

**Montana Water Center
Annual Technical Report
FY 2006**

Introduction

The Montana University System Water Center, located at Montana State University in Bozeman, was established by the Water Resources Research Act of 1964. Each year, the Center's Director at Montana State University works with the Associate Directors from the University of Montana (Missoula) and Montana Tech (Butte) to coordinate state-wide water research and information transfer activities. This is all in keeping with the Center's mission to investigate and resolve Montana's water problems by sponsoring research, fostering education of future water professionals, and providing outreach to water professionals, water users, and communities.

To help guide its water research and information transfer programs, the Montana Water Center seeks advice from an advisory council which helps set research priorities. During the 2006/2007 research year, the Montana Water Research Advisory Council members were:

Gretchen Rupp, Director and Susan Higgins, Assistant Director, Montana Water Center

Marvin Miller, Montana Bureau of Mines & Geology and MWC Associate Director

Don Potts, University of Montana and MWC Associate Director

Krista Lee Evans, Montana Legislative Environmental Council

Representative Walter McNutt, Montana House of Representatives

Steve Merritt, Montana Association of Conservation Districts

Glen Phillips, Montana Fish, Wildlife & Parks

Art Compton, Montana Department of Environmental Quality

Dan Sullivan and Amy Bamber, Montana Department of Agriculture

Larry Dolan, Montana Department of Natural Resources & Conservation

Bob Davis, US Geological Survey

Ron Nadwornick, USDA Natural Resources Conservation Service

Hal Harper, Office of the Governor

Ed Sypinski, Montana Local Government Center

This report summarizes the water research and outreach programs supported by the USGS Water Research Program. The Montana Water Center is also funded with other federal and state grants that support other initiatives, not reported here.

During FY 2006 (March 1, 2006 through February 28, 2007), the Montana Water Center developed or sponsored many programs and tools to carry out its mission. Because of support from the USGS, the Center was actively involved in these water information transfer activities:

1. Administered the 104b research grants, and promoted interest in the 104g research grant program, all with assistance from and formal communications with the Center's Water Research Advisory Committee;
2. Developed and circulated an RFP for a new competitive student fellowship program, and ultimately offered a \$5,000 research award to a University of Montana graduate student. This effort served as a model for our current and expanded student fellowship program;
3. Encouraged and enabled student involvement through internships, research opportunities, trainings, and other efforts that provide practical experience for future water professionals.

Research Program

Here we report the work of three research teams and eight student fellows who were awarded funds to conduct water research in Montana during FY 2006. A total of \$56,246 was awarded to these eleven projects.

Dr. Lisa Eby of the Department of Ecosystem and Conservation Sciences at the University of Montana, and PhD. student Magnus McCaffery received \$15,000 for their study: Impacts of beaver on invasion ecology of brook trout (*Salvelinus fontinalis*).

Dr. Chris Gammons, Professor in the Department of Geological Engineering at Montana Tech, along with Dr. Colleen Elliott of Montana Tech and John La Fave, associate research hydrologist at the Montana Bureau of Mines, were awarded \$14,600 for their study Temporal and spatial changes in the concentration of isotopic composition of nitrate in the upper Silver bow Creek drainage, Montana.

Dr. Stephen Parker, Assistant Professor and Dr. Douglas Cameron, Professor, both at Montana Tech in the Department of Chemistry and Geochemistry, were awarded \$14,646 for their study titled: Carbon cycling and the temporal variability in the concentration and stable carbon isotope composition of dissolved inorganic and organic carbon in streams.

The eight student research fellowships, of either \$1,000 or \$2,000 were presented to promising student scientists at Montana campuses. Each student participated in a competitive application process where they showed competence in addressing a regional water resource problem through research in the coming year. Awards were offered to one undergraduate, four masters, and three doctoral students. Alphabetically, they are:

Kenneth Bates, Montana Tech, Further investigation of diel cyclic changes of metals in two Montana rivers.

Teresa Cohn, Montana State University, Settlement, environment, and identity: understanding processes of vegetative change along the Wind River.

Sunni Heikes-Knapton, Montana State University, Water quality function in subalpine wetlands in response to disturbance and restoration.

Margie Kinnersley, University of Montana, A Genomic and Proteomic Approach to Characterizing Natural Variation in *E. coli*: Toward Construction of a Microbial Source Tracking Database To Identify Sources of Fecal Water Contamination in the State of Montana.

Erin Thais Riley, Montana State University, Sources of groundwater and subsurface water acquisition and utilization by conifers invading riparian communities in western Montana.

Leo Rosenthal, Montana State University, Effects of Road Culverts on Eastern Montana Prairie Fish Assemblages.

Mark Schaffer, Montana State University, Spatial and temporal variation of groundwater and surface water interaction along the Gallatin River, Four Corners Montana.

Christa Torrens, University of Montana, The effects of overwinter dewatering on brown trout redds and egg survival in a Montana creek.

Student Fellowship: Settlement, environment, and identity: Understanding processes of vegetative change along the Wind River

Basic Information

Title:	Student Fellowship: Settlement, environment, and identity: Understanding processes of vegetative change along the Wind River
Project Number:	2006MT101B
Start Date:	3/1/2006
End Date:	6/30/2007
Funding Source:	104B
Congressional District:	At large
Research Category:	Biological Sciences
Focus Category:	Ecology, Hydrology, Recreation
Descriptors:	None
Principal Investigators:	William Wyckhoff

Publication

Final Report
2006 Montana Water Center Fellowship
June 21, 2007
Teresa Cohn

In my Water Center Fellowship proposal, I outlined the general goals of my dissertation research, and particularly emphasized that I would use my funds for vegetative sampling, aerial photography work, and preparation for interviews with respect to riparian vegetation along the Wind River. The following objectives remain central to my work:

- 1) to understand the effect of two dams and a series of diversions on Wind River vegetation and hydrology, and particularly their effect on native vegetation
- 2) to reconstruct the cultural landscape of land and water use in the Wind River corridor
- 3) to investigate the “invisible landscape” of perception, traditional ecological knowledge, and place identity
- 4) to study the interaction of social and ecological landscape elements with respect to vegetation along the Wind River.

However, several unanticipated changes have taken place, which have affected both my progress and methods.

The largest change in my work took place in Fall, 2006, at my first committee meeting and extensive review of my research proposal. My committee members unanimously agreed that the scope of my project was too large, straddling the fields of both ecology and historical geography. They strongly encouraged me to choose one discipline from which to work, and build my project around this discipline’s framework. In addition, the ecologist on my committee noted that much of my vegetative sampling work was unnecessary with respect to my overall goals. As a result, I have chosen to work as an historical geographer, augmenting my work with some riparian ecology, and eliminated most of my vegetative sampling from my research.

The following, while slightly modified, is still progressing:

- 1) I have taken 25 sets of repeat photographs of the Wind River, depicting change over time
- 2) I will rely heavily on orthorectified aerial photographs to analyze vegetative change
- 3) Interview preparation is underway, and is scheduled to begin in August, as planned.

In addition, I have conducted the following research that was not outlined in my Water Center report:

- 1) I have reviewed irrigation and extension records in the Denver National Archives
- 2) I have built a much stronger foundation in historical geography.

I mentioned in my Water Center proposal that I wanted to incorporate community involvement in my work. As a result, I applied for, and received, a National Science Foundation GK-12 Fellowship through the Big Sky Institute for the 2006-2007 school year which was recently renewed for 2007-2008. This work heavily involves me in the community, as I teach science at least a week a month in a reservation school. While richly rewarding, it is well accepted that GK-12 fellows progress more slowly than they otherwise might, as a result of fellowship constraints.

In sum, my overall research objectives remain the same. Some of my research has changed, some is progressing more slowly than anticipated, and I have added substantial archival work to my methods of understanding vegetative change.

My \$1,000 fellowship, at least in part, has contributed to:

- 1) building a much stronger foundation in historical geography
- 2) preparing 25 sets of repeat photographs
- 3) beginning aerial photo analysis
- 4) conducting research in the national archives.

I sincerely appreciate support from the Water Center and look forward to the completion of my degree in Spring, 2009.

Student fellowship: Spatial and temporal variation of groundwater and surface water interaction along the Gallatin River, Four Corners Montana

Basic Information

Title:	Student fellowship: Spatial and temporal variation of groundwater and surface water interaction along the Gallatin River, Four Corners Montana
Project Number:	2006MT104B
Start Date:	3/1/2006
End Date:	6/30/2007
Funding Source:	104B
Congressional District:	At large
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Surface Water, Water Supply
Descriptors:	None
Principal Investigators:	Stephan Custer

Publication

Final Report on the Spatial and Temporal Variation of Surface and Groundwater interactions near Four Corners Montana

6/18/2007

Mark Schaffer Department of Earth Sciences Montana State University

Along the West Gallatin River as irrigated agricultural land is converted into residential developments, changes to the hydrologic system are anticipated resulting from the decline in irrigated land. The loss of irrigation along the river has been hypothesized to result in decreased aquifer recharge which will lower stream flows in late summer and fall (Kendy et al, 2006). Managing groundwater and surface water resources conjunctively, possibly by aquifer storage and recovery (injecting surface water into the aquifer for later use), has been proposed as a means of mitigating anticipated stream flow declines.

An understanding of the dynamic relationship between the West Gallatin River and the West Gallatin Alluvial Aquifer is required in order to calibrate future groundwater models which could be used to test theoretical augmentation regimes and manage the ground and surface water conjunctively.

Specific electrical conductance measured in the Gallatin River's channel, streambed, and the aquifer along with discharge and water level measurements indicate that groundwater discharge into the river varied throughout the study area on both the scale of meters and kilometers. In addition, irrigated areas along the river resulted in significant temporary rises in the water table elevations which altered the surface and groundwater exchange on a temporal scale. Included with this general report are selected figures which demonstrate some of these preliminary findings (see Appendix I).

Data collection has been completed for the project. The final product for this analysis will include a statistical analysis of the streambed data along with a geographical analysis of the irrigated area and volumes along the River's flood plain. The data from this project is currently being organized in a data base which will facilitate continued data collection and ease of transfer between interested parties. This data will be analyzed in the context of the effects of the proposed aquifer storage and recovery plans upon river flows and ecologic conditions.

Thorough analysis of the data still remains and the project is anticipated to be completed by December of 2007. In the mean time, preliminary data has been shared with interested parties which including scientist, water users, and planners. Groundwater elevations collected from the monitoring wells installed for this project are available on the internet at the Montana Bureau of Mines and Geology Groundwater Information Center (GWIC) web site. A presentation of this data is planned for the American Water Resource Association Montana Chapter meeting in October of 2007.

References

Kendy, E, Bredehoeft, J.D. 2006. Transient effects of groundwater pumping and surface-water-irrigation returns on streamflow. *Water Resources Research* Vol. 42, W08415, doi 10.1029/2005wr004792

Appendix I: Selected figures

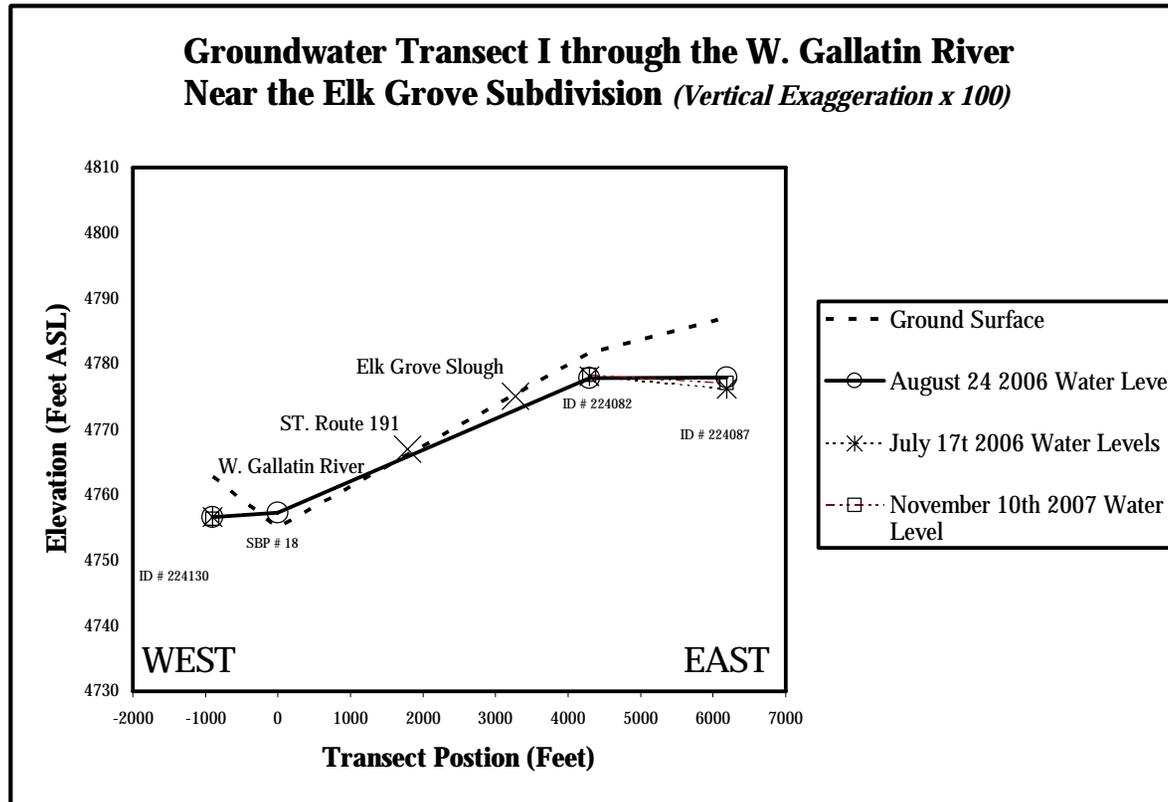


Figure 1. Groundwater elevations measured from monitoring wells and streambed piezometers located north of Axtell Bridge Road near the Elk Grove Subdivision. The potentiometric gradient along Transect I can be classified as flow through, were groundwater flows towards the river from the east but from the river towards the aquifer to the west. In addition, a groundwater divide is evident to the east of the river, the approximate crest of this divide is located below the Elk Grove Subdivision. Groundwater elevations showed little fluctuations from August to November, the greatest fluctuation occurred at well # 224087 below an irrigated field. Well and piezometer elevation where surveyed with a survey grade GPS unit provided and operated by the Montana Department of Natural Resource Conservation.

