff attended the 2018 meeting Poweshiek Skipperling Conservation Working Group in Springfield Township, MI. Research priorities and action plans were established for the 2018 season. Funding from the US Fish and Wildlife Service via the Great Lakes Restoration Initiative to the Minnesota Zoo through an Interagency Cooperative Agreement to support Poweshiek skipperling activities may result from attending this meeting. Further information can be provided in future updates.

Poweshiek Skipperling

The two Poweshiek skipperling larvae in our care successfully overwintered and in May were placed into the same protected outdoor “singleton” rearing setups as they had been during the summer of 2017. The plan is to release these individuals back at their natal site in Michigan in June 2018, hopefully using non-LCCMR funds.

Garita Skipperling

Overwintering survivorship of the garita skipperling larvae in our care was lower than expected. Of the original 57 individuals entering hibernation, 19 were healthy and normal when removed from hibernation in May, with another 12 appearing weak. Mortality of the remaining 26 larvae appears to have been recent (i.e. not at the beginning of winter). The cause of this lower survivorship is not known, but may be related to the extended hibernation that was necessary given the heavy spring 2018 snowfalls and/or decreased humidity in some hibernation chambers.

Dakota skippers rearing at the Minnesota Zoo

Transitioning of the Dakota skipper larvae into their secure winter hibernation setups began in November 2017 and had not been completed before the submission of the previous update. Recovery rates of larvae from mid-July to hibernation were consistent with prior years. We censused “General Collection” (i.e. non-Host Plant Research) pots that had contained 608 larvae in July, and recovered 469 of them in November. Some “Free Range” pots of prairie dropseed containing multiple larvae were intentionally not censused to compare survivorship across hibernation treatment methods. Given the July to December survivorship of General Collection individuals that had been censused and combined with an additional 125 individuals recovered from Host Plant Research, we extrapolated a 2017-2018 hibernating population of up to 662 Dakota skipper larvae. This represents a new high population size at the Minnesota Zoo.

As with two species previously discussed, removal of the Dakota skippers from hibernation was delayed until mid-May due to late heavy snow. Overall though survivorship was good, with 521 individuals found alive and placed back into their respective summer rearing setups to resume feeding. This 78.7% hibernation survivorship is healthy, and would likely have been higher in the absence of the Host Plant Research study, which is intentionally designed to test for differences in survivorship among grass species. The 56.9% survivorship of the 2017-2018 from neonate to post-hibernation larva is also consistent with prior years (58.3% in 2016-2017 generation to this point). We expect high survivorship through to adulthood. As in 2017, we estimate that about 150 of the resulting adults are minimally needed to maintain the Zoo’s existing insurance population (with the planned infusion of more eggs from wild females in 2018), with the remainder likely available for the reintroduction program this June. All individuals are being reared under established protocols at the Minnesota Zoo. Detailed ancestry records are maintained for all individuals.

Dakota skipper reintroductions

Multiple conference calls and meetings with key partners occurred since the last update to prepare for the 2018 releases. Data from the first year of reintroductions is helping inform federal recovery planning for Dakota skippers. A prescribed burn was planned and executed at the Dakota skipper reintroduction at Hole-in-the-Mountain Prairie Preserve by our partners with The Nature Conservancy to maintain and further enhance

habitat for Dakota skippers, in consultation with Minnesota Zoo staff and under permissions issued by the US Fish and Wildlife Service.

Project Status as of November 30, 2018:

Poweshiek Skipperling

We proudly reached a new milestone in 2018, with the first ever release of Zoo-reared Poweshiek skipperlings back into the wild, in support of the Federally-recommended population augmentation program for the last known significant U.S. population of Poweshiek skipperling. The two individuals we had been rearing since the summer of 2017 were brought back as pupae to their original population in Michigan in late June, and were released by Zoo staff as adults successfully in early July. It is hoped that this proof-of-concept can continue to expand in future years to stabilize dwindling numbers of this globally Critically Endangered species.

While in Michigan conducting these releases, Zoo staff also collected a new generation of eggs from the same location to form the basis of the 2018-2019 headstarting effort at the Minnesota Zoo. Egg collection was attempted from 12 wild females, but only five females laid eggs during their 72-hours in our care. Eighteen larvae hatched from the resulting 54 eggs (about half of which appear to have been inviable). The larvae were reared in their dedicated open air hoop house on the chilled water table (typically 1-3°C cooler than ambient) and again did not experience anomalously rapid development (as occurred in late summer 2016). Survivorship was terrific, with 16 of the 18 larvae surviving to hibernation in early November 2018. Resulting adults from this continued augmentation effort are scheduled to be released back in Michigan in June/July 2019.

Funding support for Zoo staff to execute Poweshiek skipperling work was largely provided by a new Interagency Cooperative Agreement from the U.S. Fish and Wildlife Service via the Great Lakes Restoration Initiative.

Garita Skipperling

We continued work with garita skipperling in 2018, as part of the recommended program to better understand the conditions needed to rear and hopefully breed Poweshiek skipperlings. As each other's likely closest relatives, Poweshiek skipperlings and garita skipperlings share many biological features and likely respond to conditions similarly. The nineteen garita skipperling larvae in our care in spring 2018 that have been collected as eggs from Manitoba in 2017 developed well through to adulthood in June. The adults were placed into a communal flight cage in one of the protected outdoor hoop houses at the Minnesota. Breeding occurred in this cage and three viable eggs were produced, representing a first with this genus for the Minnesota Zoo (and only the second time globally following our partner Assiniboine Park Zoo in 2017).

Zoo staff (using non-ENRTF funds) also collected eggs from five wild female garita skipperlings from western North Dakota in June 2018. Sixty-one larvae that hatched from these eggs plus the three larvae from the Manitoba-cohort breeding were reared in a similar manner to the 2017-2018 generation, under four climate conditions: 1) outside ambient in a hoop house, 2) outside on the chilled water table, 3) in a controlled climate environmental growth chamber programmed to track with the outside ambient conditions, and 4) in a controlled climate environmental growth chamber programmed to be 10% warmer than tracked outside ambient conditions. The different temperature regimes were selected to test for developmental and survivorship thresholds that might explain the unexpected accelerated development of Poweshiek skipperlings at the Minnesota Zoo and garita skipperlings at the Assiniboine Park Zoo in 2016. We did not find predictable patterns of survivorship and development between these treatments (perhaps due to small sample sizes), but we still did have accelerated development of many individuals across all treatments. Indeed, 18 individuals reached adulthood by early September – a similar pattern to 2016's anomalous rapid development. We set these new adults up in additional flight cages, and multiple breeding events again occurred. This is the first time that a second generation of *Oarisma* has been produced within one season, under human care or otherwise. Eighteen larvae hatched from this second generation eggs, and were reared in environmental growth chambers programmed to either 1) track the variability of the outdoor ambient conditions observed in early August or 2) hold a constant temperature of the average of observed early August temperatures) until October when they had reached their typical size at hibernation.

Surviving larvae (34 from the first generation and 7 from the second generation) were transitioned into winter hibernation in late October in a similar manner as the other species.

