[2018] Project Abstract
For the Period Ending June 30, 2023

PROJECT TITLE: Variable Winter Thermal Regimes and Managing Trout Streams
PROJECT MANAGER: Dr. Rebecca Swenson
AFFILIATION: University of Minnesota
MAILING ADDRESS: 249 Ruttan Hall, 1994 Buford Ave.
CITY/STATE/ZIP: Saint Paul, MN 55108

PHONE: 612-625-3866
E-MAIL: boli0028@umn.edu

WEBSITE: https://ag-ed.cfans.umn.edu/people/rebecca-swenson
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 03i as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18 as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]

APPROPRIATION AMOUNT: $400,000
AMOUNT SPENT: $317,641
AMOUNT REMAINING: $82,359

Sound bite of Project Outcomes and Results
Conservation plans are based largely on summer dynamics between fish, food sources, and water temperatures. Yet, winter-emerging aquatic insects, primarily Chironomidae, are a locally abundant and critical resource for trout. This project provides insights about winter air and water temperatures, lifecycles of aquatic insects, and impacts on stream food webs.

Overall Project Outcome and Results
Accurate identification of Chironomidae (midges) is imperative to preserve trout's winter food base. Failure to accurately identify species may result in under-representing species richness and overestimating abundances. This project provides insights about how winter weather impacts stream food webs and specific insect species, which should inform future decisions about habitat conservation and freshwater management practices.

Specifically, the research team collected water and temperature data from over 30 stream sites in the driftless region of Minnesota, which provided insights on how groundwater, air temperature, geology, and streambed conditions interact during winter. The team also identified and preserved Chironomidae specimens. From linear regressions of air and water temperature, and incubations at various temperatures of live-collected Chironomidae, our results indicate that even short-term exposure to warm air temperatures shortens lifespans of winter-active midges that emerge from groundwater dominated streams. We also found that midges can be parasitized even in good quality groundwater dominated streams, further altering their longevity.

Given that many winter species look very similar, additional molecular analysis allowed us to identify insects more accurately. Using a type of DNA analysis (MtDNA), our team examined genetic patterns and variability among insects sampled. According to sequencing data, Diamesa mendotae species were found across 6 streams (Pine Creek, Clear Creek, Miller Creek, Lawrence Creek, Winnebago Creek, and Vermillion River) and D. nivoriunda species were present in 4 of these streams. Seven mated pairs were also collected from Pine Creek, Clear Creek, and Miller Creek.

Because few insects can emerge during Minnesota's snowy and cold winters, it is important to understand how streambed conditions, geology, and temperature can impact the longevity, emergence, and reproduction of winter-emerging aquatic insects like Chironomidae. These research insights are key for maintaining healthy streams and abundant trout, and can positively impact Minnesota's fishing, outdoor recreation, and economic resources.

Project Results Use and Dissemination

This research provided the basis for a graduate student Master's thesis, chapters of a student's PhD dissertation, and an undergraduate student's honors thesis, which are available to other scholars via ProQuest. Important insights were also shared at the International Society for Freshwater Science conference, and research was published in multiple peer-reviewed academic journals. Specimens are available for public use in the University of Minnesota's Insect Museum with supplemental data files. We created communication materials and used one-on-one online interviews to evaluate messaging and to inform future outreach efforts. Our team created a website with educational materials that showcase stream food webs in the winter, the lifecycle dynamics of winter aquatic insects, and their vital importance to Minnesota streams.

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND	**Environment and Natural Resources Trust Fund (ENRTF)** **M.L. 2018 ENRTF Work Plan (Main Document)**

Today's Date: 14 August 2023

Date of Work Plan Approval: 06/05/2018

Project Completion Date: June 30, 2023

PROJECT TITLE: Variable Winter Thermal Regimes and Managing Trout Streams

Project Manager: Rebecca D. Swenson

Organization: University of Minnesota

College/Department/Division: CFANS, Agricultural Education & Communication **Mailing Address:** 249 Ruttan Hall, 1994 Buford Ave.

City/State/Zip Code: Saint Paul, MN 55108

Telephone Number: 612-625-3866

Email Address: boli0028@umn.edu

Web Address: https://ag-ed.cfans.umn.edu/people/rebecca-swenson

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Location: Southeastern Minnesota: Goodhue County, Wabasha County, Olmsted County, Winona County, Fillmore County, Houston County

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Total Project Budget: $ 400,000

Amount Spent: $317,641

Balance: $82,359

Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 03i as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18 as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]

Appropriation Language: $400,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to identify how winter groundwater flows, air temperature, and streambed conditions affect insect productivity in order to guide restoration and management efforts in southeastern Minnesota trout streams. This appropriation is available until June 30, 2021, by which time the project must be completed and final products delivered.

M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2022]
M.L. 2022 - Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30,

2023]

I. PROJECT STATEMENT:

In 2008, Trout Unlimited estimated the economic impact of recreational trout angling in the Driftless Region to exceed 1.1 billion dollars. Most management strategies for trout are based largely on summer dynamics (which are well known), however winter has a critical influence on survivorship and growth in many streams and needs to be better integrated into future management strategies. This project builds on prior research performed during winter (2010-2013) in 36 streams in the Driftless Region of southeastern Minnesota. Our previous project, which produced seven publications, showed that groundwater inputs to trout streams (1) buffers stream water in winter to prevent freezing temperatures and ice cover, (2) documents that groundwater inputs are highly variable along short stretches of stream, producing very different conditions for trout recruitment, survival, and patterns of growth during winter, (3) shows that streams support differing abundances and types of invertebrates (the trout food base) that varies substantially in relation to groundwater inputs and thermal regimes, and which are especially important for growth, survivorship and reproductive health of trout during winter,(4) confirms that the buffering of thermal regime facilitates winter-adapted invertebrate species to develop dense populations which results in increased survivorship, faster growth and greater abundance of trout, and (5) that several of the winter adapted invertebrate species are new to science or have unknown basic biology. These results were based on studies at single sites located on 36 different streams. In this project we will reduce the number of streams to be analyzed (to 21) but will assess 5 sites/stream selected from areas with differing local groundwater inputs, so that we can better understand the longitudinal patterns of thermal regimes and dynamics of winter-adapted invertebrate life-cycle variation and winter emergence. This is necessary because trout selectively feed on emerging insects. Prior to our studies, little was known about winter adapted insects in streams of the Driftless Region, but we have shown that they have the ability to produce one or more generations during winter, and we predict that local thermal regimes strongly influence the number of successive generations. We also have significant preliminary molecular data that suggest there may be cryptic species adapted to localized thermal conditions and that have developed very specialized physiological and behavioral adaptations that facilitate winter growth, development, and emergence.

Additional Background: Winter emerging aquatic insects, primarily Diptera (family Chironomidae) can be locally abundant and serve as a substantial food resource for trout. Some are active on snow at air temperatures well below freezing and are able to mate then return to the stream surface to oviposit. At least one species supercools to ~-20 degrees C, and actively grows and develops at water temperatures below 10 degrees C. Although more than 35 species are known to emerge during winter in Minnesota trout streams, the basic life history and autecology of most are not known or are only poorly understood. Several are still undescribed, and some evidence suggest that one or many cryptic species may occur. Water temperatures, however, are likely to strongly influence the life cycle dynamics of species adapted for growing, developing and emerging in winter.

II. OVERALL PROJECT STATUS UPDATES:

First Update January 31, 2019

During the period from 1- July 2018 to 31 January 2019 we initiated our research project and completed most scheduled tasks for each of our three Activities. Detailed information regarding tasks and their current status (completed or still in progress) are provided for each Activity in subsequent sections of this Update.

Second Update June 30, 2019
During the period from 1 February 2019 to 30 June 2019 we finished our first round of field sampling and completed most tasks for each of our three activities. Detailed information regarding tasks and their current status (completed or still in progress) are provided for each Activity in subsequent sections of this Update.

Third Update January 31, 2020 [Submitted October 2020]
During the period from 1 July 2019 to 31 December 2019 we selected streams to be assessed during the second round of winter field sampling and completed most tasks for each of our three activities. Detailed information regarding tasks and their status (completed or still in progress) are provided for each Activity in subsequent sections of this Update.

Fourth Update June 30, 2020 (Submitted 15 March 2021)
During the period from 1 January 2020 to 30 June 2019 we completed all biological sampling of streams selected to be assessed during the second round of winter field sampling. Travel restrictions imposed in March due to COVID 19 prevented us from retrieving automated temperature probes that were scheduled to be retrieved. As of 30 June 2020 we still have not been able to retrieve most probes. Restrictions for working on laboratory-based tasks delayed progress on other activities. In addition, the University implemented a freeze on hiring and training new personnel in the early stages of response to COVID-19. In May two of our undergraduates that were assigned to sample-processing tasks graduated and left our work group. As of the end of this report period we have not yet been able to replace them.

Detailed information regarding tasks and their status (completed, still in progress or delayed) are provided for each activity in subsequent sections of this update. Delays that have occurred because of responses to COVID-19 are noted and/or explained.

Fifth Update January 31, 2021—Waived by LCCMR 9/21/2021

Sixth Update June 30, 2021—Waived by LCCMR 9/21/2021

Due to unforeseen circumstances and the need for an emergency change in project manager from Dr. Ferrington to Dr. Swenson, updates from January 2021 and June 2021 have been combined into a single reporting interval by LCCMR staff on 9/21/2021. Dr. Swenson will provide a comprehensive update in October 2021 to bring the reporting up to date.

October 21, 2021 Update:
The team has completed all biological sampling of streams selected to be assessed during the third round of winter field sampling, has retrieved thermal probes that can be located, and has collected and processed samples. Sample processing and data analysis will continue. The team hosted in-person and virtual educational events and continued communication outreach to environmental and agricultural organizations. Outreach and outreach evaluation will continue. Detailed information regarding tasks and their status are provided for each activity in subsequent sections of this update. We are requesting the following amendments:

Amendment request 1: Dr. Rebecca Swenson will replace Dr. Len Ferrington as the project manager. The contact information above has been updated accordingly. The proper notification with the Office of Sponsored Research has been filed (attached).

Amendment approved by LCCMR 10/8/2021

Amendment request 2: Due to COVID related delays in this project, we request to update the Outcome Completion Dates as follows:

• Activity 1, Outcome 3: Change completion date from June 2021 to June 2022. • Activity 2, Outcome 3: Change completion date from June 2021 to June 2022. • Activity 3, Outcome 3: Change completion date from June 2021 to June 2022.

