2019 Project Abstract
For the Period Ending June 30, 2023

PROJECT TITLE: Conservation and Monitoring of Minnesota's Rare Arctic Plants
PROJECT MANAGER: Briana L. Gross
AFFILIATION: University of Minnesota Duluth
MAILING ADDRESS: 207 SSB, 1035 Kirby Drive
CITY/STATE/ZIP: Duluth, MN, 55812
PHONE: 218-726-7722
E-MAIL: blgross@d.umn.edu
WEBSITE: https://scse.d.umn.edu/biology-department/faculty-staff/dr-briana-gross
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 08d

APPROPRIATION AMOUNT: $135,000
AMOUNT SPENT: $133,697.60
AMOUNT REMAINING: $1,302.40

Sound bite of Project Outcomes and Results
Through three years of genetic and field study, we found that the rare arctic relict plants of Minnesota have retreated northward since the 1900s. They will likely decline into the future, and one species is threatened by an aggressive invasive species. Protection and education are critical to preserve these unique species.

Overall Project Outcome and Results
The North Shore of Lake Superior in Minnesota is home to "arctic relict" plants that are usually found in the arctic or sub-arctic. In Minnesota, they survive in rocky, cool, arctic-like microhabitats created by Lake Superior. These plants are threatened by a changing climate, declining available habitat due to tourism and development, and in one case, hybridization with a related invasive species. First, our project examined historical Minnesota Biological Survey (MBS) survey sites along the shore to determine arctic relict community change over time. Next, we studied the health of three different arctic relict species along the North Shore to determine if warmer, southern populations were showing more signs of stress than cooler, northern populations. Last, we set out to test if removing the invasive species by hand was an effective way to reduce hybridization and protect the genetic integrity of a threatened native species. The middle year of our study, 2021, was a drought year, which allowed us to monitor the effect of drought on our three target species. We found that change in community composition varied among sites, but there was a general decrease in species diversity along the shore. Additionally, the southern-most occurrence has contracted substantially northward for two of three species. The species under threat of hybridization is also most at risk of being affected by a summer drought and is projected to decline across most sites. The invasive species is an aggressive invader, and removal of it is only feasible in target areas of concern where it is near native populations but not yet well established. Our work adds to and enhances natural history data collected by the state of Minnesota and highlights the need to continue protecting these plants and educating Minnesotans about our unique, rare species.

Project Results Use and Dissemination
This project was conducted with the cooperation state and federal partners, and we hosted a meeting for land managers from across the North Shore to share our findings and promote cross-agency collaboration. This led to the creation of the "Lake Superior Arctic Relicts" information sharing group to communicate with land managers and other stakeholders. Discussion with stakeholders and community education events will continue. We are also conducting field site visits to address specific land manager needs and questions. We've submitted our re-survey results to the DNR, and the genetic data is freely available online.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2019 ENRTF Work Plan Final Report (Main Document)

Today's Date: June 30, 2023

Final Report: October 18, 2023

Date of Work Plan Approval: June 5, 2019

Project Completion Date: June 30, 2023

PROJECT TITLE: Conservation and Monitoring of Minnesota's Rare Arctic Plants

Project Manager: Briana L. Gross

Organization: University of Minnesota Duluth

College/Department/Division: Swenson College of Science and Engineering, Biology Department

Mailing Address: 207 SSB, 1035 Kirby Drive

City/State/Zip Code: Duluth, MN, 55812

Telephone Number: 218-726-7722

Email Address: blgross@d.umn.edu

Web Address: https://scse.d.umn.edu/biology-department/faculty-staff/dr-briana-gross

---

Location: Northeastern MN; St. Louis, Lake, and Cook counties (coastal regions)

Total Project Budget: $135,000
Amount Spent: $133,697.60
Balance: $1,302.40

Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 08d

Appropriation Language: $135,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to provide monitoring and invasive species removal to conserve rare and endangered arctic plants on Minnesota's North Shore. This appropriation is available until June 30, 2023, by which time the project must be completed and final products delivered.

I. PROJECT STATEMENT:

If you visit the rocky shore of Lake Superior, you will probably see plants that are not found anywhere else in the continental USA, including some of the most endangered species in Minnesota: the arctic relicts. These plants contribute to the charm of the most important tourist area in the state, but they are at risk. Of 48 arctic relicts, at least six species are endangered, four are threatened, and three are of special concern. In addition, our research recently shows that one of these rare species is hybridizing with an invasive relative and is in danger of extinction due to genetic swamping (Zlonis and Gross 2015). It has been 10+ years since the Minnesota Biological Survey (MBS) conducted a comprehensive surveyed these populations. **Our goal is to understand and ultimately learn how to protect this unique community.**

What will we do?

- Collect detailed information on the health of Minnesota's arctic relict plant communities
- Establish plots for long-term monitoring at key locations
- Implement invasive species removal
- Share our findings with managers to protect develop plans for long-term conservation of their habitats

What are arctic relicts? Species referred to as 'arctic relicts' were once common in northern Minnesota when glaciers retreated approximately 10,000 years ago, but are now the last representatives of their kind outside of the arctic. They survive along the North Shore because the lake creates a cold microclimate with disturbances that mimic an arctic environment. These communities include many species of conservation concern, such as:

- Hudson Bay eyebright (*Euphrasia hudsoniana*) (SC)
- Alpine bistort (*Bistorta vivipara*) (TH)
- Spike trisetum (*Trisetum spicatum*) (SC)
- Butterwort (*Pinguicula vulgaris*) (SC)
- Alpine woodsia (*Woodsia alpina*) (TH)
- Smooth woodsia (*Woodsia glabella*) (TH)
- Wild chives (*Allium schoenoprasum*) (EN)
- Auricled twayblade (*Listera auriculata*) (EN)
- Alpine bilberry (*Vaccinium uliginosum*) (EN)
- Small false asphodel (*Tofieldia pusilla*) (EN)
- Knotty pearlwort (*Sagina nodosa*) (EN)
- Northern paintbrush (*Castellija septentrionalis*) (EN)

SC = Special Concern; TH = Threatened; EN = Endangered

Why are they in danger? Suitable habitat mimicking arctic environments is extremely limited for these species along the North Shore. As tourism and development increase and temperatures changes, populations of these unique and beautiful species are in danger and perhaps at risk of extinction. **Our surprising new discovery also indicates that one of these species (*Euphrasia hudsoniana*) is in danger from hybridization with an invasive relative, which compromises the genetic integrity of this rare Minnesota species.** The MBS intensively surveyed plant communities on the North Shore in 1999-2005 and found new occurrences of several rare species. However, we do not have any information on the health or viability of these populations. At least one study conducted since then suggests that the communities are vulnerable to environmental change and land managers have reported the arrival of invasive species and decline of some arctic species.