Dakota skipper rearing at the Minnesota Zoo

Dakota skipper rearing at the Minnesota Zoo again proceeded successfully during the summer of 2018. We produced 401 adults, of which 244 were reintroduced back in the wild (see below). This represents a continued increase in the total population of the conservation population relative to prior years. Of the 157 adults retained at the Minnesota Zoo, at least 16 mating events were confirmed. This percentage of breeding adults is similar to prior years. From this Zoo-based breeding, 1057 new larvae were produced. Minnesota Zoo staff also collected an additional 489 larvae from 26 females from three populations in northeastern South Dakota, following protocols previously employed. Of these 1546 larvae, 1473 survived the critical first few weeks and were added to the program in the summer of 2018 – a new record high for our program. These 1473 larvae were reared in a combination of “singleton” (one caterpillar per 4” pot with prairie dropseed) and “free-range” (multiple larvae on 1-gallon pots with prairie dropseed) set-ups through the summer in the dedicated protected open-air hoop houses at the Minnesota Zoo. Transitioning of these larvae into their secure winter hibernation setups began in November 2018, and is still in progress at the time of this writing. Full results can be presented in future reports.

Dakota skipper reintroductions

We resumed reintroducing Dakota skippers back into the wild in 2018. As in 2017, Dakota skippers from the Minnesota Zoo’s population were brought as pupae in late June and early July 2018 to the Hole-in-the-Mountain Prairie Preserve (near Lake Benton, Lincoln Co., MN) and released daily into the wild as they reached adulthood. Over a three week period, 244 adults were released at the Preserve. Releases were initiated at the same location as in 2017, with 159 individuals released from this “Box 1”. Partway through the effort, a second release box was established, from which 85 more individuals were released. This “Box 2” was located about 300 m uphill from Box 1, in an area of high quality prairie habitat that we have always hoped Dakota skippers would colonize. Deployment of Box 2 was delayed relative to Box 1 because the prime blooming of the preferred nectar wildflower for adult Dakota skippers (narrow-leaved purple coneflower, *Echinacea angustifolia*) at the Box 2 location occurred about one week after the pale purple coneflower (*Echinacea pallida*) at the Box 1 location bloomed.

Zoo staff conducted intensive surveys during and after the release, recording the precise locations of all Dakota skippers seen at the Preserve in 2018. A total of 121 Dakota skippers were sighted by Minnesota Zoo staff over the 20-day survey period at the Preserve by mid-July. As in 2017, most individuals were seen in close proximity to the Box 1 release point in the large patch of pale purple coneflowers, but we also saw an increase in the number of individuals outside this area in 2018 (at 26 locations, up from 3 locations in 2017), even before Box 2 was established higher up the ridge in this area. Thus, the skippers appeared to be independently colonizing some of the best habitat in the Hole-in-the-Mountain Prairie Preserve without our assistance. As a reminder wild Dakota skippers were last recorded at this site in 2008, and were believed to be extirpated from the site prior to our current reintroduction program.

We did observe at least one wild breeding event, as well as multiple additional courtship attempts, at the Preserve in 2018. A primary goal heading into 2018 was to determine if the observed successful breeding of reintroduced Dakota skippers that we observed in 2017 resulted in detectable numbers of adults in 2018. Due to logistical hurdles that prevented Zoo staff from surveying the preserve before new Zoo-reared adults needed to be released in 2018, as well as the fact that newly-released adults in 2018 were not marked before release (due to safety concerns associated with handling the powerfully-bodied but small-winged adults), we cannot determine how many (if any) of the adult Dakota skippers observed in 2018 were the product of the observed reproduction in 2017 or were from the 2018 release. We will seek strategies to answer this question of successful establishing in 2019. Nonetheless, the expanded range of observed individuals is very encouraging.

Project Status as of May 31, 2019:

Poweshiek Skipperling

As noted above, we entered the winter of 2018-2019 with 16 Poweshiek skipperling larvae. Hibernation survivorship was stellar, with 15 of the 16 larvae surviving to early May. These larvae were transferred back onto their original singleton plant setups and will be reared as normal in their outdoor protected hoop house. The plan is to release these individuals back at their natal site in Michigan in 2019 (using non-ENRTF funds) for the population reinforcement program.

Garita Skipperling

Winter survivorship of garita skipperlings was lower than expected, with 26 of 41 surviving to early May. There was not a strong indication of survivorship of related to the 2018 temperature treatments they were assigned to, but larger larvae appeared to be less likely to survive winter hibernation. We have observed this size-related pattern previously in other skippers, which suggests that later stage caterpillars may experience hormonal changes prior to the metamorphosis to pupae that override development of key body chemistries related to freeze tolerance. Confidence in these assessments remains low however due to relatively low sample sizes.

Dakota skipper rearing at the Minnesota Zoo

Despite our high field egg collection and high breeding success in 2018, fewer than expected larvae survived to winter diapause (censused in late November 2018). As in past years, a subset of larvae are overwintered in their husbandry setup outdoors and were not censused as to not disturb their shelters so we cannot say with certainty how many Dakota skipper larvae were in our holding going into winter. Nonetheless, we estimated that there were up to 795 larvae entering winter hibernation for a hatchling to hibernation rate of about 59%. In 2017, 76% of larvae survived to diapause (not including larvae used in a host plant experiment). Though the total number of larvae in our care during the winter of 2018-2019 is still higher than in any other previous year, the lower survivorship of early instar larvae is concerning. At this time, we do not know what has contributed to this decrease in pre-diapause survivorship.

The cool spring of 2019 delayed the transition of Dakota skippers out of hibernation onto their host plants at the Minnesota Zoo to the second half of May. This effort is still in progress at the time of this writing, and more complete results can be presented in our final report. However, hibernation survivorship appears strong and consistent with prior years.

Dakota skipper reintroductions

2019 is planned to be the third of the three years of planned Dakota skipper reintroductions at the Central Unit of the Hole-in-the-Mountain Prairie Preserve. We expect to retain 100-150 adults at the Zoo to continue the insurance population breeding program, with the remainder eligible for the reintroduction effort. Our goal is to release about 400 adults to this Preserve in 2019. We continue to work closely with The Nature Conservancy and the US Fish and Wildlife Service to implement this program and to manage for high quality prairie at the Preserve.

Overall Project Outcomes and Results:

Poweshiek Skipperling

We have achieved foundational milestones to help save the Poweshiek skipperling, one of the world's most endangered species. Poweshiek skipperling was recently classified globally as "Critically Endangered" on the International Union for the Conservation of Nature's Red List of Threatened Species. This international classification is in addition to the existing US and Minnesota Endangered Species listings, and raises the global profile of Poweshiek skipperling as one of the most endangered species.

The Minnesota Zoo remains the only institution in the United States operating a conservation and rearing *ex situ* program for Poweshiek skipperlings (or even the entire *Oarisma* genus). In early July 2019, we accomplished another round of Poweshiek skipperling population augmentation, releasing 14 more Zoo-reared adults into one of the last known populations in the United States. The addition of these adults back into the wild may constitute

a 5% addition to the global population and is intended to promote breeding viability and population stability of one of the last populations. The headstarting program is highlighted by the international Poweshiek Skipperling Conservation Partnership as a key component of the conservation actions that will be needed to recover the species. Another releases of a new generation of Poweshiek skipperling back to the Michigan population is planned for 2020.

Some aspects of the Poweshiek skipperling headstarting work have also presented unforeseen and hurdles, but those challenges have led to new research and advances that are contributing directly to their conservation. For instance, the apparently higher sensitivity of Poweshiek skipperling caterpillar development rates to high temperatures than other butterfly species that we have worked with has led to a new research focus on microclimate conditions and the accumulation of “degree-days” for Poweshiek skipperlings. Similarly, we have begun exploring the conditions needed to better promote oviposition success, egg hatch rates, and early larval survival. These research programs have the potential to inform best management practices for both *ex situ* programs (like the Minnesota Zoo’s) and for habitat management in the wild.