Amendment approved by LCCMR 10/8/2021

Amendment request 3:
Due to COVID restrictions to allow for social distancing and the change in project personnel, we wish to make the following changes to the budget:

- Personnel: line 15 (Dr. Ferrington's salary) will be removed and that budget ($646) moved to line 16 (Dr. Swenson's salary).
  - Total balance of line 15 will be $0.
  - Total balance of line 16 will be $2,274.
  - Total personnel budget will remain same.
- We wish to remove $3,900 from line 31 (Category: Room Rental) for a new total of $9,171.
- $3,900 will be added to Travel Expenses in Minnesota/Vehicle Rental for a total budget of $7,000.

Amendment approved by LCCMR 10/8/2021

Seventh Update January 31, 2022 (Submitted 31 January 2022)
The team has completed biological sampling of streams and has downloaded data from temperature loggers. Adult specimens from 26 streams have been identified, labeled, and preserved. Temperature data from 15 streams and 33 sites has been downloaded and curated. Some sample and data analysis will continue. The team also developed educational materials about stream food webs in the winter, as well as new information for the Bugs Below Zero website. Postcards and flyers were developed and mailed to 288 K12 Minnesota educators to share these potential resources that can be integrated into lessons on environmental science topics.

Project Status as of June 31, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Update waived per LCCMR staff on 6/30/2022.

Project Status as of January 30, 2023:
Our team has concluded all field research connected to this phase of the grant. We were able to sample at least 33 stream sites, which provided data to better understand how groundwater, air temperature, geology, and streambed conditions interact. The team was also able to identify, label, and preserve adult fly specimens from at least 26 streams. From linear regressions of air and water temperature, and incubations at various temperatures of live-collected flies, our results indicate that

even short-term exposure to warm air temperatures shortens lifespans of winter-active midges that emerge from groundwater dominated streams. We also found winter-active midges can be parasitized even in relatively good quality groundwater dominated streams and this can also alter longevity of adult flies.

These results are important, especially since adult flies are an important energy and nutritional source for trout in winter.

Given that many winter species look very similar (especially undescribed species), molecular analysis allowed us to more accurately identify insect species that provide the most reliable energy and nutritional sources to trout in winter. Using a type of DNA analysis (MtDNA), our team examined genetic patterns and variability among insects sampled. We were able to collect 1,268 winter emerging Diamesa adult samples from 20 stream sites, and our team sequenced 261 of these specimens. According to sequencing data, D. mendotae species were found across 6 streams (Pine Creek, Clear Creek, Miller Creek, Lawrence Creek, Winnebago Creek, and the Vermillion River and *D. nivoriunda* species were present in 4 of these streams. Seven mated pairs, all of which were *D. mendotae,* were collected from Pine Creek (5), Clear Creek (1), and Miller Creek (1). This cryptic winter-emerging chironomid identification can increase the accuracy of insect identification and improve our knowledge of stream food webs, and in turn, provide more specific data for decisions about trout habitat conservation and freshwater management practices.

Not all our budget was spent, due to a partnership that we did not anticipate with Dr. Debora Paula Pires (specialist on MtDNA analytical techniques) who volunteered her time, disposable supplies, and lab space to assist in lab determinations and data interpretations. One of our graduate students also received a fellowship award from the University of Minnesota, which supported some of her time on this project. Due to Covid, some of the outreach work was conducted online and with digital materials that fit distant learning models adopted in many K-12 and University classrooms during this period, which also created some cost-savings.

The team continued to refine educational materials for Minnesota teachers about stream food webs in the winter and drafted new educational content for the Bugs Below Zero website. Emails were sent to a listserv Minnesota K12 educators to describe new Bugs Below Zero materials and how these might be integrated into science lessons.

We are entering the final phase of this project and the team will be drafting papers to publish research results in academic journals and preparing other communication materials to share insights with relevant audiences. We will also be evaluating citizen science efforts to develop insights for future work and to further refine outreach activities.

Amendment request 4:
Due to a change in status for the graduate student working on this project (Ph.D. successfully defended by Corrie Nyquist in Fall of 2022), an increase in fringe rates over the years, and due to the continued work of Dr. Swenson to direct this project, we wish to make the following changes to the budget, to allow both Dr. Swenson and Corrie Nyquist to continue working on the project until its completion:

- Personnel: Line 18 (Graduate Student 1 salary and fringe) will be reduced by $30,000 and that budget will be moved to line 17 (PostDoc salary and fringe). The PostDoc is a new position that will replace the graduate student position. This will allow Corrie Nyquist to continue working on the project during the final phase of the grant, since she has successfully defended her dissertation and must move from a graduate student to a PostDoc role.

- Personnel: Line 20 (Undergraduate student salary) will be reduced by $16,000 and that budget will be moved to line 16 (Dr. Swenson salary and fringe).
    - o The total balance of line 16 will be $15,866.
    - o The total balance of line 17 will be $35,000.
    - o The total balance of line 18 will be $4,999.
    - o The total balance of line 19 will be $1,071.
    - o The total balance of line 20 will be $125.
    - o Total personnel budget will remain the same.

Amendment Approved by LCCMR 2/9/2023

Final Report of Overall Project Outcomes and Results as of June 30, 2023:
Over the course of this project, the research team collected insect specimens and data from streams in Minnesota's driftless region, a key area for trout habitat. This work produced important insights on groundwater, air temperature, geology, streambed conditions, and insect lifecycles. We also used advanced molecular analysis to identify genetic patterns and variability among insects in these streams. This work resulted in new research insights centered on winter dynamics of aquatic insects, which is an understudied area.

This research was used as the basis for a graduate student's Master's thesis, a few chapters of a graduate student's PhD dissertation, and an undergraduate student capstone Honor's thesis, including:

Durnin, T. (2021). *Exploring the hidden diversity of winter-emerging Chironomidae (Insecta: Diptera)* [Masters Thesis, University of Minnesota, Twin Cities]. https://conservancy.umn.edu/handle/11299/226346

Nyquist, C. (2022). *Effects of environmental temperature on biology of cold-adapted Chironomidae (Diptera) from Minnesota and Iceland* [PhD Dissertation, University of Minnesota, Twin Cities]. http://conservancy.umn.edu/handle/11299/250408

Bodmer, H. (2022). *Exposure of Diamesa mendotae, a cold-adapted chironomid, to short-term high temperature.* [Undergraduate Honors Thesis, University of Minnesota, Twin Cities].

In addition, research results from this project have been published in academic, peer-reviewed journals and shared at both regional and international academic conferences. Here is a list of some of these publications, posters, and presentations:

Academic Publications

Bodmer, H., Nyquist, C. 2022. Nematodes infest winter-active chironomids in Minnesota trout streams. *CHIRONOMUS Journal of Chironomidae Research* 35: 54-57.

Bodmer, H., Nyquist, C., Vondracek, B., & Ferrington, L. C. (2023). Exposure of cold-adapted Diamesa mendotae Muttkowski, 1915 (Diptera: Chironomidae) to short-term high temperature reduces longevity and reproduction. *Aquatic Insects*, *0*(0), 1–15.

https://doi.org/10.1080/01650424.2023.2204093

Bodmer, H., Nyquist, C., Vondracek, B. Analysis of chironomid behavior in longevity experiments. [In Progress]

Research Posters and Presentations

Bodmer H., Nyquist C., Vondracek B., Ferrington L. C. Jr. 2022. Winter warm spikes reduce cold-adapted insect longevity and reproduction. *Undergraduate Research Symposium*. Minneapolis, MN. https://conservancy.umn.edu/handle/11299/227176.

Bodmer H., Durnin T., Vondracek B., Nyquist C., and Ferrington L. C. Jr. 2021. Short-term warm air temperature effects on *Diamesa mendotae* (Diptera: Chironomidae) longevity. *Goldwater Scholar Community Symposium.* Virtual Meeting.

Bodmer H., Durnin T., Vondracek B., Nyquist C., and Ferrington L. C. Jr. 2021. Short-term warm air temperature effects on *Diamesa* (Diptera: Chironomidae) longevity and behavior. *Annual Meeting of the Society for Freshwater Science.* Virtual Meeting.

Bodmer, H., Nyquist, C. and L. C. Ferrington Jr. 2020. Longevity of *Diamesa* spp. (Diptera: Chironomidae) at 22°C for 24 Hours. *Society for Freshwater Science Summer of Science Online Conference.*

Durnin, T., Nyquist, C., Bodmer, H., Hobbs, M., Perry, J., Vondracek, B. and L. C. Ferrington Jr. 2020. Simulated Air/Water Regression Models to Predict Quality of Trout Habitat. *Society for Freshwater Science Summer of Science Online Conference.*

Bodmer, H., Nyquist, C., Vondracek, B. and L. C. Ferrington, Jr. 2022. Short-term warm temperatures reduce winter-active chironomid longevity and reproduction. *Entomological Society of America Joint Annual Meeting.* Vancouver, B.C.

Bodmer, H., Nyquist, C., Vondracek, B. and L. C. Ferrington, Jr. 2022. Nematode infestations in winter-active Chironomidae (Diptera) from groundwater-dominated streams. *Joint Aquatic Sciences Meeting.* Virtual Conference.

Bodmer, H., Nyquist, C., Vondracek, B. and L. C. Ferrington, Jr. 2022. Nematode infestations in winter-active Chironomidae (Diptera) from groundwater-dominated streams. *Entomological Society of America North Central Branch Meeting.* Minneapolis, MN.

Swenson, R., Schrank, A., Ferrington, L.C., and Vondracek, B. (2021, May). *Bugs Below Zero: Engaging the public in winter research. Society of Freshwater Science International Annual Meeting (SFS)*. Virtual Conference.

This project also included an ongoing outreach effort focused on teachers, landowners, conservation professionals, and outdoor enthusiasts in order to raise awareness about Minnesota streams and the winter dynamics of aquatic insects. We successfully started a website to share educational materials and participated in media interviews, webinars, workshops, and classroom visits. This science communication work provided an important foundation and testing ground for future citizen science outreach. Some examples of these activities include:

Website

Bugs Below Zero website: https://www.bugsbelowzero.com/

Webinars and workshops

Swenson, Rebecca, Ferrington, Leonard, Schrank, Amy, and Vondracek, Bruce. (2021, February). *How citizen volunteers can improve understanding of stream food webs.* Invited presentation for Minnesota Master Naturalist Program, virtual webinar.

Hanson, Dean, Swenson, Rebecca, Ferrington, Leonard, Schrank, Amy, and Vondracek, Bruce. (2021, February). *Citizen Science and U: Learning more about insects, winter, and stream food webs.* Interactive workshop and demonstration for St. Paul students, parents, and teachers, Stillwater, Minnesota

Ferrington, Leonard, Hanson, Dean, Schrank, Amy, Swenson, Rebecca, and Vondracek, Bruce. (2020, February). Bugs Below Zero: Lectures, demonstrations, and interactive workshops on winter streams for the public. St. Paul, Minnesota.