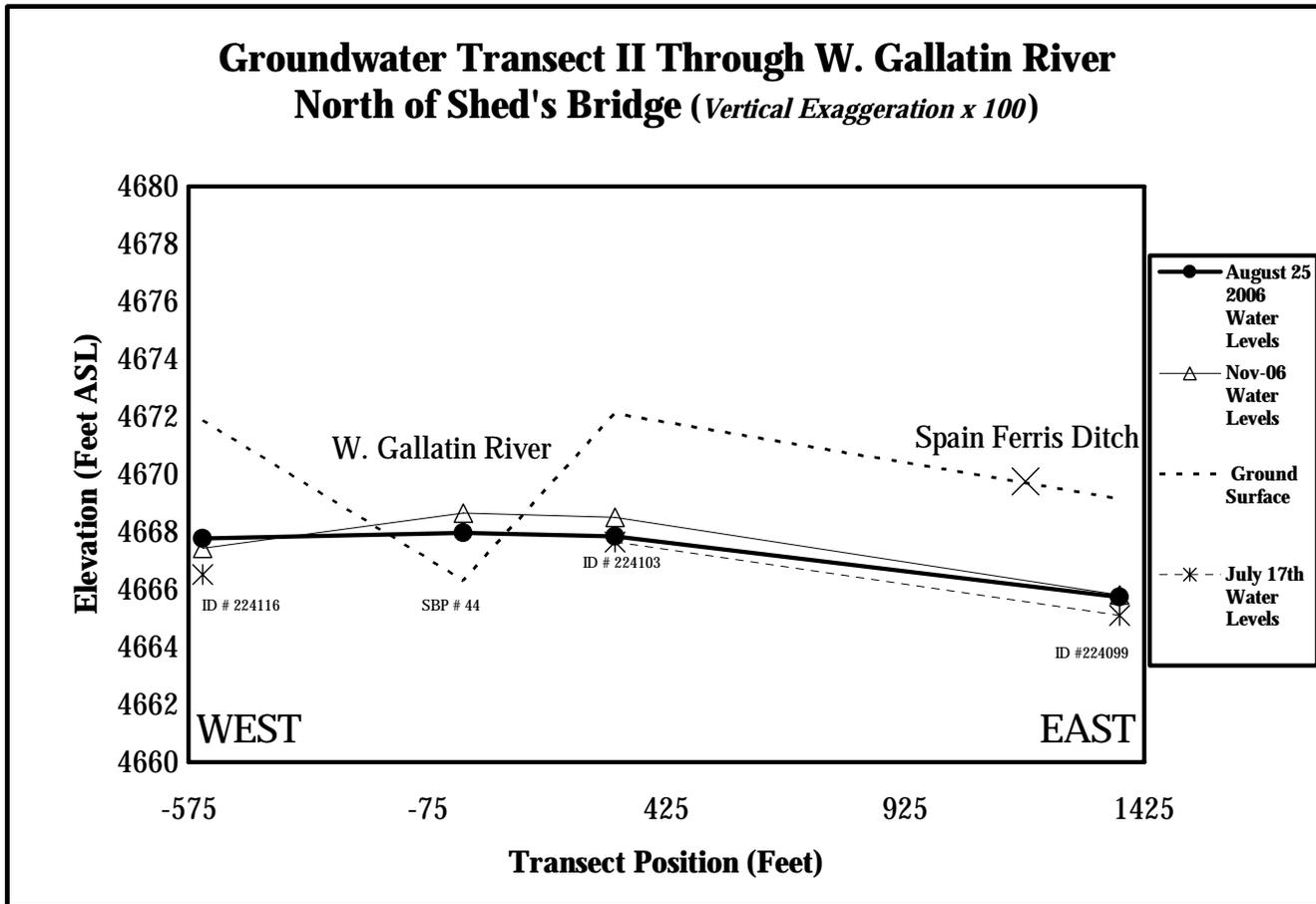


Figure 2. Groundwater water elevations measured from monitoring wells and streambed piezometers north of Shed's Bridge near Four Corners. Potentiometric gradients show water moving from the river channel to both east and west to the aquifer. The greater water level elevations in the river and monitoring wells during July and November correspond to higher stages in the West Gallatin River. Well and piezometer elevation were surveyed with a survey grade GPS unit provided and operated by the Montana Department of Natural Resource Conservation.

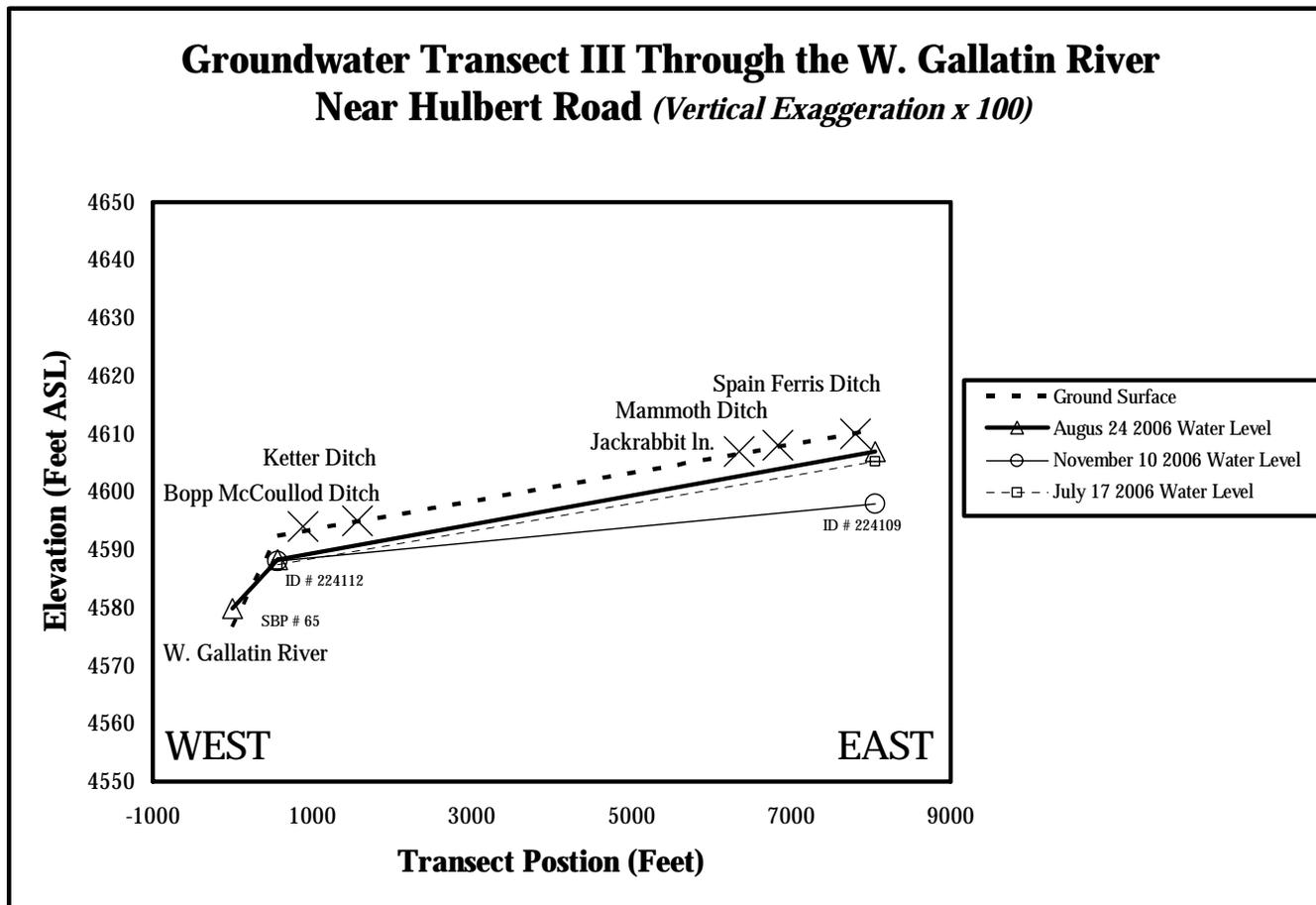


Figure 3. Groundwater elevation measurements measured in monitoring wells and streambed piezometers to the east of the Gallatin River near Hulbert Road. The dramatic fluctuation (> 7 feet) occurred below a flood irrigated field. Groundwater flow towards the river from the east decreased dramatically as the water table elevation declined below the flood irrigated field once irrigation ceased. Well and piezometer elevation were surveyed with a survey grade GPS unit provided and operated by the Montana Department of Natural Resource Conservation.

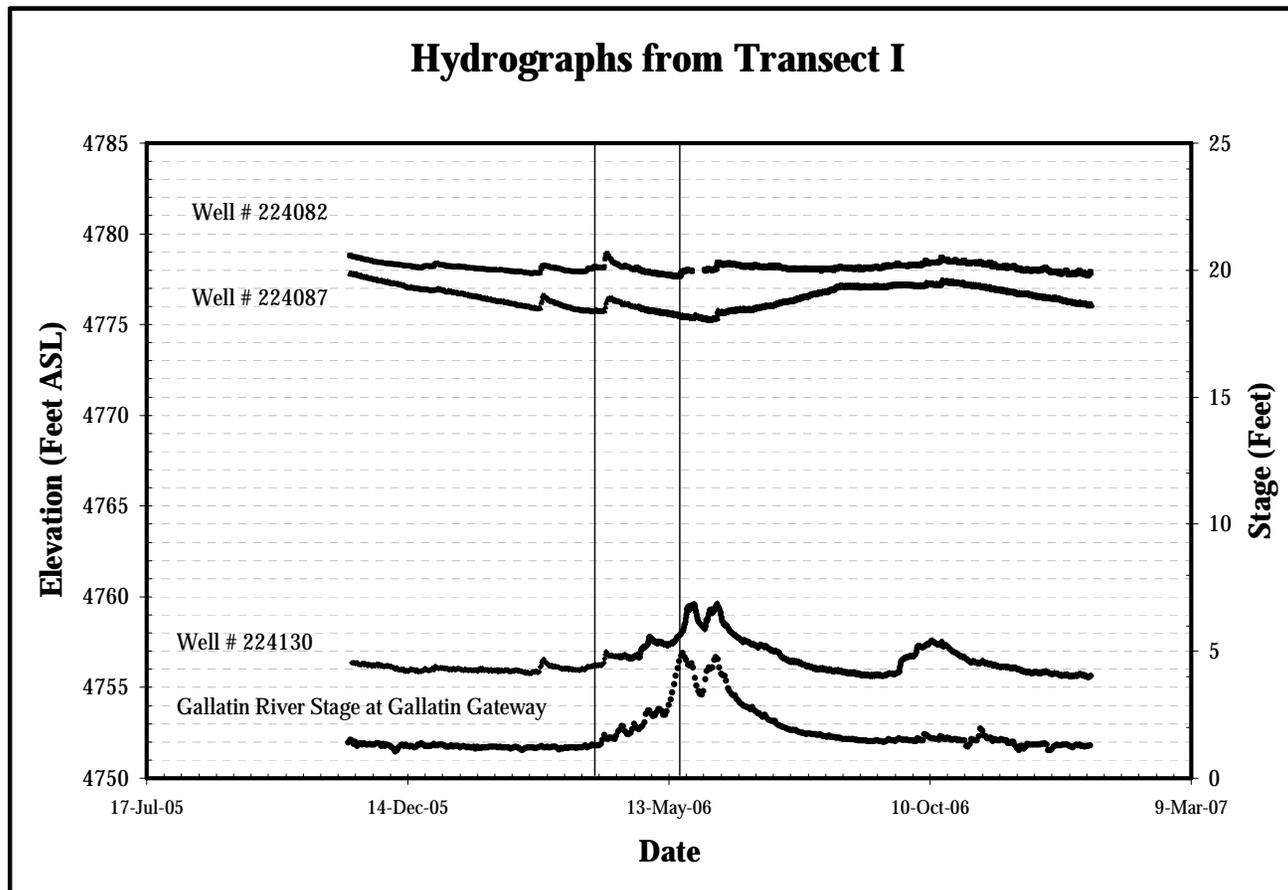


Figure 4. Ground and surface water hydrographs from the West Gallatin River at Gallatin Gateway USGS site and the monitoring wells located along Transect 1 (Figure 1). Water levels to the east of the river in wells 224082 and 224087 show little response to river stage where flow is from these wells to the river, while well 224130 resembles a slightly attenuated and delayed version of the Gallatin Gateway hydrograph. Note well 224087 located below an irrigated pasture, the water table rises during the summer and declines through the winter returning to within a foot of its' pre-rise stage by January 2007.