II. OVERALL PROJECT STATUS UPDATES:

First Update May 1, 2020

Initial work was done to establish relationships with community stakeholders and to locate additional populations of *Euphrasia hudsoniana* (special concern)*, Pinguicula vulgaris* (special concern), and *Primula mistassinica.* Removal of the invasive *E. stricta* was completed at two known hybridization locations and samples of the invasive, native (*E. hudsoniana*), and hybrids were collected for genetic analysis (fall 2019). Invasive removal will continue throughout the summer of 2020. Historical MBS Releve surveys have been collected and the timeline and identification of resurvey sites to have been outlined. Resurveying will continue during the

summer of 2020. Plots for the planned population viability study have been selected and 1st year data will be collected in the summer of 2020.

Second Update November 1, 2020

The 2020 summer field season proceeded as planned. Plots for long-term viability study were established and life history and phenotypic data was collected for the first of three consecutive years. Re-survey of the targeted releve plots was completed. A second year of the invasive removal (*E. stricta*) was completed at the two target hybridization locations. Genetic analysis was delayed as the University of Minnesota Genomic Center shifted focus to primarily address COVID-19 but genetic work will resume in the fall of 2020.

Third Update May 1, 2021

Initial phenotypic data from year one of long-term plots was analyzed. Plots will be revisited weekly during the summer of 2021. Re-surveyed releve data was compiled and initial analysis comparing historical and modern communities was completed. Final analysis will be completed by the end of the summer and sent to MBS with accompanying releve data. Genetic sequencing, to be completed by the University of MN Genomics Center still continues to be delayed due to the COVID-19 pandemic.

Fourth Update November 1, 2021

The 2021 summer field season proceeded as planned. Activity #1 was completed as planned. Plots for long-term viability study were revisited and life history and phenotypic data was collected for the second of three consecutive years. A third year of the invasive removal (*E. stricta*) was completed at the two target hybridization locations. Initial genetic sequencing was completed after long delays due to the COVID-19 pandemic.

Fifth Update May 1, 2022

Initial phenotypic data from year two of long-term plots was analyzed. Preparations were made for plots to be revisited weekly and for invasive removal to continue in the summer of 2022.

Sixth Update November 1, 2022

The 2022 summer field season proceeded as planned. The plots for long-term viability study were revisited throughout the summer for the final year, where life history and phenotypic data was collected. Analysis of the data collected over the last three years will be performed to project population growth or decline among the target populations. The final year of invasive removal (*E. stricta*) was completed at the two target hybridization sites, thereby completing Outcome 1 of Activity #3. *Euphrasia* samples were collected from the hybridization zones and along MN's North Shore, and will be sent to the University of MN Genomics Center for genetic sequencing.

Amendment Request November 1, 2022

1. We are requesting that funds be shifted within Personnel as follows:
    a. $7,123 from undergraduate hourly worker to field technician. This will fund salary/fringe for an undergraduate who received their BS degree and now works full-time as a field technician on the project. The amount will cover salary/fringe until the project end date. This technician is collecting and analyzing data for Activities 2 and 3.

b. $5,099 from undergraduate hourly worker to MS graduate student. This will fund fringe including tuition during the semester, which was higher than we originally budgeted. This MS graduate student is collecting and analyzing data for Activities 2 and 3.

2. We are requesting that $8,250 be shifted from Personnel to Professional/Technical/Service Contracts. We originally predicted we would need to hire an outside consultant to advise on the project for $8,250 but were able to complete the necessary work without their input. At the same time, costs of DNA sequencing has increased due to inflation, supply-chain issues, and increased demand related to the Covid-19 pandemic (we use the same facilities used for Covid-19 PCR testing). In addition, a preliminary analysis showed that we will need to include more samples than we originally planned in the budget. The money that is transferred will be used to generate sequence data to accomplish the genetic assessment of hybridization in Activity 3 of the project.
3. We are requesting that $2,500 be shifted from "Travel expenses" to "Equipment/Tools/Supplies". We slightly over-estimated how much we would use for mileage and travel over the course of three years and are now mainly done with travel to field sites. At the same time, while we have decided to extract DNA in our lab to save money, costs of DNA extraction kits have skyrocketed due to inflation and supply-chain issues related to the Covid-19 pandemic. The money that is transferred to will be used to purchase DNA extraction kits to accomplish the genetic assessment of hybridization in Activity 3 of the project.

Amendment APPROVED by LCCMR 11/11/22

Final Report June 30, 2023

The North Shore of Lake Superior in Minnesota is home to "arctic relict" plants that are usually found in the arctic or sub-arctic. In Minnesota, they survive in rocky, cool, arctic-like microhabitats created by Lake Superior. These plants are threatened by a changing climate, declining available habitat due to tourism and development, and in one case, hybridization with a related invasive species. First, our project examined historical Minnesota Biological Survey (MBS) survey sites along the shore to determine arctic relict community change over time. Next, we studied the health of three different arctic relict species along the North Shore to determine if warmer, southern populations were showing more signs of stress than cooler, northern populations. Last, we set out to test if removing the invasive species by hand was an effective way to reduce hybridization and protect the genetic integrity of a threatened native species. The middle year of our study, 2021, was a drought year, which allowed us to monitor the effect of drought on our three target species. We found that change in community composition varied among sites, but there was a general decrease in species diversity along the shore. Additionally, the southern-most occurrence has contracted substantially northward for two of three species. The species that is under threat of hybridization is also most at risk of being affected by a summer drought, and is projected to decline across most sites. The invasive species is an aggressive invader, and removal of it is only feasible in target areas of concern where it is near native populations but not yet well established. Our work adds to and enhances natural history data collected by the state of Minnesota, and highlights the need to continue protecting these plants and educating Minnesotans about our unique, rare species.

Amendment Request October 17, 2023

1. We are requesting that $143 be shifted from "Travel expenses" (particularly the funds dedicated to milage for land managers) to "Other". We underestimated the costs of food for group meeting of land managers at the end of the project, due to both the popularity of the meeting and the impacts of inflation, resulting in a negative balance. Re-allocating these funds from travel will correct the negative balance in the "Other" category.
2. We are requesting that $201 be shifted from "Travel expenses" to "Professional/Technical/Service Contracts". We slightly underestimated the cost of the genomic work necessary for the project, and

slightly over-estimated the amount we needed for travel expenses. Re-allocating these funds from travel will correct the negative balance in the "Professional/Technical/Service Contract" category.

