2010 Project Abstract
For the period ending June 30, 2013

PROJECT TITLE: ***Predicting and Mitigating Vulnerability of Trout Streams***
PROJECT MANAGER: Leonard C. Ferrington Jr.
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http://www.entomology.umn.edu/midge/People/Ferrington/Ferrington.htm
FUNDING SOURCE: Environmental and Natural Resources Trust Fund
LEGAL CITATION: ML 2010, Chap. 362, Sec. 2, Subd. 5i.

APPROPRIATION AMOUNT: $ 300,000.00

<u>Overall Project Outcome and Results</u>

Trout streams in southeastern Minnesota differ markedly in brown trout abundance and growth during winter. Our project objectives were to better understand stream thermal regimes, fish feeding, and fish growth patterns between November and March, so habitat management strategies can be designed to maximize trout production. Prior to this study there was very little detailed knowledge of the winter diets of trout, and virtually no knowledge of the kinds and quantitative abundances of aquatic insects growing during winter. To achieve project objectives, we assessed trout lengths and mass two or three times per winter in 36 streams (12 streams/year for three years) and determined the types of aquatic invertebrates eaten by the trout, the abundances of these dietary organisms in the streams, and the corresponding patterns of trout growth. Our findings show trout are most abundant in streams where groundwater (springs and seeps) inputs keep water temperatures significantly warmer and ice-free in winter. These thermal conditions promote high abundance or emergence of aquatic insects specifically adapted for emergence and reproduction in winter, even when air temperatures are substantially below freezing. Some species that we discovered have never been described and are new to science. We developed predictive models relating air temperatures to water temperatures in areas buffered by groundwater. The models also demonstrate linkages between groundwater input and (1) the corresponding aquatic insect composition and their abundances, (2) the trout diets during winter and (3) trout growth patterns as a function of types of aquatic insects eaten. Based on our predictive models we are able to recommend conditions under which in-stream habitat management efforts can be better spatially focused to maximize trout growth and abundance. This information is being communicated to Trout Unlimited and the MN Department of Natural Resources to help inform their programs to manage trout streams.

Project Results Use and Dissemination

Our results have been presented at local, state, regional, national and international scientific meetings and at local and state conservation planning sessions. Staff of the MN DNR assisted with much of our field work and have participated in interpreting and writing summaries and drafts of manuscripts for peer review. Consequently, they are very familiar with our findings. In addition, we are communicating our results to regional Trout Unlimited members, and hope to be able to discuss how our findings can help guide the in-stream habitat improvement programs. Two theses have been completed, and three additional graduate students will use portions of our findings as sections for their Ph.D. dissertations. One undergraduate worked on a class activity in Spanish to help serve as an “in-reach” effort to inform undergraduates in areas such as humanities and arts of our research. One newspaper article was written, and we have put videos of our field work on-line for public viewing via our Facebook sites.

Environment and Natural Resources Trust Fund (ENRTF)
2010 Work Program: FINAL REPORT

Date of Report: FINAL REPORT--- August 2013
Date of Next Progress Report: FINAL REPORT
Date of Work Program Approval:
Project Completion Date: June 30, 2013

I. PROJECT TITLE: Predicting and Mitigating Vulnerability of Trout Streams

Project Manager: Leonard C. Ferrington Jr.
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Web Site Address: The Chironomidae Research Group, on-line @ http://www.entomology.umn.edu/midge/People/Ferrington/Ferrington.htm

Location: Southeast Minnesota, counties are: Dakota, Goodhue, Rice, Wabasha, Winona, Olmsted, Dodge, Steele, Waseca, Freeborn, Mower, Fillmore, Houston

Total ENRTF Project Budget:	**ENRTF Appropriation**	**$ 300,000.00**
	Minus Amount Spent:	**$ 282,582.00**
	Equal Balance:	**$ 17,418.00**

Legal Citation: ML 2010, Chap. 362, Sec. 2, Subd. 5i.

Appropriation Language: This appropriation "$300,000 is from the trust fund to the Board of Regents at the University of Minnesota, to assess aquatic insect abundance and water temperatures as predictors of trout growth in southeastern Minnesota and assess options to minimize stream temperature changes.

II. FINAL PROJECT SUMMARY AND RESULTS: Trout require streams with excellent water quality that are fed by groundwaters which keep streams cold in summer but ice-free in winter. The trout sport-fishing industry is vulnerable to global climate changes that can increase stream temperatures, alter the cold-adapted aquatic insects that form trout diets, and affect trout reproduction. Increasing air temperatures are predicted to increase the maximum water temperatures during summer, but also are very likely to dramatically change winter thermal conditions in trout streams. Our objectives are to: (1) investigate the role of stream bank vegetation and adjacent land use to minimize changes in stream temperatures in relation to climate change during summer; (2) determine winter diets and growth of trout populations; and (3) determine kinds, abundances, and timing of growth patterns of cold-adapted insects that are essential in winter diets of trout. We will work on 36 trout streams in the Driftless Area in SE Minnesota, using GIS coupled with habitat surveys for objective (1); seining and standard diet analysis techniques for objective (2); and rapid bioassessment protocols for objective (3). The project will identify and rank the streams most vulnerable to increases in summer high temperatures, and will identify cold-adapted insects that are most critical to trout diets and growth during winter. Trout fishing annually provides more than $150 million dollars in direct expenditures to local economies in Minnesota and $654 million through the Driftless Region (Trout Unlimited, 2008). With re-circulating dollars this represents more than one-billion dollars of economic stimulus to local economies. Our results will enable us to identify streams and food species that are most vulnerable to increasing temperatures, and translate scientific results into management strategies to protect and conserve this valuable industry.

III. PROGRESS SUMMARY AS OF *[insert date of Work Program progress report]*:

December 2010

During summer 2010 we were notified that one graduate school fellowship would be awarded to one of the students recruited to work on Result #3 of this project. The graduate school fellowship includes a one-year student stipend, coverage of health care, fringe benefits and tuition (with an approximate value of $ 35,980). In addition, Ferrington received a grant from the U. S. Forest Service that provided some additional resources to work in the trout streams. Consequently, we were able to do all reconnaissance of sites for the first year, to complete field work related to the first round of sampling for Results #2 and #3, and make timely progress to completing Result #1 by this report date.

August 2011

Over the past six months we completed all field work scheduled for the first year of our project. We also began lab processing of diet and invertebrate samples, located and collated historical water temperature data for our streams that we worked in, and set temperature probes to collect data concurrent with our sampling periods. Temperature data we down loaded and archived for analysis which will be used to develop our predictive models.

December 2011

During this report period we selected 12 additional trout streams to analyze during November 2011 through March 2012. The streams were selected from a set of 16 potential streams recommended by Fisheries Biologists from the MN DNR. Our final selection of 12 streams was done after site visits to determine the streams that best fit our profiles of stream habitat and thermal conditions appropriate for the research objectives of this project. In addition, we were able to locate historical stream water temperature data for 28 more trout streams in SE MN. The twelve streams selected for analysis during this report period were included in the 28 additional streams for which historical data were located. Consequently, we now have temperature data for 40 trout streams.

June 2012

Over the past six months we completed all field work scheduled for the second year of our project. We also began lab processing of diet and invertebrate samples, located and collated historical water temperature data for our streams that we worked in, and set temperature probes to collect data concurrent with our sampling periods. Temperature data were downloaded at various intervals and archived. These newer data will be used to validate our predictive models.

December 2012

During this report period we selected 12 additional trout streams to analyze during November 2012 through March 2013. The streams were selected from a set of 15 potential streams recommended by Fisheries Biologists from the MN DNR. Our final selection of 12 streams was done after site visits to determine the streams that best fit our profiles of stream habitat and thermal conditions appropriate for the research objectives of this project. The twelve streams selected for analysis during this report period were included streams for which historical data were located. Consequently, we will have predictive temperature models for 40 trout streams and comprehensive biological data for 36 of the 40 streams.

June 2013

During this report period we completed all field work on the 12 additional trout streams that are the final twelve streams of the project. The field sampling for this last set of 12 streams was initiated in November 2012 and continued through March 2013, after our last round of sample collections. This last set of 12 streams were chosen from a set of 15 potential streams recommended by Fisheries Biologists from the MN DNR after completing reconnaissance visits during our previous report period. All 12 streams fit our profiles of stream habitat and thermal conditions appropriate for the research objectives for the third year of this project and included streams for which historical data were available. Consequently, we now have predictive temperature models for 40 trout streams and comprehensive biological data for 36 of the 40 streams. During this last report period one paper was published related to the outcome of our Result 1 in a peer-reviewed journal. A second manuscript, related to our outcomes for Result 2, was revised and resubmitted to a second peer-reviewed journal. We received formal acceptance of our revised manuscript in early August 2013. We anticipate writing at least two more manuscripts related to finding from Result 2, and at least two more manuscripts related to outcomes of our Result 3 during the remainder of this calendar year (2013).

AMENDMENT REQUEST (6/30/13)

During the performance period of this grant we have had some cost-savings as a result of sharing expenses for some equipment, disposable supplies and travel from other grants that were approved after this project was accepted for funding. We also were able to support two undergraduates with funding from the Undergraduate Research Opportunities Program at the University of Minnesota. No funds from this grant were used to support these two undergraduate students. They both provided substantial *lab help analyzing scales of brown trout to develop age estimates, and used this information to calculate growth rates for fish captured and measured during the first two years of the project. In addition, several undergraduates provided volunteer help during field work. Consequently, our project came in under budget.*

In addition to the cost-savings associated with equipment, disposable supplies, travel and undergraduate involvement, we also had some cost over-runs associated with graduate student duties during the project. These cost over-runs resulted from higher diversity of invertebrates than expected in our quantitative hess samples and, to a lesser degree, to the diversity of diet items obtained. The aquatic insect densities in several of the streams were also greater than anticipated and the time required to sort, identify and quantify the samples far exceeded our budgeted amounts. To complete these tasks in a timely manner (and within the performance period of this grant) it was necessary to incorporate three additional graduate student assistants into the lab work during the last report period. This resulted in a cost over-run in personnel wages and associated tuition expenses. The over-run in tuition expenses was also exacerbated by higher than predicted annual increases during the performance period of the grant.

IV. OUTLINE OF PROJECT RESULTS*:*

Result 1: The physical, geologic and riparian settings in which stream systems occur are known to modulate surface water temperatures. We hypothesize that specific combinations in these parameters can result in more effective buffering of summer water temperatures in trout streams of southeastern Minnesota. We will seek to identify the combinations that function on local- to landscape-levels to produce the most buffered thermal conditions.

Description: Result 1 is structured to determine present-day configurations of riparian vegetation, adjacent land use and geological setting that provide the greatest capacity to buffer changes in thermal regime of stream waters during both summer and winter over the largest longitudinal distances of stream, and thus maximize habitats appropriate for foraging and reproduction of trout. Streams not fitting this profile will be considered as "most at-risk" as global climates warm and can be targeted for management.

Summary Budget Information for Result 1: ENRTF Budget: $95,085.00
Amount Spent: $95,085.00
Balance: $00,000.00

Deliverable/Outcome for Result 1	Completion Date	Budget
1. *Analysis of first set of 12 streams* (Estimates of adjacent land use percentage, summary of local geology and type/depth to bedrock or broken rock strata, percent riparian cover,	Fall 2010	$30,760

patterns of variation in water temperature, stream width, depth in riffles and pools, and cross sectional profiles. We expect streams where trout grow fastest during winter will have unique combinations of geology, substrate compositions that interact to produce the most highly buffered water temperatures)		
2.*Analysis of second set of 12 streams* (Same details as for deliverable #1, above)	Summer 2011	$31,680
3.*Analysis of third set of twelve streams* (Same details as for deliverable #1 & #2, above)	Fall 2011	$32,645

Result 1 Estimated Completion Date: December 2011
NOTE: Result 1 took longer than anticipated to complete. The last work was completed by December 2012. The person working on this result completed a Master's Degree thesis from the concepts and data generated by this Result). At this time all research related to Result 1 has been completed and a final manuscript has been submitted and accepted for publication. A pdf copy of the publication and an electronic data base of all forty models will be submitted as the deliverables for Result 1.