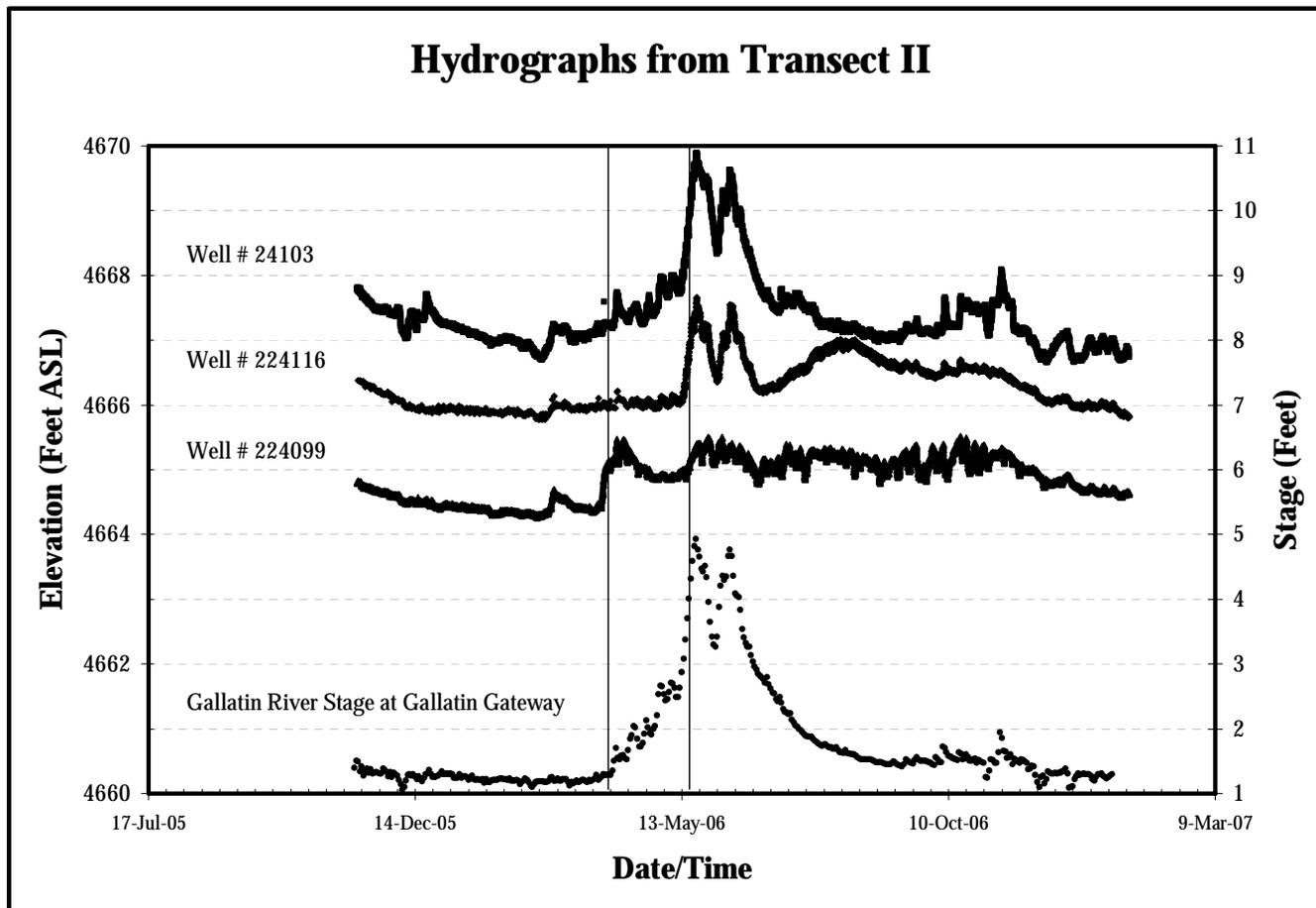


Figure 5. Ground and surface water hydrographs from the West Gallatin River at Gallatin Gateway USGS gauge and the monitoring wells located along Transect II (Figure II). At this transect water leaves the channel to the east and the west, wells 24103 and 224116 respond quickly to changes in the hydrograph, while well 224099 responds to the initial rise but does not exceed 4665 ft despite the rise in river stage. One possible explanation is that the groundwater traveling from the river east discharges into the Spain Ferris Irrigation Canal located between well 224099 and 224116.

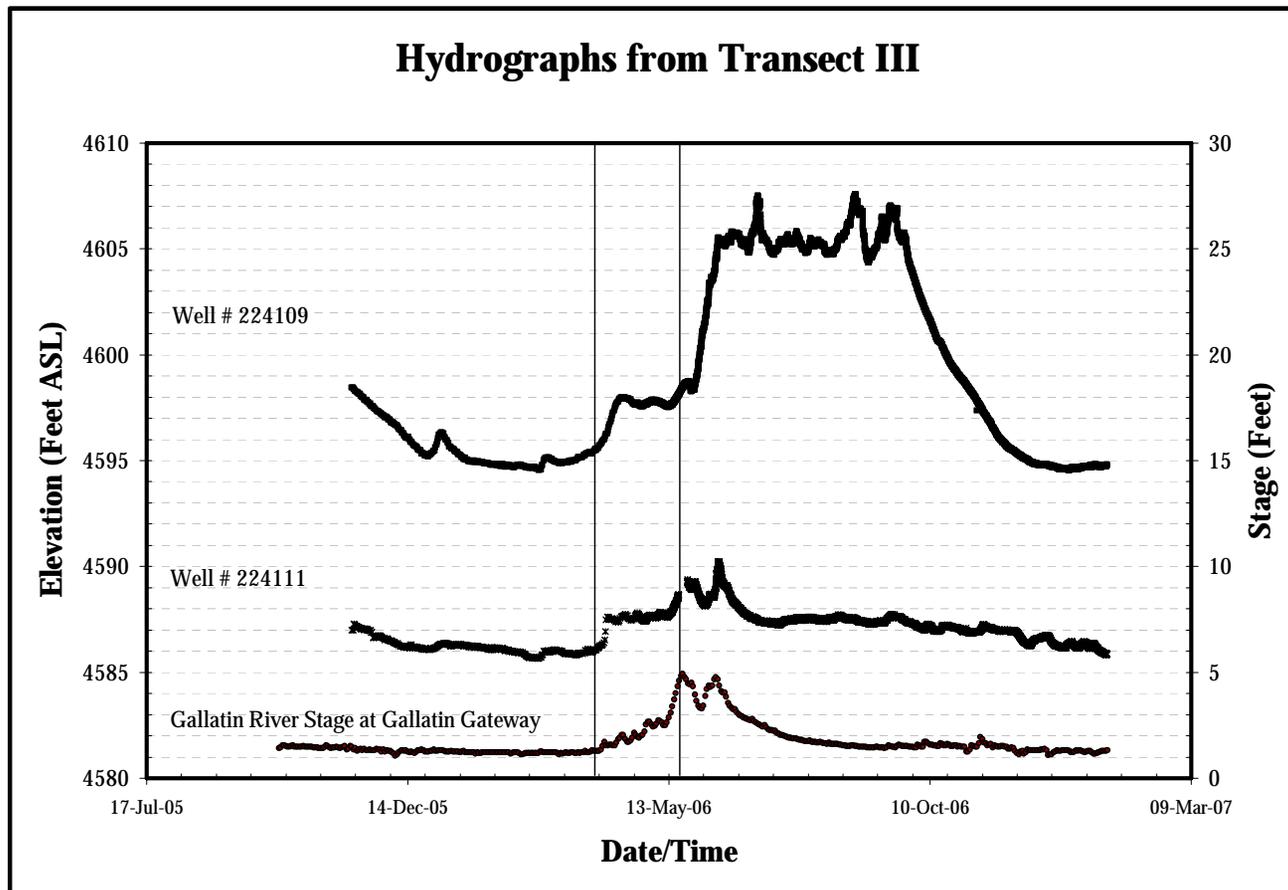


Figure 6. Ground and surface water hydrographs from Gallatin Gateway USGS Gauge and the monitoring wells located along Transect III (Figure 3). Well 224111 initially responds to changes in river stage, however during irrigation season rises despite declines in river stage, indicating that during the winter water flows from the river into the aquifer until groundwater elevations are elevated by irrigation during the summer. Well number 224109, located below a flood irrigated field shows a dramatic rise during irrigation season.

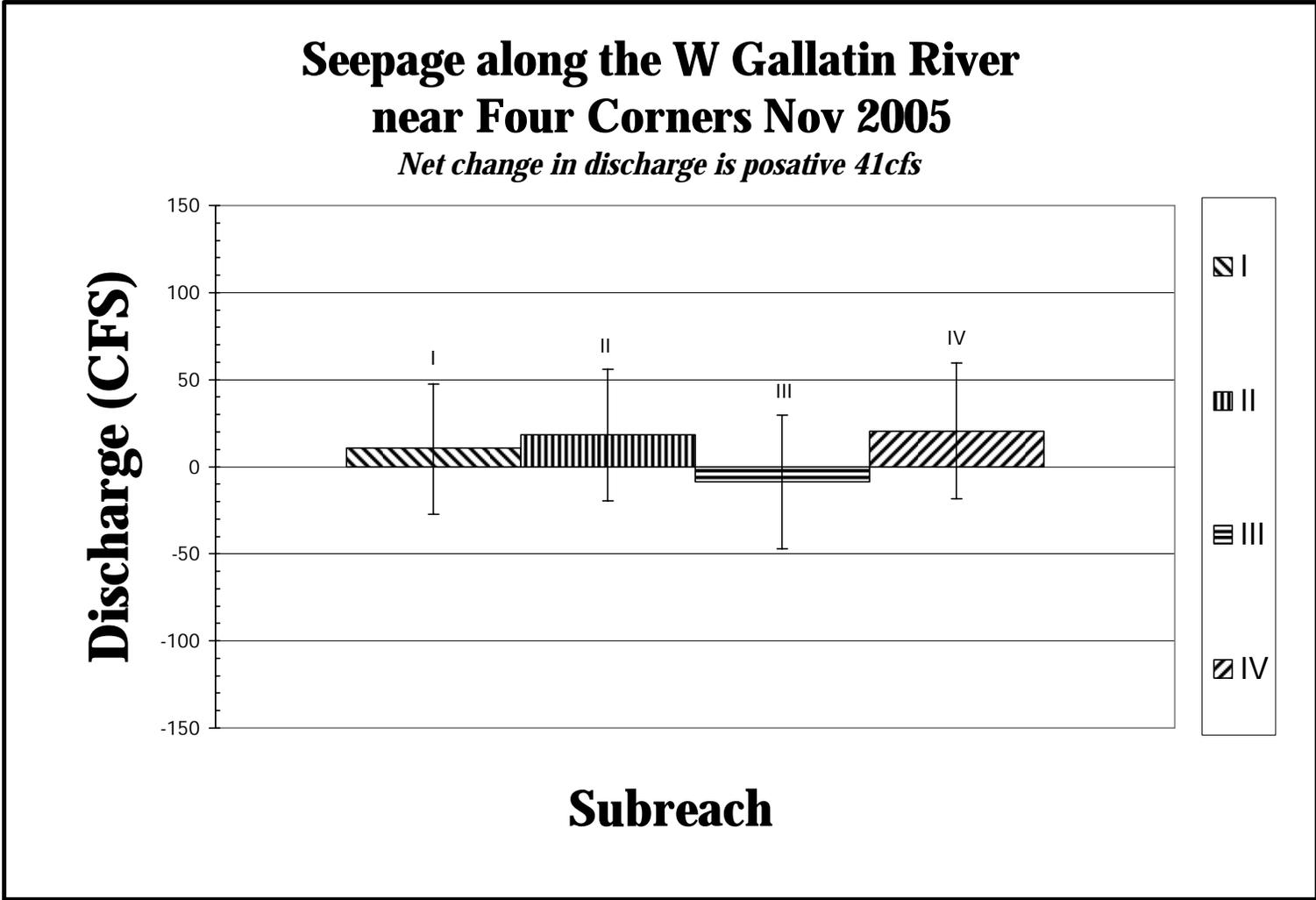


Figure 7. Change in discharge along four consecutive reaches of the West Gallatin River near Four Corners in November 2005. Note the decline in discharge across Reach III located near Shed’s Bridge.