3. We are requesting that $257 be shifted from "Travel expenses" to "Personnel". We slightly underestimated the number of hours necessary to finish the data analysis for this project, and slightly over-estimated the amount we needed for travel expenses. Re-allocating these funds from travel will correct the negative balance in the "Personnel" category.

Amendment Approved by LCCMR January 15, 2024

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1 Title: ***Survey communities and establish six long-term monitoring locations***
Description: We will systematically assess whether and how these communities have changed in the 10+ years since the MBS survey by revisiting survey locations at the same time of year as the original survey and documenting species abundance and richness according to the same methods. We will also establish six locations for long-term monitoring in future years and will establish guidelines for monitoring efforts. This activity will increase the value of past investments in these plant communities by the state of Minnesota.

ACTIVITY 1 ENRTF BUDGET: $ 49,382

Outcome	Completion Date
1. *Arctic communities visited by the Minnesota Biological Survey in 1999-2005 re-assessed for species composition and compared to previous surveys to document potential changes*	September 2021

First Update May 1, 2020

Historical MBS releve surveys containing arctic relict plants were compiled, and assistance was provided to MBS in the summer of 2019 for several re-survey efforts at multiple sites. These re-surveyed sites provided a training opportunity to ensure our field technician adheres to releve methodology and provides consistent data collection and deposition. Locations and plans for the remaining arctic relict survey sites have been outlined and will be completed during the summer of 2020. Possible locations for future long-term monitoring sites have been discussed and visited. (Outcome 1)

Second Update November 1, 2020

Selected releve re-survey sites were revisited and data collected. The Releve data collected will be submitted to the MN DNR/MBS once completed. This data will be used to track changes in community composition.

Third Update May 1, 2021

Data from the nine selected and resurveyed sites was compiled and analyzed comparing community composition of modern to historical. This analysis will be complete by the end of the summer and the data will be shared with MN DNR/MBS.

Fourth Update November 1, 2021

Data analysis comparing community composition of modern to historical sites is complete. All resurvey data was shared with the MN DNR/MBS. This Activity is complete; we will continue to share the information with interested parties.

Fifth Update May 1, 2022

Activity complete, no further updates.

Sixth Update November 1, 2022

Activity complete, no further updates.

Final Report June 30, 2023

Historical MBS relevé surveys from 2000/2001 containing arctic relicts were resurveyed in the summer of 2019/2020. The Bray-Curtis dissimilarity statistic was used to compare community composition change between the current and historical survey sites for 9 locations. There was no latitudinal trend in dissimilarity. Dissimilarity ranged from 0.14 (low change in community composition) to 0.60 (high change in community composition). Only one new invasive species was documented. Next, the Shannon Diversity Index was used to assess species diversity at each site. While one site increased in species diversity, all other sites decreased at least somewhat in diversity, which was a significant trend when removing the one site with increased diversity. We also surveyed locations of historical herbarium collection records, revisiting to search for the target species *Primula mistassinica*, *Pinguicula vulgaris*, and *Euphrasia hudsoniana*. *Primula mistassinica* was re-located at 65% of historical locations, *P. vulgaris* was re-located at 80%, and *E. hudsoniana* was re-located at 85%. The southern-most known location for *P. vulgaris* has shifted from the mouth of the Knife River to 7 miles (11 km) north, at Two Harbors. The southern-most known location for *E. hudsoniana* has shifted 10 miles (16 km) north, from Stoney Point to Two Harbors; however, it is no longer found at Two Harbors and the southern-most location has now moved a total of 22 miles (35 km) north, to Gooseberry Falls State Park (see Activity 3). Additionally, we have established 12 long-term monitoring plots for the target species at four locations along the shore (see Activity 2), and the plots can be revisited in the future to monitor changes going forward. The extirpation of the known southern populations of *P. vulgaris* and *E. hudsoniana* is evidence of stress on arctic relicts in their southern range, and calls for more monitoring and attention to the protection of these plants.

ACTIVITY 2 Title: *Determine population growth or decline for three rare arctic species*
Description: We will collect detailed information on three plant species that are characteristic of these communities including *Euphrasia hudsoniana* (special concern)*, Pinguicula vulgaris* (special concern), and *Primula mistassinica*. At several locations, we will count individuals and track their reproduction for three years to build models that can project population growth or decline over time. This will help us to determine whether populations are holding steady, increasing, or declining, which can allow managers to prioritize conservation or restoration efforts.

ACTIVITY 2 ENRTF BUDGET: $72,698

Outcome	Completion Date
1. *Assessment of the presence, number, and health of rare species across multiple years*	June 2023

First Update May 1, 2020

Locations for the multi-year collection of detailed life histories for *Euphrasia hudsoniana* (special concern)*, Pinguicula vulgaris* (special concern), and *Primula mistassinica* populations were selected. Locations are Artist Point in Grand Marais, Temperance River State Park, Gooseberry Falls State Park, and Stoney Point in Duluth. Plots at these locations will be selected and preliminary trait and demographic data will be collected in the summer of 2020. (Outcome 1)

Second Update November 1, 2020

Preliminary trait and demographic data were collected for *Euphrasia hudsoniana* (special concern)*, Pinguicula vulgaris* (special concern), and *Primula mistassinica* locations at Artist Point in Grand Marais, Temperance River State Park, Gooseberry Falls State Park, and near the Two Harbors Lighthouse. The Stoney Point location originally intended to be surveyed only contained one of the three relict populations and is now private property, therefore a site in Two Harbors was selected as the fourth site for multi-year data collection.

Third Update May 1, 2021

Phenotypic data from year one of the long-term plot project was analyzed. Year two demographic and phenotypic data will be collected from the same plots for *Euphrasia hudsoniana* (special concern)*, Pinguicula vulgaris* (special concern), and *Primula mistassinica* locations at Artist Point in Grand Marais, Temperance River State Park, Gooseberry Falls State Park, and near the Two Harbors Lighthouse in the summer of 2021.

Fourth Update November 1, 2021

Year two demographic and phenotypic data was collected from the established plots for *Euphrasia hudsoniana* (special concern), *Pinguicula vulgaris* (special concern), and *Primula mistassinica* locations at Artist Point in Grand Marais, Temperance River State Park, Gooseberry Falls State Park, and near the Two Harbors Lighthouse in the summer of 2021. Temperature data was also collected at these locations.