Result 1 Status as of: December 2010
Twelve streams were selected for investigation based on availability of long term water temperature data. All sites were visited and adjacent land use recorded. Descriptions of in-stream substrate compositions were compiled and stream width, depth in pools and profiles were determined. Existing data layers for local geology, land use/ land cover were located and downloaded. We began to compile a series of regression analyses of air temperatures (as independent variable) versus water temperatures (dependent variable) to determine the influence of air temperature on water temperatures. For each stream we will determine the slope and intercept. Slope of the least-squares regression line will describe the response of stream temperature to ambient water temperatures. Intercepts will describe the average water temperatures when mean air temperatures at zero degrees centigrade. The buffering effect of local groundwaters can be compared with the regression models, and we will be able to rank each stream according to its susceptibility to changing climate conditions.

One presentation of preliminary results for 12 streams was given by Lori Krider (student assigned to this Result) during this report period. Authors, title and meeting details are:

Krider, L. A., J. Perry, J. A. Magner, B. Vondracek, & L. C. Ferrington, Jr. "Air-water Temperature Relationships in the Trout Streams of Southeastern Minnesota's Carbonate - Sandstone Landscape". Presented at the 2010 Midwest Fisheries and Wildlife Conference; Minneapolis, MN.

Result 1 Status as of: August 2011

Computer analysis of air and stream water temperatures showed that the greatest predictive power resulted from using data that are aggregated at weekly temporal scales, with mean weekly water temperatures versus mean weekly air temperatures, respectively, as dependent and independent variables. In our models, a slope of 1 would indicated that changes in mean weekly air temperatures and mean weekly water temperatures vary in a 1-to-1 relationship, and the y-intercept predicts mean stream water temperature when mean weekly air temperatures equal zero. Our results demonstrate that both slopes and intercepts showed variability across the twelve streams. In all cases, however, the models for each stream yielded slopes were less than 1, demonstrating amelioration of stream water temperature by input of groundwaters, and intercepts were higher than zero. We are on-schedule with the outcome for Result 1, and expect to build another set of twelve models during the next report period, after we select the next set of twelve streams to work on during year two of our project.

Result 1 Status as of: December 2011

During this reporting period we located stream water temperature data for 28 additional trout streams in SE MN. We used the same modeling approach as was used for our set of twelve streams that were worked on during the last reporting period. This has resulted in 40 comprehensive models of surface water temperature relationships to air temperatures (again using data aggregated at weekly temporal scales) of trout streams in SE MN.

Twelve of the streams that we have modeled were sampled for fish and invertebrates during winter of our first year of this project (July 2010-June 2011), and twelve of the streams are streams that have been selected for analysis during winter of year 2 (July 2011-June 2012). We anticipate that streams which we will consider as appropriate for sampling during winter of year three of this project (July 2012-June 2013) will be among the remaining 16 streams that we have modeled. Consequently, all 36 streams to be used in this project will have a comprehensive, empirically-based model. We will them be able to rank all the 36 streams with regard to the influence of groundwater on thermal regime (using slope and intercept values). This will enable us to compare and contrast trout diets and invertebrate composition and abundance as a function of slope and intercept to quantitatively interpret how groundwater input relates to trout growth during winter.

During the next reporting period we will summarize our results for all forty streams in manuscript form, and will select a professional journal for possible publication of our model results. As a consequence, we may not complete this outcome for Result 1 according to our anticipated time schedule provided in our work plan.

One presentation of comprehensive results for forty trout streams was given by Lori Krider (student assigned to this Result) during this report period. Authors, title and meeting details are:

Krider, L. A., J. Perry, J. A. Magner, B. Vondracek, & L. C. Ferrington, Jr. "Air-water Temperature Relationships in the Trout Streams of Southeastern Minnesota's Carbonate - Sandstone Landscape". Present at the 2011 Water Resources Conference; St. Paul, MN

Result 1 Status as of: June 2012
One refined and final presentation of comprehensive results for forty trout streams was given by Lori Krider (student assigned to this Result) during this report period. Authors, title and meeting details are:

Krider, L. A., J. Perry, J. A. Magner, B. Vondracek, & L. C. Ferrington, Jr. "Air-water Temperature Relationships in the Trout Streams of Southeastern Minnesota's Carbonate - Sandstone Landscape." Present at the 2012 Driftless Area Symposium; LaCrosse, WI

Ms. Lori Krider used the findings from Result 1 as the primary content for her thesis, and received her M. S. degree in June 2012. Her thesis was titled: "Air-water Temperature Relationships in the Trout Streams of Southeastern Minnesota's Carbonate-sandstone Landscape: Implications for Climate Change, Brown Trout Biological Processes, and Land Management." Lori Krider was advised by Jim Perry and Joe Magner, both of whom are collaborators on this project.

After completion of her M. S. degree, Lori Krider continued to contribute to our research effort and worked on a manuscript for publication through the end of this report period. Progress on the manuscript went a little slower than anticipated, however it is expected that the manuscript draft will be completed and submitted for publication during the next reporting period.

Result 1 Status as of: December 2012
Lori Krider continued to contribute to our research effort during this report period. She finished a draft, obtained input from Magner, Perry, Vondracek and Ferrington, and integrated our suggestions into a final draft which was submitted to the Journal of the American Water Resources Association (JAWRA) in October 2012. The manuscript was tentatively accepted for publication (pending revisions based on suggestions from peer review). The manuscript was revised and re-submitted and has been accepted for publication. An electronic copy of the publication is included with this report.

Final Report Summary: June 2013
All field and lab research for Result 1 was completed prior to this report period. The final outcomes for Result 1 are provided below.

Outcomes of Result 1 include:
(1) Successful completion of a Master's Degree and Master's Thesis by Ms. Lori Krider

(2) Quantitative models relating air temperatures to water temperatures in 40 trout streams of southeastern Minnesota. The models will help prioritize trout stream restoration efforts by state and non-profit organizations by indication conditions where in-stream modifications will be most effective versus areas where riparian modifications are likely to result in better habitat for brown trout

(3) Three presentations of our research findings for Result 1 at local, national or international conferences over the performance period of this project.

(3) One publication of our research finding in a peer-reviewed national journal. Citation for this publication follows:

Lori A. Krider, Joseph A. Magner, Jim Perry, Bruce Vondracek, and Leonard C. Ferrington, Jr. 2013. *AIR-WATER TEMPERATURE RELATIONSHIPS IN THE TROUT STREAMS OF SOUTHEASTERN MINNESOTA'S CARBONATE-SANDSTONE LANDSCAPE. JOURNAL OF THE AMERICAN WATER RESOURCES ASSOCIATION. Vol. 49, No. 4: 896-907.*

Summary of What Was Learned and Potential Benefits: The local geological setting influences groundwater movement and controls areas where the water –resurfaces as springs of bed-seepage from bank margins or directly into the stream channel. The quantity of spring water related to surface water in a trout stream varies spatially as the stream valley cuts through differing geological strata. Consequently, the amount of groundwater entering into the stream influences the thermal regime differentially along the length of a stream, and varies from stream-to-stream. Our regression modeling allows us to use the empirically-derived relationships between air temperatures and water temperatures to guide decisions where either (1) instream habitat modifications or (2) riparian zone modifications will likely be most effective in terms of improving conditions for trout growth during winter. In our publication (Krider *et al.,* 2013) we define and differentiate areas of trout streams that where "*temperatures are more meteorologically than groundwater controlled*" and provide the following recommendation for management strategy:

> *"Thus, we suggest restoration for streams that fall on the meteorological control end of the spectrum and protective measures for streams that fall on the hydrological control end of the spectrum (Figure 4). Streams on the hydrological control portion of the spectrum are most likely fed by relatively large quantities of groundwater, and could be targets for protection because they fulfill the basic requirement for producing ideal coldwater habitat"*

We will work with the MN DNR and Trout Unlimited to determine if, or to what extent, this recommendation can help inform and prioritize their habitat improvement activities in trout streams.

Result 2: Diets of trout during winter are poorly documented and often reported at taxonomic levels that mask the importance of individual species (e.g. Chironomidae are known to be common prey, but more than 50 genera could be included in the diet). Without more detailed knowledge of the insect taxa that trout consume in winter, and the thermal preferences and life-history biology of these prey insects, it is not possible to predict how increasing thermal regimes will influence trout diets. We hypothesize that differing thermal preferences and life histories of prey will be important controls on winter growth and yield of trout, and that these differences in prey have potential to account for a large amount of the variability in trout yield that is presently known for streams in southeast Minnesota.

Description: Trout will be obtained using routine electro-shocking methods during December through March in each of 12 streams/year for the three years of our study. The streams will be the same as investigated for Objectives 1 and 3. Diet will be determined using a gastric-lavage technique, modified for use in winter. We have successfully used our Standard Operating Procedure (SOP) for the technique over the past two winters and are confident that it is an appropriate technique for this objective. After identification and quantification of diet items, the resulting data will be analyzed with a fish bioenergetics model (Hanson *et al*. 1997) to determine the extent to which patterns of increasing UCS aquatic insect species can be quantitatively related to caloric density.

Field work for Result 2 will be coordinated with field work for Result 3 and, when possible, will be completed concurrently. Field work each year will be done on the same 12 streams as were analyzed in Result 1. Field work will be initiated in mid-November and completed by mid-March. Sample processing, data analyses and summary will be completed by the end of June for each of the three years of the grant. The summary prepared during year three will cover results from all three years and will included a full-project synthesis of results obtained.

Summary Budget Information for Result 2: **ENTRF Budget:** **$ 96,487.00**
Amount Spent: **$ 90,316.00**
Balance: **$ 6,171.00**

Deliverable/Outcome for Result 2	Completion Date	Budget
1.Analysis of fish diets in first set of 12 streams **(Quantification of types and quantities of food items consumed, scientific names and trophic habits of invertebrates that are eaten, analysis of monthly variation and variation across streams in compositions of diets, summaries of life stage for each species of food eaten by fish. We expect winter diets will consist primarily of ultra-cold adapted, winter developing aquatic insects such as Chironomidae and Plecoptera in streams**	Summer 2011	$31,213

where trout grow fastest during winter)		
2.Analysis of fish diets in second set of 12 streams **(Same details as for deliverable #1, above)**	Spring 2012	$32,150
3.Analysis of fish diets in third set of 12 streams (**Same details as for deliverable #1 and #2, above)**	Spring 2013	$33,124

Result Completion Date: March 2013

Result 2 Status as of: December 2010
Fish were collected using electroshocking in all 12 streams. Up to 30 specimens were subjected to gastric lavage to recover food items in the guts of the fish. The gut contents were sieved and preserved in the field for analysis and identification. Total length, mass and age class determinations were performed and recorded for each fish before returning them to the water.
A presentation of preliminary results for 12 streams was given by Jennifer Cochran-Biederman during this report period. Jennifer Cochran-Biederman is employed by Saint Mary's College and is a Ph.D. degree student in the Water Resources Sciences Program at the University of Minnesota. Jennifer is funded through another grant (Bruce Vondracec, PI) but her research interests include dynamics of trout through winter, and she volunteers her time (as available) to assist with field work and laboratory tasks. She will collaborate with us on presentations and publications of information learned from Result 2. Authors, title and meeting details are:

Cochran-Biederman,Jennifer, Leonard Ferrington, Bruce Vondracek, James Perry, Joe Magnar, William French, Jane Louwsma, Lori Krider & Petra Kranzfelder. "Mitigating the Effects of Climate Change on Coldwater Streams in Southeastern Minnesota". December 13, 2010. Midwest Fish and Wildlife Conference. Minneapolis, MN.