Seepage along the Gallatin River July 2006

Net Flux for the entire reach is *positive 39 CFS*

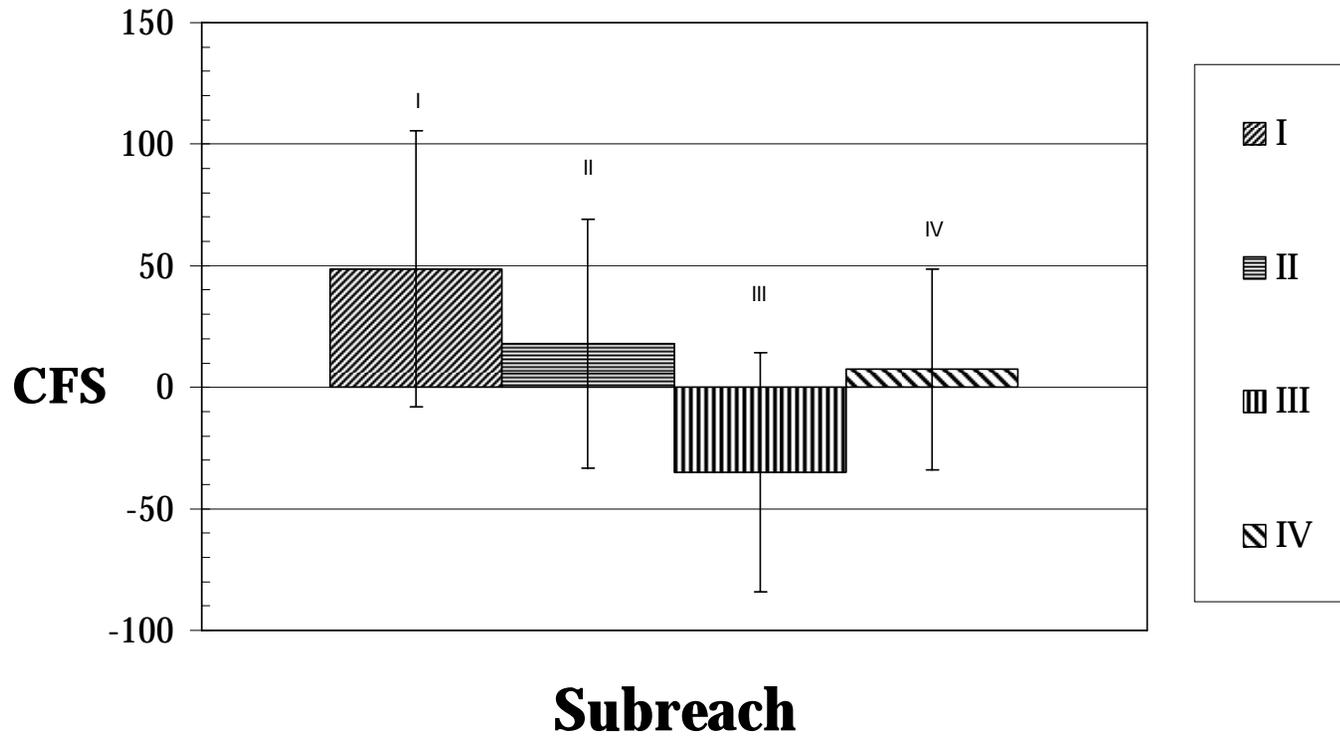


Figure 8. Change in discharge along four consecutive reaches of the West Gallatin River near Four Corners in July 2006. Note the decline in discharge across Reach III located near Shed's Bridge.

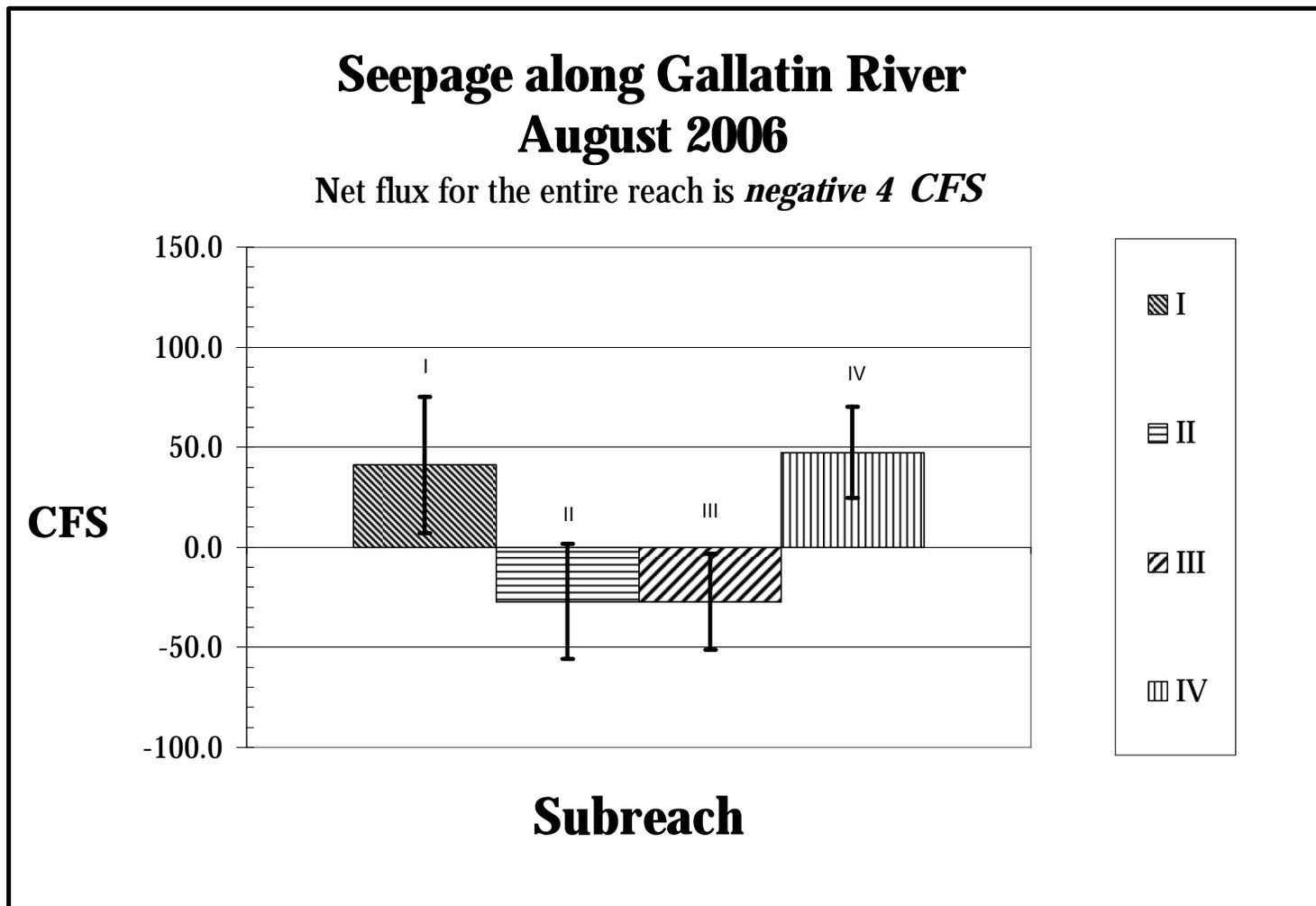


Figure 9. Change in discharge along four consecutive reaches of the West Gallatin River near Four Corners in August 2006. Note the decline in discharge across Reaches III and IV located near Shed's Bridge.

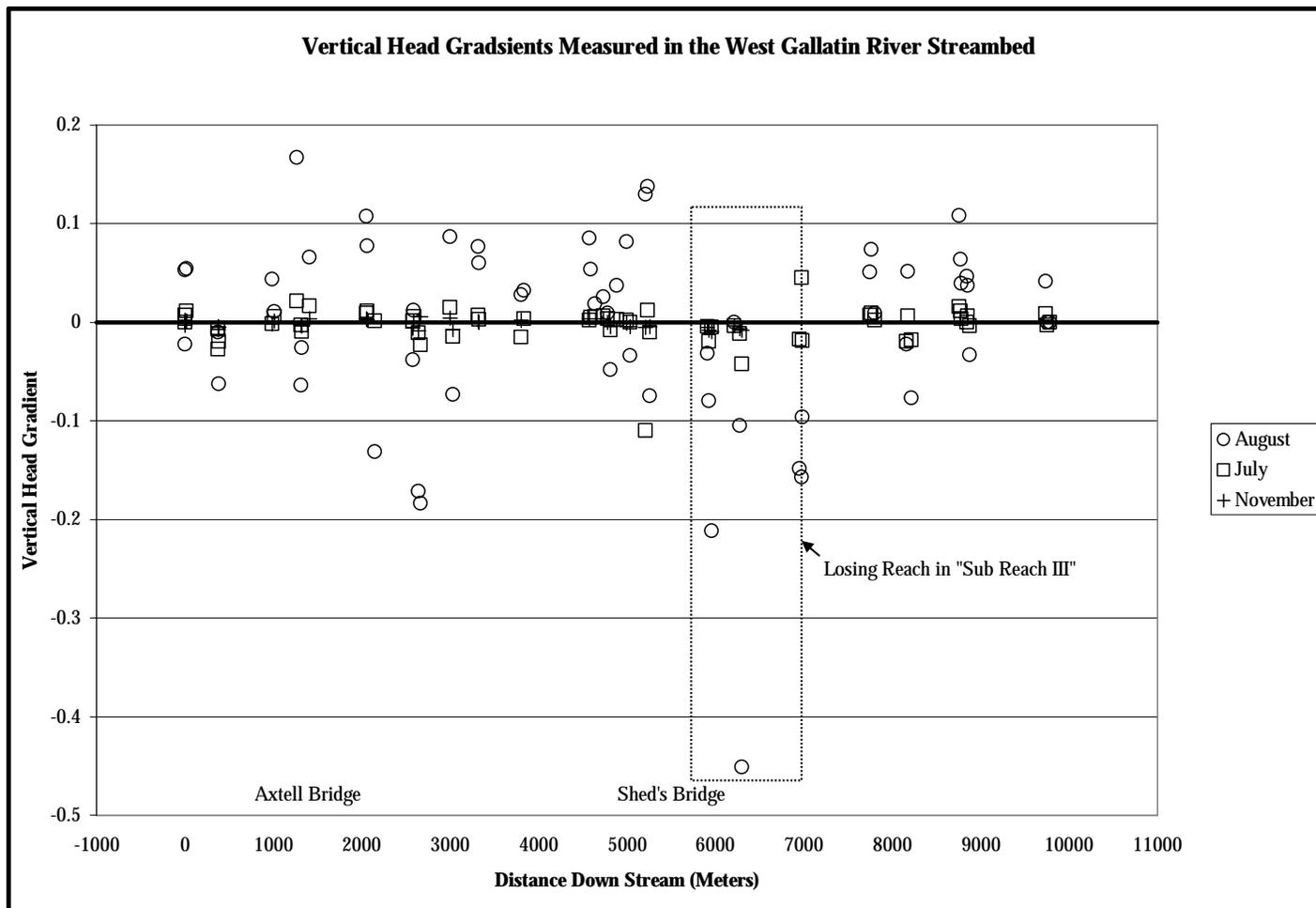


Figure 10. Vertical head gradients measured in streambed piezometers in the West Gallatin Riverbed. Note the absence of upward gradients in Reach III, which showed losses in November, July and August (Figure 7, 8, and 9). However in the other reaches (I, II, and IV) which show net gains (Figures, 7, 8, and 9) vertical gradients indicate gains and losses on the scale of meters indicative of hyporheic exchange.