The Poweshiek skipperling was once widespread in Minnesota, but is unfortunately now suspected to be extirpated from Minnesota. As an Agency of the State of Minnesota, we sought support from non-Minnesota state resources (such as the ENRTF) for Minnesota Zoo staff to execute the necessary out-of-state Poweshiek skipperling headstarting program. We have secured short-term funding from the US Fish and Wildlife Service and from the National Geographic Society to support out-of-state travel, supplies, and personnel costs associated with the release of Zoo-reared Poweshiek skipperlings in Michigan, and the collection of additional eggs from wild Poweshiek skipperlings from Michigan to begin the a generation of headstarted individuals. These external funding sources are ephemeral, limited in scope, and short-term. As a result of these external grants, there are remainders in the out-of-state travel budget and in the supplies budget for this ENRTF appropriation that would have been otherwise expended.

Garita Skipperling

Garita skipperling is a key species in the Minnesota Zoo’s Prairie Butterfly Conservation Program, with the primary goal to improve husbandry protocols for the closely related Poweshiek skipperling. We have been similarly successful achieving our objectives. In addition to providing insights into breeding behavior of *Oarisma* skipperlings, with the first ever breeding of *Oarisma* in the United States under controlled conditions at the Minnesota Zoo in both 2018 and 2019, garita skipperling caterpillars have been a key component of the microclimate experiments, providing significantly more replication than is possible with the Critically Endangered Poweshiek skipperling. As a result of the successful breeding efforts, as well as the additional infusion of new individuals from wild North Dakota females in the summer of 2019, over 200 garita skipperling caterpillars are currently being reared at the Minnesota Zoo in five microclimate experimental treatments to test for sensitivities in development rates and survivorship. This research is also being conducted in collaboration with Assiniboine Park Zoo (Winnipeg, Manitoba) and the University of Winnipeg where additional garita skipperling caterpillars are being reared under variable microclimate regimes.

The garita skipperling is a Minnesota Threatened Species, and therefore our efforts to better understand its life history is major contribution to its conservation prospects. Garita skipperling is however likely also extirpated from Minnesota, so as with the Poweshiek skipperling conservation program, work with garita skipperling at the Minnesota Zoo requires significant investment of resources outside of Minnesota. To support this work without Minnesota State resources, the US Fish and Wildlife Service and National Geographic Society funding that we secured for the Poweshiek skipperling headstaring program also has supported the garita skipperling research program.

Dakota skipper

The Dakota skipper conservation program has been very successful, and support from the ENRTF has been critical. Achieving our objections, we have successfully reared and then released over 900 adult Dakota skippers over three years back into a Minnesota prairie as part of the world’s first re introduction program for this US Threatened and Minnesota Endangered species. In addition to the roughly 200 Zoo-reared adults in 2017 and 250 in 2018, we released approximately 480 more in July 2019 back into The Nature Conservancy’s Hole-in-the-

Mountain Prairie Preserve near Lake Benton, Minnesota. As in 2017 and 2018, dozens Dakota skippers were re-sighted in 2019 at the release points, utilizing the native wildflowers and exhibiting behaviors typical of other wild populations. Released Dakota skippers have been observed utilizing an expanding portion of the Hole-in-the-Mountain Prairie Preserve through the course of this project. Happily, we observed five more mating pairs of Dakota skippers at the release sites in 2019, indicating that Zoo-reared Dakota skippers were breeding again successfully in the wild.

As detailed in the 2017 Dakota skipper reintroduction plan, additional releases of Zoo-reared Dakota skippers will now be paused for two years at the release sites within the Hole-in-the-Mountain Prairie Preserve and those areas will be monitored for Dakota skippers in 2020 and beyond. If Dakota skippers can be found at the site without the yearly additions of Zoo-reared individuals, then we are optimistic for the prospects of the first ever re-establishment of a wild Dakota skipper population as a result of this ENRTF-support. The Dakota skipper reintroduction effort that we have been undertaking is the first of its kind, and as such, is providing foundational information on dispersal patterns and habitat usage that will provide key insights to future efforts.

All individuals released as part of this reintroduction effort were the product of the Minnesota Zoo's rearing and breeding program. We retained about 100 more Dakota skipper adults for breeding at the Minnesota Zoo during the summer of 2019 also obtained hundreds more individuals from the same natal populations in South Dakota to extend the conservation insurance program into the future. At the time of this writing, over 2000 more Dakota skippers are being reared at the Minnesota Zoo, many of which may form the core of new reintroduction efforts in 2020 and beyond.

A small portion of the total Activity 1 budget was not utilized: \$11,126 of the budgeted \$386,000. About half of the unused remainder is the result of slightly over-projecting rises in personnel costs across the three years. The travel and supplies budgets have remainders because 1) we later secured additional funding through external grants from the US Fish and Wildlife Service and from the National Geographic Society to support the heavily out-of-state Poweshiek skipperling and gairta skipperling programs, and 2) the 2019 cold wet spring delayed Dakota skipper field work into July 2019 and beyond the final fiscal year of this appropriation.

ACTIVITY 2: Pesticides Research – Phase 2

Description:

The historically vast 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. Since 2014, the Minnesota Zoo has used support from the ENRTF (and leveraged that support for matching US Fish and Wildlife Service funds) to begin assessing the degree of pesticides drift onto three prairies in Minnesota and one in South Dakota. These four prairies are designated as critical habitat for the Poweshiek skipperling and Dakota skipper by the U.S. Fish and Wildlife Service. As described in semi-annual updates for the Minnesota Zoo's M.L. 2014 ENRTF support, we have documented the presence of several insecticides along the edges (within 10 m) and within the interiors (>100 m away from an agricultural edge) of these prairies. These insecticides (primarily chlorpyrifos, cyhalothrin, and bifenthrin) are primarily associated with applications for the control of invasive soybean aphids.

Building on our current research, the Zoo will continue to collect field samples for pesticides screening from native prairie remnants, especially those designated as critical habitat for Poweshiek skipperling and Dakota skippers and/or those sites where reintroductions may occur. Final site selection and sampling regime will be coordinated with the US Fish and Wildlife Service and other relevant parties. Based on current cost structures and depending on the scope of analyses, the current budget would allow for testing of about 75-200 more samples.

Grass skippers spend the majority of their lives as caterpillars, and aerial insecticide spraying against soybean aphids usually occurs in mid-August during the first 1-2 months of a caterpillar's life. However, the consequences of exposure to aerial sprays of insecticides against soybean aphids on butterflies is generally unknown, and is completely unknown for prairie grass skippers like Poweshiek skipperling and Dakota skipper. Dakota skipper

larvae construct shelters at the bases of their host grasses, but Poweshiek skipperling larvae do not and may be more exposed to aerial drift. Our field sampling research will be paired with experimental tests on the effects of these soybean aphid insecticides on the survivorship and growth rates of grass skipper butterfly caterpillars, pupae and adults. 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.

Depending on the availability of planned future facilities at the University of Minnesota, we plan to expose young larvae and the plants that they feed on to aerial applications of these compounds. The formal experimental design and the needed resources will be determined in 2016 pending further discussions with all relevant parties.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 35,000
Amount Spent: \$ 35,000
Balance: \$ 0

Outcome	Completion Date
1. Complete field sampling of prairie remnants for pesticides	September 2018
2. Complete pesticides exposure experiments	June 2019

Project Status as of November 30, 2016:

Field Sampling:

Field sampling and pesticide residue analysis occurred during May and August, 2016, and is currently being supported by funds from ENRTF funds appropriated under the Minnesota Zoo's M.L. 2014 ENRTF (M.L. 2014, Chp. 226, Sec. 2, Subd. 05j-1). Results of sampling are reported in that project update. Sampling and analyses will be funded by this appropriation after the 2014 funds have been completely expended.