Result 2 Status as of: August 2011
All field sampling (including electroshocking and gastric lavage) during this report period was completed as scheduled. Up to 30 specimens (including a variable number of recaptured specimens) per stream were subjected to gastric lavage. Total length, mass and age class determinations were performed and recorded for each fish before returning them to the water. Gut contents for fish collected during the preceding report period were identified and quantified, and all data for total length and mas of the fish were computerized and summary statistics were computed. Age class determinations were predicted from length data, but still need to be independently confirmed through scale analyses. We anticipate that scale analyses will be initiated during the next report period, and age-class determinations will be confirmed or refined. We are on-schedule with all tasks related to these outcomes for Result 2.

Presentations of preliminary results for 12 streams were given by Will French (student assigned to this Result) and Jennifer Cochran-Biederman during this report period. Authors, titles and meeting details are:

French, W.E., L. Ferrington, B. Vondracek, J. Perry, J. Magnar, J. Biederman, J. Louwsma, L. Krider, P. Sherman, P. Kranzfelder. " Winter diets and dynamics of brown trout in groundwater dominated streams. 2011. 141st Annual meeting of the American Fisheries Society. Seattle, WA.

French, W.E., L. Ferrington, B. Vondracek, J. Perry, J. Magnar, J. Biederman, J. Louwsma, L. Krider, P. Sherman, P. Kranzfelder. Mitigating the effects of climate change on cold water streams in southeastern Minnesota. 2011. Minnesota Chapter of the American Fisheries Society Annual Meeting. Sandstone, MN

Cochran-Biederman, Jennifer, Leonard Ferrington, Bruce Vondracek, James Perry, Joe Magnar, William French, Jane Louwsma, Lori Krider & Petra Kranzfelder "Mitigating the Effects of Climate Change on Coldwater Streams in Southeastern Minnesota." March 16, 2011. Driftless Area Stream Restoration Symposium. La Crosse, WI.

Result 2 Status as of: December 2011

During this reporting period we continued to process, identify and quantify the gut contents collected through gastric lavage of fish collected during our field sampling events during winter of year one. All length/weight data were computerized and age classes predicted based on patterns in the data set.

In September of this year, we integrated another student into our project to analyze samples of scales taken from fish. This student is an honors student in the Department of Fisheries and Wildlife, and the project will serve as the basis for his undergraduate honors project that is required of all students seeking to graduate with honors status in our college. This student has also obtained an Undergraduate Research Award from the University of Minnesota to cover his research costs and provide him with a stipend, so there is no additional cost to our project for his efforts. We anticipate that his results will be used to help refine our estimates of growth patterns as a function of fish age across 12 streams with differing amounts of groundwater input as measured by our predictive models generated from Result 1.

The graduate student that works on this portion of our project was successful in obtaining a small exploratory research grant to use a newly developed technique to determine feeding status of trout using isotopic signatures that can be obtained from mucus of trout. This innovative technique in non-invasive to fish, and is anticipated to provide additional insight into feeding patterns of trout with differing growth rates. We are very excited about trying this technique, and if it yields reproducible results we will seek additional funding from other sources to expand this type of analysis during year three of this grant.

Results from year one of our project show that two sampling events is sufficient to provide quantitative estimates of change in mass and length of trout during winter. Consequently, we are focusing our field efforts related to this Result in November-December and February-early March. Most field work related to trout that was *scheduled for November-December has been completed and we anticipate that the remainder will be completed within two weeks of the next reporting period (i.e., early January 2012). At this point in time we are very close to schedule with field and lab work for Result 2.*

Presentations of preliminary results for 12 streams were given by Will French (student assigned to this Result) during this report period. Authors, titles and meeting details are:

French, W.E., L. Ferrington, B. Vondracek, J. Perry, J. Magnar, J. Biederman, J. Louwsma, L. Krider, P. Sherman, P. Kranzfelder. Winter diets and dynamics of brown trout in groundwater dominated streams. 2011. 72nd Annual meeting of the Midwest Fish and Wildlife Conference, Des Moines, IA.

French, W.E., L. Ferrington, B. Vondracek, J. Perry, J. Magnar, J. Biederman, J. Louwsma, L. Krider, P. Sherman, P. Kranzfelder. Not your father's field season: Winter foraging and growth of brown trout in the Driftless Ecoregion. 2011. Fish Biology and Fisheries Seminar, University of Minnesota. St. Paul, MN.

French, W.E., L. Ferrington, B. Vondracek, J. Perry, J. Magnar, J. Biederman, J. Louwsma. Winter stream electrofishing techniques. 2011. Guest lecture Biol 248 St. Olaf College Northfield, MN.

Result 2 Status as of: August 2012
During this report period we continued to do field work, completing our second round of sampling, measurement and gastric lavage. We also collected, preserved and completed initial preparations of collections of mucus from brown trout for isotopic analysis, and collected and preserved quantities of the most abundant groups of aquatic insects and other macroinvertebrates from one of our streams (Badger Creek).

The data from this innovative non-invasive analysis of feeding patterns will be used to complement our assessments of food items collected from fish using gastric lavage. The gastric lavage data provide detailed information of the kinds and abundances of insects and other aquatic invertebrates in the guts of the fish, but are point estimates in time. The isotopic analyses provide integration over a longer time period of the trophic level the fish are consuming, but lacks the details of the actual species of insects and other macroinvertebrates the fish have eaten. Consequently, these two techniques provide complementary information that allows a more robust picture of dietary patterns to be formed for the brown trout.

Analysis of fish scales was completed in May 2012 for 12 of the streams sampled during the first year of our project. The analyses were completed by Andrew Carlson, an undergraduate in CFNAS majoring in Fisheries and Wildlife. Andrew completed the research and submitted his findings to satisfy the original research requirement for graduating with honors. Andrew graduated with honors at the end of summer, and was accepted into the Master's Degree Program in Fisheries at South Dakota State

University, where he is currently studying. The results of his research will help confirm our estimates of growth patterns among different year classes of trout across the first twelve streams that we have worked on during this project. This research was completed by a grant from the UROP Program at the University of Minnesota for Andrew's stipend and some of the disposable supplies. Our grant covered the remaining costs for disposable supplies, but otherwise the research was completed without additional direct support from our grant.

During this report period we also began initial discussions with our collaborators in the MN DNR to identify a short list of potential streams to sample for our next round of field work.

Presentations of preliminary results for 24 streams were given by Will French (student assigned to this Result) during this report period. Authors, titles and meeting details are:

French, W. E., J. Mazack, J. Biederman, L. Krider, P. Sherman, B. Vondracek, and L. Ferrington Jr. Winter diet and growth of brown trout in SE Minnesota. 2012. Minnesota DNR Summer Research Meeting. Lanesboro, MN.

French, W. E., J. Mazack, J. Biederman, L. Krider, P. Sherman, B. Vondracek, and L. Ferrington Jr. Winter foraging and growth of brown trout in southeastern Minnesota streams. 2012. 5th Annual Driftless Area Symposium. LaCrosse, WI.

Result 2 Status as of: December 2012
During the early part of this report period we finalized our selection of 12 streams to be sampled during year three of the project based upon input from the MN DNR Fisheries Biologists and field reconnaissance by our staff.

In November we initiated field work and completed nearly all of our first round of sampling by the end of this report period. We anticipate finishing the remainder of the field work for round during the first 10 days of our next report period. All field procedures were accomplished in streams sampled during this field season.

We identified and recruited another undergraduate student to continue working on fish scale analyses from samples collected during our second field season. The student was trained in lab safety and scale analysis techniques and she started analyses by the end of this report period.

The graduate student that works on this portion of our project was successful in processing mucus and numerically dominant aquatic invertebrates from Badger Creek to be analyzed for isotopic analysis. Samples were prepared for analysis, and costs for some supplies and other processing were covered by a small exploratory research grant to use the newly-developed technique to determine feeding status of trout. This innovative technique is non-invasive to fish, and is anticipated to provide additional insight into feeding patterns of trout with differing growth rates. Samples were shipped to a contracting laboratory for final determinations and results were received during this report period. The results showed predicable shifts in feeding status of trout and we prepared a manuscript outlining the results and our interpretations. After revisions and peer review by staff of the USGS, we submitted the manuscript for publication.

Presentations of preliminary results for 24 streams were given by Will French (student assigned to this Result) during this report period. Authors, titles and meeting details are:

French, W. E., J. Mazack, J. Biederman, L. Krider, P. Sherman, B. Vondracek, and L. Ferrington Jr. Winter diets and growth of brown trout in groundwater dominated streams. 2012. 142nd Annual meeting of the American Fisheries Society. St. Paul, MN.

Final Report Summary: June 2013

During the last report period we continued with all field and laboratory procedures related to Result 2. The last round of fish measurement and gastric lavage was completed, and diets were determined. A second undergraduate student applied for and was awarded a UROP grant from the Undergraduate Research Opportunities Program and completed scale-aging analyses for fish assessed during our second field season Our results show that brown trout rely predominantly in winter-growing aquatic insects, including larval and pupal stages, and that most individuals consume copious amounts of emerging adults during periods when winter-emergence is occurring. This conclusion is based on diets and date obtained for our Result 3 (discussed in detail in the next section of this report).

Outcomes of Result 2 include:

(1) Data base of marked and recaptured brown trout for 36 trout streams located in southeastern Minnesota.
(2) Detailed diets for up to 30 individual fish per stream per sample date.
(3) Estimates of growth during winter for successful recaptures of tagged brown trout in 36 streams.
(4) Data base that links diets to growth rates for recaptured brown trout.
(5) One completed UROP project and a poster presentation during the Undergraduate Research Opportunities Symposium in April at the University of Minnesota
(6) Recruitment of two graduate students, and successful field sampling that will generate data for two Ph.D. dissertations in Fisheries, Wildlife & Conservation Biology and the Water Resources Sciences Programs at the University of Minnesota.
(7) Ten presentations of our research findings for Result 2 at local, national or international conferences over the performance period of this project.
(8) One peer-reviewed paper accepted for publication in a national scientific journal. The results of the publications will show successful application of a new technology to determine integrated responses to winter diets of brown trout in one of our streams.
The partial citation for the manuscript follows:

William E. French, Bruce Vondracek, Leonard C. Ferrington Jr., Jacques C. Finlay, Douglas J. Dieterman. (Accepted for Publication, August 2013) *Winter feeding, growth and condition of brown trout Salmo trutta in a groundwater-dominated stream.* The Journal of Freshwater Ecology.

Two cost-saving opportunities were achieved during the work to complete this project Result. One cost savings was associated with the expenses for two undergraduate students that applied for and were awarded UROP grants from the Undergraduate Research Opportunities Program and completed scale-aging analyses for fish assessed during our first and second field seasons. The second cost-saving was associated with purchase of pit-tags for marking fish. We were able to cost-share 50% this expense with another source of funding, thereby achieving cost-savings for disposable supplies.

Summary of What Was Learned and Potential Benefits:

Our study of brown trout diets has provided quantitative estimates of the importance of winter-growing and winter-emerging aquatic insects in their diet during winter. This is important when attempting to manage for fast winter growth of trout, and means that it will be necessary to create in-stream conditions that result in high densities of the winter-developing aquatic insects. We now know the groundwater input conditions that result in most favorable conditions for the insects to emerge and be available for the trout to efficiently capture and consume and, as a consequence, can recommend that instream modifications of substrate can be targeted to areas where the thermal regime of a stream segment is most strongly groundwater rather than meterorologically controlled (in the sense of Krider *et al*., 2013). This approach is considered to be more cost-effective than just randomly placing instream modifications at random points in a trout stream. In our publication (French, *et al, in press*), we conclude:

> “Although winter can be stressful for brown trout in some systems, trout in groundwater dominated streams may benefit from stabilized annual temperature regimes and increased prey availability. Fish mucus was a useful tissue to evaluate temporal variation in SIA signatures during a period of reduced growth, especially when combined with fin tissue, which has a slow turnover rate. Brown trout in a groundwater-dominated stream continued to feed, maintained or increased their condition, and grew during the winter. Allochthonous inputs and aquatic macrophytes were the most significant sources of primary production in the winter aquatic food web of Badger Creek, supporting the majority of aquatic invertebrates and brown trout”

We will work with the MN DNR and Trout Unlimited to determine if, or to what extent, this recommendation can help inform and prioritize their habitat improvement activities in trout streams.