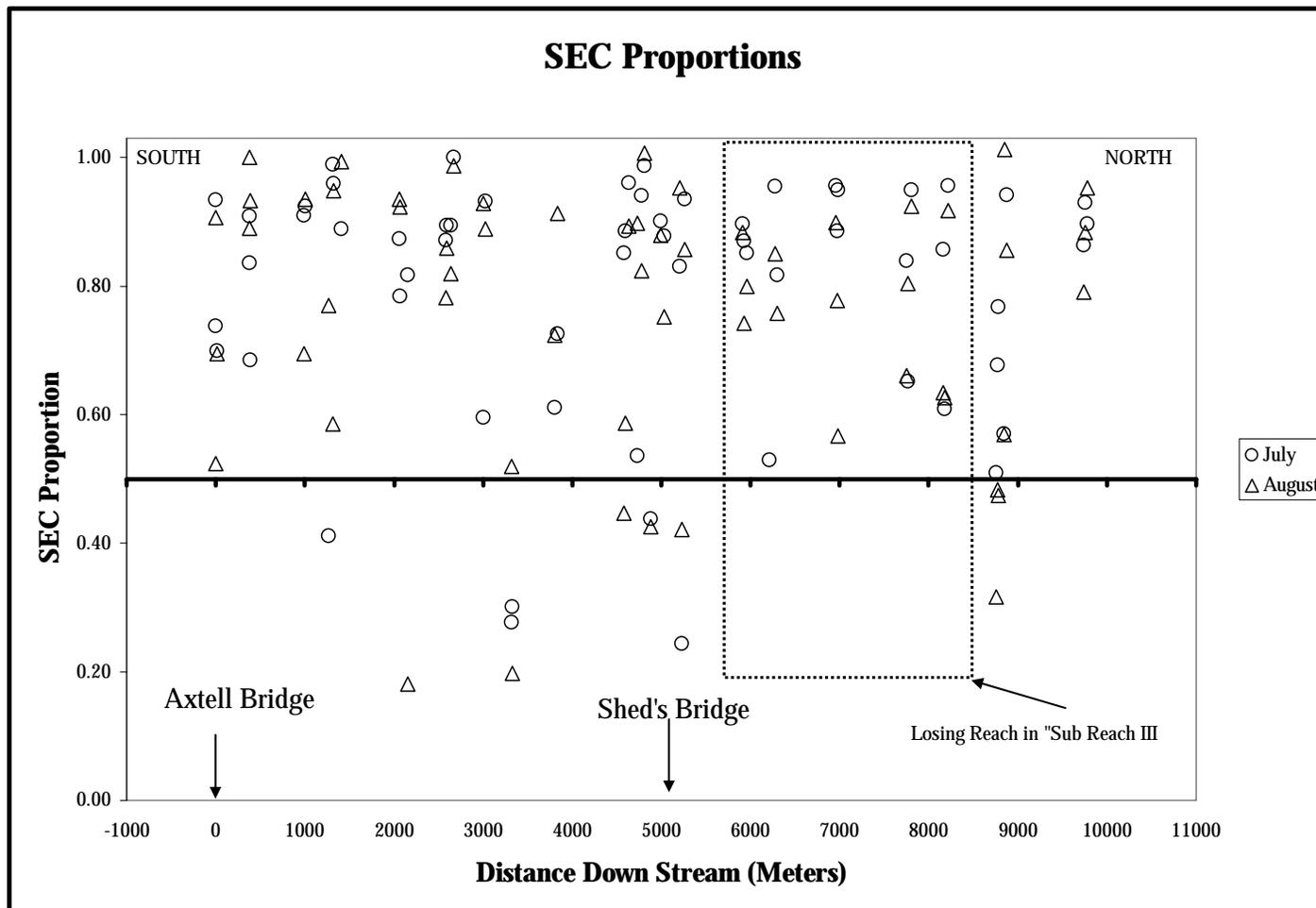


Figure 11. SEC proportions measured in streambed piezometers in the West Gallatin River. The SEC proportion is a measure of the mixing of surface and groundwater, values of 1 are pure river water, values of 0 are pure groundwater, and values greater than 0.5 are more than 50 % river water while values less than 0.5 are more than 50% groundwater. Note the absence of groundwater in Reach III, which shows losses in November, July and August (Figure 7, 8, and 9).

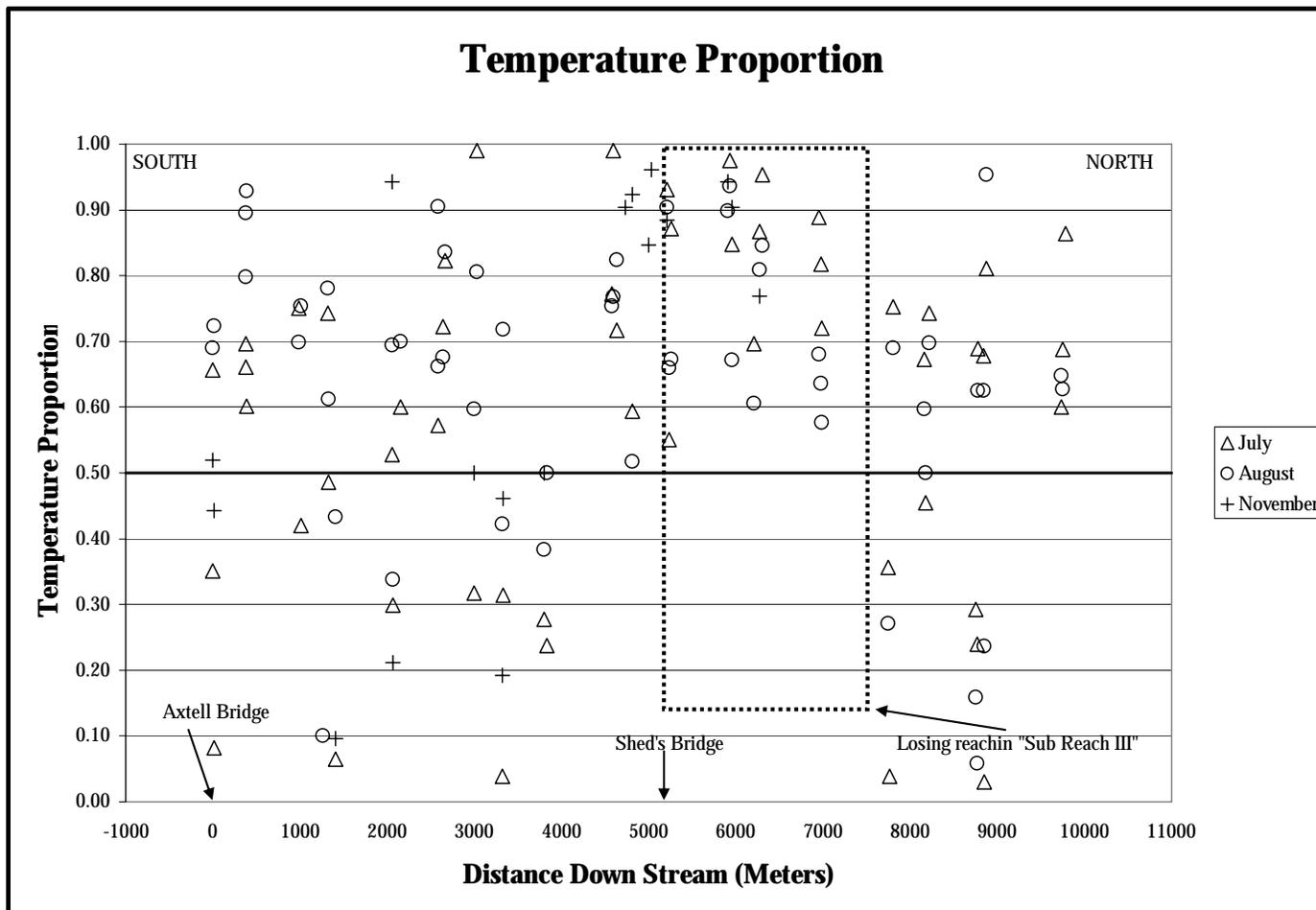


Figure 12. Temperature proportions measured in streambed piezometers in the West Gallatin River. The temperature proportion is a measure of the mixing of surface and groundwater, hyporheic cooling aside, values of 1 are pure river water, values of 0 are pure groundwater, and values greater than 0.5 are more than 50 % river water while values less than 0.5 are more than 50% groundwater. Note the absence of groundwater in Reach III, which shows losses in November, July and August (Figure 7, 8, and 9). The absence of cooling from groundwater discharge and hyporheic exchange has important ecologic implications.

Student fellowship: Water quality function in subalpine wetlands in response to disturbance and restoration

Basic Information

Title:	Student fellowship: Water quality function in subalpine wetlands in response to disturbance and restoration
Project Number:	2006MT105B
Start Date:	3/1/2006
End Date:	6/30/2007
Funding Source:	104B
Congressional District:	At-large
Research Category:	Climate and Hydrologic Processes
Focus Category:	Wetlands, Hydrology, Management and Planning
Descriptors:	None
Principal Investigators:	Duncan T. Patten

Publication

Water quality function in subalpine wetlands in response to disturbance and restoration

Final Report January 18, 2007

*Sunni Heikes-Knapton - Master's of Science candidate at Montana State University,
Land Resources and Environmental Sciences*

Upon being granted a Montana Water Center Student Fellowship Grant, awardee Sunni Heikes-Knapton and advisor Dr. Duncan Patten commenced with site investigation for the research project titled; Subalpine Fen Wetlands: Environmental Drivers and Response to Human Perturbation and Restoration. Throughout the early summer of 2006, numerous wetlands were located and evaluated for research potential within a subalpine region of Southwest Montana. Discussion was held to determine the best options for the direction of the study and to ensure that the chosen sites were able to address the research questions. By mid July, 28 wetlands were identified to be part of the study, all within the boundaries of Moonlight Basin ski area.

Following site selection, appropriate research methods were chosen to examine the parameters of interest for the study. A hand held field meter with 2 pH probes and 2 oxidation/reduction probes were purchased for use in examination of the soils. A small diameter soil core probe was also purchased to take soil samples with the least disturbance to the study area. To examine the water table parameter of the study, multiple sections of 2 inch slotted PVC pipe and associated caps and couplers were purchased to construct the shallow monitoring wells. Three auger heads, two handles, and three 1 meter extensions were purchased to core into the soil to install the monitoring wells. Two 50 meter tapes were purchased to examine the landscape parameters with the use of an eye level. A digital camera was also purchased for recording images of the research. Funds from the Fellowship grant were used to purchase some of this equipment, and also used to offset costs of traveling and working in the field during the 2006 season.

The remaining equipment needs for the study are a soil probe for measuring moisture content, and miscellaneous field gear for recording data. Numerous unmentioned pieces of equipment are available for use through Dr. Patten's Hydroecology Lab. Additional expenses will be associated with analysis of soil organic C content and vegetation biomass.

Through the remainder of the 2006 field season, over 70 monitoring wells were manually installed by Dr. Patten and Sunni Heikes-Knapton. During the installation of the wells, the sites were evaluated for characteristic similarities in vegetation, hydrology, soils, and landscape parameters. A field tour was also performed with the environmental compliance officer from Moonlight basin to gain background information on the restored/constructed wetland sites. Data collection sheets have been composed, and limited initial data collection was performed including preliminary water table

measurements, soil profile data, and identification of wetland plant species. Further field work was prevented by weather in mid September.

Enrollment in LRES 500 was completed in Fall 2006. The course requirements included a presentation of the research topic with some additional preliminary findings. Sunni Heikes-Knapton received a grade of A- for the class. Shortly thereafter, a similarly structured presentation was given at the 2006 Montana AWRA conference in Polson, Montana. This presentation was awarded the first prize student presenter award. The MSU news service also interviewed Sunni for a story on the project to be featured in the "Research Roundup" section of the web publication.

Tasks for spring 2007 include enrollment in 5 thesis credits. A formal proposal of the research was written, and will be reviewed by the graduate committee on January 24, 2007. Following this meeting, a clear set of objectives for the remainder of the research will be laid out, as well as a job description for a field assistant during the 2007 field season. Ideally, the field assistant will be assigned and paid by MSU's Undergraduate Scholars Program.