Controlled Exposure Experiment:

We have been coordinating with soybean aphid researchers at the University of Minnesota and other organizations to plan for the implementation of the experiment. Study design and logistics to assess the impacts of soybean aphid pesticide exposure on skipper butterflies will be finalized in winter and spring 2017.

Project Status as of May 31, 2017:

There continues to be strong interest in the results of this on-going ENRTF-funded pesticides study, and for what those results may mean for wild prairie butterflies that may be exposed to them. For example, and as noted below, the Minnesota Zoo's Butterfly Conservation Biologist, Dr. Erik Runquist, co-organized a symposium on behalf of the Minnesota Invasive Terrestrial Plant and Pest Center at the University of Minnesota in November 2016. The finds of the research program to date were summarized and incorporated into a broader discussion of the state of knowledge about potential impacts of soybean aphid insecticides on prairie butterflies. The proceedings of the symposium, "Potential Causes of Declines in Minnesota's Prairie Butterflies with a Focus on Insecticidal Control of the Soybean Aphid" (Runquist and Heimpel, March 20, 2017; available at https://mitppc.dl.umn.edu/sites/g/files/pua746/f/media/mitppc_soybean.final_.pdf) summarizes the findings of the symposium, and identifies new research needs to better understand the consequences of exposure to these compounds to wild skipper larvae. The controlled exposure experiment we are proposing to conduct this summer is identified as one of these research needs.

Field Sampling:

No new field sampling (and therefore any associated pesticides residue testing) has occurred since the last update, but more is planned for late summer. A small limited amount of funds from this appropriation were used to pay for a small portion of the analysis samples collected in August 2016, particularly those collected at the Hole-in-the-Mountain Preserve where Dakota skipper reintroductions are planned for 2017. In summary, we

found low levels of chlorpyrifos (a commonly applied broad-spectrum insecticide against soybean aphids) at several points scattered across the Hole-in-the-Mountain Preserve, as well as at the nearby Prairie Coteau Scientific and Natural Area. These observations fit a similar pattern that has been observed at all other sites sampled in late summer since August 2014. More detail on these findings is outline in the project updates in our ML 2014 ENRTF grant.

Controlled Exposure Experiment:

We have developed a draft study design and research plan with soybean aphid pesticides researchers at the University of Minnesota for the controlled exposure experiment. We will also be consulting with scientists and regulatory personnel with the Minnesota Department of Natural Resources, US Fish and Wildlife Service, and Environmental Protection Agency to refine the study. The experiment(s) may be limited initially, but likely will involve the spraying of a range of known dilution treatments of a soybean aphid insecticide (like chlorpyrifos) over potted grasses containing appropriately aged larvae of a common surrogate species of closely-related skipper (likely the Long Dash, *Polites mystic*) that possesses similar natural history features to the imperiled skippers. Samples will be collected to determine the resulting pesticide concentrations landing on the plants. Survivorship data and other health metrics of the exposed larvae will be tracked through time. The experiment is planned to begin in August 2017.

Project Status as of November 30, 2017:

Field Sampling:

We again sampled native prairie grasses for pesticides in late August at the same four Minnesota prairies that we have been sampling since 2014. The same sampling protocols were utilized, and samples were once again sent to the US Department of Agriculture's National Sciences Laboratory for pesticides residue concentration analysis. Similar to prior years, chlorpyrifos (the organophosphate insecticide widely applied against soybean aphids) was again widespread, present in all but one of the prairie samples and at relatively consistent levels. There was also no apparent edge (within 10 meters of an agriculture x prairie border) vs. interior (100+ meters inside a prairie preserve from any edge) differences in compounds within sites. There were also several notable differences between our 2017 and prior year (2014-2016) results. For the first time, the large and relatively buffered Glacial Lakes State Park had the highest average observed levels of chlorpyrifos. Interestingly, bifenthrin and cyhalothrin, the two pyrethroid soybean aphid insecticides we had commonly observed in previous years, was not detected in any of the 24 samples. Their absence could mean these pyrethroid insecticides were not included in soybean aphid sprays this year (unlikely) or had degraded in the environment before sampling (also unlikely), but it is most likely attributable to decreased Levels of Detection (LOD) for these two insecticides compared to prior years. Many new compounds were also detected in these samples, particularly fungicides. The USDA laboratory has improved protocols to a) allow for additional compounds to be tested and b) improve minimum LODs for many more. Of the 12 compounds detected in this year's data, 11 are new to our dataset. Three of these 11 compounds had not been tested for previously. All of these field pesticides findings have been shared with our partners with the US Fish and Wildlife Service, Minnesota DNR, and Minnesota Department of Agriculture. Charges for these August 2017 samples have not been received as of the time of this writing, and thus will be reported in future updates.

Controlled Exposure Experiment:

In August, we began experimental testing of the effects of exposure of agricultural insecticides that we have observed in the wild on a common (non-imperiled) surrogate species of grass skipper. This work was conducted in collaboration with researchers at the University of Minnesota. The goal is to estimate the risk that wild skipper caterpillars (especially the federally protected species) may experience from occasional exposure to agricultural insecticides. The first step of this research was to assess the survivorship of skippers through direct contact with a range of exposures to those compounds. We used a commonly utilized exposure study method using glass vials coated with precisely known amounts of the insecticides for this first round of experiments. Larvae of the widespread Sachem (*Atalopedes campestris*) were placed individually in replicate vials coated with the

commonly observed pyrethroid insecticide bifenthrin, as well as bifenthrin-free control vials. Individuals were then observed at 4, 18, and 24 hours, with survivorship and behaviors recorded at each time step for each caterpillar. Analyses of this work are still in progress, but the range of bifenthrin dosages coating the vials (10 treatments in all) was appropriate to encompass the full range of responses (all survived to all are killed). This experiment will inform additional work in the future.

Project Status as of May 31, 2018:

Field Sampling:

As planned, no new data or samples have been collected since the last update. Additional sampling is being planned for August 2018 and will be described in future updates. Charges for the August 2017 sample analyses described in our previous update were almost entirely paid for using private donations received through the Minnesota Zoo Foundation, with only \$115 paid through the ENRTF.

Controlled Exposure Experiment:

As planned, no new studies have been implemented since the last update. Additional research is being planned with partners for late summer 2018, and will be described in future updates.

The Sachem larvae that had been used in the bifenthrin toxicity exposure experiment in September 2017 were placed into hibernation along with all the larvae of all of the other skipper species currently maintained in the Prairie Butterfly Conservation Program. None of these Sachem larvae survived winter however, so lifetime sublethal effects of exposures will not be possible to measure for these individuals. This result is not surprising though since Sachem is only a temporary colonist of Minnesota from the southern half of the United States and it likely is unable to tolerate extended cold temperatures as the permanent resident species can.