Media Interviews

Broberg, J. (2021, March 15). Where Wonders Never Freeze. *Agate*. http://www.agatemag.com/2021/03/where-wonders-never-freeze/

The Wandering Naturalist Podcast: Snow Bugs: Episode 153 (~34,000 listeners/year) hosted by the Three Rivers Parks District, Minnesota (2022): (https://thewanderingnaturalist.libsyn.com/episode-153-snow-bugs-bugs-below-zero-project)

Explore, Teach, Conserve Podcast: Bugs Below Zero, April 1, 2021 hosted by University of Minnesota Extension (https://exploreteachconserve.transistor.fm/episodes/bugs-below-zero)

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Develop Quantitative Thermal Models Relating Air Temperatures to Water Temperatures

Description: For this activity, we will build on Calvin Alexander's spring mapping work in the Driftless Region and leverage the MN DNR's Long Term Monitoring program to create thermal models (Krider *et al*. 2013) to predict how groundwater inputs buffer water temperatures of trout streams from winter cold and freezing temperatures. In these regression models the slope of the regression line indicates the relationship between air temperatures (as the independent variable) and water temperatures (as the dependent variable), and the Y-intercept indicates the predicted water temperature when mean daily air temperatures reach 0 degrees C.

Our goal for thermal modeling is to quantify the ranges of thermal conditions available to trout, and to demonstrate how the thermal conditions vary longitudinally over pre-defined spatial scales within streams of approximately 5 to 10 kilometers in most streams. It may also be necessary to work at finer spatial scales in some streams, perhaps 1 to 2 kilometers when sharp increases or decreases in discharge occur over short stream distances. This type of modeling will enable managers to locate areas where transitions are occurring between groundwater controlled thermal regimes and meteorologically controlled thermal regimes within stream systems, so that management plans can be developed to position and tune restoration efforts to have the greatest impact in extending the moderating influence of groundwater on stream temperature.

We will intensively map and sample seven streams/year at each of five locations/stream that span a wide range of groundwater input intensities, to generate results leading to improved habitat management guidance. Our specific objectives are to:

- identify how groundwater, air temperature, geology and streambed conditions interact to determine optimal winter habitat for invertebrates that trout feed on and are critical for trout in winter;
- understand how changes in geology, groundwater input, and vegetation affect stream temperatures and therefore influence food availability during winter, and ultimately trout growth and productivity;
- create quantitative models relating winter thermal regimes to food availability for trout consumption;
- recommend ways that quantitative models we develop can guide efforts to enhance trout productivity via habitat modifications to protect or improve stream temperature, with a focus on the winter period.

We will test the following hypothesis--- **Hypothesis 1:** ***Thermal variability*** *in water temperatures will vary longitudinally in relation to ground water inputs and over relatively short spatial scales in winter.*

Methodology: Each stream will be evaluated based on reconnaissance by foot to select sample sites in region where thermal regimes are likely to change most dramatically. After determining the appropriate spatial scale, we will use air and water temperatures (7 streams/year, 5 sites/stream) to map groundwater influences at fine spatial scales. Water temperature probes will be inserted into each stream at each site and will be pre-set to record data at 15-minute intervals. Daily mean temperatures will be averaged for seven-day periods and used as the dependent variable to create each site-specific regression relationship to air temperature as the independent variable. We will use records of air temperatures from local air monitoring stations as was done during our previous modeling.

Activity 1 Workflow (Tasks and Timing):

Year 1: 2018-2019 ***Tasks***	***Timing: (Initiate)***	***(Complete by)***
Re-train existing staff and/or recruit & train new staff	July 2018	end of July 2018

Review and select possible streams to investigate	July 2018	end of Aug. 2018
Consult with MN DNR and Trout Unlimited	Aug. 2018	mid Sept. 2018
Field reconnaissance of possible study streams	Aug. 2018	end of Sept. 2018
Final selection of streams and study sites within	Sept. 2018	early Oct. 2018
Develop sample design and sampling schedules	Oct. 2018	end Oct. 2018
Assign tasks and duties to staff, review existing SOP's	Oct. 2018	mid Oct. 2018
Refine existing SOP's & write new project-specific SOP's	Sept. 2018	mid Oct. 2018
Purchase equipment and disposable supplies	Oct. 2018	end Oct. 2018
Complete final field and lab training activities	Oct. 2018	end Oct. 2018
Practice field sampling event in local stream	Oct. 2018	end Nov. 2018
Deploy water temperature probes	Nov. 2018	end. Nov 2018
Download first sequence of water temperature data	Dec. 2018	end Dec. 2018
Re-set and re-deploy water temperature probes	Dec. 2018	end Dec. 2018
Write and submit first 6-month Progress Report	**Dec. 2018**	**end Jan. 2019**
Begin data analysis, check for outliers, malfunctions, etc.	Jan 2019	end Jan. 2019
Download second sequence of water temperature data	Jan. 2019	end Jan. 2019
Re-set and re-deploy water temperature probes	Jan. 2019	end Jan. 2019
Begin data analysis, check for outliers, malfunctions, etc.	Feb. 2019	Feb. Jan. 2019
Download third sequence of water temperature data	Mar. 2019	end Mar. 2019
Re-set and re-deploy water temperature probes	Mar. 2019	end Mar. 2019
Begin data analysis, check for outliers, malfunctions, etc.	Apr. 2019	end Apr. 2019
Write and submit second 6-month Progress Report	**Jun. 2019**	**end Jun. 2019**
Download last sequence of water temperature data	Oct. 2019	end Oct. 2019
Remove and re-set water temperature probes	Oct. 2019	end Oct. 2019
Finish data analysis & construct all 7 regression models	Nov. 2019	end Dec. 2019
Write and submit third 6-month Progress Report	**Dec. 2019**	**end Jan. 2020**

Year 2: 2019-2020 ***Tasks***	***Timing: (Initiate)***	***(Complete by)***
Re-train existing staff and/or recruit & train new staff	July 2019	end of July 2019
Review and select possible streams to investigate	July 2019	end of Aug. 2019
Download fourth sequence of water temperature data	Jul. 2019	end Jul. 2019
Re-set and re-deploy water temperature probes	Jul. 2019	end Jul. 2019
Begin data analysis, check for outliers, malfunctions, etc.	Aug. 2019	end Aug. 2019
Consult with MN DNR and Trout Unlimited	Aug. 2019	mid Sept. 2019
Field reconnaissance of possible study streams (year 2)	Aug. 2019	end of Sept. 2019
Final selection of streams and study sites (year 2)	Sept. 2019	early Oct. 2019
Develop sample design and sampling schedules (year 2)	Oct. 2019	end Oct. 2019
Assign tasks and duties to staff, review existing SOP's	Oct. 2019	mid Oct. 2019
Refine existing SOP's & write new project-specific SOP's	Sept. 2019	mid Oct. 2019
Purchase equipment and disposable supplies	Oct. 2019	end Oct. 2019
Complete final field and lab training activities	Oct. 2019	end Oct. 2019
Download last sequence of water temperature data (year 1)	Oct. 2019	end Oct. 2019
Remove and re-set water temperature probes (year 1)	Oct. 2019	end Oct. 2019
Practice field sampling event in local stream (year 2)	Oct. 2019	end Nov. 2019

Deploy water temperature probes (year 2)	Nov. 2019	end. Nov 2019
Finish data analysis & construct all 7 regression models (year 1)	Nov. 2019	end Dec. 2019
Write and submit third 6-month Progress Report	**Dec. 2019**	**end Jan. 2020**
Download first sequence of water temperature data (year 2)	Dec. 2019	end Dec. 2019
Re-set and re-deploy water temperature probes	Dec. 2019	end Dec. 2019
Begin data analysis, check for outliers, malfunctions, etc.	Jan 2020	end Jan. 2020
Download second sequence of water temperature data	Jan. 2020	end Jan. 2020
Re-set and re-deploy water temperature probes	Jan. 2020	end Jan. 2020
Begin data analysis, check for outliers, malfunctions, etc.	Feb. 2020	Feb. Jan. 2020
Download third sequence of water temperature data	Mar. 2020	end Mar. 2020
Delayed because of COVID-19		
Re-set and re-deploy water temperature probes	Mar. 2020	end Mar. 2020
Delayed because of COVID-19		
Begin data analysis, check for outliers, malfunctions, etc.	Apr. 2020	end Apr. 2020
Delayed because of COVID-19		
Write and submit fourth 6-month Progress Report	**Jun. 2020**	**end Jun. 2020**
Download fourth sequence of water temperature data	Jul. 2020	end Jul. 2020
Re-set and re-deploy water temperature probes	Jul. 2020	end Jul. 2020
Begin data analysis, check for outliers, malfunctions, etc.	Aug. 2020	end Aug. 2020
Download last sequence of water temperature data	Oct. 2020	end Oct. 2020
Remove and re-set water temperature probes	Oct. 2020	end Oct. 2020
Finish data analysis & construct all 7 regression models	Nov. 2020	end Dec. 2020
Write and submit fifth 6-month Progress Report	**Dec. 2020**	**end Dec. 2020**

Year 3: 2020-2021 Tasks	*Timing: (Initiate)*	*(Complete by)*
Re-train existing staff and/or recruit & train new staff	July 2020	end of July 2020
Review and select possible streams to investigate	July 2020	end of Aug. 2020
Consult with MN DNR and Trout Unlimited	Aug. 2020	mid Sept. 2020
Field reconnaissance of possible study streams	Aug. 2020	end of Sept. 2020
Final selection of streams and study sites within	Sept. 2020	early Oct. 2020
Develop sample design and sampling schedules	Oct. 2020	end Oct. 2020
Assign tasks and duties to staff, review existing SOP's	Oct. 2020	mid Oct. 2020
Refine existing SOP's & write new project-specific SOP's	Sept. 2020	mid Oct. 2020
Purchase equipment and disposable supplies	Oct. 2020	end Oct. 2020
Complete final field and lab training activities	Oct. 2020	end Oct. 2020
Practice field sampling event in local stream	Oct. 2020	end Nov. 2020
Deploy water temperature probes	Nov. 2020	end. Nov 2020
Download first sequence of water temperature data	Dec. 2020	end Dec. 2020
Re-set and re-deploy water temperature probes	Dec. 2020	end Dec. 2020
Begin data analysis, check for outliers, malfunctions, etc.	Jan 2021	end Jan. 2021
Download second sequence of water temperature data	Jan. 2021	end Jan. 2021
Re-set and re-deploy water temperature probes	Jan. 2021	end Jan. 2021
Begin data analysis, check for outliers, malfunctions, etc.	Feb. 2021	Feb. Jan. 2021
Download third sequence of water temperature data	Mar. 2021	end Mar. 2021
Re-set and re-deploy water temperature probes	Mar. 2021	end Mar. 2021

Begin data analysis, check for outliers, malfunctions, etc.	Apr. 2021	end Apr. 2021
Write and submit sixth 6-month Progress Report	**Jun. 2021**	**end Jun. 2021**
Write and submit results of all models for publication	**Jun. 2021**	**end June. 2021**

Anticipated Results and Deliverables: We will use the regression results for identifying highest priority management actions like our earlier models published as a result of our previous LCCMR funded project (Krider *et a*l. 2013). Highest priority areas will be defined as lengths of stream where thermal regimes transition most rapidly from groundwater control of thermal regime to meteorological control of thermal regime. The analytical approach will be identical to the approach described in Krider et al. (2013) and will enable managers to pinpoint areas of stream where the buffering influence of ground water transitions to meteorological control of water temperature.