Remaining time under the Water Center's Fellowship Program will be spent on literature review and outlining and formatting of the draft thesis. Additionally, an application may be submitted for acceptance as a presenter at the 2007 National Society of Wetland Scientists conference early June in Sacramento, California. Weather dependent, the field season and data collection will commence either shortly before or after this conference. Data analysis and additional writing will commence during or directly after the 2007 field season. Defense of the thesis is intended to take place by December 2007.

It is with much appreciation that I have been able to accomplish the previously listed tasks. The Water Center's Fellowship Program has undoubtedly increased the productivity and progress of the project.

Sunni Heikes Knapton

Student fellowship: The effects of overwinter dewatering on brown trout redds and egg survival in a Montana creek

Basic Information

Title:	Student fellowship: The effects of overwinter dewatering on brown trout redds and egg survival in a Montana creek
Project Number:	2006MT106B
Start Date:	3/1/2006
End Date:	6/30/2007
Funding Source:	104B
Congressional District:	At large
Research Category:	Biological Sciences
Focus Category:	Ecology, Water Supply, Conservation
Descriptors:	None
Principal Investigators:	Vicki Watson, Vicki Watson

Publication

Christa Torrens
Research progress briefing
Montana Water Center –
Student Research Fellowship Center
Feb 10, 2007

Project Title: The effects of overwinter dewatering on brown trout redds and egg survival in a Montana creek

For the 2005-2006 field season, 12 baskets containing eggs from wild brown trout specimens native to the site were placed at the study site in Warm Springs Creek. Each basket contained 100 eggs dispersed in gravel that had been sieved to 10-50 mm diameter. The baskets were buried in 6 artificial redds, or fish nests, with two baskets per redd, for a total of 6 sampling locations at the site. Redd locations were selected to replicate the substrate, flow and depth conditions where actual brown trout redds are built. This stretch of Warm Springs Creek is an active spawning location for brown trout, and one member of the study team has been conducting an annual redd count for several years. There was no industry dewatering this year, and streamflow was at its natural level.

The baskets were a modified version of those used by Maret et al (1993) and Rubin (1995), made of rubberized wire and lined with a fiberglass mesh to prevent newly hatched fish from escaping. A metal standpipe was placed in between each pair of baskets for drawing water samples from egg depth (9-12 cm below streambed surface), to measure DO and ammonia. iButton temperature loggers were placed in each standpipe, to record hourly temperatures at egg depth. These were designed to be removed and replaced at each sampling visit; the original study plan was to visit the site semimonthly, sample for DO and ammonia at egg depth, exchange temperature data loggers, and record stream stage and flow data at the head of each artificial redd.

Persistent, bottom-to-surface ice and the disruption of the standpipes led to a modification of the study design. Some ice for much of the wintertime study period was expected, as was total freezing for some parts of the study period. However, there was an early cold snap and by Dec 5 much of the site was iced in. It was found that the metal standpipes became a locus for ice formation, and facilitated bottom-to-surface freezing at almost every basket site. This resulted in a thick layer of ice within the standpipes, hindering data collection; extreme measures to remove the ice, such as pouring heated water down the standpipe, were determined to be too disruptive to the study environment and potentially harmful to the nearby eggs. The standpipes were also frequently scoured out by ice movement, resulting in lost iButtons and holes in the temperature data set. Some sites were occasionally rendered inaccessible due to stream conditions. Temperature, stage and flow data were collected at each accessible redd and, using an existing USGS gauge, just above the study site.

In April 2006, the eggs hatched; the baskets were removed and the number of live fry, live eggs and dead eggs in each basket were tallied. There were very few dead eggs in the samples; almost all showed all live fry. Curiously, up to 60% of the eggs in each basket were simply not accounted for. It is unlikely that any fry escaped, as the mesh lining the basket was tight and the seams were well-sealed. Dead eggs were present and

were quite noticeable, even at advanced stages of decomposition; given the cold instream temperatures, it seems unlikely that more dead eggs existed but decomposed beyond recognition. Stonefly, mayfly and caddisfly larvae were present in some but not all of the baskets, so predation is one possibility for the diminished return. Predation/cannibalism of unhatched eggs, dead eggs and new hatch by other new hatch is a second possibility.

For the 2006-2007 field season, another 12 baskets with 100 eggs each were placed at the same site locations. One site required slight modification to avoid disrupting wild redds. The new location is within .8 meters of the original placement and still within the range of depth and flow typical of brown trout redds. It appears there will not be an overwinter dewatering this season, although it has happened without notice in the past. This season, Hobo temperature loggers were buried in each basket with the egg samples, to be left in place all season. The use of standpipes was discontinued. Regular flow, stage and instream DO and temperature measurements will be taken. When site conditions permit, water samples will also be collected from egg depth in the gravel environment. In April, the baskets will be removed and the numbers of fry/live egg/dead egg will be tallied.

In the summer of 2007, a groundwater and intragravel flow component will be added to the study, to determine how intragravel flow is affected by different stream stage and streamflow conditions as well as the locations of any downwelling or upwelling points at the study site. Intragravel flow is essential for flushing metabolic wastes and carrying oxygen-rich water to the egg environment; it will serve as an approximation for DO and ammonia measurements with developing eggs, which will not be available in the summer. Both instream and intragravel measurements of DO and temperature will be taken. Any variation between the two environments will be noted and examined for significant correlation with stream stage and flow. All DO measurements will be calculated in terms of percent saturation to permit comparison between summer and fall-winter conditions. Combining these results with the winter measurements will allow a more complete description of how ambient conditions at egg depth are likely to shift with changes in stage and streamflow. It will also allow for measurements at very low flows, in case there is not an overwinter dewatering during the course of this study.

Due to funding from another source, the study will continue another year, through the 2007-2008 field season. It is expected that the study design for next season will remain the same as for this season; modified groundwater/intragravel monitoring may continue through the winter season as well.

Again, the outcome of this study will help determine the effects of overwinter dewatering on egg development and survival, and will be used by the Butte branch of Trout Unlimited to determine their position on the practice of industrial overwinter dewatering of Warm Springs Creek. Thanks again for your assistance; the Montana Water Center fellowship has been invaluable to my 2005-2006 research, coursework and fieldwork, making this project possible.

Student fellowship: Effects of road culverts on eastern Montana prairie fish assemblages

Basic Information

Title:	Student fellowship: Effects of road culverts on eastern Montana prairie fish assemblages
Project Number:	2006MT108B
Start Date:	3/1/2006
End Date:	6/30/2007
Funding Source:	104B
Congressional District:	At large
Research Category:	Biological Sciences
Focus Category:	Water Quality, Ecology, Nutrients
Descriptors:	None
Principal Investigators:	Thomas McMahon

Publication

2006 Water Center Fellowship: Progress Report

Effects of Road Culverts on Eastern Montana Prairie Fish Assemblages

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Graduate Committee:
Thomas McMahon, Joel Cahoon, Robert Bramblett



Abstract

Road culverts can serve as obstacles to fish migrating between seasonal habitats. The development of new roads, as well as the repair and upgrade of existing roads has led to research addressing the effects culverts have on fish populations. The majority of this research has focused on salmonid species, but the total effect road culverts have on species continuity in small, prairie streams is largely unknown. This study examines the effects road culverts have on prairie fish assemblages in the lower Yellowstone River drainage. Because many of the diverse number of fish species found in prairie streams are small bodied, and likely poor swimmers, culverts may act as significant barriers to passage. Culvert characteristics that limit passage include outlet drop, high water velocity, and insufficient water depth. Several tributaries of the Yellowstone River with a variety of culvert crossings will be examined. Passage abilities of prairie-fish species will be assessed indirectly using software models, and directly using mark-recapture experiments. The longitudinal distribution of fish species will also be examined for trends related to restricted passage. This study will ultimately provide insight into the effects culverts are having on an assemblage of fish that not only represents a recreational resource, but also contributes the overall diversity of a "healthy" ecosystem. Fish managers and engineers alike could also gain valuable information on the relationships of culvert type and discharge on fish passage efficiency. This could lead to more effective culvert designs and installations.

Accomplishments for 2006

Objective 1: Examine the physical and hydraulic characteristics of culverts associated with fish passage.

- Installed water height data loggers at five culverts in PVC stilling wells. Stream discharge was recorded throughout the summer to create a stage-discharge relationship. These data will be used to estimate water velocities in the culverts, and will be used for both the indirect and direct assessments of fish passage.
- Physical dimensions and channel cross sections associated with each culvert were measured. These data will be used for the FishXing software model (indirect assessment).
- Mark-recapture experiments were conducted at two different flow levels at all five culverts. This was done to examine the effects of water depth and water velocity on fish passage.

⇒ Preliminary results show that fish movement was not significantly different through culvert versus natural reaches ($P > 0.05$). This suggests that water depths and velocities found in these culverts were similar to those of natural stream reaches.

Objective 2: Examine how species and total length of fish affect passage capabilities.

- As mentioned above, mark-recapture experiments were conducted twice at each culvert crossing. The predominant three to four species captured in the vicinity of the culvert were used for each experiment. Species included: creek chub *Semotilus atromaculatus*, brassy minnow *Hybognathus hankinsoni*, flathead chub *Platygobio gracilis*, longnose dace *Rhinichthys cataractae*, sand shiner *Notropis stramineus*, and white sucker *Catostomas commersoni*.
- ⇒ Preliminary results show that fish movement through culverts was similar to that of natural reaches for all species tagged throughout the study. One exception to this finding occurred during very low flow conditions. In this case, movement of longnose dace was lower through a culvert reach than through its corresponding reference reach. During this experiment, other species (creek chub and white sucker) successfully passed the culvert. This suggests that passage conditions may be different for each species of fish.
- Fish of different size classes were used during each mark-recapture experiment to examine the effects of body length on passage capability. Because some species' maximum length was equal to the minimum tag length, only creek chub and white sucker were able to be broken down into different size classes.
 - The FishXing software will be used to indirectly assess fish passage for each species used in the mark-recapture experiments (where available) to compare against the results of our direct observations (mark-recapture).

Objective 3: Examine how passage capabilities influence the longitudinal distribution of prairie fish.

- A total of 13 sites were sampled for fish species composition and relative abundance in both Clear (10 sites) and Sand Creeks (3 sites). The sites were sampled twice throughout the summer to account for some species recruiting to the gear as the summer progressed. Sites were 300m in length, and were sampled using 6.35mm mesh seines. The sites were selected so that three equally spaced sites were located above and below each stream crossing. The exception to this was in Sand Creek, where only one site was established above the crossing due to lack of water. Additionally, on Clear Creek only one site can be found above the uppermost crossing due to access complications. At each site, habitat variables including thalweg depth, wetted width, and dominant substrate were measured as well.
- ⇒ Preliminary results show few differences in species richness and relative abundance above/below each culvert crossing. This suggests that in these streams, culverts are having little effect on the spatial distribution of fish.

⇒ Preliminary results show little difference in the habitat variables measured above/below culvert crossings.

Additional work conducted:

- Capture efficiency using seines and backpack electrofishing appeared to vary in relation to in-stream habitat and turbidity. Therefore, capture efficiency was measured at a subset of mark-recapture sites. To determine capture efficiency, reaches upstream and downstream of the culvert were closed at either end using 6.35-mm mesh block nets. 30 fish per reach were then marked with a pelvic fin clip, and placed in their respective reaches. Duration of these studies was the same as the direct assessment experiments. After 48 hours, the same method of recapture (seining and electrofishing) was used to collect the fish in each reach. Fish were counted and examined after each pass with the seine and with the electrofisher. Percent recapture efficiency was calculated as the total proportion of fish recaptured after three passes of seining and three passes of electrofishing.
 - Visible Implant Elastomer (VIE) tags were chosen as the method of marking because of their adaptability to a number of species and size classes, and because we felt they would have the least effect on fish swimming capability. Interspecific body type and color difference, as well as tagging error, can affect the retention of VIE tags. The unknown loss of tags can adversely affect mark-recapture experiments. Therefore, a pilot study to determine the retention of VIE tags was necessary. This study involved tagging 30 fish representing the predominant species and size classes, and placing them in a cage with 30 unmarked fish in the stream for 48 hours. Fish were then examined by a field technician for VIE tags, and a percentage representing retention rates after 48 hours was calculated.
- ⇒ Results from this pilot study show 100% retention and easy identification of VIE tags after 48 hours. Species tagged included creek chub and white sucker.

Student fellowship: Sources of groundwater and subsurface water acquisition and utilization by conifers invading riparian communities in western Montana

Basic Information

Title:	Student fellowship: Sources of groundwater and subsurface water acquisition and utilization by conifers invading riparian communities in western Montana
Project Number:	2006MT110B
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Principal Investigators:	Clayton Marlow

Publication

ROOTING DISTRIBUTION OF TWO TREE SPECIES IN UPLAND AND RIPARIAN AREAS IN WESTERN MONTANA

Final Report for Water Research Fellowship

Erin Thais Riley, PhD Candidate
Clayton Marlow, Main Advisor



Picture by Erin Thais Riley at Pony Canyon, January 26, 2007.