Project Status as of November 30, 2018:

Field Sampling:

In August, we collected our fifth year of sampling for pesticides from grasses at the same four Minnesota prairies. The same sampling protocols were utilized, and samples were once again sent to the US Department of Agriculture's National Sciences Laboratory for pesticides residue concentration analysis. With one exception, there were again not strong differences in pesticides loads between prairie edges (within 10 meters of an agriculture x prairie border) vs. interior (100+ meters inside a prairie preserve from any edge) within sites. Chlorpyrifos (the organophosphate insecticide widely applied against soybean aphids) was again widespread, and was detected in all 25 sampled points across the four prairies. The levels of chlorpyrifos detected were slightly higher than prior years, which may be a response to the increasing resistance by soybean aphids to pyrethroid insecticides (like bifenthrin, which now has not detected for the third year in a row). The most noteworthy detection was an Edge sample within the north side of Glacial Lakes State Park, where 2290 parts per billion of chlorpyrifos was detected in a 3-gram grass sample. This amount is more than 8 times higher than the previous highest detected level of chlorpyrifos at any site since we began sampling in 2014. This point also had the highest level of the fungicide azoxystrobin (93 ppb), and the second highest levels of the pyrethroid cyhalothrin (80 ppb) and the fungicide chlorothalonil (269 ppb) in any of our samples. An adjacent Edge point within Glacial Lakes State Park had much lower levels of these four compounds, so any detrimental effects may have been relatively local.

Six fungicides were detected in the 2018 samples, two of which were new to our study: chlorotalonil and diflubenzuron.

All of these field pesticides findings have been shared with our partners with the US Fish and Wildlife Service, Minnesota DNR, and Minnesota Department of Agriculture. The invoice (comprising the remainder of the P/T Services budget) for these August 2018 samples have not been received as of the time of this writing, and thus will be reported in future updates.

Controlled Exposure Experiment:

We conducted a large, foundational insecticide exposure experiment in September 2018, building upon the 2017 experiments. While the glass vial experiment conducted in 2017 is a vital step to understand toxicity of the insecticide bifenthrin to larvae of grass-feeding skippers under controlled conditions that remove as many variables as possible in an artificial setting, it may also over-estimate the risk of bifenthrin exposure to wild skipper larvae because the larvae are unable to find refuge from the insecticide inside the glass vials when refuge may be possible in the wild. Wild larvae inside their shelters may be more protected from exposures to these foliar applied insecticides (and other environmental stressors). To better understand how larvae are affected under more natural situations (i.e. inside their shelters at the bases of their host grasses), we partnered with Dr. Robert Koch at the University of Minnesota to spray plants containing established skipper larvae with a range of concentrations of bifenthrin. The goal is to determine if the levels of the insecticides we have detected on prairie grasses pose a risk to wild skipper larvae.

After recording mass, instar, and head cap size, we placed 110 Sachem and 46 Dakota skipper larvae (under written permission from the U.S. Fish and Wildlife Service; comprised of genetically over-represented individuals from the Zoo population) placed on a separate potted little bluestem plants (1 gallon pot size). The plants containing the 95 Sachem and 30 Dakota skipper larvae were then sprayed 2-3 days later with a four concentrations of bifenthrin (100%, 10%, 1%, 0.1%, and 0% of the labelled field application rate). The remaining 15 Sachem and 16 Dakota skipper larvae were not sprayed but were maintained and tracked similarly as additional controls. The day after spraying, grass samples were collected from the plants and sent to the USDA National Sciences Laboratory for parts per billion analysis. The larvae on all plants were censused for survivorship at 24 hours, 7 days, 14 days, 21 days, and weekly thereafter as needed until pupation.

Results from this experiment are still being processed, and we are awaiting the critically important corresponding parts per billion amounts of bifenthrin that resulted on each sprayed plant to explicitly test the correlation between bifenthrin quantity and larval survivorship. Additional details can be provided in future updates. In summary though, none larvae in the 100% and 10% spray treatments were found alive after the 24 hour census. Three of 20 of Sachem survived in the 1% treatment and 13 of 20 survived in the 0.1% treatment. Survivorship in the control groups was nearly universal. Dakota skippers may have fared slightly better than Sachem, with higher overall survivorship in the 1% and 0.1% treatments (Dakota skippers were not placed in the 100%, 10% and 0% treatments due to sample size limitations), and perhaps a delayed mortality in the 1% treatment relative to Sachem. The differences between species are interesting, and may be related to 1) the more elaborate basal plant shelters constructed by Dakota skippers and 2) the slower metabolisms of Dakota skippers (one generation annually) than Sachem (2+ generations per year).

We plan on publishing these results in peer-reviewed scientific literature. There are no published or available studies of the responses to prairie skipper larvae to the observed insecticides. Experiments testing responses are needed across multiple related species, particularly given the imperiled state of Dakota skippers and Poweshiek skipperlings. Furthermore, multiple forms of exposure studies need to be conducted, ranging from direct contact studies under artificial conditions that remove as many variables as possible to studies that are designed to capture as much natural history as possible to provide inferences of the “real world” risks.

Project Status as of May 31, 2019:

Field Sampling:

No additional field sampling has occurred since the last update, and all ENRTF funding for this activity has been expended. Results of this study are anticipated to be submitted for peer-reviewed publication in the near future.

Controlled Exposure Experiment:

Since our last report, we have received the data on the quantities of bifenthrin present on the plants that were sprayed in the exposure experiment described above. We assumed that the No Spray treatment would be comparable to 0% bifenthrin spray treatment, and that any plant with bifenthrin “Not Detected” was equal to 0 ppb bifenthrin since the minimum Level of Detection reported by the USDA National Sciences Laboratory was

reported as 1 ppb for all plants. We hypothesized that each successive 10% solution of the 100% field rate application of bifenthrin would result in quantities of bifenthrin residues detected on each plant that were 10% of the previous solution and that the 0% and No Spray plants would have no bifenthrin detected. Generally, we did indeed find corresponding 10-fold differences between treatment groups, with the exception of the two control treatments. Bifenthrin was reported detected on 25 of the 31 No Spray treatment plants and on all of the 0% Treatment plants. The levels reported for these two control groups were similar, and with only a few exceptions did not overlap the ppb reported for the 0.1% treatment plants. The source of the contamination of bifenthrin in the No Spray and 0% spray control plants is unknown at this time. Multiple sources of contamination cannot be ruled out, including handling during sample collection, direct inadvertent contact with contaminated secondary sources (such as clothing or equipment), aerosolized contaminated droplets, cross-contamination during sample processing, contamination of laboratory equipment, and analysis error due to lower than desired masses of submitted tissue samples. Nonetheless, the ranges of ppbs reported within and across treatment groups are reasonably close to expectation.

As noted above, bifenthrin has not been observed in our field collections since 2015. Thus, the risk associated with non-target applications of bifenthrin on wild grass skippers may be currently reduced, particularly in light of the increasing resistance to bifenthrin and other pyrethroid insecticides by soybean aphids. To date, most of the field observations of bifenthrin have been in range of the 0.1% treatment group of our exposure experiment, where some limited mortality was observed in both species. The highest observed level of bifenthrin in our field sampling was on little bluestem grass with 76 parts per billion, which is mid-range between our 0.1% and 1% treatments. Further analyses are pending, but our early results suggest that some mortality of wild grass skipper caterpillars residing in their constructed shelters may be expected at this level.

We plan to submit these results for publication in peer-reviewed scientific literature. There are no published or available studies of the responses to prairie skipper larvae to the observed insecticides. Experiments testing responses are needed across multiple related species, particularly given the imperiled state of Dakota skippers and Poweshiek skipperlings. Furthermore, multiple forms of exposure studies need to be conducted, ranging from direct contact studies under artificial conditions that remove as many variables as possible to studies that are designed to capture as much natural history as possible to provide inferences of the “real world” risks.