ENRTF BUDGET: $ 81,504

Outcomes for Activity 1: The following outcomes will be accomplished at 5 sites in each of the 7 streams/year (total of 105 sample sites over three years)	**Completion Date**
1. Specific, measurable outcome: Develop thermal models (TM) 7 streams, 5 sites/str, year 1	*June 2019*
2. Specific, measurable outcome: Develop TM for 7 more streams, 5 sites/str, year 2	*June 2020*
3. Specific, measurable outcome: Develop TM for 7 more streams, 5 sites/str, year 3	*June 2023*

First Update January 31, 2019

Re-training of existing staff (Graduate student RA, Ms. Corrie Nyquist) was completed in July August 2018. It was not necessary to recruit & train any undergraduate hourly students during this report period.
Review and selection of 30 possible streams to investigate was initiated in July 2018 and continued until the end of September 2018. Our short list of 12 possible streams was sent to MN DNR for vetting in October 2018 and we received review and suggestions from Mark Nemeth in mid-October. Field reconnaissance of the short list of possible study streams was completed in October – November.

During this report period we also scheduled and held meetings with Will French (MN DNR), Will Bouchard (MN PCA) and Alex Egan (US NPS) to obtain their input and advice on suitability of streams to be included. This input occurred in September – November. Final selection of streams and study sites was completed by the end of November. Development of the sample design and tentative sampling schedules were completed by the end of October. Actual field sampling schedules are largely influenced by weather conditions, but the tentative times serve as target for preferred dates.

Review and refining of existing SOP's and writing of a new project-specific SOP was completed by mid-November. A practice field sampling event was completed in a local stream in November. Deployment of water temperature probes was delayed but occurred in December.

Second Update June 30, 2019

Field collection of water temperature data for the winter period of the first year of this was completed. After downloading, we converted the files to EXCEL format and began data analysis, checked for outliers, malfunctions, and any indications the probes were out of the water. All data readings looked accurate, and development of regression models was initiated. Probes were re-set and re-deployed to continue to measure water temperatures. The file formatting that we are using is a little different than the format we have used in the past, so the SOP was modified to match the slightly different format. This is only a minor adjustment and is not a cause for concern but is necessary to maintain GLP.

During this time period we also met with John Hoxmier and Doug Dieterman to learn about their on-going research on brook and brown trout in streams of the Driftless Region. The intent was to keep each other informed, and to explore possible partnering to achieve our project goals. We also met with Bruce Vondracek on a regular basis to seek his input and guidance on interpretation of newly gathered data. We also me with Kent Johnson (Trout Unlimited, Kiap-TU-Wis Chapter) to review their progress on stream restoration in Driftless Region trout streams in Wisconsin, and to learn about their progress developing an online app for use by citizen volunteers.

Third Update January 31, 2020 [Submitted October 2020]

All modeling of water temperature data for the winter period of the first year of this project was completed. This consisted of manipulation of data in files converted earlier to EXCEL format and checking for outliers, malfunctions, and any indications the probes were out of the water. Site-specific regression models were developed. Probes were retrieved from sites used during the first field season and, when battery power was sufficient, probes were re-set and stored in lab so that they could be re deployed in November/December of 2019 to measure water temperatures in streams selected for assessment during our second winter field season. The file formatting has not changed, so the SOP that was modified previously is appropriate for continued model construction.

During fall of 2019 we met with Dr. Mark Nemith (MN DNR) to learn about his on-going research and assessments of brook and brown trout in streams of the northern portion of the Driftless Region and more urban trout streams near the Minneapolis/Saint Paul metropolitan region. We met with Ms. Vicki Sherry of the Minnesota River Valley National Wildlife Reserve in Bloomington in Fall to learn about mitigation procedures that have been implemented to protect Ike's Creek (brook trout stream close to the Mall of America) as land use changes associated with local infrastructure development near the Light Rail Corridor adjacent to the Reserve. The intent was to stay informed of recent activities, and to explore possible partnering to achieve our project goals.

We also met with Bruce Vondracek and Jim Perry on a regular basis to seek their input and guidance on interpretation of newly gathered data. We also met several with Kent Johnson (Trout Unlimited, Kiap-TU-Wis Chapter) to review their continued expansion of stream restoration in Driftless Region trout streams in Wisconsin, and to review their progress developing an on-line app for use by citizen volunteers. Selection of streams and study sites for the second field season was completed by the end of October and field reconnaissance of sites was completed in November. Development of the sample design and tentative sampling schedules also were completed by the end October. In practice, actual field sampling schedules are largely influenced by weather conditions, but the tentative times serve as target for preferred dates. Review and refining of existing SOP's and writing of a new project site-specific SOP's were completed by mid-November. A practice field sampling event was completed in a local stream in November.

Deployment of water temperature probes occurred in November and December. In October we learned of a new funding opportunity provided by the Institute on the Environment at the University of Minnesota. The opportunity was through their Mini-Grants program, and we prepared a successful proposal to develop three workshops for citizen science volunteers that are interested in conservation and/or remediation of trout streams. Our first workshop targeted trout fishing members of local Trout Unlimited Chapters and was held at the University of Minnesota in February. We used data from this project and an earlier LCCMR-funded project to Ferrington for content of the workshop.

Fourth Update June 30, 2020 (Submitted 15 March 2021)
Development of thermal models was completed for a subset of three streams. Weather conditions in early March limited our ability to retrieve thermal probes so data could be downloaded and analyzed to just three streams by mid-March. Travel restrictions due to lock down response to COVID-19 prevented travel to the remaining sample sites, and as of 30 June the thermal probes were still recording stream temperatures. The probes have sufficient capacity to store temperature readings over the extended time period that probes remained in the streams, so no data will be lost.

Fifth Update January 31, 2021—waived by LCCMR 9/21/21
Sixth Update June 30, 2021—waived by LCCMR 9/21/21

October 2021
Fieldwork was completed on additional six streams. We were able to collect a portion of the thermal probes from last season and retrieve the data (at least 16 streams to date). The data regressions have been completed for a portion of streams and sites. The team will continue to retrieve, download, and curate the data.

Seventh Update January 31, 2022 (Submitted 31 January 2022)
Temperature data from 15 streams (33 sites) has been downloaded off temperature loggers and curated. From this data, six linear regressions of air and water temperature have been analyzed. Adult specimens from 26 streams have also been identified, labeled, and preserved. Community composition in relation to groundwater input and water temperature will be analyzed for 6 of these streams.

From one of the streams, Pickwick Creek, 148 adult flies were collected from the upper site and 145 adult flies from the lower site over the winter of 2021. Most of these flies were identified to the species Diamesa mendotae, three were Diamesa nivoriunda, and 3 were non-Diamesa. These flies were divided between incubations at constant 6 and 22 degrees C for 24 and 48 hours in the lab. This was to test how short-term exposure to warm air temperatures affects lifespan for winter-active midges that emerge from groundwater dominated streams. Water temperatures were also recorded for this site and used to compare to lifespan (longevity). Results indicate that even short-term exposure could shorten lifespans of cold-adapted midges. Eight flies were also infected with nematode parasites. These caused earlier death in the infected flies since nematodes emerge killing their host, and one fly was infested with up to 17 nematodes. High parasitization such as this has not been documented for winter-active chironomids, and the temperature treatments also affected nematode emergence and longevity. Thus, these results indicate that winter-active midges can be parasitized even in relatively good quality groundwater dominated streams, and this can alter longevity of adult flies. The parasitism may also be compounded by warming winter temperatures since warming temperatures also alter timing of nematode emergence from their hosts.

Project Status as of June 31, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Update waived per LCCMR staff on 6/30/2022.

Project Status as of January 30, 2023:
Our team completed analysis of air and water temperature data and adult specimens. We completed the final examination of community composition for the final 6 streams. Our results indicate that the longevity of winter-emerging midges is impacted by warm air and water temperatures. Our thermal models can help identify areas where transitions between groundwater controlled thermal regimes and meteorologically controlled thermal regimes occur within stream systems. This will help conservation management plans and restoration efforts more accurately consider the moderating influence of groundwater on stream temperature. One team member completed her dissertation and this data served as the foundation for some of her chapters. Over the next semester, the research team will be drafting academic publications, and other forms of communication, to share all research results with relevant audiences.

Final Report Summary as of June 30, 2023:
Over the course of this project, the research team collected water and temperature data from over 30 stream sites in the Driftless Region, which provided insights on how groundwater, air temperature, geology, and streambed conditions interact during winter. The team also identified and preserved Chironomidae (midge) specimens. From linear regressions of air and water temperature, and incubations at various temperatures of live-collected Chironomidae, our results indicate that even short-term exposure to warm air temperatures shortens lifespans of winter-active midges that emerge from groundwater dominated streams. We also found winter-active midges can be parasitized even in good quality groundwater dominated streams, further altering their longevity.

ACTIVITY 2: ***This activity has two inter-dependent parts. They are: Relate changes in macroinvertebrate abundances (PART 1) and macroinvertebrate genetics (PART 2) at the same 5 sites in each of the same 7 streams/year used for thermal models developed in Activity 1.***
This activity links stream conditions to trout through assessment of food density, availability, and nutritional quality. Given that many winter species look the same (especially undescribed species), we will use a type of DNA analysis (MtDNA) to efficiently and accurately identify insects that provide the most abundant and reliable energy and nutritional sources to trout in winter. We will determine how genetic patterns differ among the abundant insects, and how genetic variability aligns with abundances as a function of water temperatures across streams.