Result 3: Winter dynamics, including species composition and abundances, of aquatic insects strongly control patterns of productivity and yield of trout that have been documented in streams of southeastern Minnesota, and we propose to focus our efforts toward developing a better understand winter dynamics. We hypothesize that factors identified in Objective 1 will also be critical in controlling the types and abundances of aquatic insects in the streams. We will focus on UCS winter-developing species to better understand how in-stream habitat can be structured to increase abundances and growth of UCS species that are shown to be important in trout diets as demonstrated by results of Objective 2.

Description:
Comprehensive studies at lower latitudes in the Central Plains have shown that more than 50 species of aquatic insects grow and emerge as adults during winter (Ferrington 2000, 2007). At least 25 species are now known to occur in trout streams in SE MN, and most that are UCS species are exclusively constrained to development and emergence during winter (Ferrington, unpublished data). It appears that UCS species are most diverse and possibly most abundant in trout streams that have fastest growth rates and yields of trout. Several of the UCS insects are undescribed species. The focus of this objective will be to quantify the patterns of diversity and population abundances of UCS species across the 36 streams used for Objective 1 & 2, and that represent a gradient of trout growth and yield. We will use routine methods to quantify abundances (PIBS samplers, lab sorting & quantification) combined with lab rearings in cold growth chambers to assist in identification and description of unidentified species. We will also use a method for collecting surface floating pupal exuviae of Chironomidae to profile the emergence periods and phenologies of USC species. This method has be developed by Ferrington *et al*. (1991) and utilized successfully in a variety of pollution assessment projects and basic ecological research by him over the past 29 years.

Summary Budget Information for Result 3: **ENTRF Budget** **$108,428.00**
Amount Spent: **$ 97,181.00**
Balance **$ 11,247.00**

Deliverable/Outcome	Completion Date	Budget
1.*Analysis of composition and abundances of UCS species that are potential items for fish to feed on in first set of 12 streams* (Will collect, estimate species composition, abundance and tolerances of UCS on substrates in the streams. Will differentiate by type of stream substrate, feeding habits of UCS and types of life cycles, and maximum size when mature. We expect the aquatic insects that will be most abundant will consist primarily of ultra-cold adapted, winter developing aquatic insects such as Chironomidae and Plecoptera in streams where	Fall 2011	$35,070

trout grow fastest during winter)		
2. *Analysis of composition and abundances of UCS species that are potential items for fish to feed on in second set of 12 streams* (Same details as for deliverable #1, above)	Summer 2012	$36,132
3. *Analysis of composition and abundances of UCS species that are potential items for fish to feed on in third set of 12 streams* (Same details as for deliverable #1 & #2, above)	May 2013	$37,226

Result Completion Date: May 2013

Result 3 Status as of: December 2010
The first round of sampling was completed, consisting of five quantitative Hess samples/stream, one dip net sample per stream and one SFPE sample per stream. Samples were process and specimens sorted for identification. Volumetric estimates of selected groups were obtained. All material is labeled and curated for long terms storage. Some data has been entered into spread sheets for analysis.

Result 3 Status as of: August 2011
The second and third rounds of sampling were completed for year one, consisting of five quantitative Hess samples/stream during each round, one dip net sample per stream per round and one SFPE sample per stream round. Samples were all field-preserved and we began processing the samples in-lab. Specimens from samples taken during the second round of sampling for 3 streams were sorted for identification. Volumetric estimates of selected groups have not yet been obtained. All sorted and identified material is labeled and curated for long term storage. All data generated during the previous report period has been entered into spread sheets for analysis. Collections of surface-floating pupal exuviae have been processed for all three sample rounds for year one, and data are computerized for analysis. These data will be used by one of our graduate students for inclusion in her Master's thesis. Sample sorting of Hess samples has taken longer than anticipated for several of the streams. Otherwise, we are on-schedule with tasks related to these outcomes for Result 3.

Presentations of preliminary results for 12 streams were given by Jane Mazack (student assigned to this Result) during this report period. Authors, titles and meeting details are:

Mazack, Vondracek, Perry, Biederman, French, Krider, and Ferrington, Jr. "Predicting and Mitigating Vulnerability of Trout Streams to Climate Change." (Poster presentation) February 2011 Upper Midwest Stream Restoration Symposium, Oconomowoc, WI

Result 3 Status as of: December 2011
We finished processing and counting the remaining Hess samples from our first field season during this report period.

We started our second field season in November of this report period. All samples were collected for our first round of sampling for 10 of the 12 selected for our second field season. We anticipate that the remaining streams will be sampled during the first 10 days of our next report period.

Specimens from samples taken during the first round of sampling for several streams were sorted for identification during this report period. Volumetric estimates of selected groups have been obtained. All sorted and identified material is labeled and curated for long term storage. All data generated during the previous report period has been entered into spread sheets for analysis. Collections of surface-floating pupal exuviae have been analyzed. These data will be used by one of our graduate students for inclusion in her Master's thesis. Sample sorting of Hess samples continues to take longer than anticipated for several of the streams. Otherwise, we are on-schedule with tasks related to these outcomes for Result 3.

Presentations of detailed results for 12 streams were given by Jane Mazack (student assigned to this Result) during this report period. Authors, titles and meeting details are:

Mazack, French, Biederman, Sherman, Krider, Perry, Vondracek, Ferrington, Jr. "Winter invertebrate dynamics in trout streams of southeastern Minnesota." (Oral presentation) October 2011, Water Resources Conference, St. Paul, MN

Result 3 Status as of: August 2012

We completed the first round of sampling in our last two streams in early January of this report period, and the second and third rounds of sampling were completed on schedule. In each stream our sampling consisted of five quantitative Hess samples/stream during each round, one dip net sample per stream per round and one SFPE sample per stream round. Samples were all field-preserved and we began processing the samples in-lab.

Specimens from samples taken during the first round of sampling for 8 streams were sorted for identification. Volumetric estimates of selected groups have not yet been obtained for samples collected during our second field season. However, all sorted and identified material is labeled and curated for long term storage. All data generated during the previous report period has been entered into spread sheets for analysis. Collections of surface-floating pupal exuviae have been processed for all three sample rounds for year two, and data are computerized for analysis. These data also will be used by one of our graduate students for inclusion in her Master's thesis. Sample sorting of Hess samples has taken longer than anticipated for most of the streams. Otherwise, we are on-schedule with tasks related to these outcomes for Result 3.

Presentations of detailed results for 24 streams were given by Jane Mazack (student assigned to this Result) during this report period. Authors, titles and meeting details are:

Mazack, French, Biederman, Sherman, Krider, Perry, Vondracek, Ferrington, Jr. "Winter invertebrate dynamics in trout streams of southeastern Minnesota." (Oral presentation) March 2012, Stream Restoration Symposium, Minneapolis, MN

Mazack, Krider, Vondracek, Ferrington, Jr. "Winter invertebrate community dynamics in groundwater-fed streams of southeastern Minnesota." (Oral presentation) March 2012, 5th Annual Driftless Area Symposium, LaCrosse, WI

Mazack, Krider, Vondracek, Ferrington, Jr. "Winter invertebrate community dynamics in groundwater-fed streams of southeastern Minnesota (Oral presentation)." May 2012, Society for Freshwater Science, Louisville, KY

Mazack, Krider, Vondracek, Ferrington, Jr. "Winter invertebrate community dynamics in groundwater-fed streams of southeastern Minnesota." (Oral presentation) July 2012, Minnesota DNR Summer Research Meeting

Result 3 Status as of: December 2012

We finished processing and counting the remaining Hess samples from our first and second rounds of field season during our second field season toward the end of this report period.

We started our third field season in November of this report period. All samples were collected for our first round of sampling for 11 of the 12 selected for our second field season. We anticipate that the remaining streams will be sampled during the first 7 days of our next report period.

Processing of Hess samples continues to take longer than anticipated, and we will likely have to recruit and train more staff to make up our back log in this task. Processing of surface-floating pupal exuviae continues on schedule. Results for our third year of sampling will not be used as part of the MS thesis by Jane Mazak, but will be reserved to validate the models that she will develop from data generated during the first two field seasons. Results from the first two field seasons have been computerized and most analyses were completed by the end of this report period.

Presentations of detailed results for 24 streams were given by Jane Mazack (student assigned to this Result) during this report period. Authors, titles and meeting details are:

Mazack, Krider, Vondracek, Ferrington, Jr. "Winter invertebrate community dynamics in groundwater-fed streams of southeastern Minnesota." (Oral presentations) August 2012, American Fisheries Society, St. Paul, MN

Final Report Summary: June 2013

During this report period we completed all remaining field work related to Result 3 for our third and final year of this project. All samples of surface-floating pupal exuviae were sorted, identified, quantified and computerized. Data from Result 3 were incorporated into a Master's thesis by Jane Mazack, who is the graduate student working on this project result. The pupal exuviae data were used to develop an empirical predictive model of emergence across different trout streams sampled during the first and second years of the project. The model uses the slope and intercept relationships developed for Result 1 to predict the large temporal scale emergence of the most abundant aquatic insect that emerges in winter in the streams we investigated. Adults of this insect comprise a large proportion of emerging insects that were found in our study of diets in Result 2. These results formed 50% of the total thesis findings. The thesis was successfully defended in May, 2013 and received in final revised form in June 2013.

The title of the thesis is:
Mazack, J. E. 2013. "Emergence, survival, and longevity of adult *Diamesa mendotae* Muttkowski (Diptera: Chironomidae) in groundwater-fed streams." Masters Thesis, Water Resources Sciences Program, University of Minnesota. 40 pp + Appendix.

Data for winter emergence is being used for calibration of the model proposed in the thesis, and we have a draft manuscript prepared for internal review before submitting to the peer reviewed journal ***Aquatic Insects***, with submission anticipated by October 2013. We also anticipate submitting for publication of at least one additional manuscript reporting results related to winter emergence dynamics over the next 3-5 months.

Jane Mazack has applied to and been accepted for additional graduate study to the Ph.D. degree in the Water Resources Sciences program. She will continue to work on data generated by the Hess sampling that was accomplished for Result 3 of the project. It is expected that these data will comprise the primary research findings for her Ph.D. dissertation.

During this report period one presentation related to Result 3 was completed. The authors and title are:
Mazack, J., B. Vondracek & L. C. Ferrington, Jr. *"OVERWINTER INVERTEBRATE COMMUNITY DYNAMICS IN GROUNDWATER-FED STREAMS OF SOUTHEASTERN MINNESOTA."* Abstract presented at Annual meeting of the Society for Freshwater Sciences, May 2013.

Outcomes of Result 3 include:
(1) Data base of winter emergence patterns for 34 species of aquatic insects encountered in 36 trout streams located in southeastern Minnesota.
(2) Data base of winter-growing aquatic macroinvertebrates for 36 trout streams in southeastern Minnesota.
(3) Quantitative estimates of population abundances for 113 taxa of aquatic macroinvertebrates for 36 streams per sample date.

(4) Estimates of mass for most common aquatic invertebrates in 36 streams.
(5) Estimates of winter emergence as a function of mean weekly stream temperatures for 34 species of aquatic insects.
(6) Data bases that can be used for UROP projects by undergraduate students at the University of Minnesota
(7) Completion of graduate research leading to Master's thesis in the Water Resources Sciences Program at the University of Minnesota
(8) Recruitment of one Ph.D. candidate in the Water Resources Sciences Programs at the University of Minnesota.
(9) Eight presentations of our research findings for Result 3 at local, national or international conferences over the performance period of this project.