Fifth Update May 1, 2022

Preparations for a third year of demographic and phenotypic data to be collected from the same plots for *Euphrasia hudsoniana* (special concern)*, Pinguicula vulgaris* (special concern), and *Primula mistassinica* locations at Artist Point in Grand Marais, Temperance River State Park, Gooseberry Falls State Park, and near the Two Harbors Lighthouse in the summer of 2022.

Sixth Update November 1, 2022

Year three demographic and phenotypic data was collected from the established plots for *Euphrasia hudsoniana* (special concern), *Pinguicula vulgaris* (special concern), and *Primula mistassinica* locations at Artist Point in Grand Marais, Temperance River State Park, Gooseberry Falls State Park, and near the Two Harbors Lighthouse in the summer of 2022. Temperature data was also collected at these locations. The demographic and phenotypic data from the last three years will be analyzed to project population growth or decline over time.

Final Report June 30, 2023

For each of three target arctic relict species, *Primula mistassinica*, *Pinguicula vulgaris*, and *Euphrasia hudsoniana*, three 1x1 meter plots were established at four sites along the shore (near the Two Harbors

lighthouse, Gooseberry Falls State Park, Temperance River State Park, and Artist Point in Grand Marais). These plots were established in 2020 (except for *P. mistassinica* and *P. vulgaris* at Two Harbors, where plots were established in 2021), and measured through the summer of 2022. Plots were measured and revisited weekly while plants were flowering and initially fruiting, with each individual tracked using a grid system. Measurements included flowering date, diameter, height, number of flowers, and fruit size (used to estimate seed counts per individual), and other characteristics. Survival and fecundity/reproduction over the three years were tracked in order to build Population Viability Analysis (PVA) models to project if populations were growing, declining, or remaining stable. The summer of 2021 was a drought year, while the summers of 2020 and 2022 had more typical precipitation, allowing us the opportunity to see how drought affects these plants and how they recover the following year. We conducted two different PVA analyses (count-based and matrix-based) and discuss both of these below.

The count-based PVA is a relatively simple model, using the number of individuals counted during each year to determine current population growth and predict future population extinction risk. Although it is less informative and precise than the matrix-based PVA, we include the results here because it is easier to replicate and therefore will provide a useful baseline for future studies. We found that the drought had a less severe effect on the two perennial species (*P. mistassinica* and *P. vulgaris*) than on the annual species (*E. hudsoniana*). For the perennials *P. mistassinica* and *P. vulgaris*, the effects of the 2021 drought were reflected in an increase in mortality and general decrease in flowering in 2022. Despite this, the count-based PVA model shows that 3 out of 4 *P. mistassinica* and *P. vulgaris* populations were stable or increasing, with a decline at only one site each. For *P. mistassinica*, the site that shows decline (Temperance River) had a cumulative extinction risk of 90% in 10 years. However, this decline was not drought related, and was instead driven by rapid snow-melt and above average spring rain that flooded two of the three plots in 2022. While this is another example of an extreme weather event that may become more common in the future, it is also important to note that there are estimated to be thousands of individuals at this site outside of our plots. This highlights the importance of conducting full population surveys, which we discuss later. The one site where population declined for *P. vulgaris* (Gooseberry Falls) in 2022 did show signs of drought stress in 2021, with a majority of individuals entering their winter dormancy stage about a month or more earlier than is typical, and many individuals dying by 2022. This population rebounded strongly in 2023, but the count-based PVA predicts a cumulative extinction rate of 60% in the next 20 years at this site. Contrary to these two perennial species, the count-based PVA for the annual *E. hudsoniana* shows that three of the four populations were declining (Two Harbors, Temperance River, and Artist Point). It should be noted that the Two Harbors *Euphrasia* plots are now only composed of invasive and hybrid individuals (see Activity 3). Since *E. hudsoniana* is an annual species, it relies on producing seed every year in order to ensure the population returns the following year. *Euphrasia hudsoniana* primarily grows and flowers beginning in July and lasting through part of September, which is when the 2021 drought was at its height. As a result, *E. hudsoniana* was the most affected by summer drought - most populations declined in 2021 and either declined further or existed at a reduced size in 2022. The count-based PVAs for *E. hudsoniana* predict a 90 to 100% cumulative extinction risk in 10 years at Temperance River and Artist Point.

The matrix-based PVA model is more complex and incorporates the other characteristics we measured, such as survival and fecundity, to make more informed, accurate predictions of future population sizes. The matrix-based PVAs reveal populations of *P. mistassinica* and *P. vulgaris* to be less stable than they appeared in the count-based PVA. *Primula mistassinica* only shows one population (Gooseberry Falls) to be stable and not declining over time. Based on survival and reproduction, Artist Point had a slowly declining growth rate both before and after the drought. The population is projected to be stable for about 15 years, and then cumulative extinction risk rapidly increases and reaches 100% in 20 years. Temperance River and Two Harbors are also both projected to be unstable, although Temperance River plots were affected by localized flooding in 2022, and Two Harbors only has two years of data, not three, so the results of these sites should be viewed with caution. For *P.*

vulgaris, all sites are seen to be stable before the drought in 2021, but unstable afterwards, highlighting the impact of the drought. While Gooseberry Falls is still the most unstable and reaches 100% cumulative extinction risk by 10 years, both Artist Point and Temperance River are predicted to start increasing extinction risk in 5 years, and reach 100% extinction risk by 20 years. Again, Two Harbors does not have enough years of data to make future predictions about extinction risk. A matrix-based PVA is not appropriate for an annual species like *E. hudsoniana* where growth through life-stages within a season are not recorded, so a matrix-based PVA was not created for this species. Across both the count-based and matrix-based PVA models, we found no latitudinal trends in population viability over time.

It is important to keep in mind some of the limitations of the PVAs, and how they affect the results. First, the projections created by the PVAs are based on plots with small sample sizes compared to the entire population of a species at each site. If full site population counts are conducted, that data can be incorporated into these projections to provide better estimates of extinction risk over time. Second, count-based PVAs show positive growth rates and no extinction risk in 20 years at most sites of *P. mistassinica* and *P. vulgaris*, while matrix-based PVAs show current and projected decline. One important difference between the two models is the incorporation of germination rates in matrix-based PVAs. Germination rates have a large impact in the matrix-based models, and changes in those rates can create differences in extinction risk outlook. If true germination rates differ from what was observed, that would alter the extinction risk outlook for that species/site. Last, while the 2021 drought provided an opportunity to track how arctic relicts were affected by and recovered from drought, it also means that this study does not reveal population growth rates during multiple good years in a row, which could have potential to balance or slow population declines in and after drought years. Without the ability to include the positive effects that multiple good years can have on population size into the model, the projected population declines may be more extreme than the plants will experience in reality.