PART 1: Abundances--- Previous studies have shown that 35 or more species of Chironomidae can emerge from trout streams in Minnesota during winter and serve as food for trout (Anderson and Ferrington 2012, Anderson *et al*. 2016). The species *Diamesa mendotae* is one of the most abundant and widespread species encountered in the thermally buffered streams of the Driftless Region in

Minnesota (Mazak *et al*. 2015). This species has been intensively investigated (Bouchard and Ferrington 2009) and has been shown to produce two or more generations in Valley Creek near Afton, MN. It has served as the "test organism" for understanding and modeling how thermal variability may influence voltinism. A similar study on *Diamesa cheimatophila* in one stream in PA (Ferrington and Masteller 2015) has also been informative for developing this hypothesis. From these studies, we predict that optimal growth and development rates will occur at water temperatures close to the average groundwater temperatures, approximately +8 degrees C in streams of the Driftless Region. Growth and development is predicted to decline at cooler temperatures, especially when lower than 3-4 degrees C. It has also been shown that these two species do not complete their lifecycle and emerge at higher water temperatures, exceeding mean daily values of 10-12 degrees C, and it is assumed that growth and development rates also decline when water temperatures increase to these upper values. Consequently, stream segments that are most strongly buffered by ground water (and will have the lowest slopes and highest Y-intercepts from the thermal models) will provide thermal conditions for fastest growth and development rates, and result in greater number of generations per year. Less strongly buffered segments of stream with higher regression slopes and lower Y-intercepts will allow for colder water temperatures in mid-winter and higher water temperatures later into Autumn and earlier in Spring, which will result in lower growth and development rates, and fewer generations.

We will test the following hypothesis--- **Hypothesis 2: *Thermal variability*** *in streams will influence the abundance, timing, composition, and life cycles of winter-emerging Chironomidae.*

Methodology: We will use a standard collection protocol that has been shown to be effective to assess winter emergence (Ferrington *et al.* 1991, Kranzfelder *et al*. 2015). We will sample at each site on each of seven streams/year on six dates between December and February to assess species composition, relative abundance, and timing of emergence of winter-emerging species. Our field and laboratory methods will be identical to those used in our past research projects, so that detailed comparisons can be made to previous studies.

Activity Two Work Flow (Tasks and Timing):

Year 1: 2018-2019 ***Tasks***	***Timing: (Initiate)***	***(Completed by)***
Re-train existing staff and/or recruit & train new staff	July 2018	end of July 2018
Review and select possible streams to investigate	July 2018	end of Aug. 2018
Field reconnaissance of possible study streams	Aug. 2018	end of Sept. 2018
Final selection of streams and study sites for year 1	Sept. 2018	early Oct. 2018
Develop sample design and sampling schedules	Oct. 2018	end Oct. 2018
Assign tasks and duties to staff, review existing SOPs	Oct. 2018	mid Oct. 2018
Refine existing SOPs & write new project-specific SOPs	Sept. 2018	mid Oct. 2018
Purchase equipment and disposable supplies	Oct. 2018	end Oct. 2018
Complete final field and lab training activities	Oct. 2018	end Oct. 2018
Practice field sampling event in local stream	Oct. 2018	end Nov. 2018
First round of sampling of SFPE	Dec. 2018	mid. Dec. 2018
Second round of sampling of SFPE	mid Dec.2018	end Dec. 2018
Write and submit first 6-month Progress Report	**Dec. 2018**	**end Dec. 2018**
Begin sample processing and data analysis SFPE samples	Jan 2019	end Jan. 2019
Third round of sampling of SFPE	Jan. 2019	mid Jan. 2019
Fourth round of sampling of SFPE	mid Jan. 2019	end Jan. 2019

Continue sample processing & data analysis SFPE	Jan. 2019	end Jan. 2019
Fifth round of sampling of SFPE	Feb. 2019	Mid Feb. 2019
Sixth round of sampling of SFPE	mid Feb. 2019	end Feb. 2019
Continue sample processing & data analysis SFPE	Feb. 2019	end May 2019
Write and submit second 6-month Progress Report	**Jun. 2019**	**end Jun. 2019**

Year 2: 2019-2020 Tasks	***Timing: (Initiate)***	***(Completed by)***
Re-train existing staff and/or recruit & train new staff	July 2019	end of July 2019
Review and select possible streams to investigate	July 2019	end of Aug. 2019
Consult with MN DNR and Trout Unlimited	Aug. 2019	mid Sept. 2019
Field reconnaissance of possible study streams	Aug. 2019	end of Sept. 2019
Final selection of streams and study sites for year 2	Sept. 2019	early Oct. 2019
Develop sample design and sampling schedules	Oct. 2019	end Oct. 2019
Assign tasks and duties to staff, review existing SOP's	Oct. 2019	mid Oct. 2019
Refine existing SOP's & write new project-specific SOP's	Sept. 2019	mid Oct. 2019
Purchase equipment and disposable supplies	Oct. 2019	end Oct. 2019
Complete final field and lab training activities	Oct. 2019	end Oct. 2019
First round of sampling of SFPE	Dec. 2019	mid. Dec. 2019
Second round of sampling of SFPE	mid Dec.2019	end Dec. 2019
Write and submit third 6-month Progress Report	**Dec. 2019**	**September 2020**
Begin sample processing & data analysis SFPE samples	Jan 2020	end Jan. 2020
Third round of sampling of SFPE	Jan. 2020	mid Jan. 2020
Fourth round of sampling of SFPE	mid Jan. 2020	end Jan. 2020
Continue sample processing & data analysis SFPE	Jan. 2020	end Jan. 2020
Fifth round of sampling of SFPE	Feb. 2020	Mid Feb. 2020
Sixth round of sampling of SFPE	mid Feb. 2020	end Feb. 2020
Continue sample processing & data analysis SFPE	**Feb. 2020**	**end May 2020**
NOTE: The above task was partially delayed due to restriction associated with COVID-19		
Write and submit fourth 6-month Progress Report	Jun. 2020	end Jun. 2020

Year 3: 2020-2021 Tasks	***Timing: (Initiate)***	***(Complete by)***
Re-train existing staff and/or recruit & train new staff	July 2020	end of July 2020
Review and select possible streams to investigate	July 2020	end of Aug. 2020
Consult with MN DNR and Trout Unlimited	Aug. 2020	mid Sept. 2020
Field reconnaissance of possible study streams	Aug. 2020	end of Sept. 2020
Final selection of streams and study sites for year 3	Sept. 2020	early Oct. 2020
Develop sample design and sampling schedules	Oct. 2020	end Oct. 2020
Assign tasks and duties to staff, review existing SOP's	Oct. 2020	mid Oct. 2020
Refine existing SOP's & write new project-specific SOP's	Sept. 2020	mid Oct. 2020
Purchase equipment and disposable supplies	Oct. 2020	end Oct. 2020
Complete final field and lab training activities	Oct. 2020	end Oct. 2020
First round of sampling of SFPE	Dec. 2020	mid. Dec. 2020
Second round of sampling of SFPE	mid Dec.2020	end Dec. 2020
Write and submit fifth 6-month Progress Report	**Dec. 2020**	**end Dec. 2020**

Begin sample processing & data analysis SFPE samples	Jan 2021	end Jan. 2021
Third round of sampling of SFPE	Jan. 2021	mid Jan. 2021
Fourth round of sampling of SFPE	mid Jan. 2021	end Jan. 2021
Continue sample processing & data analysis SFPE	Jan. 2021	end Jan. 2021
Fifth round of sampling of SFPE	Feb. 2021	Mid Feb. 2021
Sixth round of sampling of SFPE	mid Feb. 2021	end Feb. 2021
Continue sample processing & data analysis SFPE	Feb. 2021	end May 2021
Write and submit sixth 6-month Progress Report	**Jun. 2021**	**end Jun. 2021**
Write and submit results of emergence for publication	**Jun. 2021**	**end Jun. 2021**

Anticipated Results and Deliverables: For each sample site we will interpret the outcomes (species richness, abundances, numbers of generations) relative to the corresponding thermal regression model for the site. Our predictions are that species richness, abundances, and number emergence peaks (representing successive generations of individual species) will be greatest at sites that have the lowest slopes and highest Y-intercepts, and that the results relate to the site-specific buffering effects of groundwater input.

PART 2: Genetic Variability---Recent studies have shown the benefits of molecular analyses for studying the biology of Chironomidae (Broden *et al*. 2013, Ekrem *et al.* 2007, 2010, Kranzfelder *et al.* 2016, Krosch and Cranston 2012, Sinclair and Gresens, 2008, Stur and Ekrem, 2011) including revealing cryptic species (Anderson et al 2013). However, we are not aware of any molecular studies on winter emerging insects that focus on genetic variability. We earlier completed a very preliminary study of variability in adults of winter collected *Diamesa mendotae* but have not evaluated any other of the remaining 34 species that we know emerge from trout streams of the Driftless Region. Our preliminary studies were designed to test a hypothesis that the severity of emerging into winter conditions, and having evolved specialized physiological and behavioral mechanisms to survive sub-freezing air temperatures, would provide selective force to favor a single genotype or narrow range of genotypes that is specialized to survive in winter. Therefore, molecular variability should be low within populations at a given site and also among sites within a given stream. At larger spatial scales, we also predicted that there would be genetic homogenization across streams over intermediate geographic scales. An alternative to the last prediction, however, could be that lack of effective dispersal in winter by adults from stream-to-stream would either result in (1) genetic drift increasing molecular variability, or (2) differing selective forces that are stream-specific could result in increasing molecular variability among streams, but low variability within sites of the same stream. Although our preliminary sample sizes are small, the results are counter to either of our alternative predictions.

For the preliminary study we generated and analyzed sequence data from the mtCOI target region for seven adults from each of two populations from a stream in Minnesota (MN: Valley Creek at Belwin, 12/20/2002) and a second stream in Wisconsin (WI: Kinnickinnic River, 03/12/2003). Whole genomic DNA was extracted from the entire body using the QIAamp DNA Micro Kit (Qiagen) following the manufacturer's protocol. The last two abdominal segments and genital structures were separated prior to tissue lysis and stored in 96% Ethanol as vouchers. The target region was amplified via PCR. We used the primers and cycling conditions described in Hebert *et al.* (2003) to amplify a slightly shorter stretch from the target region. PCR was carried out using Ready-to-Go PCR beads (GE Health Systems). 2.5pmol of each primer, 3µl genomic DNA and 17µl ddH2O were added to single bead. PCR products were then run on a 1% agarose gel and subsequently gel purified using the peqGOLD Microspin Gel Extraction Kit (Peqlab). Purified products were cycle sequenced using the DTCS Quick Start Kit

(Beckman-Coulter) and run on a CEQ8800 sequencer (Beckman-Coulter). SCF traces were edited in Seqman 4.03 (Lasergene) and sequences subsequently manually aligned in BioEdit (Hall 1999).