Two cost-saving opportunities were achieved during the work to complete this project Result. One cost savings was associated with the expenses for undergraduate students to assist in our filed work. During this grant we were able to utilize undergraduates that volunteered their time in exchange for costs of transportation to and from our sample sites to gain field experience in fish and macroinvertebrate collection during harsh winter periods. The second cost-saving was associated with purchase of some field gear and other disposable supplies. We were able to cost-share up to 50% this expense with another source of funding, thereby achieving cost-savings for selected items of disposable supplies.

Although we had some cost-savings associated with equipment, disposable supplies, and undergraduate involvement, we also had significant cost over-runs for graduate student duties during the project. The cost over-runs resulted from greater than expected diversity of invertebrates in our quantitative hess samples. Aquatic insect densities in several streams were also greater than anticipated, and the corresponding time required to sort, identify and quantify the samples far exceeded our budgeted amounts. To complete these tasks in a timely manner (and within the performance period of this grant) it was necessary to incorporate three additional graduate student assistants into the lab work during the last two months of this report period. This resulted in a cost over-run in personnel wages and associated tuition expenses. The over-run in tuition expenses was exacerbated by higher than budgeted annual increases in tuition during the full performance period of the grant.

Summary of What Was Learned and Potential Benefits:
At least 10 of the 34 species of aquatic insects that we have discovered emerging and active as adults in the winter appear to be restricted to trout streams or at least are more common in trout streams. Several are not described and are new to science, so were not know to form a significant part of the diet of brown trout during winter. For these same species nothing was previously known about their life cycles and habitat requirements, and it was not possible to begin to develop management strategies to conserve or increase their abundances so that they can become a larger reserve of prey items for the trout. We now know that several of the species do not grow during summer, but still do not understand their over-summering stages.

We now feel confident, however, that the thermal regimes during winter caused by groundwater input facilitate higher abundances of some of the winter emerging species. Consequently, we have put together a descriptive model that relates the findings of Krider *et al*. (2013) to the potential patterns of emergence by these species. Trout are effective visual-cue predators, and can efficiently consume aquatic insects that are in the process of completing the aquatic phases of their life cycle and are in the process of emergence. Our descriptive model for thermal regime suggests the water temperature conditions are maximized in segments of stream where the thermal regime of the stream water is most “groundwater controlled” (in the sense of Krider *et al*., 2014), and may result in more winter time emergence. Consequently, we can recommend that instream habitat management efforts should be designed to create substrate conditions that favor these species in areas of groundwater inputs. However, we presently do not understand the substrate conditions that may favor these species (it was not one of our project objectives), and we did not know prior to this research that these species would strongly track the buffered thermal regime produced by groundwater input. As a result, we will seek additional funding to do winter field research in the future that is designed to better understand the substrate conditions under which the winter developing species will develop the most-dense populations.

We will work with the MN DNR and Trout Unlimited to determine if, or to what extent, this recommendation can help inform and prioritize their habitat improvement activities in trout streams.

V. TOTAL ENRTF PROJECT BUDGET:

Personnel: $ 117,454 (salary for 2 graduate students each for 3 years) plus $ 69,792 Tuition (three years for each of two grad students). Category total = $ 187,246
Personnel: $ 16,983 (fringe benefits for 2 graduate students each for 3 years)
Personnel: $ 23,182 (salary for 3 undergraduate students each for 3 years. First year salary @ $ 10/hour for $ 10 hours/week/person for 26 weeks/person. Second and third years hourly salary increased by 3% to cover increases necessary to retain trained and experienced undergraduates that gain skills from year-to-year)
Contracts: $ NONE
Equipment/Tools/Supplies: $ 41,801 (Disposable field supplies, chemicals and lab supplies)
Travel: $ 30,788 (ALL IN-STATE TRAVEL, includes mileage & lodging & meals)
(Lodging & meals reimbursed at actual amount up to @ $75/day for 5 people for 20 days per year = $7,500 for first year. Second and third years increased by 3% to cover predicted inflationary increases)
(Vehicle rental cost @ $63/day x 20 days/year = $1260 for first year. Second and third years increased by 3% to cover predicted inflationary increases)

(Vehicle mileage cost @ $0.23/mile for 5221 miles = $1201 for first year. Second and third years increased by 3% to cover predicted inflationary increases)
Additional Budget Items: NONE

TOTAL ENRTF PROJECT BUDGET: $ 300,000

Explanation of Capital Expenditures Greater Than $3,500: NONE

VI. PROJECT STRATEGY:

A. Project Partners: No partners or subcontractors identified at this time.

B. Project Impact and Long-term Strategy:
Management strategies to slow or reverse conditions associated with global climate change optimistically will require a decade or more to develop and implement strategies that can be applied on scales large enough to provide world-wide protection of trout streams. In the intervening time, conditions in most vulnerable trout streams in SE Minnesota will continue to deteriorate. Consequently, our proposal is focused on learning how to identify the characteristics of the most vulnerable streams in southeast Minnesota where high concentrations of productive trout streams provide an array of streams with potentially differing vulnerabilities in a small geographic area. We will investigate the role of riparian vegetation and adjacent land use as potential modulators or controlling factors that minimize changes in stream thermal regimes as air temperatures vary in contrasting landscapes. Consequently, we expect that our findings will provide a road map for how to prioritize conservation and management activities, rather than address mechanisms to reduce or reverse large-scale patterns of climate change. By developing methods to identify highly vulnerable streams with high trout productivity and diverse cold-adapted, winter developing invertebrates that form the trophic basis for trout, it will be possible to more effectively allocate efforts to conserve genetic and biological diversity. We will work with state agencies and Non-profit conservation organizations, Watershed District and Water Management Organizations to try to develop conservation resource management plans and to help implement management recommendations based on scientific findings.

ADDITIONAL BACKGROUND AND CONTEXT: Minnesota has 689 designated trout streams that represent a valuable natural resource with high economic, sport and esthetic importance. Concentrated in the Arrowhead Region of the northeast and the Driftless Region in the southeast, the sport fishing industry in trout streams annually provides more than $150 million dollars in direct expenditures to local economies in Minnesota (Gartner *et al.* 2002) and $654 million throughout the Driftless Region of MN, WI, IL and IA (Trout Unlimited, 2008). In terms of direct and recirculating dollars in today's market place this natural resource likely generates more than $1.1 billion dollars per year of additional economic value to the state. In SE Minnesota, the trout sport fishing industry provides economic diversification and alternative sources of vitality to numerous small towns that otherwise predominantly rely on agriculture for their economic fabric.

Global climate change models predict Minnesota freshwater systems will warm to levels that can radically change the composition and productivity of their aquatic fauna and flora (NRDC 2002, Eaton and Scheller 1996) over the next 20+ years if trends in climate change are not modified. Cold-water adapted trout and other Salmonids are dependent on low summer stream temperatures and corresponding high dissolved oxygen levels for successful reproduction, and are among the most vulnerable freshwater water fish species to anthropogenic stresses. Trout streams located in SE Minnesota, and other similar mid-latitudes across the globe, are in areas where summer thermal regimes are nearly marginal in terms of conditions for cold water fish species. Although these streams currently support harvestable yields of trout, many are highly vulnerable to warming climates. Only subtle increases in ambient air and water temperatures undoubtedly will cause trout to experience reduced reproductive success. Under such conditions, trout streams will undergo decreased productivity and yield, and may even experience extirpation of populations (Clark *et al.* 2001, Meisner 1990) that can irreplaceably decrease genetic variability of populations in isolated watersheds within the next 20 years.

Because of their vulnerability to altered thermal regimes and other human-induced pollution stresses, the trout streams in southeast Minnesota are ideal field-based systems in which to study insipient effects of global warming on a resource that has high economic, sport and esthetic importance, both in Minnesota and elsewhere across the globe. Recent reports by the Minnesota Department of Natural Resources show a wide range in growth rates and total fish yield in southeastern streams (Dieterman *et al.* 2006, Dieterman *et al.* 2004) based on studies during warmer months of the year. Although the summer conditions are relatively well-understood, processes and patterns during warmer months do not adequately account for substantial amounts of the variability in growth and yield of trout (Dovciak and Perry 2002). It is therefore likely that differences in thermal regimes and availability of food resources in winter strongly constrain trout productivity, resulting in differential growth rates and yields.

In recent years, an insect fauna capable of growing at low water temperatures has been discovered (Ferrington 2000, 2003, Bouchard and Ferrington, 2009). Several species are fast-growing and appear to be capable of producing multiple generations in winter, and this fauna is especially well-represented in trout streams but are not common in warmer-water streams. For example, our recent research has shown that the most productive trout streams are strongly thermally buffered by groundwater sources and springs that feed into the stream at 9° C, and result in open water though winter (Ferrington, unpublished data). During winter, temperatures in these streams range from 2° through 8° C and the streams harbor unusual aquatic insects that are ultra-cold stenotherm (UCS) species that are able to survive freezing in water (as larvae), but also survive exposure to air temperatures lower than -20°C (Carillo et al. 2004, Bouchard et al. 2006a, 2006b) as adults. We predict that increases as small as 2°C in average water temperatures can reduce productivity of larvae of several of these UCS species, and posit that the winter dynamics of the UCS insects strongly control patterns of trout productivity and yield that have to date primarily been documented only during summer.

We propose two additional research objectives designed to provide better understanding of the winter dynamics of the valuable stream systems. We expect that modifications of winter ecosystem dynamics will serve as initial evidence of insipient responses to altered thermal regimes related to climate warming. We will work as a coordinated, inter-disciplinary team consisting of three faculty, two graduate students and several undergraduate student technicians, to better understand winter dynamics.

C. Other Funds Proposed to be Spent during the Project Period: None

D. Spending History: *This project builds on research findings of a Ph.D. dissertation by Dr. R. W. Bouchard (graduated 2008) that discovered some of the unusual ultra-cold stenothermic aquatic insects in trout streams near the Minneapolis/Saint Paul Metro Area. Total funding for this research came from a variety of sources including grants, in-kind contributions and scholarships from the University of Minnesota Graduate School and private donors. Total amount estimated to average $ 34,000/year for five years. More recently, Ferrington has received a Minnesota SeaGrant to work on similar, but not identical, patterns of seasonal dynamics of aquatic insects in trout streams near Duluth in relation to land use/land cover characteristics of impervious substrates instream catchments. This project is still ongoing, but has been funded for $ 35,000 in direct expenses and an additional award of $ 35,732 for salary, tuition and fringe benefits for one graduate student research assistant.*

VII. DISSEMINATION:

Web Site Development--- A World Wide Web site for the project will be established and maintained through the on-line resources of the Chironomidae Research Group, Department of Entomology, College of Foods, Agricultural and Natural Resources Sciences at the University of Minnesota. The web site will have a link to data bases that are built through this project for use by ecologists, conservationists, policy makers, and the public. The web site will provide additional and regularly updated information not contained in full in peer-reviewed publications and will synthesize past, current, and future research in this area. The information will be presented through text, multimedia (e.g. photos, figures, video), and links to relevant websites.

As part of the project web site, a separate page will be produced for the public and educators. It will be less technical and provide information on the emergence of insects from trout streams, field trip possibilities, educational experiments, information for use in lesson plans, and links to additional information and organizations.

Funding for this project comes at a propitious time for Leonard Ferrington in terms of outreach potential. Ferrington previously was awarded a Faculty Fellowship from the Digital Media Center, Office of Information Technology at the University of Minnesota (Twin Cities). The proposal is titled "*From Verification to Modeling: Adding Complexity and Realism to Web-Based Environmental Assessment Tools*" and the full text of the proposal is available on-line. Activities completed or planned during the fellowship include developing assessment tools to judge use and effectiveness of interactive digital media. The techniques learned during the fellowship tenure will be integrated into digital media resulting from this project.

During the first half of the project efforts will be completed to teach citizen volunteer groups in southeastern Minnesota the mechanics of making collections of surface-floating pupal exuviae of Chironomidae, and the benefits and short-comings of using the method as part of their monitoring activities. We also will contact fly-fishing groups, Trout Unlimited and private businesses of colleagues and friends such as Streamside Adventures (www.streamsideadventures.com) to assist in advertising our outreach activities.