Project Synopsis

This current project is an effort to provide insight into a plant-soil interaction that may be fundamental to the understanding of landscape scale effects of global climate change, disrupted fire cycles, and stream flow. While it is believed larger trees are tapping into deeper groundwater sources and smaller sized trees are using water in the upper 1-2 m of the forest floor, it is possible that small Douglas-fir are capturing shallow ground water before it can enter the subsurface flow path that recharges streams. In contrast, small aspen may be sharing deeper water sources with conspecific larger trees (Arno 1986).

Identifying those variables that affect lateral runoff is key to understanding the water budget and ultimately the affect of fire suppression and climate change on stream flow. This research will help management agencies to adjust their management objectives for vegetation management under the constantly changing pressures of climate change, fire suppression, and grazing.

The Beaverhead-Deerlodge forest south of Boulder, MT is dominated by lodgepole pine and Douglas-fir in the uplands with Quaking aspen in the riparian systems. Following a century of aggressive wildfire suppression, local ranchers and government officials are seeing a decline in stream flow that wildlife and livestock use for water sources. The degree of interaction between climate change and disrupted fire cycles and stream flow has become a focal point for forest, range, and landscape ecological research. Among the management agencies interested in this work is Bureau of Land Management, Lewistown Field Office. A fire ecologist at this field station acknowledges immediate benefits from this research.

Vegetation management requires knowing which species and age class to manipulate to achieve landscape goals. The goal of this project is to determine if Douglas-fir, aspen, and herbaceous communities extract water from the same or different depths within the top 1-2 m of the soil column. To achieve this goal we will: 1) ascertain rooting zone within the upper soil column in Douglas-fir, Aspen and grass/forb communities; 2) confirm rooting depth stratification by using a DNA fingerprinting tool (AFLP's) to match roots from a specific depth to their counterpart; 3) compare the impact of the forb and grass community on soil water decline in non-forested areas. The hypotheses of this study are as follows: 1) smaller sized Douglas-fir and aspen use water within the upper profile where as larger sized Douglas-fir use deeper water sources and 2) grasses and forbs have less affect on soil water status than small-size classes of trees.

Douglas-fir size classes 1,2 vs. 3 are significantly different ($P=0.07$). Conversely, aspen was not showing significance among different size classes with $n=15$. The initial results are based on four sites, two were Douglas-fir and two were aspen out of the 24 total sites.

Research Approach:

This research project will aim to achieve the goal of determining if Douglas-fir, aspen, and herbaceous communities extract water from the same or different depths within the top 1-2 m of the soil column. Predawn water potentials (Ψ_{predawn}) coupled with soil water potentials (Ψ_{soil}) extrapolated from soil water content (θ) measured with a neutron moisture meter will give estimations about what depths Douglas-fir and Aspen are acquiring their water from. Measuring leaf conductance with a porometer will allow us to see if the trees are water stressed. AFLPs which is a DNA fingerprinting tool, will allow us to definitively say if those tree roots are found at that depth in the soil.

Study Design:

Individual trees will be sampled for three seasons within 24 pre-selected sites represented by four drainages. Samples will be collected during the months of April, May, June, July, August, September and October. Each of the 24 sites have two Neutron Moisture Meter tubes inserted to a 154 cm depth and soil water volume at 20 cm intervals will be collected Monthly during the spring and fall and weekly during the summer months. One

of the Neutron Moisture Meter tubes is undisturbed and one has the entire vegetative understory removed to account for water uptake by grasses and forbs. At each site 12 trees will be selected, tagged, and classed into three size categories. Class size one is between 0 and 120 cm, class size two is between 120 and 240 cm and class three is any tree above 240 cm. Twelve trees at each site will be paired within 2 meters (Moore and Owens 2006) of one another and sampled using the predawn water potential and porometer on the sides as close to each other as possible to avoid environmental variability that can occur from atmospheric, edaphic, topographic, and vegetation variables (Wambolt 1973). To be sure roots are present at certain depths, DNA from aboveground plant tissue such as leaves will be isolated and digest the DNA using Amplified Fragment Length Polymorphism technique then matched to their roots. Soil cores will be taken with a two inch circumference soil core to 154 cm depth or bedrock, extracted and fine roots will be removed, their depth recorded in the soil, and matched to their above ground counterpart. Drs. Luther Talbert and Mike Giroux at Montana State University have offered the use of their labs to conduct this portion of the research project.

Neutron Moisture Access Tubes

24 forested sites were chosen to install two Neutron Moisture Meter access tubes. These sites were chosen by location within watersheds and tree community. 12 of the sites are dominated by aspen and 12 sites are dominated by Douglas-fir. These access tubes will be 15 meters apart and one will have all understory vegetation removed with roundup. 24 of these access tubes were inserted 154 cm or bedrock, whichever came first, and the remaining will be inserted at the same depth this coming summer. The access tubes were dug with a 5 cm hand auger and thin walled PVC pipe was installed for the access tubes. Rubber stoppers were inserted in the bottom of each tube to keep moisture from entering at the bottom of the tubes. About 31 cm were left above ground to set the Neutron Moisture Meter on top of.

Soil water potential

Soil water potential (Ψ_{soil}) will be calculated through soil retention curves developed for the study site. Samples from different horizons visible within soil pits dug will be collected. Soil water volume (θ) is measured at 20 cm intervals to the bottom of the neutron moisture access tubes. Soil water potential will be developed by Midwest Industries Corp. from the soil samples collected from the different soil horizons, and soil water potentials (Ψ_{soil}) will be extrapolated from soil retention curves by using soil water content collected with the Neutron Moisture Meter. This method of extrapolating soil water potentials from soil water content has shown to have a very high correlation (Fahey and Young 1984).

Xylem Water Potential

In this study, leaf petioles from Quaking aspen trees are used to get an estimate of xylem water potential and twigs are used to get xylem water potential for Douglas-fir. Pressure chamber determinations are estimates of the total water potential of the xylem sap (Ritchie and Hinckley 1975). The twig or leaf is removed from the tree and cut with a razor blade at an angle to allow for more surface area of the xylem. The instant water is seen at the end of the leaf petiole or twig, the pressure gauge is read and recorded. This value measured in bars is the xylem water potential for the twig and will be used to represent the xylem water potential for the tree.

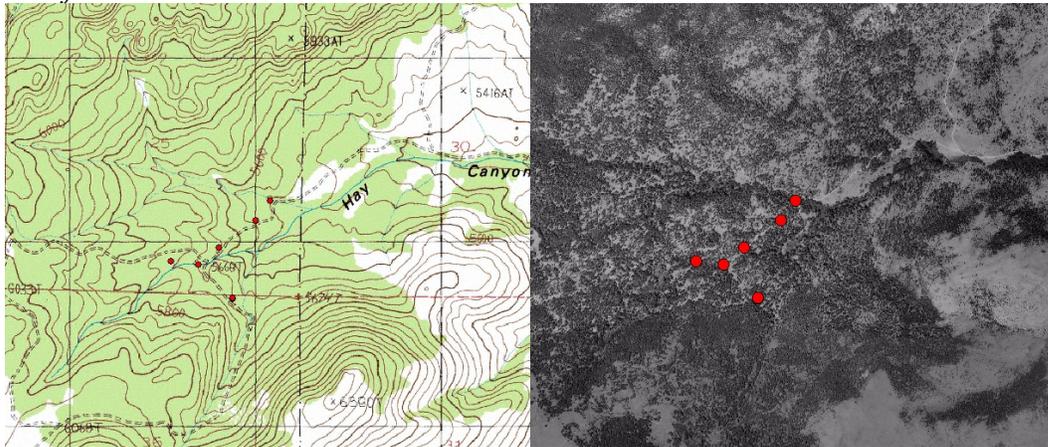
Leaf Conductance

A steady state leaf porometer (Decagon Devices) is used to measure stomatal conductance. This is a measure of the passage of carbon dioxide (CO₂) or water vapor through the stomata of the leaf. The leaf porometer calculates the resistance between the inside and outside of the leaf with a measurement in mmol/m² s⁻¹ (Millimoles per meter squared seconds). This measure how much conductance or exchange is taking place between the atmosphere and the leaf of the tree. If the tree is transpiring more, it has access to water, but when the tree does not have access to water, it will close its stomates. The same twelve trees that were measured for xylem water potential will be measured for leaf conductance.

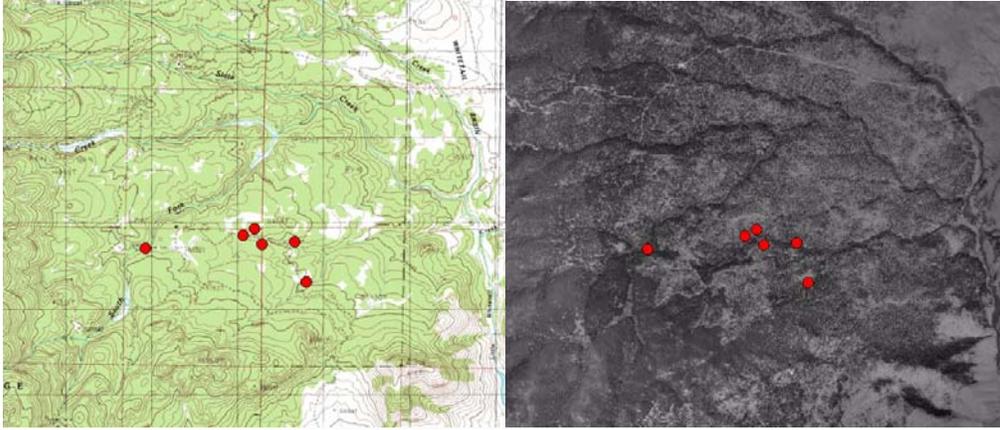
Amplified Fragment Length Polymorphisms

This DNA fingerprinting tool allows DNA polymorphisms to be determined between individual plants. We will use this technique to determine the identity of fine root tissue and match them to their aboveground counterpart at different depths. The use of AFLP kit from Invitrogen Life Technologies will be used to do the isolation and amplification following three major steps: restriction endonuclease digestion of the DNA and ligation of adapters, amplification of the restriction fragments, and gel analysis of the amplified fragments. This technique usually creates 50 to 100 restriction fragments in each AFLP, making it very powerful in detecting DNA polymorphisms and a good means to identify individuals. High quality DNA must be used with this technique which is why small roots will be used in fingerprinting (Jackson 2000). Douglas-fir plots will be sampled by individuals while fingerprinting aspen may be difficult since they are all one organism.

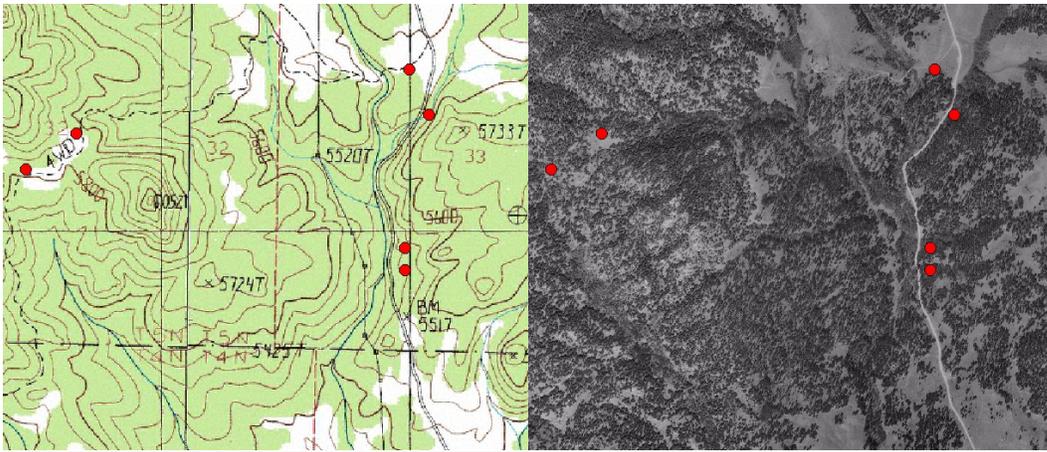
Study area



Hay Canyon with neutron access wells as the red points.



State Creek with neutron access wells as the red points.



Boulder Divide with neutron access wells as the red points.



Pony Reject with neutron access wells as the red points.

This study site is in Jefferson County, Montana north of Whitehall, Montana. It is located on Route 16 that goes from Whitehall, MT to Boulder, MT. The site is located on the west side of the road between long 45°00' and Lat 112°00, 46°00' and 112°15', 112°00' and 46°15', 112°15' and 46°15'. Land resources within the study are administered by the Bureau of Land Management, Butte Area Office and the Beaverhead-Deerlodge National Forest. The sub-watersheds under consideration lie within the northern portion of the Upper Jefferson TMDL Planning Unit.