The resulting alignment contained 581 unambiguous base pairs with 14 positions variable. Ten haplotypes were identified and are shown in a median joining network calculated using the default settings in Network 4.2 (Fluxus Technologies) (Figure 1, below).

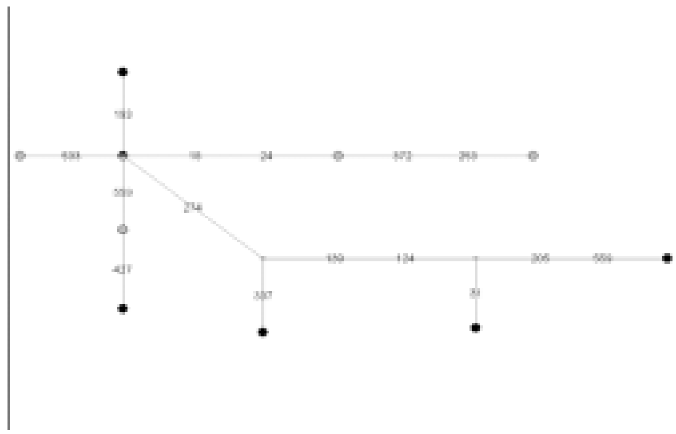


Figure 1. Median joining network of 10 haplotypes identified for *Diamesa mendotae*. Haplotypes are coded by population origin. MN: grey; WI: black; median vectors: white. Numbers indicate mutated positions in the alignment.

One haplotype is shared between individuals of both populations. The other haplotypes are only found in one or the other population (4 in MN, 5 in WI), but there is no clear pattern of similar haplotypes dominating each population. We calculated uncorrected pairwise distances (p) using the DNADist function as implemented in BioEdit V7.0 (Hall1999). Maximum p within populations was 0.87% for MN and 1.04% for WI and 1.57% between haplotypes of different populations.

Based on our preliminary data the number of haplotypes found in *D. mendotae* was high. Although Martin et al. (2002) found a similar number of haplotypes in the target region for a similar number of specimens in three Holarctic species of Chironomus sp., the maximum intraspecific haplotype differences ranged between 0.8% and 6.4% across the Holarctic.

While our preliminary data do not allow in depth analysis, the degree of variability between the populations appears to be relatively high when compared to the values reported by Martin et al, especially considering the geographic scope. The values in *D. mendotae* are similar to values observed for isolated populations of the cold tolerant montane caddisflies *Drusus discolor* and *D. romanicus* in Europe (Pauls 2004, Pauls *et al*. 2006). Thus, the marker appears sufficiently variable to detect intraspecific population structure and/or the presence of cryptic species in *Diamesa mendotae,* and we conclude it is suitable for the proposed project.

The degree and extent of haplotype occurrences over the broader Driftless Region is unknown at this time. To facilitate a more extensive data set, we will focus our effort to bar code adults collected from on snow at all sites on all streams selected for this project. This will provide a large-scale assessment of both within stream and across-streams variability of haplotypes.

We will test the following hypothesis--- **Hypothesis 3: *Genetic variability*** *in Diamesa mendotae will be* *low at an individual sample site but may vary across sample sites within a given stream in relation to the variability of site-specific thermal variability.*

Anticipated Results and Deliverables: We will summarize the genetic variability by sample site, date of emergence, within and across streams, and in relation to the thermal model. Relationships of genetic variability in relation to one or more of the variables will be determined from non-metric multi-dimensional scaling analyses. All sequence data will be uploaded and stored in BOLD (*sensu* Zhou *et al.* 2009). We will determine how genetic patterns differ among the abundant insects, and how genetic variability aligns with abundances as a function of water temperatures across streams. If reliable relationships are found as a function of sample site or thermal regime, we will collect adults, keep them alive, allow them to mate and oviposit in lab environmental chambers set at 6 degrees C. so that laboratory test cultures can be started. After mating, the adult males and ovipositing females will be analyzed to determine if morphological features can be discovered to differentiate the different molecular form or forms. We will also determine if longevity, oviposition behavior and egg-hatching success in laboratory-controlled temperature cabinets is different among different haplotypes. We will also test for differing growth and development rates at 6 degrees C.

This activity also has potential to link stream conditions to trout dynamics through better assessment of food density, availability, and nutritional quality. Given that many winter species look very similar (especially undescribed species), we expect that bar coding and molecular analysis will allow us to efficiently and accurately identify the insect species that provide the most reliable energy and nutritional sources to trout in winter.

ENRTF BUDGET: $ 220,354

Outcomes for Activity 2: 5 sites in each of 7 streams/year (total of 105 sites over 3 years)	**Completion Date**
1. Assessment of density & genetic variability of the most abundant invertebrate species	*June 2019*
2. Assessment of density & genetic variability of the most abundant invertebrate species	*June 2020*
3. Assessment of density & genetic variability of the most abundant invertebrate species	*June 2022*

First Update January 31, 2019
Part 1 and Part 2 of this activity are summarized in this update. These are two interdependent parts in terms of field and most lab-related activities.

Training of existing staff in field and lab techniques for assessing densities and performing genetic analyses occurred from July through January. Our review of existing SOPs in the primary literature was initiated in mid-October and continued through January. Purchase of equipment and disposable supplies was not initiated because we have not yet determined the best literature protocols for the analyses. We have consulted with Dr. Debora Paula Pires (specialist on MtDNA analytical techniques)

and she has volunteered her time to assist in lab determinations and data interpretations.
She has also volunteered disposable supplies and lab space for our use. This is still an on-going activity, and we will continue to consult and collaborate with Dr. Pires.

Sampling of SFPE and collection of adults for analyses were initiated in December and continued through January but were not as effective as we predicted because of reduced winter emergences associated with colder weather on sample dates. We will continue our efforts into the next report period.
Samples that we were able to obtain, however, were preserved (SFPE) and adults collected live from snowbanks near the trout streams were assessed for longevities, then placed in freezer for preserving until analysis.

Second Update June 30, 2019
Sampling of SFPE and collection of adults for analyses that was initiated in December continued through early March but was more effective than during our last project report period. All samples of (SFPE) that were planned were obtained. Adults were collected live from snowbanks near four trout streams, including on two separate dates for two of the streams. All adults were successfully assessed for longevities and, in the case of females, for oviposition and egg development. Adults were placed in freezer within 24 hours of death and maintained frozen until analysis.

We have continued to consult with Dr. Debora Paula Pires (specialist on MtDNA analytical techniques) and she continues to volunteer her time to assist in lab determinations and data interpretations. She also has continued to volunteer some disposable supplies, and significant lab space for our use. This is still an on-going activity, and we will continue to consult and collaborate with Dr. Pires.

Third Updates: Due January 31, 2020 [October 2020]
Site selection and field reconnaissance were completed in October-November of 2019. Field samples for the December sample period collected at all sites in December.
Once again, these activities were more effective than during our last project report period. Adults were collected live from snowbanks near five trout streams; however, adults were not present at all sites. All adults that were collected were placed into environmental chambers to assessed for longevities and, in the case of females, for oviposition and egg development. As of the end of this report period, most adults were still alive, and will continue to be checked until all have died.

Adults collected for molecular analyses were placed in freezer within 24 hours of death and maintained frozen until analysis. Dr. Debora Paula Pires (specialist on MtDNA analytical techniques) has continued to assist with specimen preparation and DNA isolation and amplification. She has confirmed that she will continue to volunteer her time to assist in refining lab techniques and data interpretations. She also has continued to volunteer some disposable supplies however most supplies used for these analyses have been purchased through this grant. Dr. Pires continue to provide significant lab space for our use and has dedicated a workspace and desk for the graduate student that is leading the lab work. We have been able to develop primers that are specific for our species and have refined techniques to focus on three distinct genes. This represents a major step forward in terms of potential to detect population or species-specific markers. The lab work on frozen specimens is still an on-going activity and we will continue to consult and collaborate with Dr. Pires through the remainder of this field season and will provide more detailed summaries in our next interim report.

Fourth Update June 30, 2020 (Completed 15 March 2021)
All field work scheduled for winter 2019-2020 (November-February) was completed and samples were preserved according to SOPs for storage until processing in lab. Restrictions related to lab access due to COVID-19 prevented significant progress with processing of the samples during March May. However, Ferrington was able to convert garage space at private residence into make-shift work area and some progress processing samples was made during late May and June.

We continued to be effective collecting adults for longevity, oviposition, and molecular analyses, as was the case during our last project report period. More than 800 additional adults were collected live from snowbanks near all trout streams assessed during winter 2019-2020. However once again adults were not present at all sites. All adults that were collected during January-February were placed into environmental chambers to assessed for longevities and, in the case of females, for oviposition and egg development. As of the end of this report period, all adults have died, and longevity and oviposition analyses have been completed.

Adults collected for molecular analyses were placed into a freezer within 24 hours of death and maintained frozen until analysis. Restrictions on use of lab space due to COVID-19 stopped progress on this aspect of our project from mid-March through May, and we were only able to resume limited lab work in June.

Dr. Debora Paula Pires (specialist on MtDNA analytical techniques) continued to assist via ZOOM to make suggestions related to preparation of specimens and DNA isolation and amplification in June. She has once again agreed to continue to volunteer her time to assist in refining lab techniques and data interpretation. All adults collected but not processed during this report period have been frozen at - 80 degrees C. and will be processed as time permits into the future.

Fifth Update January 31, 2021—waived by LCCMR 9/21/21
Sixth Update June 30, 2021—waived by LCCMR 9/21/21

October 2021:
Adults have been collected from an additional four streams and samples have been sorted. Longevity has been recorded for adults placed into incubation chambers. One mitochondrial gene and two nuclear DNA genes have been extracted, amplified, and quantified per individual. Samples have been sent to be Sanger sequenced at UMGC. The sequences have been edited, contigs formed, and all sequences aligned for phylogenetic analysis. The team will continue to process the data, including phylogenetic analysis: (dendrograms created, genetic distances verified.)

Seventh Update January 31, 2022
All sequencing data has been processed and analyzed. The team discovered two putative cryptic species hiding as *Diamesa nivoriunda,* using ABGD and phylogenetic analysis. One team member successfully completed her Master's thesis and published some results from this work with ProQuest with the title, "Exploring the hidden diversity of winter-emerging Chironomidae (Insecta: Diptera)." These molecular methods of analysis help us better understand the genetic diversity of winter-emerging flies; more clear knowledge of the genetic diversity of stream food sources might influence future decisions about conservation and management practices.