This analysis shows that *P. vulgaris* and especially *E. hudsoniana* struggle to recover from a drought-induced decline and indicates that multi-year droughts will pose an extreme threat. The population of *E. hudsoniana* that did not decline during the summer drought (Gooseberry Falls) actually had population increases in both 2021 and 2022. The location of *Euphrasia* at this site is an example of good habitat that is more resilient to the effects of drought. Refugia-like locations like this represent the potential for being seed sources, allowing for the re-seeding of less suitable habitat that lost individuals in drought years but can recover in better years. Locations such as these offer more opportunities for research and protection.

One insight we gained from this study is the importance of conducting full population counts at each site alongside monitoring the plots, due to the variable nature of these microclimates along the shore. For example, some of our *P. mistassinica* plots at Temperance River were flooded due to extreme, stochastic weather events, which resulted in skewing the PVAs for that site. While monitoring the plots was a crucial component of this study because tracking the same individuals over time allowed us to understand survival and reproduction rates, future studies should include a full population count alongside the plot monitoring to put these micro-site findings related to the plots in the context of the larger population. The total populations at a site are small enough to be counted within a half day to a day, so future studies will include a census survey to better track the true population dynamics over time. We plan to initiate this during the summer of 2023, and we are in discussion with land managers and stakeholders about how to turn this into this a long-term, sustainable monitoring program.

This work directly monitored three arctic relict species along the shore and found one, *P. vulgaris*, to be vulnerable to a drought at one southern location according to a count-based PVA. According to the matrix-based PVAs, *P. vulgaris* is susceptible to droughts at all sites, and both *P. mistassinica* and *P. vulgaris* are projected to decline at most sites over the next 20 years. Additionally, this study highlighted the elevated risk to *E.*

hudsoniana, which was found to be particularly susceptible to droughts. Because it takes more than one year to recover from a drought, it is especially vulnerable to multi-year droughts which could decimate populations. In this regard, managers should focus on protecting the areas where individuals are currently found, particularly high-quality habitat, in order to preserve them as much as possible.

ACTIVITY 3 Title: ***Remove invasive species threatening a rare species***
Description: An introduced species, *Euphrasia stricta*, is invading habitat occupied by the rare arctic species *Euphrasia hudsoniana*. In 2015, we found genetic evidence of hybridization between the native and non-native species at two locations, which represents a threat to the genetic integrity of the native species. Fortunately, the low level of hybridization suggests that removing *E. stricta* now will leave the native species genetically intact. We have shared this information with managers on the North Shore, but the agencies lack the time and resources necessary to address this pressing issue. We will coordinate with the local community in Grand Marais, including the Cook County Invasives Team and volunteers, to remove *E. stricta* at each of these sites by hand every year for three years, followed by another genetic assessment of hybridization between native and non-native plants during the final year of the project to evaluate the impact of this work. During this process, we will also collect data on the morphology of pure and mixed populations to see how they change over this time period.

ACTIVITY 3 ENRTF BUDGET: $13,461

Outcome	Completion Date
1. *Removal of invasive E. stricta from arctic communities during each summer of funding*	Sept 2022
2. *Hybridization in 2022 measured using genetic techniques and compared to 2015 study*	June 2023

First Update May 1, 2020

In the fall of 2019, invasive populations of *E. stricta* were removed to prevent future hybridization at Artist Point in Grand Marias, MN and Horseshoe Bay in Hovland, MN. Hybrid plants, based on trait observations, were left intact so that we could get a better understanding of the genetic composition of these populations to better inform future removal decisions. (Outcome 1)
In order to determine the level of hybridization taking place between the rare native *Euphrasia hudsoniana* and the non-native invasive congener *Euphrasia stricta*, representative hybrid specimens from Artist Point were collected for DNA analysis (currently underway). Additional samples of the native and invasive were also collected from various geographic locations along MN's Northshore to provide reference (Outcome 2).

Second Update November 1, 2020

Invasive populations of *E. stricta* were removed in the fall of 2020 to prevent future hybridization at Artist Point in Grand Marias, MN and Horseshoe Bay in Hovland, MN. Populations of the invasive *E. stricta* found outside of these hybrid zones were observed and phenological and phenotypic data was collected to better understand differences between native and invasive populations.
Additional samples of possible hybrids between the native *Euphrasia hudsoniana* and the non-native invasive congener *Euphrasia stricta* were collected at hybrid zones. These samples will be the added to the previous samples collected for DNA analysis.

Third Update May 1, 2021

All samples for initial genetic analysis have been submitted to the University of Minnesota Genomics Center for analysis. Samples have yet to be processed by the UMGC due to COVID related delays. Removal of the invasive *E. stricta* will continue in the summer of 2021.

Fourth Update November 1, 2021

Invasive populations of *E. stricta* continued to be removed in the late summer and fall of 2021 to prevent future hybridization at Artist Point in Grand Marias, MN and Horseshoe Bay in Hovland, MN. Populations of the invasive *E. stricta* found outside of these hybrid zones were observed and additional phenological and phenotypic data was collected to better understand differences between native and invasive populations. Initial genetic data for *E. hudsoniana* was generated.

Fifth Update May 1, 2022

Phenological and phenotypic data for new *E. stricta* populations were analyzed. Preparations were made for a final year of invasive removal and collection of a final set of samples for genetic analysis.

Sixth Update November 1, 2022

Invasive populations of *E. stricta* continued to be removed in the fall of 2022 to prevent future hybridization at Artist Point in Grand Marias, MN and Horseshoe Bay in Hovland, MN. Populations of the invasive *E. stricta* found outside of these hybrid zones were observed and additional phenological and phenotypic data was collected to better understand differences between native and invasive populations. Having finished invasive population removal for the final summer of funding, Outcome 1 of this Activity is complete.
The final set of *Euphrasia* samples were collected for genetic analysis from the two hybridization zones, as well as other various geographic locations along MN's North Shore to provide reference. All samples will be submitted to the University of Minnesota Genomics Center for analysis.