Overall Project Outcomes and Results:

Field Sampling, Exposure Experiments, and Next Steps:

Overall, we have observed widespread occurrences of pesticides within prairies designated as Critical Habitat for Dakota skipper and Poweshiek skipperling in Minnesota by the US Fish and Wildlife Service. The results of the sampling under the scope of this ENRTF appropriation in 2017 and 2018 are consistent with results we have also collected using comparable methods from 2014 through 2016. Within these protected prairies, we have detected commonly insecticides applied against soybean aphid in late summer, particularly the organophosphate chlorpyrifos. The declines of Poweshiek skipperling, Dakota skipper, and other prairie-dependent insects are correlated with the invasion of the economically-damaging soybean aphid, and subsequent increases in the applications of these pesticides against it. We have also observed two herbicides and eight fungicides during the course of this ENRTF appropriation. It is critical to note that the observed occurrences of these pesticides inside prairies that once maintained robust populations of now imperiled and extirpated butterfly species is correlative at this time. Mechanistic determinations of the causes for the declines are needed, with multiple factors likely contributing and interacting to population persistence. Nonetheless, our findings suggest a possible causal connection between pesticides occurrences and prairie butterfly declines. The experimental testing we conducted in 2017 and 2018 are the first steps to understanding the potential risks to pesticide exposures. Preliminary estimates from our controlled exposure experiments suggest that some of the observed concentrations of one of the insecticides that we have historically observed in the wild can lead to some mortality to Dakota skipper caterpillars under scenarios very similar to wild conditions, and therefore could be a contributing stressor.

Additional field sampling and controlled exposure research experiments are absolutely needed in the near future, particularly for chlorpyrifos. We are planning to begin submitting these results for peer-reviewed publication as early as 2020. The LCCMR and the ENRTF will be credited.

V. DISSEMINATION:

Description:

The activities and results of the Minnesota Zoo's husbandry, reintroduction, population supplementation, and research operations will be shared with all named partners through annual reports. The outcomes of 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.

Project 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.

Project Status as of May 31, 2017:

A detailed report of all of the activities of the Minnesota Zoo's Prairie Butterfly Conservation Program (largely supported by this appropriation and the Program's ML 2014 ENRTF grant) was distributed to our various federal, state, and local permittees and partners in January 2017. We have remained active in outreach through the winter months. These activities are discussed in greater detail in the May 2017 status update for our ML 2014 ENRTF appropriation, but include large presentations before the Minnesota Native Plant Society and the Minnesota Prairie Chicken Society.

Most relevant to the activities of this appropriation, Cale Nordmeyer (Butterfly Conservation Specialist) and Dr. Seth Stapleton (Field Conservation Supervisor) met in March with landowners adjacent to The Nature Conservancy's Hole-in-the-Mountain Preserve where Dakota skipper reintroductions are scheduled to begin this June. They also presented the formal reintroduction plan to the Lake Benton City Council. Additional outreach in

Lake Benton associated with the reintroduction, and prairie butterflies and the disappearing habitats they depend upon, is scheduled for June and July.

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.

Project Status as of November 30, 2017:

We continue to coordinate all of our work with our partners at the US Fish and Wildlife Service, Minnesota DNR, and others across the US and Canada. This coordination includes monthly conference calls and in-person meetings. Annual reports of our work, including the information described here, will be submitted in coming months.

The Dakota skipper reintroduction effort garnered significant media coverage this summer, including:

- The Nature Conservancy's Cool Green Science blog (September 27, 2017): <https://blog.nature.org/science/2017/09/27/rare-butterflies-return-home-dakota-skippers-prairie-restoration/?intc3=nature.science.lp.news1>
- 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 ENRTF), 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 #ButterflyBrew Dakota Skipper Endangered Reserve promotion through Fair State Brewing Cooperative.

Dr. Runquist provided two webinars in October for prairie biologists, researchers, and land managers for the Minnesota Department of Natural Resources and for personnel associated with and/or interested in the Minnesota Prairie Conservation Plan. These webinars, co-presented with Dr. Robert Dana (PI on the DNR's portion of this joint ENRTF), discussed the status of Minnesota's prairie butterflies, the Minnesota Zoo's conservation efforts for Dakota skippers and Poweshiek skipperlings, and on-going research into the potential mechanisms for their declines. ENRTF support for all this work was highlighted in both webinars.

Dr. Runquist presented a summary of the ENRTF-supported pesticides research to date and then participated in a panel discussion at the North American annual meeting of the Society for Environmental Toxicology and Chemistry in Minneapolis in mid-November. This session was attended by over 50 scientists and policy managers from a broad range of federal, state, local, corporate, and private agencies.

Mr. Nordmeyer presented information about our program and participated in a panel discussion at the Invertebrate Education and Conservation Conference in Tucson, Arizona in July. He also provided a general audience talk about the program to the staff of Think Mutual Bank (a Minnesota Zoo sponsor) in Rochester, MN in August.

The Minnesota Zoo's Prairie Butterfly Conservation Program also again hosted a popular booth at the Minneapolis Monarch Festival in September. Zoo staff talked with an estimated 2000 people at the Festival about prairie butterflies and the LCCMR-supported Zoo's work with them. We distributed copies of the "Plant For Pollinators" and "Your Butterfly Neighbors" pamphlets that were produced in both English and Spanish through the Zoo's M.L. 2014 ENRTF grant.

Project Status as of May 31, 2018:

A detailed report of all of the activities of the Minnesota Zoo's Prairie Butterfly Conservation Program was distributed to our various federal, state, and local permittees and partners in January 2018. We participate in frequent conference calls and planning meetings with key State, federal, and non-profit partners, on all aspects of the Program aspects described here.

The Minnesota Zoo is a member of the Interagency Pollinator Protection Team, as designated by Governor's August 25, 2016 Executive Order 16-07 "Directing Steps to Reverse Pollinator Decline and Restore Pollinator Health in Minnesota". Zoo staff regularly attend all IPPT meetings and provide constructive feedback on IPPT planning and decisions. Zoo staff also regularly attend the meetings of the Governor's Committee for Pollinator Protection. Dr. Runquist also provided an update on all Program activities for the annual meeting of ENRTF-funded pollinator projects, held at the University of Minnesota in March 2018.

We have remained active in public outreach through the winter months. Since our last update, we have presented to several general public audiences, and at two undergraduate courses (University of Minnesota and Macalester College). In February, Dr. Erik Runquist was a featured speaker at the annual Best Practices for Pollinators Summit, hosted by the Pollinator Friendly Alliance. He was also a speaker at a Mendota Heights public town hall forum on pollinators for Representative Rick Hansen in March. He also provided a general audience talk about the program to the staff of 3M (Maplewood, MN) in December 2017 and for the staff of Thomson Reuters (Eagan, MN) in May.

Butterfly Conservation Associate Emily Royer (currently supported using non-LCCMR funds) also hosted a hands-on interactive butterfly science station at the Bakken Museum's annual "Discovery Days: Women in Science" event, in March.

Project Status as of November 30, 2018:

We remain actively involved in federal, state, local, and international recovery planning and risk assessment working groups for Poweshiek skipperling and Dakota skippers. We share all results with these partner organizations through written reports, conference calls, and in-person meetings.

Butterfly Conservation Specialist Cale Nordmeyer and Butterfly Conservation Biologist Dr. Erik Runquist co-authored an important new open-access peer reviewed scientific article entitled "Improving Standards for At-Risk Butterfly Translocations" (*Diversity* 2018, 10(3), 67; <https://doi.org/10.3390/d10030067>). Building upon the processes we used to develop and initiate the programs supported by this ENRTF, we provide guidance to improve other butterfly conservation translocations.