Project Status as of January 30, 2023:

Our team completed the molecular research portion of this grant and shared results. Voucher specimens were slide-mounted and deposited in the University of Minnesota Entomology Department Insect Museum (219 Hodson Hall, 1980 Folwell Avenue, St Paul, MN 55108) for public use. We also shared supplemental files and data sequences for future research. In all, the species identification research conducted for this activity can have implications on our understanding of the biodiversity and evolution of organisms. Failure to accurately identify species may result in underrepresenting species richness and overestimating abundances. Accurate identification and management of Chironomidae is imperative for preservation of the food base of trout.

Final Report Summary as of June 30, 2023:
Given that many winter species look very similar, molecular analysis allowed us to identify insects more accurately. Using a type of DNA analysis (MtDNA), our team examined genetic patterns and variability among insects sampled. According to sequencing data, D. mendotae species were found across 6 streams (Pine Creek, Clear Creek, Miller Creek, Lawrence Creek, Winnebago Creek, and Vermillion River) and D. nivoriunda species were present in 4 of these streams. Seven mated pairs, all of which were D. mendotae, were collected from Pine Creek, Clear Creek, and Miller Creek.

ACTIVITY 3: *Develop a communication and educational outreach program*.

The goal of the communication and outreach program is to improve public engagement with science and to increase understanding of how specific actions impact trout stream conditions, which have important economic implications for Minnesota areas relying upon tourism revenue. Landowners in SE MN, agricultural stakeholders, and conservation organizations are a critical audience for this project, especially as we must gain access to privately owned land to collect data. We will conduct focus group and survey research with these groups to better understand their knowledge of trout stream research, attitudes towards the importance of this research, and barriers to granting access to researchers or serving as citizen volunteers that aid in data collection. It will help us identify best communication strategies, channels, and content to reach SE MN landowners and best ways to partner with stakeholder groups. Our communications plan will help us share goals, results, and impact of this research with key audiences. The plan will use social media channels, media organizations in SE MN to communicate events to leader organizations (e.g. MN Farm Bureau, FFA, MN Farmers' Union, Trout Unlimited).

ENRTF BUDGET: $ 98,142

Outcomes: Development of a communication and educational outreach program	**Completion Date**
Coordinate with key audiences and design communication campaign plan	*August 2019*
Develop communication and outreach materials and begin implementing campaign plan	*August 2020*
Implement communication campaign, evaluate impact of education and outreach messages	*June 2023*

First Update January 31, 2019
During this reporting period we scheduled a meeting with Ms. Laurie Sovell (Citizens Monitoring Program Coordinator, MPCA) to determine points of connection between her program and our needs for this Activity. We refined a strategy for coordinating with our preferred audiences, and Ms. Sovell agreed to publish a note in her upcoming Newsletter for Citizens Volunteers describing our project and possible ways in which citizens could become involved. Ferrington wrote the communication, and it is to be included in the newsletter.

Swenson continued to work with our research team to refine our concepts for citizen communication and started developing the assessment protocols for the final campaign instrument. Swenson also began recruitment and training of a Graduate Student RA to work on this Activity. The student is Ms. Sonja Hakanson, and it is possible that she will use this activity (in full or part) for her graduate research.

Second Update June 30, 2019
During this reporting period, faculty member Rebecca Swenson and student Sonja Hakanson worked with the Minnesota Farm Bureau and the Ag Water Resource Center to identify a list of landowners in SE Minnesota that have worked on water projects in the past and who might be willing to participate in phone interviews or focus groups to learn more about the trout stream research. Phone interviews have been completed with 6 of the 20 farmers on the list and insights about interest, knowledge, communication preferences, and barriers/motivations are in development.

Swenson also developed a survey and phone interview guide to better understand motivations/barriers to participation in citizen monitoring programs and engagement with environmental science research. We worked with Laurie Sovell and Shannon Martin at the Citizens Water Monitoring Program to gather insights from their current and past program participants, in order to better inform communication efforts and to understand how these insights might translate to involving Minnesota citizens in current trout stream research. To date, Swenson and Hakanson have engaged 408 Minnesota residents in the survey research and are currently setting up follow-up phone interviews with segments of this group to develop more in-depth insights.

During the Spring 2019 semester, an interdisciplinary group of undergraduate students, graduate students, and faculty from the Agricultural Communication & Marketing, Fisheries & Wildlife, and Environmental Science and Policy Management programs went on a field trip to visit the Valley Creek stream research site in Afton, MN. The group captured science stories about the snowy life of midges, the process of field work, and how winter dynamics impact trout populations. In addition, 61 undergraduate students learned more about the LCCMR trout stream research project and the MPCA Citizen Water Monitoring programs, and used this information to practice developing science communication materials and strategies, as part of a “Communicating Science to the Public” course. This is a “value added” benefit of our Activity 3 that provides real world practical experience for students with our respective academic degree programs and is consistent with our collegiate goal to provide “experiential learning experiences” for our students.

Third Update January 31, 2020 [Submitted October 2020]
During this reporting period, faculty member Rebecca Swenson and student Sonja Hakanson continued their efforts to conduct interview landowners in SE Minnesota that have worked on water projects in the past and who might share insights about their involvement in our research. Phone interviews with

have farmers have provided useful insights about stakeholder interest related to trout streams, potential interest in participating in research or allowing field site access, and some information about communication preferences.

Swenson previously developed a survey and phone interview guide which was used to better understand motivations/barriers to participation in citizen monitoring programs and engagement with environmental science research. She has reviewed the data compiled from the survey to understand how these insights might promote involvement of Minnesota citizens in current trout stream research. Swenson and Hakanson have continued to involve Minnesota residents in survey research and are currently reviewing information from follow-up phone interviews with subset of this group to develop more in-depth insights.

As reported in our previous interim report, during the Spring 2019 semester an interdisciplinary group of undergraduate students, graduate students, and faculty from the Agricultural Communication & Marketing, Fisheries & Wildlife, and Environmental Science and Policy Management programs went on a field trip to visit the Valley Creek stream research site in Afton, MN. Swenson and Dr. Troy McKay accompanied the group and took photographs and videos that captured our activities and were edited and developed into an on-line video by Ms. Kayla Leiding and Ms. Krista Styer (as undergraduate student projects) under his supervision to produce a documentary about our research on the "snowy life of midges." The video also provides insight into how we do our winter field work, and why the winter dynamics of aquatic insects important to the health and vigor of trout populations. A copy of the video can be viewed at: https://youtu.be/GTkoNGex8CE

During the report period Dr. Swenson spearheaded the development of the beta version of a web site that will be targeted to citizen volunteers and fly-fishing enthusiasts. At the end of this report period the web site was not fully developed for external viewing, but the goal is to have it ready for review and comment by an external interest group during the next report period. Our tentative name for the website is "Bugs Below Zero (bugsbelowzero.com/contact).

Fourth Update June 30, 2020 (submitted 15 March 2021)
In February we conducted an outreach event on-campus targeting citizen science volunteers and fly-fishing groups. The event was attended by 22 persons, and videos of the activities we recorded. Videos were trimmed and edited, then added to a web site that we have developed called "Bugs Below Zero." We worked on editing the site for content and functional attributes before final placement online. As of 30 June, the web site has not yet been advertised to prospective stakeholder groups, but the URL is active and the site can be viewed at: https://www.bugsbelowzero.com/ The website includes an overview of research and its importance for Minnesota resources, details about the research team and funding organizations, educational materials about winter emerging insect species and their role in stream health, and directions for citizens to get involved.

As a result of our outreach event in February we have started receiving information about winter active insects on snowbanks around trout streams in SE Minnesota from some of the participants of the event. We anticipate that we will continue to experience increasing participation among citizen volunteers after we have completed final edits to our "Bugs Below Zero" web site and advertised it to prospective stakeholder groups.

Fifth Update January 31, 2021—waived by LCCMR 9/21/21

Sixth Update June 30, 2021—waived by LCCMR 9/21/21

October 2021:
Our team has developed new materials for the "Bugs Below Zero" website, refined content, accessibility, and navigation features of the website, and shared educational materials with key stakeholder groups. We also hosted both in-person and virtual events, including an interactive winter workshop at Brown's Creek near Stillwater, Minnesota for St. Paul science teachers, students, and parents which included streamside demonstrations to connect insects, water, and academic research. Dr. Ferrington, Dr. Swenson and other members of the research team also hosted a webinar in partnership with the Minnesota Master Naturalist program, which had 46 attendees in the live session and additional audiences reached online. Dr. Ferrington and Dr. Swenson spoke about this grant project on the Explore, Teach, Conserve podcast by Extension Natural Resources programs, which can be heard here: https://share.transistor.fm/s/d8701aea.

In addition, written summaries of the research and Bugs Below Zero resources have been shared with agricultural organizations, which passed them on to their members via newsletters and other internal communication channels. This includes Minnesota Farm Bureau, Midwest Organic and Sustainable Education Service (MOSES), and the Minnesota Farmers Union. We also contacted outdoor recreation groups like snowmobiling clubs, fishing groups, conservation organizations, and Audubon societies, which shared the Bugs Below Zero website and resources with their members on social media channels and other communication channels. The team will continue to implement and evaluate science communication activities.

Seventh Update January 31, 2022 (Submitted 31 January 2022)
The team developed educational activities for K12 classrooms that help students learn about the winter life of bugs and stream food webs. We wrote, designed, and mailed postcards and flyers that shared information about the Bugs Below Zero website and related classroom resources with 288 Minnesota educators, who teach courses related to agricultural and environmental topics. The team plans to continue expanding resources on the Bugs Below Zero website and share insights from this work.

Project Status as of June 31, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Update waived per LCCMR staff on 6/30/2022.

Project Status as of January 30, 2023:
The team continued to share communication campaign materials with Minnesota teachers. New educational content about stream food webs in the winter was drafted for the Bugs Below Zero website, and outreach to Minnesota K12 educators continued. We plan to continue evaluating citizen science activities, so we can improve this program and expand future outreach, especially to classrooms. We will be conducting research on the effectiveness of community science efforts and share insights to strengthen future work in this area.

Final Report Summary as of June 30, 2023:

Throughout this project, our team interacted with a variety of stakeholders, including landowners, education professionals, academics, conservation organizations, teachers, students, and outdoor enthusiasts to share research insights. We also focused on creating new educational materials and events that can help audiences learn more about the life cycles of winter aquatic insects and their importance to Minnesota streams. Much of this work is shared on a website created for this project that includes educational content, webinars, videos, lesson plans, photos, and other materials that raise awareness about stream food webs in the winter. Our team plans to continue adding content to this website and sharing updated educational resources with classrooms and other stakeholder groups.