Final Report June 30, 2023

In a previous study in 2015, we found genetic evidence of hybridization between the native *Euphrasia hudsoniana* and invasive *Euphrasia stricta* at two locations, which represented a threat to the genetic integrity of the native species. We planned to 1) remove the invasive species in the contact zones with the native at Artist Point in Grand Marais and Horseshoe Bay in Hovland, and 2) generate genetic data from the native, invasive, and hybrids to compare to the 2015 study. Because *Euphrasia* can be phenotypically variable and difficult to ID, we decided it would be beneficial to collect some samples in 2019, before removal began, to better inform our removal efforts and match phenotypic characteristics to genetics. The genetic results were not received until summer 2022 (due to delays associated with the Covid-19 pandemic), so we focused our removal efforts on areas that were heavily invaded by *E. stricta*, and contained no *E. hudsoniana*. We made this choice to avoid accidentally removing any native *E. hudsoniana* plants that resembled hybrids. The genetic results showed this to be a wise decision, as many individuals that phenotypically appeared to be hybrids were, in fact, genetically *E. hudsoniana*. Additionally, there was little change in morphology of individuals at each plot over the years.

At Artist Point, *E. stricta* occupies all the land surrounding the large parking lots, as well as being nearby and within *E. hudsoniana* habitat. The genetic results did continue to show relatively low levels of hybridization at Artist Point, which is a positive sign that hybridization could be controlled and prevented in areas where *E. stricta* is not well established and could still be removed. However, *E. stricta* is a strong invader, and consistent removal many times throughout the season would be needed to effectively remove it. At locations such as Artist

Point, it is likely too well established and it would be too resource intensive to remove enough to significantly reduce the population. This means that the most critical problem at Artist Point is an overwhelming invasion and physical replacement of the native by the invasive. At Horseshoe Bay, *E. stricta* occupies the edges of a short road, as well as being among the *E. hudsoniana* habitat. The *E. stricta* at Horseshoe Bay is well established, but ultimately does not take up a large area, and a strong effort should be made to remove it from this location. After three years of study, it is clear that time and resources would be best spent on removing *E. stricta* where it occurs at lower density or smaller numbers, particularly when it occurs near *E. hudsoniana* sites and could eventually spread there. Gooseberry Falls represents such a location, and we are focusing on this summer as a target for consistent removal.

To complete Outcome 2, additional samples were collected during 2022 to assess hybridization compared to the 2014 and 2019 datasets. This data was combined with the data from 2019 and analyzed to assess the extent of hybridization at Artist Point and Horseshoe Bay. The results for Artist Point revealed an increase in the percentage of hybrids (21% in 2019 to 38% in 2022) and a decrease in the percentage of pure species present (64% in 2019 to 40% in 2022), despite similar sampling strategies. Although the fact that the native species is still strongly present at Artist Point, the massive presence of the invasive at that site and the increasing presence of hybrids (despite our removal efforts) suggests that the native species may eventually be lost at this location. However, the results at Horseshoe Bay were more encouraging. The 2022 study revealed no hybridization at this location, indicating that the population is safe from genetic contamination and making invasive removal at this site (along with sites like Gooseberry Falls, mentioned above) a top priority.

We included other populations of native and invasive *Euphrasia* in our study as references in the genetic study, which lead to multiple surprising results. First, we learned that the "species" we have been referring to as a single invasive, *E. stricta*, actually separates out as two or three distinct genetic groups, with varying degrees of mixing at different locations. It is unclear what these invasive groups truly are at this point. They might represent multiple waves of invasion by *E. stricta* from Eurasia, or the spread of a different invasive or native *Euphrasia* species. It seems possible that one additional group is the species *E. nemorosa*, which is common in herbarium records throughout the east coast. There is some confusion about whether *E. nemorosa* is a native or invasive species in the upper Midwest. For example, the state of Michigan recognizes it as a native and classifies it as threatened. However, herbarium records show it is common throughout Europe, and the taxonomic description indicates that it is introduced to North America rather than being native. In order to understand the history and status of *E. stricta* and *E. nemorosa* in North America, we mapped the location and date of herbarium record occurrences for both species. *Euphrasia stricta* is a documented invasive species, and a spread across the US from east to west over decades can be seen in these records, with a handful of *E. stricta* specimens in Minnesota recorded before 1999, but the majority being reported in 2000 or later. A similar spread of *E. nemorosa* can be seen in the records, with an older center on the east coast, a westward spread over decades, and new records in Minnesota since 2000. The similarity in the *E. stricta* and *E. nemorosa* records showing a westward spread over time is consistent with *E. nemorosa* being an invasive species. Overall, consider it most likely that *E. stricta* and *E. nemorosa* make up two of the three invasive groups we detected in our study. The third group of invasive *Euphrasia* will require more research, and we are planning future projects to address this. Despite the mystery surrounding the taxonomic identity of these invasive groups, we emphasize that the native species can be distinguished from all of them using genetic techniques and through careful observation in the field. Therefore, land managers can continue to remove anything that resembles what has traditionally been call "*E. stricta*" and there is no need to change strategies to preserve the native species.

The genetic analysis also revealed that two locations where we expected to find the native species (Two Harbors and a segment of Tettegouche State Park) consisted of invasives or hybrids between the native and invasive. At Two Harbors, the hybrids were almost entirely composed of invasive genetic material, with only about four

native individuals present in 2022. This means that the southern-most known location of *E. hudsoniana* is farther north than was previously reported in Activity 1, yielding an additional range contraction of 13 miles (21 km), and a total range contraction of 22 miles (35 km), from Stoney Point to Gooseberry Falls State Park. There are also a few native individuals at Tettegouche State Park, but again they represent an extremely small proportion of the plants that are present. This highlights the severity of range contraction of arctic relicts, and in the case of *E. hudsoniana*, the double threat of climate change and hybridization with an invasive congener. The genetic results are at these two sites represent critical information for land managers – since these two populations are no longer made up of the native species, managers should focus their time and resources on the remaining native populations at other locations along the shore. Based on our surveys of *E. hudsoniana* during the course of this project, we have developed a tentative prioritization of which *E. hudsoniana* sites are most valuable for preservation, although we emphasize that this proposed ranking must be vetted with land managers to integrate their feedback. Our results indicate that the highest priority populations are: Gooseberry Falls State Park, Sugarloaf Cove, Temperance River State Park, and Horseshoe Bay.

IV. DISSEMINATION:

Description:

Sharing with community stakeholders: Plant survey results from Activities 1 and 2 will be provided to the DNR to include in the state's relevé (native plant community) and natural heritage databases. Results from Activities 1, 2, and 3 will be shared with the managers of the arctic plant communities along the North Shore (Cook County Invasives team, Grand Portage Band of Lake Superior Chippewa, US Forest Service, Sugarloaf Cove Nature Center, and the MN DNR) at a meeting that we will host to facilitate discussion and planning for the future based on the results of these activities.