Activities Related to Dissemination:

Ms. Amy Maas participated in an activity we term as "in reach" whereby we try to connect with undergraduates at the University of Minnesota that are not studying in the sciences disciplines using digital media. Amy was in our Environmental Sciences, Policy and Management Degree program and graduated with "Honors" in the Environmental Education and Communication Track. To complete the Honors requirements she developed and conducted an independent research project. The title of Amy's research topic was "Integration of Environmental Education into Spanish Language Learning at the University of Minnesota: A Case Study"

The research project consisted of preparing a "Tortulia" (in Spanish) that focused on the importance of our research project to citizens volunteers. The Tortulia consists of approximately three minutes of audio and video of two persons talking about citizen science in a coffee shop. The science they talk about relates to our project. Student taking introductory conversational Spanish access the Tortulia, listen to the conversation, then are quizzed on their understanding and interpretation of the subject, thereby learning about citizen science involvement. Many of the students taking the Spanish class were not science major, so we are able to connect our science to this student audience as "in reach." As part of her research efficacy assessment, Amy did a follow-up evaluation of the students that viewed the Tortulia and found that they had markedly increased their understanding of the implications of our research topic, and that several had retained moderate to rather sophisticated knowledge of, and interest in the topic of the conversation of the Tortulia. This modules is still being used in the Spanish class, and continues to introduce our science topic to students that are nonscience majors within the University of Minnesota. Amy Maas graduate with "Honors" in May 2012. The costs of this activity were deferred through a stipend from the University of Minnesota Undergraduate Research Opportunity Program and not from our grant, so we benefitted from this value-added activity.

During the past two years one of our research group (LCF) has periodically met with and discussed our results with a citizen volunteer working with the Kiap-TU-Wish Chapter of Trout Unlimited. This chapter has completed several river restoration projects, and has several more being considered for the future. They have developed a comprehensive biomonitoring and assessment program to help judge the effectiveness of their river restoration projects. The biomonitoring program is focused on summer dynamics, but they, too are concerned about trout survivorship and patterns of growth during winter. Consequently, our results, which focus on winter dynamics, are of interest to them as they develop plans for the future.

During the past two years, we have also coordinated with citizens groups elsewhere within the Driftless Region and students at Saint Mary University. Recently we have been contacted by a citizens group near Ely, Minnesota, which is also interested to learn more about winter dynamics of trout growth and emergence of aquatic insects. We will meet with persons from this group during spring of 2013.

At present, we have started to develop a preliminary conceptual design for a web site related to our project findings. We will complete the page and post it by the end of our grant.

During this project our personnel participated in numerous conferences and workshops and shared their interim results with participants in both formal presentations and informal discussions. Summed across all Results and year, we gave 21 formal presentations. This provided another significant outlet for disseminating our findings.

VIII. REPORTING REQUIREMENTS: Periodic work program progress reports will be submitted not later than 31 December 2010, 31 August 2011, 31 December 2011, 31 August 2012, 31 December 2012, 15 April 2013 and 15 May 2013. A final work program report and associated products will be submitted between June 30 and August 1, 2013 as requested by the LCCMR.

Attachment A: Budget Detail for 2010 Projects - Summary and a Budget page for each partner (if applicable)

Project Title: ***Predicting and Mitigating Vulnerability of Trout Streams***

Project Manager Name: ***Leonard C. Ferrington Jr.***

Trust Fund Appropriation: ***$ 300,000***

1) See list of non-eligible expenses, do not include any of these items in your budget sheet

2) Remove any budget item lines not applicable

2010 Trust Fund Budget	**Result 1 Budget:**	**Amount Spent** *6/30/13*	**Balance 6/30/13**	**Result 2 Budget:**	**Amount Spent** *6/30/13*	**Balance 6/30/13**	**Result 3 Budget:**	**Revised Result 3 6/30/13**	**Amount Spent** *6/30/13*	**Balance 6/30/13**	**TOTAL BUDGET**	**TOTAL BALANCE**
	Quantifying Physical, Geological and Riparian Settings of Trout Streams in Relation to Thermal Regimes			*Quantifying and Modeling Winter Diets of Trout*			*Determination and Quantification of Dynamics of UCS Aquatic Insect Species that Grow and are Active in Winter*					
BUDGET ITEM												
PERSONNEL: wages (Of two Graduate Students @ 50% FTE for 3 years)	39,152	39,152	0	36,704	36,704	0	41,598	51,380	51,380	0	127,236	0
PERSONNEL: benefits (academic tuition for 2 graduate students for 3 years)	26,173	26,173	0	21,810	21,810	0	21,810	35,558	35,558	0	83,541	0
PERSONNEL: Fringe benefits	6,367	6,367	0	5,308	5,308	0	5,308	5,308	5,308	0	16,983	0
PERSONNEL: wages for 3 Undergraduates (@ $10.00/hour for 10 hour/week for 25 weeks/year)	2,782	2,782	0	7,418	4,458	2,960	12,982	0	0	0	10,200	2,960
Supplies: Disposable field and lab supplies (Including preservatives, sample jars, storage containers, nets sieves, slides, coverslips, mounting medium, forceps, probes, dissecting scalpel, petri dishes, labels, markers, pencils, pens, field & lab notebooks, chestwaders, field gloves, purchase remote sensing and LU/LC data)	13,933	13,933	0	13,934	10,723	3,211	13,934	3,386	0	3,386	31,253	6,597
Travel expenses in Minnesota (Includes meals, lodging, four-wheel drive vehicle rental, and mileage)	6,678	6,678	0	11,313	11,313	0	12,796	12,796	4,935	7,861	30,787	7,861
COLUMN TOTAL	**$95,085**	**$95,085**	**$0**	**$96,487**	**$90,316**	**$6,171**	**$108,428**	**$108,428**	**$97,181**	**$11,247**	**$300,000**	**$17,418**

Winter feeding, growth and condition of brown trout *Salmo trutta* in a groundwater-dominated stream.

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[1] The Unit is jointly sponsored by the U. S. Geological Survey, the University of Minnesota, the Minnesota Department of Natural Resources, the U. S. Fish and Wildlife Service, and the Wildlife Management Institute

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Abstract

Winter can be a stressful period for stream dwelling salmonid populations, often resulting in reduced growth and survival. Stream water temperatures have been identified as a primary mechanism driving reductions in fitness during winter. However, groundwater inputs can moderate water temperature and may reduce winter severity. Additionally, seasonal reductions in prey availability may contribute to decreased growth and survival, although few studies have examined food webs supporting salmonids under winter conditions. This study employed diet, stable isotope, and mark-recapture techniques to examine winter (November through March) feeding, growth, and condition of brown trout *Salmo trutta* in a groundwater-dominated stream (Badger Creek, Minnesota USA). Growth occurred during the winter season and was greater for fish $\leq$ 150mm (mean = 4.1 mg*g^{-1} *day^{-1}) than for those 151 - 276mm (mean = 1.0 mg*g^{-1} *day^{-1}). Overall condition from early winter to late winter did not vary for fish $\leq$ 150mm (Mean relative weight (Wr) = 89.5) and increased for those 151 - 276mm (Mean Wr = 85.8 early, 89.4 late). Although composition varied both temporally and by individual, brown trout diets were dominated by aquatic invertebrates, primarily Amphipods, Dipterans, and Trichopterans. Stable isotope analysis supported the observations of dominant prey taxa in stomach contents, and indicated the winter food web was supported by a combination of allochthonous inputs and aquatic macrophytes. Brown trout in Badger Creek likely benefited from the thermal regime and increased prey abundance present in this groundwater-dominated stream during winter.

Keywords: Brown trout, winter, diet, growth, stable isotope analysis

Introduction

Winter can be a stressful time for stream dwelling salmonids, as evidenced by reduced growth rate, condition, and survival (Quinn and Peterson 1996; Schultz and Conover 1999; Post and Parkinson 2001). Winter ice formation can be a significant stressor for stream dwelling trout. Surface and anchor ice can decrease the amount of available habitat for trout through reductions in physical space and the formation of ice dams (Brown et al. 2011; Chisholm et al. 1987). Biro et al. (2004) found overwinter mortality of age-0 fish (60-80%) because of depleted lipid reserves was a primary limiting factor for rainbow trout *Oncorhynchus mykiss* recruitment. Winter conditions often cause stream dwelling salmonids to alter behaviors and habitat preferences, and can lead to reductions in foraging and general activity levels (Hussko et al. 2007).

Brown trout *Salmo trutta* are a stream dwelling salmonid that inhabit a range of habitats encompassing a wide variety of physical variation in winter stream conditions. As such, brown trout populations can experience a range of winter severity dependent on the physical characteristics of a particular stream. Brown trout in the Credit River, Ontario (Canada) and the River Dodder (Ireland) experienced depressed growth rates and reductions in condition over winter (Cunjak and Power 1987; Cunjak et al. 1987; Kelly-Quinn and Bracken 1990). Age-0 brown trout had significantly reduced monthly survival rates during winter than during summer (0.65 vs. 0.99) in a small southeastern Norwegian stream (Lund et al. 2003).

In contrast, some studies suggest winter may be no more stressful for brown trout than other seasons. Survival rates of stream-dwelling brown trout in winter were equal to or greater than other seasons in 11 of 16 cases reviewed by Carlson et al. (2008). Similarly, brown trout experienced positive growth and low over-winter mortality in three groundwater-dominated streams in southeastern Minnesota (USA) (Dieterman et al. 2012). Although winter severity appears to vary, differences in stream thermal regime have the potential to influence the degree to which brown trout may be affected in winter.

Reductions in growth and condition during winter are generally attributed to the effects of decreased temperature on brown trout physiology, with a minimum temperature of ~3.6°C required for growth (Elliot et al. 1995). Water temperatures in surface water-dominated streams closely track air temperatures, and often drop below 3.6°C in temperate locales during winter (Pilgrim et al. 1998). However, the temperature of ground water is approximately equal to mean annual air temperature (Erickson et al. 2000). Ground water input has a buffering effect on stream thermal regime, and can maintain water temperatures within acceptable ranges for brown trout growth even when air temperatures drop below freezing (Power 1999; O'Driscoll and DeWalle, 2004, 2006; Krider et al. 2013). The elevated winter temperatures of ground-water dominated streams may allow brown trout to maintain higher activity levels and more efficient functioning of metabolic processes.

Although water temperature may directly affect fish during winter, reductions in prey availability and quality (e.g., terrestrial invertebrate and aquatic invertebrate emergence and drift) may have additional implications for brown trout growth and condition. Summer diets of brown trout frequently include a significant proportion of terrestrial invertebrates (Kelly-Quinn and Bracken 1990; Bridcut 2000; Kawaguchi and Nakano 2001), but these prey are often unavailable to trout during the winter. Aquatic invertebrates comprise the bulk of stream trout diets during winter (Cunjak et al. 1987; Kelly-Quinn and Bracken 1990), but aquatic invertebrate abundance can be reduced during the winter season (Newman and Waters 1984; Gislason 1985; Rundio and Lindley 2008). Dieterman et al. (2004) suggested differences in annual growth among brown trout populations in groundwater-dominated southeastern Minnesota streams were driven by differences in prey availability. Thus, declines in aquatic invertebrate availability have the potential to negatively affect stream trout foraging and growth.

The presence of seasonally available aquatic invertebrate species can increase the relative abundance of aquatic invertebrates in groundwater-dominated streams relative to surface-water dominated streams during winter (Bouchard and Ferrington 2009). The contributions of these seasonally available aquatic invertebrates to winter groundwater-dominated stream food webs are not well understood, but an increase in the relative abundance of potential prey may benefit brown trout within these systems.