IV. DISSEMINATION:

A large part of our dissemination plan is outlined in the description of Activity 3. As mentioned in the description of Activity 3, we seek to develop a highly effective communication and outreach program to inform Minnesota stakeholders of our findings related to the trout sport-fishing enterprise centered on the Driftless Region. The goal of the communication and outreach program is to improve public engagement with science and to increase understanding of how specific actions impact trout stream conditions, which have important economic implications for Minnesota areas relying upon tourism revenue. Landowners in SE MN, agricultural stakeholders, and conservation organizations are a critical audience for this project, especially as we must gain access to privately owned land to collect data. We will conduct focus group and survey research with these groups to better understand their knowledge of trout stream research, attitudes towards the importance of this research, and barriers to granting access to researchers or serving as citizen volunteers that aid in data collection. It will help us identify best communication strategies, channels and content to reach SE MN landowners and best ways to partner with stakeholder groups. Our communications plan will help us share goals, results, and impact of this research with key audiences. The plan will use social media channels, media organizations in SE MN to communicate events to leader organizations (e.g. MN Farm Bureau, FFA, MN Farmers' Union, Trout Unlimited). We will also develop a website and link it to our existing site at http://midge.cfans.umn.edu/research and will post up-to-date results, photos and videos to the page at http://midge.cfans.umn.edu/research/winter-active-chironomidae. Additional details about our research focus, mission, resources, facilities and publications can be accessed at http://midge.cfans.umn.edu/research/coldbiology.

Description: Not repeated here because it is described in detail in Activity 3.

Final Report:

This research provided the basis for a graduate student Master's thesis, chapters of a student's PhD dissertation, and an undergraduate student's honors thesis, which are available to other scholars via ProQuest. Important insights were also shared at the International Society for Freshwater Science conference, and research was published in multiple peer-reviewed academic journals. Specimens are available for public use in the University of Minnesota's Insect Museum with supplemental data files. We created communication materials and used one-on-one online interviews to evaluate messaging and to inform future outreach efforts. Our team created a website with educational materials that showcase stream food webs in the winter, the lifecycle dynamics of winter aquatic insects, and their vital importance to Minnesota streams.

V. PROJECT BUDGET SUMMARY:

Explanation of Capital Expenditures Greater Than $5,000: *Not applicable*

Explanation of Use of Classified Staff: *Not applicable*

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

Total Estimated Personnel Hours: 8320	Divide by 2,080 = TOTAL FTE: 4.0

Other Funds:

SOURCE OF AND USE OF OTHER FUNDS *None, not applicable*	**Amount Proposed** *None*	**Amount Spent**	**Status and Timeframe**
Other Non-State $ To Be Applied to Project During Project Period:			
	$ *None*	$	
Other State $ To Be Applied to Project During Project Period:			
	$ *None*	$	

Past and Current ENRTF Appropriation:			
Past appropriation to Ferrington fully spent. No remaining $$	$ *None*	$	
Other Funding History:			
None for this project	$ *None*	$	

VI. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

Name	**Title**	**Affiliation**	**Role**
Not applicable/None			

B. Partners NOT receiving ENRTF funding

Name	Title	Affiliation	Role
Not applicable/None			

VII. LONG-TERM- IMPLEMENTATION AND FUNDING:

VIII. REPORTING REQUIREMENTS:

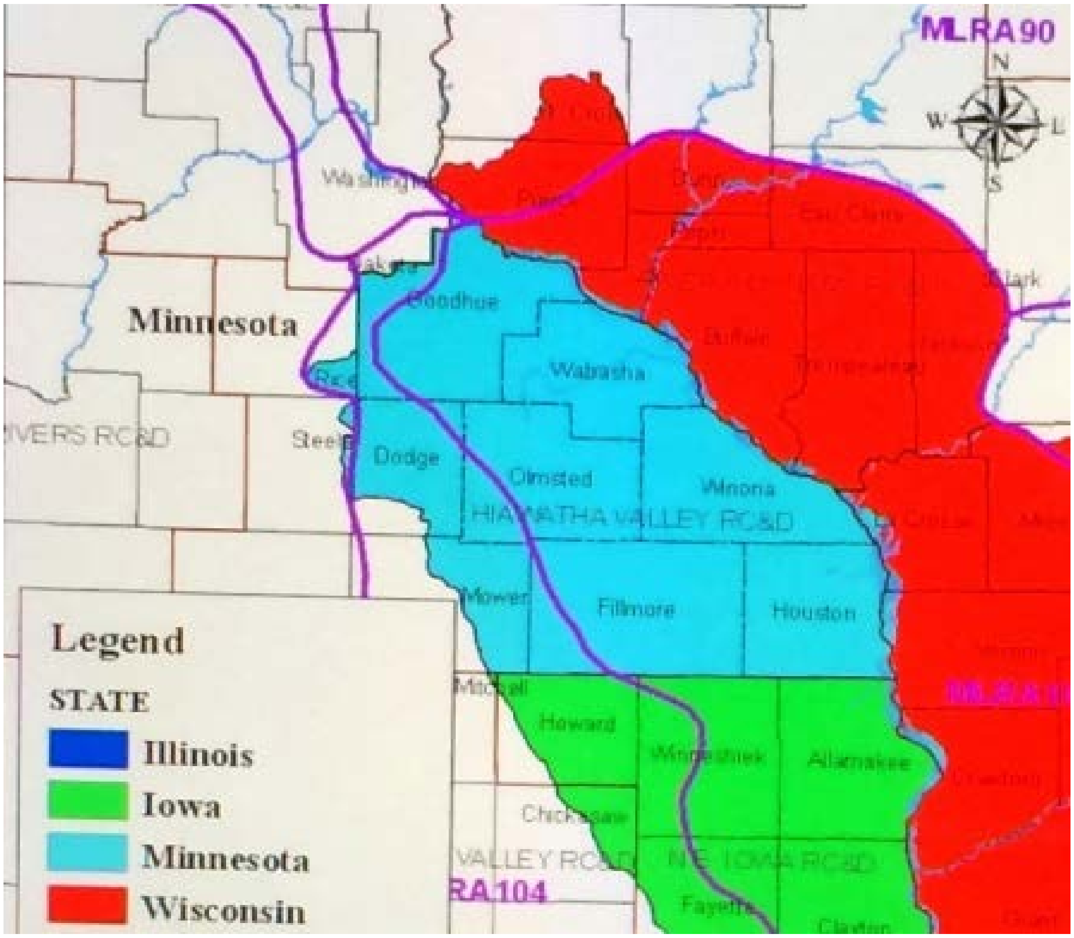
- **The project is for 3 years, will begin on July/01/2018, and end on June/30/2023. • Periodic project status update reports will be submitted January/30 and June/30 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2023.**

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet: Follows
B. Visual Component or Map: Follows
C. Parcel List Spreadsheet: Not applicable
D. Acquisition, Easements, and Restoration Requirements: Not applicable
E. Research Addendum: Follows

PROJECT TITLE: Variable Winter Thermal Regimes and Managing Trout Streams Principal Investigator: Len Ferrington, Rebecca Swenson

Map of area in which project activities will occur. The area shown in blue (below) roughly corresponds to the counties that are part of the Driftless Region in Minnesota. Streams to be used in the study all occur in the Driftless Region in Minnesota, and will be located in Goodhue, Dodge, Wabasha, Olmsted, Winona, Mower, Filmore and/or Houston counties. We have previously worked in 40 streams in these counties. Final selections of streams to be used for this project will be made after extensive consultations with our partner organizations and stakeholder groups (e.g., MN DNR, USFWS, Trout Unlimited, FFA, Farm Bureau and local citizen monitoring and county extension groups).



(NOTE: This map has been modified from a larger map prepared by David C. Wilson, as a resource for the Driftless Area Initiative in Minnesota, Wisconsin, Iowa and Illinois, and credit for the product is acknowledged).

Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2018 Budget Spreadsheet Final
Project Title: Improve Trout-Stream Management by Understanding Variable Winter Thermal Conditions
Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 03i
Project Manager: Rebecca Swenson
Project Title: Variable Winter Thermal Regimes and Managing Trout Streams
Organization: University of Minnesota
College/Department/Division: Department of Agricultural Education & Communication
M.L. 2018 ENRTF Appropriation:
Project Length and Completion Date: Five years, June 2023
Date of Report: March 15, 2024

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Amended Budget 1/30/23	Amount Spent	Balance
BUDGET ITEM			
Personnel (Wages and Benefits) - Overall	**$283,498**	**$276,265**	**$7,233**
Ferrington: 5% annually, salary (Total estimated amount $17,854), Ferrington: fringe benefits (33.5%)(Total estimated amount $5,981)			
Swenson: Salary, 2 months/year first & send year, one month third year (Total estimated amount $48,280), Swenson: fringe benefits (33.5%) (Total estimated amount $16,174)			
PostDoc, Jan-May Salary and Fringe			
Graduate Student #1: salary (50% time)(Total estimated amount $87,655), fringe benefits (Total estimated amount $13,150), tuition (Total estimated amount $47,038)			
Graduate Student #2: salary (25% time), fringe benefits, tuition (Total estimated amount $25,288)			
Undergraduate student technician #1 (39 weeks/year @ 10 hours/week at $ 10.25/per hour (Total estimated amount $11,034)			
Undergraduate student technician #2 (39 weeks/year @ 10 hours/week at $ 10.25 per hour (Total estimated amount $11,034)			
Temp/Casual employee			
Equipment/Tools/Supplies			
Miscellaneous disposable lab supplies and chemicals	*$14,500*	*$11,594*	*$2,906*
MtDNA Analysis (860 samples per year at $12/sample)	*$31,740*	*$8,212*	*$23,528*

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Amended Budget 1/30/23	Amount Spent	Balance
temperature recording devices (35/yr @ $135/device)	*$14,532*	*$7,144*	*$7,388*
Printing			
Internal reports, interim external communications and final summaries to stakeholder groups, focus group reports, non-technical summaries for general public such as Trout Unlimited, and technical publications in journals	*$15,900*	*$2,410*	*$13,490*
Travel expenses in Minnesota - overall			
Vehicle Rental (21 days @ $ 48.0	*$7,000*	*$6,976*	*$24*
Travel (mileage = 9000 miles @ $	*$14,809*	*$4,228*	*$10,581*
Room rental (25 nights @ $ 85.00/night X 2 people)(Total estimated amount $13,071)	*$9,171*	*$178*	*$8,993*
Per diem ($ 36/day, 26 days, X 2	*$5,758*	*$83*	*$5,675*
Other			
Licenses and state park fees	*$692*	*$0*	*$692*
Travel for interviews (by focus gro	*$1,000*	*$0*	*$1,000*
Participant incentive for focus gro	*$1,200*	*$550*	*$650*
Participant incentive for survey - $	*$200*	*$0*	*$200*
COLUMN TOTAL	**$400,000**	**$317,640**	**$82,360**