Publicly available genetic data: Genetic data generated in Activity 3 will be made publicly available via the Cyverse Data Commons (http://datacommons.cyverse.org/) or other publicly accessible database; a link to the data will be made available in project updates and upon presentation and publication of results from the genetic study.

Scientific publications: We expect that Activities 1, 2, and 3 will result in at least two peer-reviewed journal articles in the fields of natural resource conservation and plant biology.

The Minnesota Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENRTF Acknowledgement Guidelines.

First Update May 1, 2020

Preliminary observations were publicly shared at Sugarloaf Cove Nature Center as the keynote presentation at their annual open house in the fall of 2019. Current work was also shared with the MN-DNR and US Forest Service during the 2020 permit application process. Initial contact with the Cook County Invasives team has been made.

Second Update November 1, 2020

Re-surveyed Releve data will be submitted to the MN-DNR when completed. Season field reports required by permits will be shared with the Cook County Invasives team, Grand Portage Band of Lake Superior Chippewa, US Forest Service, Sugarloaf Cove Nature Center, and the MN DNR.

Third Update May 1, 2021

Reports and updates of the 2020 field season where long-term plots were established were supplied for the MN DNR and US Forest Service. Planned summer work was also shared with US Forest Service and MN DNR through yearly permit process.

Fourth Update November 1, 2021

Season field reports required by permits will be shared with the US Forest Service and the MN DNR and future 2022 permit applications will be submitted to continue this work.

Fifth Update May 1, 2022

Information was shared with the US Forest Service and MN DNR through the permit process.

Sixth Update November 1, 2022

Season field reports required by permits will be shared with the US Forest Service and the MN DNR.

Final Report June 30, 2023

In April we held a meeting with land managers from the shore to share our findings of Activities 1, 2, and 3, and facilitate discussion of future steps. The meeting including 18 participants from the Cook County Invasives team, the Grand Portage Band of Lake Superior Chippewa, the US Forest Service, Sugarloaf Cove Nature Center, the MN DNR, the Lake County Soil & Water Conservation District, the National Park Service, and the Nature Conservancy. A written summary of our findings and the ideas generated during the meeting and discussion was shared with all parties. The meeting also resulted in the formation of the "Lake Superior Arctic Relicts" information sharing group on Google Groups to communicate with land managers and other stakeholders. Discussion with land managers will continue throughout the summer, along with field site visits to address specific needs at individual locations. We are also pursuing funding options to continue working with arctic relicts alongside our partners. Final reports of our work will also soon be shared with the MN DNR and US Forest Service as per our research permits. The results of Activity 1 and 2 have been provided to the DNR to include in the state's relevé (native plant community). In 2019 information was shared with Sugar Loaf Cove Nature Center for their fall keynote presentation, and we currently plan to give a presentation there in August this year as well. Various findings from this study have also presented at scientific meetings, including the Botany meetings in 2021, 2022, and 2023 meeting, the Natural Areas Association conference in 2022, and the National Parks Service Great Lakes Network "Great Lakes Science for Parks Symposium 2023". The genomic data generated for this project is available through the Data Repository for the University of Minnesota.

V. ADDITIONAL BUDGET INFORMATION:

A. Personnel and Capital Expenditures

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

Explanation of Use of Classified Staff: N/A

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

Enter Total Estimated Personnel Hours for entire duration of project:	Divide total personnel hours by 2,080 hours in 1 yr = TOTAL FTE:
Field Technician: 3120 (2 summers + 1 year)	1.5
Undergraduate Hourly Worker: 2,040 (17 wks/year for 3 years)	0.98

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: *None*

Enter Total Estimated Contract Personnel Hours for entire duration of project:	Divide total contract hours by 2,080 hours in 1 yr = TOTAL FTE:

VI. PROJECT PARTNERS:

A. Partners outside of project manager's organization receiving ENRTF funding

None

B. Partners outside of project manager's organization NOT receiving ENRTF funding

Name	Title	Affiliation	Role
Chel Anderson	Botanist	MN DNR, MBS	Consultation
Jack Greenlee	Botanist	USFS Superior National Forest	Consultation
Molly Thompson	Executive Director	Sugarloaf Cove Nature Center	Outreach, Consultation
Tia Parks	Forester	Cook County Invasives Team	Invasive removal

VII. LONG-TERM- IMPLEMENTATION AND FUNDING:

The results of all three activities in this project will be provided to the DNR to include in the state's relevé (native plant community) and natural heritage databases, and to the managers of the arctic plant communities along the North Shore (Cook County Invasives team, Grand Portage Band of Lake Superior Chippewa, US Forest Service, Sugarloaf Cove Nature Center, and the MN DNR) so that they are aware of any changes in the arctic communities, and can prioritize management accordingly. We will host a meeting with all interested parties to facilitate discussion and planning for the future based on the results of our study. This proposal leverages the previous years of surveys by the MBS, and we will seek additional funding for long-term monitoring efforts.

VIII. REPORTING REQUIREMENTS:

- Project status update reports will be submitted May 1 and November 1 each year of the project; these reporting dates match with the seasonal nature of the work conducted over the summer.
- A final report and associated products will be submitted **June 30, 2023.** Note: we are requesting a fourth year for the project (but no extra funding) to allow us to complete work through the full summer of 2022 (past June 30th, 2022), corresponding to the life-cycle of the plants we are studying. We will complete work in the field by fall 2022 and genetic work by winter 2022/2023 to submit a final report by June 30th, 2023.

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet – See Excel file.

B. Visual Component or Map – See PDF file. This visual component shows the northward retreat of two arctic relict species compared to their historical locations.