Analysis of stomach contents has traditionally been used to examine trophic relationships, which allows for quantification of the contribution of specific prey taxa. However, stomach contents offer only a snapshot (dependent on stomach evacuation rate) of long-term patterns in diet. Conversely, stable isotope analysis (SIA) offers a time-integrated method of examining trophic relationships between consumers and their prey by examining ratios of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes incorporated into the consumer's tissue (Peterson and Fry 1987). $\delta^{13}C$ is commonly used to determine energy sources in fishes (Peterson and Fry 1987), because $\delta^{13}C$ signatures of prey are passed on to predators with a predictable enrichment of ~ 0.4‰. Thus, $\delta^{13}C$ can be used to track energy flow through the food web and compare the relative importance of various prey taxa. Trophic level can be inferred via $\delta^{15}N$ with about a 3.4 ‰ increase in $\delta^{15}N$ typically observed between predators and prey (Vander Zanden et al. 1997). The combination of taxonomically specific diet data from stomach contents with energy flow information from SIA provides a more complete picture of an organism's position in the food web (Chipps and Garvey 2002).

The goal of this study was to evaluate the effects of winter conditions (here defined as November through March) on a brown trout population in a groundwater-dominated stream. Brown trout growth and condition were chosen as metrics to examine the effects of winter. Additionally, brown trout diet and relative position in the winter aquatic food web were quantified to examine potential mechanisms affecting growth and condition. The objectives of this study were to: (1) quantify and compare patterns of brown trout growth and condition during the winter season in a groundwater-dominated stream, (2) quantify the contributions of aquatic invertebrates to brown trout diets during winter using stomach contents and stable isotope analysis, and (3) quantify the relative position of brown trout within the winter aquatic food web of a groundwater-dominated stream using stable isotope analysis. Diet composition and growth were tracked by uniquely marked individuals, allowing relationships between diet and growth to be examined.

Methods

Study site

Badger Creek is a groundwater-dominated (i.e. receives enough groundwater input to significantly alter stream thermal regime and prevent over-winter freezing) tributary of the Root River, located in southeastern Minnesota, USA (Krider et al. 2013). The region is characterized by karst geology, including a large number of groundwater-dominated streams that support cold-water fish assemblages. Brown trout are the most abundant fish species in Badger Creek, but native brook trout *Salvelinus fontinalis* and slimy sculpin *Cottus cognatus* are also present. The sampling site consisted of a 125 m reach of stream containing multiple pools, riffles, and runs located in a forested headwater section of Badger Creek. Stream wetted width was ~3 m, and mean depth was < 1 m. Stream water temperature (7-9°C) was measured hourly from November 2011 through March 2012 by a remote logger device in the study site (HOBO™, Onset Computer Corporation Pocassat, MA) and remained within ranges suitable for brown trout growth throughout the winter (Elliot et al. 1995). The logger device was positioned 20cm below the surface at the head of a pool in the approximate middle of the sampling reach.

Fish collection, growth and condition

Brown trout were collected from Badger Creek on 19 November 2011 (early winter; 131 fish) and 15 March 2012 (late winter; 139 fish) using a Smith Root LR 20B backpack electric fisher (Smith Root, Vancouver, WA). Fish were placed in in-stream holding pens, anesthetized with an immobilizing dose of tricaine methanesulfonate (MS 222; Argent Chemical Laboratories Redmound, WA), weighed ± 1 g and measured ± 1 mm. All fish collected on 19 November were tagged in the anterior portion of the body cavity with 9mm passive integrated transponder (PIT) tags (Biomark Inc.; Idaho, USA) to track growth ($mg*g^{-1} *day^{-1}$) and condition between sampling events. Relative weight (Wr) compares the weight at length of a fish to a regionalized standard for that species and was used as an index of fish condition. Values between 80 and 100 are generally considered acceptable for healthy populations (Anderson & Neumann 1996). Condition was only analyzed for fish $\geq$140mm TL because of limitations of the standard weight equations for lotic brown trout established by Milewski & Brown (1994).

Diet analysis

Gastric lavage was used on 30 fish per sampling date to examine diet composition. The subsample of 30 fish was selected randomly on 19 November, but 23 fish with PIT tags were preferentially selected on 15 March to quantify diet and growth. Stomach contents were preserved in 95% ethanol in the field, and later processed in the laboratory. Aquatic invertebrates were identified to family or genus and counted. Dry weight of aquatic invertebrates in the diet were estimated with equations from Benke et al. (1999) and Méthot et al. (2012). Mean morphological measurements of aquatic invertebrates (body length, shell width) were calculated from sub samples of 20 individuals per taxon randomly selected from brown trout diets and used to estimate dry weight. Dry weight estimates were multiplied by taxa counts to obtain dry weight composition of diet for each fish.

Stable isotope analysis

The ability of SIA to integrate consumer diet history over a broad time interval can provide a comprehensive food web depiction when used with stomach contents. Growth rates can affect assimilation and turnover rates of C^{13} and N^{15} (Church et al. 2009), and the specific tissue to use for SIA must be carefully considered. Use of SIA in winter has been rare because of the potential for low tissue turnover rates. Muscle and fin tissue have C^{13} and N^{15} turnover rates >140 day half-life, whereas mucus is especially suited to slow growth conditions because of more rapid turnover rate (~30 day half-life) and continual regeneration (Church et al. 2009, Hanisch et al. 2010). Thus, use of mucus and fin tissue with differing turnover rates allow for temporal comparisons of brown trout diet. The faster turnover rate of mucus reflects consumption during winter, whereas the slower turnover rate of fin tissue reflects material consumed within winter, autumn, and late summer.

Pectoral fin tissue and mucus were collected following Church et al. (2009) from the subsample of 30 fish subjected to gastric lavage on 15 March for SIA. Additionally, SIA was conducted for 20 individuals on 15 March from each of the five most common prey taxa observed on 19 November, and samples of allochthonous (leaf litter) and autochthonous (*Spirogyra* sp., *Nasturtium* sp.) primary producers collected throughout the sampling reach.

Prior to analysis, fish fin, invertebrate, and plant samples were rinsed with deionized water, placed in individual aluminum trays and dried at 55°C for 72 h. After drying, samples were homogenized into a fine powder with a stainless steel rod, and stored in individually labeled glass scintillation vials. Subsamples were weighed for SIA and placed into individual tin capsules. Fish mucus was prepared according to the process outlined in Church et al. (2009). A single, composite sample was prepared for each aquatic invertebrate and plant taxon from individuals collected throughout the sampling reach, whereas fish fin and mucus samples were analyzed individually. Samples were sent to the University of California Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/index.html) and analyzed for $\delta^{13}C$ and $\delta^{15}N$

using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). A subset of samples were analyzed at the University of Minnesota Stable Isotope Laboratory (http://www.geo.umn.edu/orgs/sil/index.html) using a Costech 4010 Elemental Analyzer interfaced to a Finnigan MAT252 Mass Spectrometer (Costech Analytical Technologies Inc., Valencia, CA) as a quality control measure.

Statistical analysis

Growth, condition and diet were compared for small ($\leq$150 mm) and large (151 - 276mm) brown trout. These size categories correspond to age 0 and age 1+ fish collected from similar streams in the region (Dieterman et al. 2012). Growth rate was measured directly for PIT tagged individuals by calculating the change in mass between sampling events and compared between small and large brown trout with a Student t-test. The relationships between growth and dry weight of prey consumed, as well as growth and brown trout total length (TL) were examined with linear regression. The differences of Wr for small and large brown trout were compared between early and late winter with a paired t-test.

Comment [vV1]: How does a Student t-test differ from a paired t-test at the end of the paragraph?

Diet composition was examined by size class and sampling date and non-metric multidimensional scaling (NMS) was used to examine patterns in diet composition by number. Nine variables (size class, sample period, and mean consumption of Chironomidae, *Gammarus*, *Physella*, Limnephilidae, *Glossosoma*, Tipulidae, and other invertebrates consumed) were used in the NMS ordination.

$\delta^{15}N$ and $\delta^{13}C$ for small and large brown trout were analyzed separately by tissue (mucus and fin) and compared with an ANOVA (model: N or C = size class). NMS was performed in PC-ORD (V. 6.0). All other analyses were performed in Program R (V 2.15.1). Statistical significance was declared at $\alpha = 0.05$. Although sample size for some treatment groups was small (n= 10-12), data were evaluated for normality before analyses were conducted with a series of Shapiro-Wilks normality tests (Shapiro and Wilks 1965). All treatment groups were normally distributed.

Results

Growth, condition and water temperature

Growth during the winter was measured for the 23 fish recaptured during the late sampling event. Both small (n=11) and large (n=12) brown trout grew throughout the winter season, and there was a negative relationship between growth and TL (Figure 1). There was a positive relationship between growth and dry weight of prey consumed by fish on the March sampling date (Figure 2A). Small brown trout (mean = 4.09 $mg*g^{-1}*day^{-1}$) grew significantly faster than large brown trout (mean = 1.00 $mg*g^{-1}*day^{-1}$; $t_{22}=5.19$, $P<0.001$; Figure 2B). Overall condition from early winter to late winter did not vary for small brown trout (Mean Wr = 89.5, Figure 2C) and increased for large brown trout (Mean Wr = 85.8 early, 89.4 late; $t_{11}=2.31$, $P=0.042$; Figure 2D). Water temperature was within the range suitable for brown trout growth throughout the study (minimum temp = 5.5°C, maximum temp = 11.1°C, mean temp = 7.8°C).

Diet analysis

Brown trout diets in Badger creek were dominated by aquatic invertebrates, but small numbers of fish (cannibalized brown trout, *n*=2) and terrestrial invertebrates (i.e. annelid worms) were also present (Table 1). Only 1.6% of all sampled fish had empty stomachs. Brown trout diet composition varied temporally, but was similar between size classes. NMS (Stress = 11.02; Instability < 0.001; Iterations = 71) explained significant variation along the first two axes (Axis 1, R^2= 0.468; Axis 2, R^2 = 0.191; Figure 3). Significant variables for Axis 1 included sample period (r= -0.76), Chironomidae consumed (r= -0.75), other invertebrates consumed (r= -0.61), *Glossosoma* consumed (r= -0.68), and Limnephilidae consumed (r= 0.345). Significant variables for Axis 2 included *Glossosoma* consumed (r= 0.66), and Chironomidae consumed (r= -0.56).

Stable isotope signatures of brown trout varied by tissue type and size class and supported the overall patterns observed in gastric lavage samples. Leaf litter had higher $\delta^{13}C$ (-29.4‰) compared to *Spyrogyra* (-35.3‰) and *Nasturium* sp. (-32.0‰). Aquatic invertebrate $\delta^{13}C$ indicated reliance on leaf litter and/or *Nasturium* sp., with the exception of *Glossosoma*, which had low values (-37.6‰) consistent with an algal based diet in headwater streams (Finlay 2001). The similarity of brown trout $\delta^{13}C$ to *Gammarus,* Chironomidae, *Physella,* and Limnephilidae $\delta^{13}C$ suggests the importance of these taxa to brown trout diets, whereas the dissimilar $\delta^{13}C$ of *Glossosoma* suggests lesser importance. $\delta^{15}N$ and $\delta^{13}C$ between large (mean $\delta^{15}N$ = 7.9‰, $\delta^{13}C$= -30.2‰) and small (mean $\delta^{15}N$ = 7.2‰, $\delta^{13}C$= -31.1‰) brown trout were not significantly different for fin tissue samples. However, $\delta^{13}C$ values were significantly different for large (mean $\delta^{13}C$ = -27.6‰) and small (mean $\delta^{13}C$ = -30.2‰) brown trout mucus samples ($F_{1,23}$=10.61, P< 0.01) indicating a greater reliance on more enriched prey during winter (e.g. *Physella*) by large brown trout. Large (mean $\delta^{15}N$ = 6.3‰) and small (mean $\delta^{15}N$ = 5.8‰)

brown trout $\delta^{15}N$ values were not significantly different suggesting both size classes occupy similar trophic levels.

