Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2018 ENRTF Work Plan (Main Document)

Today's Date: February 26, 2018

Date of Next Status Update Report: 31 January 2018

Date of Work Plan Approval:

Project Completion Date: June 30, 2021

Does this submission include an amendment request? No

PROJECT TITLE: Variable Winter Thermal Regimes and Managing Trout Streams

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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: $0

Balance: $ 400,000

Legal Citation: M.L. 2018, Chp. xx, Sec. xx, Subd. xx

Appropriation Language:

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 histories and autecologies 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

Second Update June 30, 2019

Third Update January 31, 2020

Fourth Update June 30, 2020

Fifth Update January 31, 2021

Final Update June 30, 2021

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 Drifless 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 a 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 Work Flow (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
Re-set and re-deploy water temperature probes	Mar. 2020	end Mar. 2020
Begin data analysis, check for outliers, malfunctions, etc.	Apr. 2020	end Apr. 2020
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 similar to 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 pin point 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, measureable outcome:* Develop thermal models (TM) 7 streams, 5 sites/str, year 1	*June 2019*
2. *Specific, measureable outcome:* Develop TM for 7 more streams, 5 sites/str, year 2	*June 2020*
3. *Specific, measureable outcome:* Develop TM for 7 more streams, 5 sites/str, year 3	*June 2021*

First Update January 31, 2019

Second Update June 30, 2019

Third Update January 31, 2020

Fourth Update June 30, 2020

Fifth Update January 31, 2021

Final Update June 30, 2021

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 (PART 1) Work Flow (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
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 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
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)***	***(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
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**	**end Dec. 2019**
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
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 medotae*, 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).

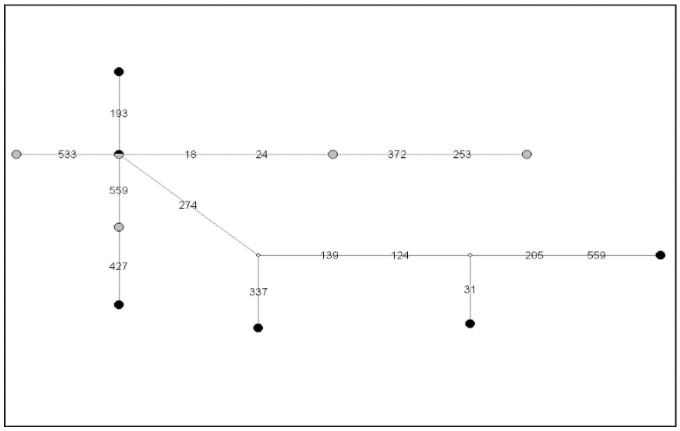

Figure 1. Median joining network of 10 haplotypes identified for *Diamesa mendotae*.
Haplotypes are coded by population origin. MN: grey; WI: black; median vectors: white.
Numbers indicate mutated positions in the alignment.

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 2021*

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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 2021*

First Update January 31, 2019

Second Update June 30, 2019

Third Update January 31, 2020

Fourth Update June 30, 2020

Fifth Update January 31, 2021

Final Update June 30, 2021

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.

First Update January 31, 2019

Second Update June 30, 2019

Third Update January 31, 2020

Fourth Update June 30, 2020

Fifth Update January 31, 2021

Final Update June 30, 2021

V. PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview: See attached budget sheet

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:

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

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

Enter Total Estimated Personnel Hours: 0	Divide by 2,080 = TOTAL FTE: 0

B. 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:

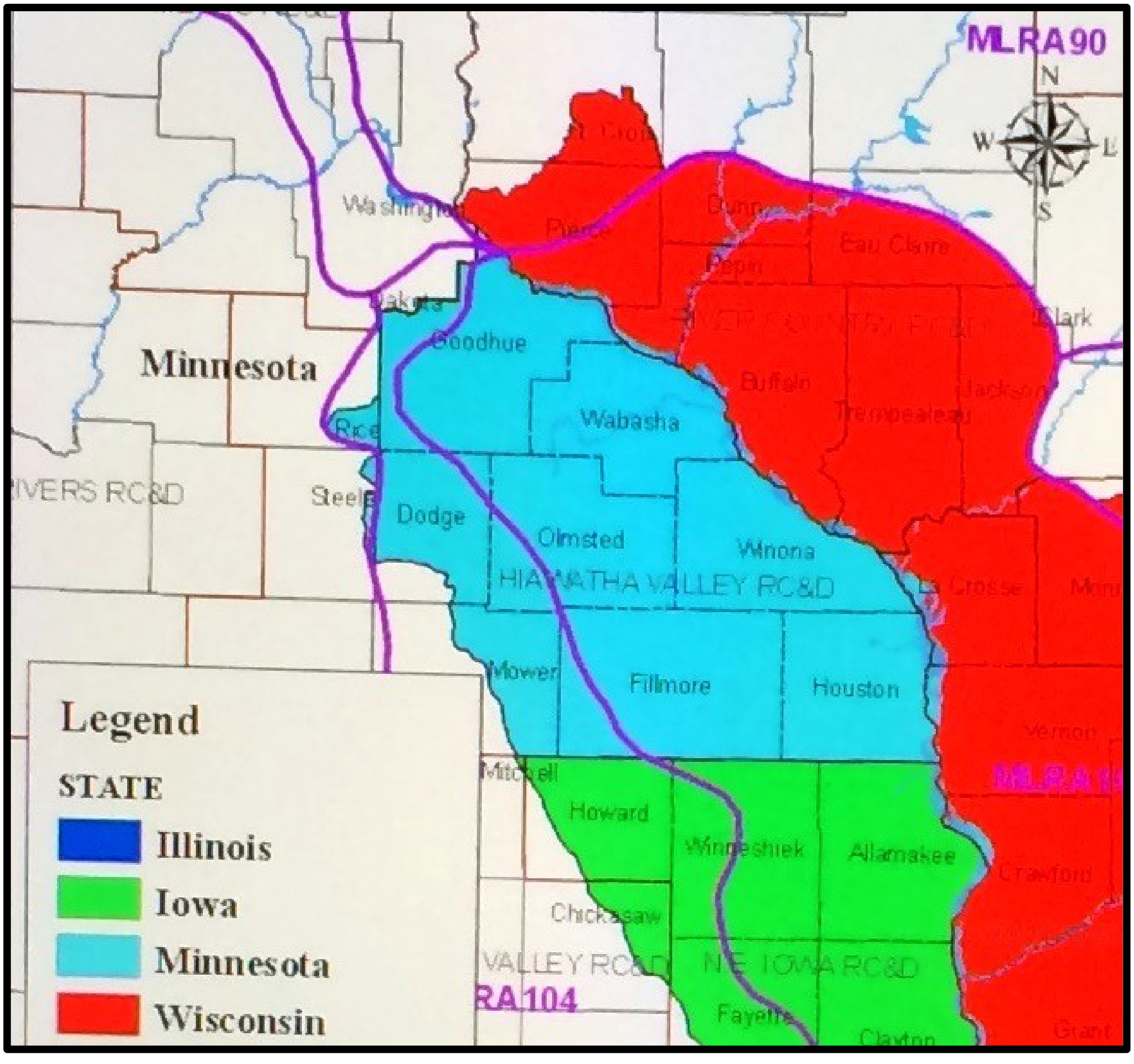
- **The project is for 3 years, will begin on July/01/2018, and end on June/30/2021.**
- **Periodic project status update reports will be submitted January/30 and June/30 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2021.**

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

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 (eg., 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

Project Title: Variable Winter Thermal Regimes and Managing Trout Streams
Legal Citation:
Project Manager: Leonard C. Ferrington Jr.
Organization: University of Minnesota
College/Department/Division: Department of Entomology
M.L. 2018 ENRTF Appropriation:
Project Length and Completion Date: Three years, June 2021
Date of Report: February 26, 2018

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Budget	Amount Spent	Balance
BUDGET ITEM			
Personnel (Wages and Benefits) - Overall	$283,498	$0	$283,498
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)			
Graduate Student #1: salary (50% time)(Total estimated amount $87,655)			
Graduate Student #1: fringe benefits (Total estimated amount $13,150)			
Graduate Student #1: 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)			
Equipment/Tools/Supplies:			
Miscellaneous disposable lab supplies and chemicals	$14,500	$0	$14,500
MtDNA Analysis (860 samples per year at $12/sample)	$31,740	$0	$31,740
temperature recording devices (35/yr @ $135/device)	$14,532	$0	$14,532
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	$0	$15,900
Travel expenses in Minnesota - overall	$36,738		$36,738
Vehicle Rental (21 days @ $ 48.00/day) (Total estimated amount $3,100)			
Travel (mileage = 9000 miles @ $ 0.535/mile)(Total estimated amount $14,809)			
Room rental (25 nights @ $ 85.00/night X 2 people)(Total estimated amount $13,071)			
Per diem ($ 36/day, 26 days, X 2 people)(Total estimated amount $5,758)			
Other			
Licenses and state park fees	$692	$0	$692
Travel for interviews (by focus groups of landowners and stakeholders)	$1,000	$0	$1,000
Participant incentive for focus groups - $50 for 24 participants = $1200	$1,200	$0	$1,200
Participant incentive for survey - $100 x 2 = $200	$200	$0	$200
COLUMN TOTAL	**$400,000**	**$0**	**$400,000**