C. Parcel List Spreadsheet - NA

D. Acquisition, Easements, and Restoration Requirements - NA

E. Research Addendum – See separate Word document.

Attachment A:

Environment and Natural Resources Trust Fund

M.L. 2019 Propjet Budget -Final

Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 08d

Project Manager: Briana L. Gross

Project Title: Conservation and Monitoring of Minnesota's Rare Arctic Plants

Organization: University of Minnesota Duluth

Project Budget: $135,00

Project Length and Completion Date: 4 years, June 30th 2023

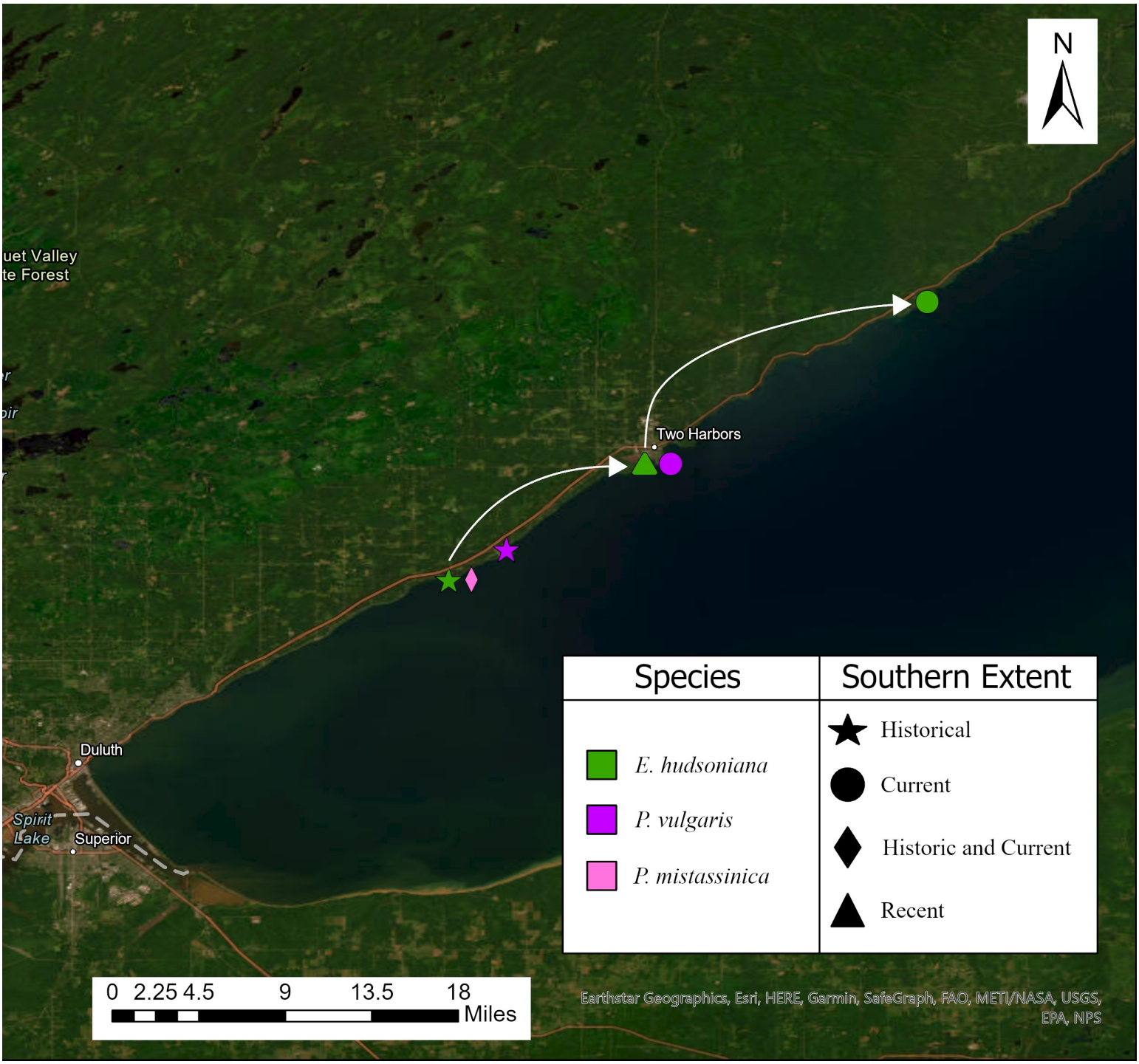
Today's Date: August 14, 2023

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Budget 10/17/2023	Amount Spent	Balance
BUDGET ITEM			
Personnel (Wages and Benefits)	$ 95,412	$ 95,412	$ -
Field technician: $60,778 (68% salary/32% fringe) 100% FTE for 1.2 years.			
MS graduate student: $26,599 (27% salary/73% fringe including tuition) 18% FTE for 3 years			
Undergraduate hourly worker: $7,778 (100% salary/0% fringe) 20% FTE for 2 years.			
Professional/Technical/Service Contracts			
UMN Genomics Center - Genotyping-by-sequencing services (enzyme optimization, DNA digestion and ligation, and Illumina sequencing) = genetic data generation for assessment of hybridiztion, $59.25/sample for 250 samples + 3% inflation (work will occur at the end of the grant)	$ 18,424	$ 18,424	$ -
Equipment/Tools/Supplies			
General field equipment (leaf pressing supplies, surveyor's tape, field flagging, write-in-the-rain notebooks, kneepads, envelopes for samples; $653), 2 GPS units + batteries ($1068), and DNA extraction kits	$ 4,221	$ 3,869	$ 352
Travel expenses			
Camping fees, per diem, car rental and mileage for travel to North Shore sites from Duluth, MN, for surveys and invasive species removal per UMN Policy.	$ 16,205	$ 15,494	$ 711
Mileage for land managers to attend group meeting to discuss findings at the conclusion of the project.	$ 383	$ 144	$ 239
Other			

Expenses for group meeting of land managers to discuss findings at the conclusion of the project (parking, modest food allowance)	$ 355	$ 355	$ -
COLUMN TOTAL	$ 135,000	$ 133,698	$ 1,302

OTHER FUNDS CONTRIBUTED TO THE PROJECT	**Budget**	**Spent**	**Balance**
Non-State: N/A	$ -	$ -	$ -
State: N/A	$ -	$ -	$ -
In kind: Etterson/Gross (1/2 month salary during academic year x 3 years for project advisement and data analysis)	$ 26,691	$ -	$ 26,691
In kind: Unrealized indirect cost return from this proposal	$ 67,500	$ -	$ 67,500
PAST AND CURRENT ENRTF APPROPRIATIONS		**Spent**	**Balance**
Current appropriation: N/A		$ -	$ -
Past appropriations: N/A		$ -	$ -


NMENT
RESOURCES
FUND



A map showing the loss of two arctic relict species from their historical southern locations. *Pinguicula vulgaris* (purple) has shifted 7 miles (11 km) north, from Knife River to Two Harbors. *Euphrasia hudsoniana* (green), has sifted first 10 miles (16 km) north from Stoney Point to Two Harbors (first white arrow), and then again to Gooseberry Falls State Park (second white arrow) for a total retreat of 22 miles (35km). The second shift of *E. hudsoniana* was caused by hybridization with an invasive species.