We have remained active in public outreach. Since our last update, we have presented to several general public audiences, other government agencies, and at scientific conferences. Dr. Erik Runquist presented at the Minnesota Pollution Control Agency in June, for the staff of U.S. Senator Tina Smith in July, and for the Eagan Rotary in September. He was a featured speaker at the Minnesota Landscape Arboretum's 2018 Pollinators Summit. He was also an invited speaker about the pesticides studies supported through this ENRTF at the joint annual meeting of the Upper Midwest Invasive Species Conference / North American Invasive Species Management Association Conference in Rochester in October. He also presented about the Dakota skipper and Poweshiek skipperling reintroduction/reinforcement efforts at the International Union for the Conservation of Nature's 2nd International Wildlife Reintroduction Conference, in Chicago in November. The Program's Butterfly Conservation Specialist Cale Nordmeyer presented information about our program and participated at the annual meeting of the Association of Zoo Horticulturalists in Winnipeg, Manitoba in August.

Our work was highlighted in an article in the Economist for World Environment Day: <https://perspectives.eiu.com/sustainability/what-prairie-butterfly-can-tell-us-about-emerging-environmental-risk>.

A film crew from the Prairie Public TV's Prairie Sportsman show came to the Hole-in-the-Mountain Prairie Preserve during the Dakota skipper reintroduction effort and then later to the Minnesota Zoo. A segment is in production now for airing on Prairie Sportsman on public TV statewide beginning in February 2019.

The Minnesota Zoo's Prairie Butterfly Conservation Program also again hosted a popular booth at the Minneapolis Monarch Festival in September 2018. Zoo staff talked with an estimated 2500 people at the Festival about prairie butterflies and the LCCMR-supported Zoo's work with them. We distributed copies of the "Plant For Pollinators" and "Your Butterfly Neighbors" pamphlets that were produced in both English and Spanish through the Zoo's M.L. 2014 ENRTF grant.

Project Status as of May 31, 2019:

Minnesota Zoo staff remain highly engaged with the Dakota skipper and Poweshiek skipperling conservation working groups, which are composed of dozens of US Federal, State, Canadian Federal, Provincial, local, and non-profit agencies. We participate in regular conference calls and multiple in-person meetings. We hosted the 2019 annual in-person meeting for the international Poweshiek skipperling conservation partnership, in February.

The Minnesota Zoo partnered with the Minnesota DNR to host and co-teach a Skipper Identification Workshop in May 2019 as part of Activity 4 of this joint Zoo-DNR collaborative project. Comprised largely of state land managers and other interested parties, about 30 individuals attended from all across Minnesota, as well as North Dakota. Participants learned techniques to identify more than a dozen species of skippers, particularly Dakota skipper and Poweshiek skipperling along with those prairie species that they may co-occur with. They also learned about ecological settings, management of prairies as it relates to skippers, survey techniques, and permitting requirements.

A summary of our ENRTF-supported pesticides exposure research was presented to dozens of scientists and managers at the Prairie Conservation and Endangered Species Conference in Winnipeg, MB in February.

We have also been active with outreach to the general public. This included hosting tables about prairie butterfly conservation and the activities supported by this ENRTF at the Super Bee Weekend (February) and the Great Minnsect Show (April) events at the University of Minnesota. Butterfly Conservation Specialist Cale Nordmeyer lectured to a conservation biology course at the University of Minnesota. Project Manager Dr. Erik Runquist also provided a similar free talk for about 100 people about pollinator conservation and ENRTF support at the Minnesota Zoo's Adult Night Out - Our World Speaker Series in May.

Project Manager Dr. Erik Runquist provided invited testimony to the Minnesota House Environment and Natural Resources Finance Committee in January, largely focused on prairie butterfly conservation and the activities supported by this ENRTF.

Final Report Summary:

Our work has garnered attention and the Minnesota Zoo has become a key agency in the international conservation programs for Poweshiek skipperling and Dakota skipper. We remain active in multiple planning processes and multi-party action teams for both species and have hosted multiple workshops and meetings, including the 2019 meeting for the International Poweshiek Skipperling Conservation Partnership. Similarly, the field component of the Skipper Identification Workshop of this joint Zoo-DNR ENRTF appropriation (Activity 4; to be discussed in greater detail in the Update provided by DNR) was held in late June 2019 at the Hole-in-the-Mountain Prairie Preserve at the site of the Dakota skipper reintroduction program as well as several other sites in southwest Minnesota.

Across the duration of this project, there have been five TV stories, two radio segments, three newspaper articles, two major blog posts, dozens of social media posts, and dozens of speaking engagements. The Dakota skipper reintroduction effort has recently been the subject of four new TV segments (including two live) and one newspaper story that was widely shared across other news outlets across Minnesota and the Northern Plains:

Public TV's Prairie Sportsman, February 17, 2019: <https://www.pbs.org/video/return-dakota-skipper-oenx2w/>

Pioneer Press, July 6, 2019: <https://www.twincities.com/2019/07/06/minnesota-zoo-to-release-400-endangered-dakota-skippers-butterflies/>

KDLT-TV (Sioux Falls, SD), July 9, 2019: <https://www.kdlt.com/2019/07/09/minnesota-conservationists-release-endangered-butterflies-back-into-wild/>

KSFY-TV (Sioux Falls, SD), July 10, 2019: <https://www.ksfy.com/content/news/Endangered-butterflies-reintroduced-in-Minnesota-512560981.html>

KARE11-TV (Minneapolis, MN), July 11, 2019: <https://www.kare11.com/video/news/local/kare11-sunrise/sven-explains-the-plight-of-the-dakota-skipper-butterfly/89-5a9cf971-5738-42cf-81ca-7eedd3a48bcf>

The research generated by this Project will be submitted for publication a peer-reviewed scientific journals.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	\$ Amount	Overview Explanation
Personnel:	\$ 350,000	1 State Program Administrator Principal at 100% FTE for 2 years; 1 Project Analyst/Specialist at 100% FTE for 2 years and 35% FTE for 1 year; 1 Temporary Student Worker at 25% FTE for 2 years
Professional/Technical/Service Contracts:	\$30,000	1 contract (RFP) pesticide residue testing
Equipment/Tools/Supplies:	\$29,000	Supplies needed to support butterfly rearing and breeding operations as well as pesticides research, including tables, rearing cages, butterfly nets, collecting supplies, plants, and laboratory supplies
Travel Expenses in MN:	\$5,000	Mileage, lodging, meals for travel to and between prairie sites for data collection and breeding operations
Other:	\$7,000	Travel expenses outside of MN. Mileage, lodging, meals for travel to and between prairie sites to obtain individuals for the Zoo conservation breeding program. All known viable populations of the Minnesota-native endangered butterflies are now outside of Minnesota in Wisconsin, Michigan, North Dakota, South Dakota, and Manitoba, necessitating out of state travel to obtain founder stock.
TOTAL ENRTF BUDGET:	\$421,000	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than \$5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 4.842