Discussion

Growth

Both small and large brown trout in Badger Creek fed and grew throughout the winter season. Dieterman et al. (2012) also observed overwinter growth (~0.1mm * day^{-1}) in three groundwater-dominated southeastern Minnesota streams. In contrast, brown trout did not grow overwinter in the Credit River, a Canadian tributary to Lake Ontario (Cunjak and Power 1987), and in a tributary of the River Dodder, Ireland (Kelly-Quinn and Bracken 1988). Brown trout in West Brook (Massachusetts, USA) also experienced little or no growth between September and March (Carlson et al. 2007). Dissimilar stream temperature regimes in Badger Creek, the Credit River, and West Brook may explain differences in growth. Water temperature in Badger Creek remained significantly warmer (minimum temperature >5.9°C) throughout the winter than in the Credit River (minimum temperature 0.1 °C) and West Brook (minimum temperature < 0.0°C). Water temperatures were not recorded in the River Dodder, but mean January air temperature was 3.9 °C during the study. Although elevated water temperatures prevented ice formation in Badger Creek, Cunjak and Power (1987) documented surface ice cover of up to 22% in the Credit River during their study period. The prevention of ice formation in Badger Creek may have benefited brown trout by eliminating associated reductions in available habitat and foraging opportunities.

Growth in large brown trout may slow as they approach maximum size. Brown trout up to 388mm were collected from the sampling reach during this study. The largest individual used in our analyses was 276mm, whereas the majority of large brown trout were between 150mm and 250mm. Growth rates for large brown trout in Badger Creek may have decreased as trout approached their maximum size; however, the largest fish used in analyses was only ~70% of the TL of the largest fish captured from within the sampling reach.

Mature brown trout invest substantial amounts of energy into gamete production, and sexual maturation may have influenced growth and condition of large fish in our study. Brown trout in southeastern Minnesota typically spawn in October, and the majority of redds are constructed by early November (Stefanik and Sandheinrich 1999; Doug Dieterman, Minnesota DNR, unpublished data). Late spawning between sampling events may have reduced growth rates of some mature fish because of gamete production and increased activity levels. However, variation in growth rates of large brown trout was considerably less than in small (immature) brown trout, suggesting that most large brown trout had similar resources available for growth.

Condition

Condition of brown trout in Badger Creek remained stable or increased during winter for small and large fish (late winter mean Wr = 90.3 for small and 89.4 for large fish). In contrast, brown trout experienced a substantial decrease in condition by the end of winter in the Credit River (Cunjak et al. 1987), and River Dodder (Kelly-Quinn and Bracken 1990) where stream thermal regimes approached freezing. As an autumn spawning species, mature brown trout condition should be reduced following spawning. Surprisingly, no significant change in condition was observed for small brown trout, and large brown trout increased condition between sampling dates in Badger Creek. The ability of brown trout to maintain condition and recoup potential body mass losses from spawning may have reduced demand on energy reserves and provided trout with an advantage to continue growth in spring.

Diet

Diets of both small and large brown trout were dominated by aquatic invertebrates, although the abundance of specific taxa varied by trout size class and sampling date. Empty stomachs were rare in our study (1.6%), but rates as high as 15% were observed in the River Dodder during winter, suggesting possible differences in prey availability (Kelly-Quinn and Bracken 1990). Brown trout often display size selectivity, preferentially feeding on larger prey items (Newman and Waters 1984). In Badger Creek, large-bodied taxa (*Gammarus*, Limnephilidae, Tipulidae and *Physella*) comprised the majority of prey consumed by dry weight during both early and late winter. However, smaller bodied prey such as *Glossosoma* and Chironomidae were often abundant in the diet, especially during late winter. Small and large brown trout consumed a greater abundance of small-bodied prey items in March than November.

The increase in small-bodied prey in brown trout diets during late winter may reflect shifts in aquatic invertebrate abundances between early and late winter. An increase in the abundance of small-bodied prey items may have increased their attractiveness to foraging brown trout, and large emergences of Chironomidae were observed before and during March sampling. Alternatively, stream trout can affect the aquatic invertebrate community's abundance and composition through predation pressure (Lepori et al. 2012). Brown trout predation pressure may have reduced the abundance of large-bodied prey taxa during winter in Badger Creek, forcing brown trout to consume greater numbers of small-bodied prey. Notably, although brown trout showed a substantial increase in the relative number of *Glossosoma* and Chironomidae consumed in late winter, these taxa contributed little dry weight in the diet because of their small size.

The significant relationship between growth and the mass of prey consumed may indicate that prey availability in winter has the potential to constrain brown trout growth in Badger Creek. Bioenergetics modeling of brown trout populations in southeastern Minnesota suggested that prey quality and availability may limit growth in groundwater-dominated streams (Dieterman et al. 2004). Dry weights of prey used in this analysis were obtained from diet samples collected on a single sampling date, whereas growth rates incorporate changes in mass from November through March. The ability of a snapshot of the diet to represent brown trout consumption over the entire time period during which growth was measured is a legitimate concern. Additionally, the lack of prey density estimates did not allow comparisons between prey availability and consumption in early and late winter.   However, the relationship between prey consumption and overwinter growth is an interesting observation that warrants further investigation.

Stable isotope analysis and winter food web

The stable isotope analyses generally supported the results of stomach content observations. Brown trout diets in Badger Creek were dominated by aquatic invertebrates during winter, primarily by taxa using allochthonous or aquatic macrophyte based food sources. The relatively enriched brown trout $\delta^{13}C$ (-30.1‰ to -27.6‰) indicate *Gammarus* (-31.6‰), Chironomidae (-30.8‰), and *Physella* (-26.5‰) may be important prey taxa. *Glossosoma* do not appear to contribute significantly to brown trout diet, as evidenced by the $\delta^{13}C$ of *Glossosoma* (-37.6‰) and the low biomass of *Glossosoma* consumed by brown trout (mean dry weight= 0.4mg) compared to *Gammarus* (mean dry weight= 5.14mg) or *Physella* (mean dry weight= 3.09mg). Although *Glossosoma* are often abundant in small streams, they are typically not primary taxa in predator diets, as their stone cases may render them less vulnerable to predation (McNeely et al. 2007).

$\delta^{13}C$ of the selected prey taxa and brown trout suggest that energy in the Badger Creek food web may have been derived from a combination of autochthonous and allochthonous sources, primarily leaf litter (-29.4‰) and aquatic macrophytes (*Nasturtium sp.; -32.0‰*). Watercress (*Nasturtium sp.*) was abundant throughout the sampling site during the early winter and late winter sampling events, and contributed to primary production for the winter aquatic food web. Autochthonous algal growth (*Spirogyra;* -35.3‰) likely played a lesser role in the Badger Creek food web as consumer $\delta^{13}C$ was more enriched than would be expected from an algal-based diet. Groundwater input within the site may have contributed to aquatic macrophyte growth by maintaining higher water temperatures and preventing the formation of ice cover, which allowed light to reach aquatic macrophytes during winter.

Brown trout often become piscivorous as they grow, sometimes as early as 150mm TL (Jonnson et al. 1999; Garman and Nielson 1982). Although only two fish (both cannibalized brown trout) were found in the 60 diet samples, the large amount of energy supplied by a single instance of piscivory makes the relative importance of fish prey to brown trout diets difficult to determine from stomach content data alone. If piscivory were an important component of brown trout diet in Badger Creek there should have been more than one trophic level of separation between brown trout and primary consumers, but brown trout $\delta^{15}N$ indicated one trophic level (~3.4 ‰) or less separation above *Gammarus, Glossosoma*, Limnephilidae, Chironomidae and *Physella*. Additionally, there was no difference in $\delta^{15}N$ between small and large brown trout, which would accompany an ontogenetic shift to piscivory. $\delta^{15}N$ of brown trout from Badger Creek supported the stomach content observation that piscivory was rare, and aquatic invertebrates were the primary prey source for small and large brown trout.

Two tissue types with differing turnover rates for C and N allowed for temporal comparisons of diet of large and small brown trout. The faster turnover rate of mucus (half-life ~30 days) reflects consumption occurring during winter, whereas the slower turnover rate of fin tissue (>140 days) reflects material consumed in late summer, autumn and winter (Church et al. 2009). Large brown trout had more enriched mucus $\delta^{13}C$ than small brown trout, but $\delta^{15}N$ was not significantly different between size classes or tissue types. The difference in mucus $\delta^{13}C$ suggests diets of large and small brown trout may have diverged during winter, possibly due to *Physella* being more prominent in late winter diets of large brown trout. Alternatively, the differences may reflect the importance of other taxa that were not collected for SIA (e.g.

Comment [vV2]: In the previous sentence you indicated no significant differences, but in the following sentences you indicate a difference.

Tipulidae), or a combination of *Physella* and other aquatic invertebrate taxa. As no corresponding increase in *Physella* consumption was observed in late winter stomach contents, a shift to Tipulidae or other unidentified taxa is better supported by the data. Although higher trophic level prey taxa have been observed in brown trout diets in other southeastern Minnesota streams (e.g. sculpin, *Rana* sp., fish eggs; W. French, unpublished data), these prey likely did not contribute substantially to brown trout diets in Badger Creek, as there was no corresponding enrichment in brown trout $\delta^{15}N$.

Conclusions

Although winter can be stressful for brown trout in some systems, trout in groundwater-dominated streams may benefit from stabilized annual temperature regimes and increased prey availability. Fish mucus was a useful tissue to evaluate temporal variation in SIA signatures during a period of reduced growth, especially when combined with fin tissue, which has a slow turnover rate. Brown trout in a groundwater-dominated stream continued to feed, maintained or increased their condition, and grew during the winter. Allochthonous inputs and aquatic macrophytes were the most significant sources of primary production in the winter aquatic food web of Badger Creek, supporting the majority of aquatic invertebrates and brown trout. These findings illustrate the need for further research of dynamics of trout and aquatic invertebrates of groundwater-dominated streams in winter, particularly the effects of varying amounts of groundwater input on trout population dynamics and aquatic winter food webs.

Acknowledgements

We thank the following for assistance in the field: Jane Mazack, Jenna McCullough, Jessica Miller, Lori Krider, Pat Sherman, Catherine DeGuire (University of Minnesota) and Dan Spence (MN DNR). We thank three anonymous reviewers for their beneficial comments on a previous version of this manuscript. All animals used in this study were handled according to animal use and care guidelines established by the University of Minnesota IACUC committee. Funding for this study was provided by Environment and Natural Resources Trust Fund administered by the Legislative Citizens Committee for Minnesota Resources, and the Kalamazoo Valley Chapter of Trout Unlimited. Any use of trade names is for descriptive purposes only and does not imply endorsement by the U.S. Government

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576

27

Figure Captions

Figure 1. Relationship between growth rate ($mg*g^{-1}*day^{-1}$) and total length (TL) of brown trout recaptured in Badger creek on 15 March 2012.

Figure 2. (A) Relationship between growth rate ($mg*g^{-1}*day^{-1}$) and dry weight ($mg*g\ trout^{-1}$) of prey consumed., (B) Mean growth rates of large (150 - 276mm TL) and small (≤150mm TL) brown trout, (C) Mean relative weight (Wr) of small (≤ 150mm TL) brown trout in early winter and late winter. (D) Mean relative weight (Wr) of large (>150 – 276mm TL) brown trout in early winter and late winter. All data derived from marked and recaptured fish in Badger Creek. * indicates $P<0.05$.

Figure 3. Non-metric multidimensional scaling ordination of diet composition for small (≤ 150mm) and large (151 - 276mm) brown trout in Badger Creek. Closed squares represent large fish in early winter; closed circles represent small fish in early winter. Open squares represent large fish in late winter; open circles represent small fish in late winter.

Figure 4. Carbon nitrogen bi-plot of mean (± 1 SD) $\delta^{13}C$ and $\delta^{15}N$ signatures for small (≤150mm TL) and large (151 – 276mm TL) brown trout, and integrated $\delta^{13}C$ and $\delta^{15}N$ signatures for common invertebrate prey taxa, and primary producers for (A) fin tissue and (B) mucus samples.