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

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
Minnesota Zoo Foundation	\$25,000	\$ 18,000	Private funds to support the Prairie Butterfly Conservation Program. Support extra supplies and travel to non-State or Federally-supported events.
US Fish and Wildlife Service	\$72,128 <u>+\$25,353</u> <u>+\$90,500</u> <u>+\$128,000</u>	\$206,573	Three separate "CFDA Endangered Species – Candidate Conservation Action Fund Cooperative Agreements" have been issued to support the Minnesota Zoo's Prairie Butterfly Conservation Program in 2014 and 2015. These provide funding for facilities expansion for Poweshiek skipperling operations and a limited number of associated supplies, current pesticides research, personnel costs for unsupported Outside of Minnesota work, and support to host the Conservation Breeding Specialist Group <i>ex situ</i> planning workshop. A fourth interagency cooperative agreement fund was awarded to the Minnesota Zoo in March 2017, to support new microclimate control equipment and to retain personnel through the 2017 and 2018 seasons. <u>A fifth grant was awarded in June 2018 to support out-of-state travel and supplies for the Poweshiek skipperling reinforcement program.</u>
Association of Zoos and Aquariums Conservation Grants Fund	\$22,467	\$22,467	Competitive grant to the Minnesota Zoo in 2015 to provide needed facilities expansion for Dakota skipper and a limited number of associated supplies associated with a larval hostplant performance experiment.
National Geographic Society	\$29,600	\$19,000	Competitive grant to support travel, equipment upgrades, and supplies costs for the Poweshiek skipperling and garita skipperling program, particularly the microclimate experiment research.
State			
ENRTF (ML 2014)	\$380,000	\$368,768	Portions of the FY17 personnel budget are beginning supported by the existing ENRTF granted to the Minnesota Zoo in 2014.
Legacy Clean Water Arts and Cultural Heritage Fund, grant to MN Zoo for FY17	\$37,200 <u>+\$20,000</u> <u>+\$35,000</u>	\$ 95,000	Supports unfunded 62.4% of existing Project Analyst/Specialist salary and benefits, <u>as well as a portion of the pay for temporary staff.</u>
TOTAL OTHER FUNDS:	\$ 865,248	\$ 741,808	

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, permissions for egg collection and reintroduction efforts
- The Nature Conservancy of Canada: Access to prairie preserves
- University of Minnesota: Collaborative pesticides-associated mortality research
- New College of Florida: Collaborative conservation genetics research

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 for Poweshiek skipperlings and Dakota skippers have pointed to steep recent declines in both species, to the point that the Poweshiek skipperling may now be extinct in Minnesota 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 provide the necessary support to allow the Minnesota Zoo to initiate the world's first and only reintroduction and population supplementation efforts for Minnesota endangered Dakota skippers and Poweshiek skipperlings and to reduce their risk of extinction in Minnesota and globally. The complementary monitoring efforts by the Minnesota DNR of individual populations, including those sites in Minnesota where reintroductions may occur, will provide the foundation for a higher-resolution tracking of population trends and reintroduction success.

The wild reintroductions and population supplementation programs and the wild population monitoring programs are both long-term commitments, and this ENTRF project will constitute only the beginning for them. We intend for our work to develop husbandry, reintroduction, and monitoring protocols that will be used long-term. We will be working on strategies for funding the work in the long-term.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	\$ Amount
Environment and Natural Resources Trust Fund - M.L. 2014, Chp. 226, Sec. 2, Subd. 05j-1: Appropriation of cash funds to support the Zoo's Prairie Butterfly Conservation Program operations and experiments	FY15-FY17	\$ 380,000
Legacy Clean Water Arts and Cultural Heritage Fund: Appropriation of cash to MN Zoo to support all operations and staff of the Prairie Butterfly Conservation Program since its inception.	February 2012-November 2015	\$ 349,000

US Fish and Wildlife Service CFDA Endangered Species – Candidate Conservation Action Fund Cooperative Agreements: Two separate cash grants have been issued to support the Minnesota Zoo’s Prairie Butterfly Conservation Program. These provide funding for facilities expansion for Poweshiek skipperling operations and a limited number of associated supplies, current pesticides research, personnel costs for unsupported Outside of Minnesota work, and support to host the Conservation Breeding Specialist Group <i>ex situ</i> planning workshop.	FY15-FY17	\$ 72,128
In-kind donations, managed by the Minnesota Zoo Foundation	FY14-FY16	\$ 34,385
Association of Zoos and Aquarium Conservation Grants Fund – grant of cash funds to build Dakota skipper rearing facilities and conduct hostplant experiment.	October 2015-September 2016	\$ 22,467
		\$ 857,980

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): See attached graphic of Minnesota’s imperiled prairie butterflies, the history of Dakota skipper and Poweshiek skipperling observations in Minnesota, and sites to be surveyed for prairie butterflies by MN DNR.

X. RESEARCH ADDENDUM: N/A, per communication with LCCMR staff

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than November 30, 2016; May 31, 2017; November 30, 2017; May 31, 2018, November 30, 2018, and May 31, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.

Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget



Project Title: Prairie Butterfly Conservation, Research, and Breeding – Phase II

Legal Citation: M.L. 2016, Chp. 186, Chp. 2, Sec. 2, Subd. 03c1

Project Manager: Dr. Erik Runquist

Organization: Minnesota Zoo

M.L. 2016 ENRTF Appropriation: \$ 421,000 to the Minnesota Zoo

Project Length and Completion Date: 3 years, June 30, 2019

Date of Report: August 30, 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 12/7/18	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	Zoo Breeding, Headstarting, and Reintroduction Program			Pesticides Research - Phase 2				
Personnel (Wages and Benefits)	\$350,000	\$343,116	\$6,884				\$350,000	\$6,884
Butterfly Conservation Biologist Erik Runquist (State Program Administrator Principal at 100%, salary & benefits for FY18 & FY19 - \$180,000).								
Butterfly Conservation Specialist Cale Nordmeyer (Project Analyst/Specialist at 34.2% salary and benefits FY17 and 100% salary & benefits for FY18 and FY19 - \$153,800).								
Seasonal Temporary Student Worker (1 unclassified at 100% time for 6 months total during FY18 and FY19 - \$16,200)								
Professional/Technical/Service Contracts								
Pesticides residue analysis contract. Contractor and actual amount subject to RFP, but the USDA National Sciences Lab was selected in the Zoo's first ENRTF and is likely to be used again. Current rates are \$176-\$396/sample, depending on analysis.				\$30,000	\$30,000	\$0	\$30,000	\$0
Equipment/Tools/Supplies								
Breeding program supplies: butterfly plants, rearing cages, tables, collecting supplies	\$24,000	\$23,125	\$875				\$24,000	\$875
Pesticides research supplies: plants, chemicals, and space rental at the University of Minnesota or other facilities.				\$5,000	\$5,000	\$0	\$5,000	\$0
Travel expenses in Minnesota								
Mileage, lodging, meals for travel to and between prairie sites for data collection and husbandry/reintroduction operations	\$8,000	\$6,107	\$1,893				\$8,000	\$1,893
Other								

Zoo Travel expenses outside of MN. Mileage, lodging, meals for travel to and between prairie sites to obtain individuals for the Zoo conservation program and to conduct wild supplementations/reintroductions. 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. November 2018 amendment request: We seek to transfer \$3,000 of the remaining out-of-state funds to the in-state travel budget to support the breeding and reintroduction activities.A portion of previously budgeted out-of-state travel is now supported by a new Federal grant. See update text for details.	\$4,000	\$2,526	\$1,474				\$4,000	\$1,474
COLUMN TOTAL	\$386,000	\$374,874	\$11,126	\$35,000	\$35,000	\$0	\$421,000	\$11,126



Many prairie butterflies are declining, and some are in danger of extinction. With ENRTF support (M.L. 2016, Chp. 186, Sec. 2, Subd. 03c1), the Minnesota Zoo is supporting disappearing populations of Dakota skipper and Poweshiek skipperling through wild reintroductions and releases and Zoo-based insurance populations. We are researching stressors to wild populations and the conditions needed for them to thrive.