The drainages empty to the east to south east and were chosen due to similarity in aspect. The upper portion of the larger watershed area is dominated by Lodgepole pine (*Pinus contorta*) forest which transitions into Douglas-fir (*Pseudotsuga menziesii*) forest and ends in a sagebrush/grassland type in the valley bottom. The watershed that contains the target sub-watersheds drains over 11,200 ha (28,800 acres) or about 36% of the Whitetail Basin.

Vegetation and soil sampling

The vegetation changes from site to site but the dominant vegetation are: Bearberry (*Arctosaphylos uva-ursi*), Baltic Rush (*Juncus balticus*), Big Sagebrush, (*Artemesia tridentata* ssp. *Veseyana*), Bluebunch Wheatgrass (*Pseudoroegneria spicata*), Clover (*Trifolium repens*), Common Horsetail (*Equisetum arvense*), Idaho Fescue (*Festuca idahoensis*), Kentucky Bluegrass (*Poa pratensis*), Oatgrass (*Danthonia spp.*), Red Top (*Agrostis stolonifera*), Smooth Brome (*Bromus inermis*), Snowberry (*Symphoricarpos occidentalis*), Tailcup lupine (*Lupinus caudatus*), Wild Geranium (*Geranium viscosissimum*), and Woods Rose (*Rosa woodsii*)

The soils in this area are predominately sandy soils with some silt. This changes in the riparian systems where more clay is present. This is not expected considering the granite parent material of the boulder batholith area.

The parent material of the Whitehall site is intrusive igneous rock that probably originated 10-20 miles beneath the surface during the late Cretaceous and early Tertiary period. (78-69 million years ago) During the Eocene Epoch the overlying rock was removed (1 mile above the surface) exposing the igneous rock below. The major exposure in the study site is the Boulder batholith with smaller satellite bodies connected to the primary extrusion but are similar in composition and texture. The dominant type of material is light gray coarse to medium grained quartz throughout the watershed. Large boulders can be found which are called tor piles, and were formed from weathering and erosion along joint planes. The material that is found in the stream beds in the Quaternary period is Alluvium composed of silt, sand and gravel in the stream valleys (Arno 1986).

Environmental Measurements

Relative humidity, temperature, and precipitation will be measured in each of the sub-watersheds by placing a probe at 2/3rd canopy height. Vapor pressure deficit will be calculated using air relative humidity and temperature at a height corresponding to two thirds of the canopy height. This will give us a good indication of RH measures and effects on transpiration. Temperature and precipitation are recorded with digital recorders within one mile of the sample site. These are encased in fencing to keep wildlife from destroying them. Soil volumetric water content will be taken using a

Neutron Moisture Meter by wells throughout the sample units. Soils will be taken back to the lab, weighed, dried, and weighed again to get soil mass. Bulk density and volumetric water content will be calculated for the sites.

Supporting Hydrologic Data

This data will provide important information about vegetative effects on streamflow and at what depths different tree species are affecting the soil water moisture. In tangent of this study, fifteen monitoring wells have been placed in the riparian zone of 4 drainage. The wells are arranged in rows of three with the two outermost wells in the upland ecotone on the sides of the stream and one well adjacent to the stream channel. Since 2003, measurements of groundwater elevation have been taken from April to November. In 2004 pressure transducers were installed inside stilling wells on each stream above and below the prescribed burn sites to monitor surface flow. In the spring of 2006 Hay Canyon was burned with a prescription of 70% small Douglas-fir to be removed. We are currently looking how the groundwater wells and surface water runoff are affected by this burn.

Results to date:

Initial review of neutron and groundwater monitoring in 2006 indicated the following. Douglas-fir size classes 1,2 vs. 3 are significantly different ($P=0.07$). Conversely, aspen was not showing significance among different size classes with $n=15$. A sample adequacy test was used to get an idea of sample size using our initial results. This test indicated that we need close to $n=45$ samples to get a $P=0.05$ for the Douglas-fir size classes 1,2 vs.3. The sample adequacy for the aspen when comparing size class 1 vs. 3 is with a $P=.1$ we need approximately $n=91$ and we currently have approximately 15 (Kupper and Hafner 1989). The initial results are based on four sites, two were Douglas-fir and two were aspen out of the 24 total sites.

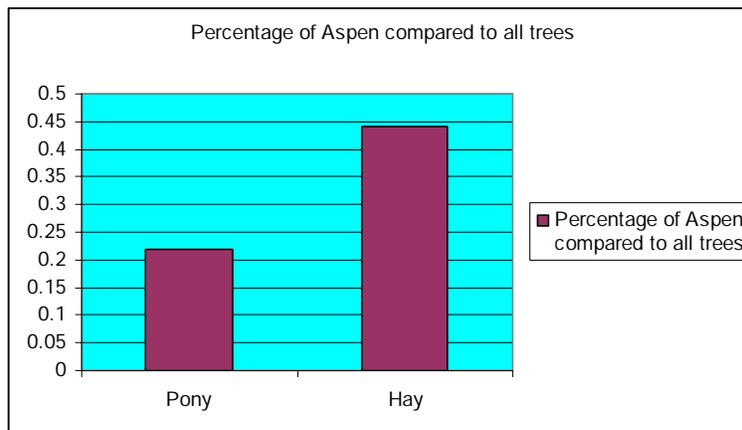


Figure 3

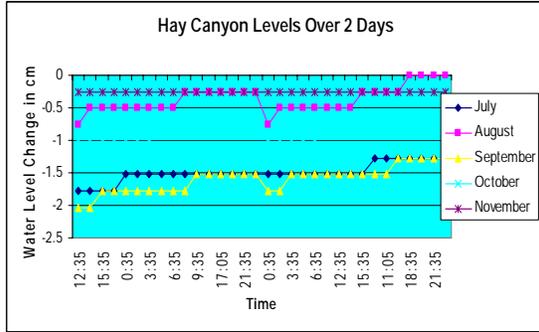


Figure 4

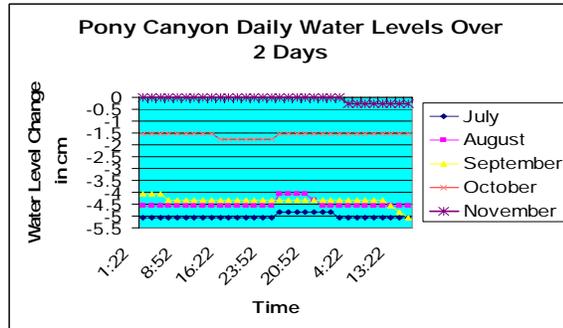


Figure 5

The two canyons sampled are Hay and Pony Canyon. Pony Canyon has considerably less aspen than Hay Canyon (fig. 3) which could explain the greater amount of oscillation in Hay Canyon than we see in Pony Canyon. If we look at the difference of surface water levels between Hay (fig. 4) and Pony (fig. 5) Canyon every hour and a half over two days, we see there is a much greater amount of fluctuation in the Hay drainage than in the Pony drainage. The more negative the number, the lower the water level. This relationship needs to be investigated in more detail to confirm or reject the hypothesis that tree encroachment can affect stream flow.

Research Products:

The expected products of this research are two journal articles, two posters presented at professional meetings, three departmental presentations and a paper for the Society of Range Management.



Outreach Activities:

As a Ph.D. student Erin Thais Riley has the opportunity to help design and implement a curriculum for a class given at Little Big Horn College on the Crow Reservation. This class was implemented this past summer and was a five day field school. A portion of the class was riparian ecology and management that Ms. Riley developed a course curriculum for. This course will continue for the next two summers with Ms. Riley’s help. The Crow, as well as other Native Americans, are concerned about the affects of off-reservation land uses on water resources within the reservation. Riparian tree species, like aspen, have cultural significance to native peoples so the Crow are concerned about management options that involve tree removal. Consequently, the information generated

from this study will be incorporated into the LBHC natural resource field school curriculum in 2007 and 2008. The results of this research will be used by the Forest Service and BLM in land management prescriptions to help regenerate water in systems that are lacking.

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Temporal and spatial changes in the concentration and isotopic composition of nitrate in the upper Silver Bow Creek drainage, Montana.

Basic Information

Title:	Temporal and spatial changes in the concentration and isotopic composition of nitrate in the upper Silver Bow Creek drainage, Montana.
Project Number:	2006MT86B
Start Date:	3/1/2006
End Date:	12/31/2007
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Congressional District:	At large
Research Category:	Water Quality
Focus Category:	Nitrate Contamination, Hydrogeochemistry, Surface Water
Descriptors:	None
Principal Investigators:	Chris Gammons, John LaFave

Publication

Temporal and spatial changes in the concentration and isotopic composition of nitrate in the upper Silver Bow Creek drainage, Montana.

Interim Progress Report

prepared for

The Montana Water Center and the U.S. Geological Survey

April 27, 2007

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Background

The upper Clark Fork River, Montana, is currently undergoing extensive and costly reclamation activities that are unprecedented in scope within the US. Whereas much progress continues to be made removing sources of heavy metal and arsenic contamination stemming from historic mining and smelting activities in the Butte-Anaconda area, a new problem has emerged that seriously threatens the water quality of the watershed: too many nutrients. As early as the 1990s the Tri-State Water Quality Council examined this problem and made detailed recommendations for the institution of a Voluntary Nutrient Reduction Program that would involve several municipal centers and industrial users in the Clark Fork watershed. Specific targets were set for standing algae crops (measured in terms of mg/m² of chlorophyll a), total P (20 µg/L upstream of Missoula), and total N (300 µg/L). Nuisance algae is known to be a major problem in the upper Clark Fork River between Deer Lodge and Missoula, and there are concerns about nutrient loads to Lake Pend Oreille in Idaho. However, the scope of the nutrient problem in Silver Bow Creek, the main headwater stream of the upper Clark Fork River, has largely gone unnoticed.

In Year 1 of this project, our research group has documented numerous sources of nutrient (nitrate, ammonia, phosphate) loading in the upper Silver Bow Creek watershed, focusing on the area upstream of the town of Rocker. The situation is worse than this writer realized when our Year 1 proposal was written. The Butte waste water treatment plant (WWTP) is the largest point source polluter of upper Silver Bow Creek. Based on our work to date, the nutrient species of greatest concern is not nitrate, but rather ammonia (NH₃ or NH₄⁺, depending on pH). Ammonia is toxic to fish, is a potential source of chemical oxygen demand (leading to lethal drops in dissolved oxygen during summer nights), and also is highly bio-available, promoting extreme summertime blooms of algae and aquatic macrophytes. The ultimate goal of the ongoing cleanup of Silver Bow Creek is to restore the stream to fully-functioning status in terms of its ability to support aquatic life and ideally a trout fishery that would have recreational and economic benefits to local communities. However, because Silver Bow Creek is a small stream (typical baseflow is < 30 cfs), the quantity of clean water coming from mountain runoff is insufficient to dilute the nutrient loads from the Butte WWTP and other sources. Consequently, despite 100's of millions of dollars in lawsuits and restoration efforts, upper Silver Bow Creek remains much too polluted to support a trout fishery.

Site Description

The city of Butte (pop. 33,000) is located in the Summit Valley, a 60-square mile alluvial-filled intermontane basin at the head of the Clark Fork River watershed (Fig. 1). The main streams flowing north through the valley are Blacktail Creek and the smaller Basin Creek. The upper reaches of Silver Bow Creek coming into the valley from the north are diverted by active mining operations. The ancestral uppermost Silver Bow Creek channel in the study area is now occupied by a much-diminished flow termed the Metro Storm Drain (MSD, Fig. 1). The recently re-engineered MSD has virtually no surface flow during the cold months, and receives less than 1 cfs of clean imported water from Silver Lake (west of Anaconda, MT) during the summer to enhance the esthetics of the area which includes walking trails and interpretative signs explaining some of the reclamation activities that have taken place. Our Year 1 monitoring indicates that the MSD is a very minor source of nutrient loading to Silver Bow Creek. More importantly, three point sources enter Silver Bow Creek before it exits the Summit Valley to the west. These include shallow groundwater collected and treated from the south (Montana Pole) and north

(Lower Area One) sides of Silver Bow Creek, as well as the effluent from the Butte waste water treatment plant (WWTP) (Fig. 1). The discharge from the Montana Pole and LAO facilities averages around 1 cfs during normal baseflow conditions. The volume of the WWTP effluent typically falls in the range of 3 to 10 cfs, and tends to crest in the late morning and afternoon hours in response to patterns of water use by the residents of Butte.

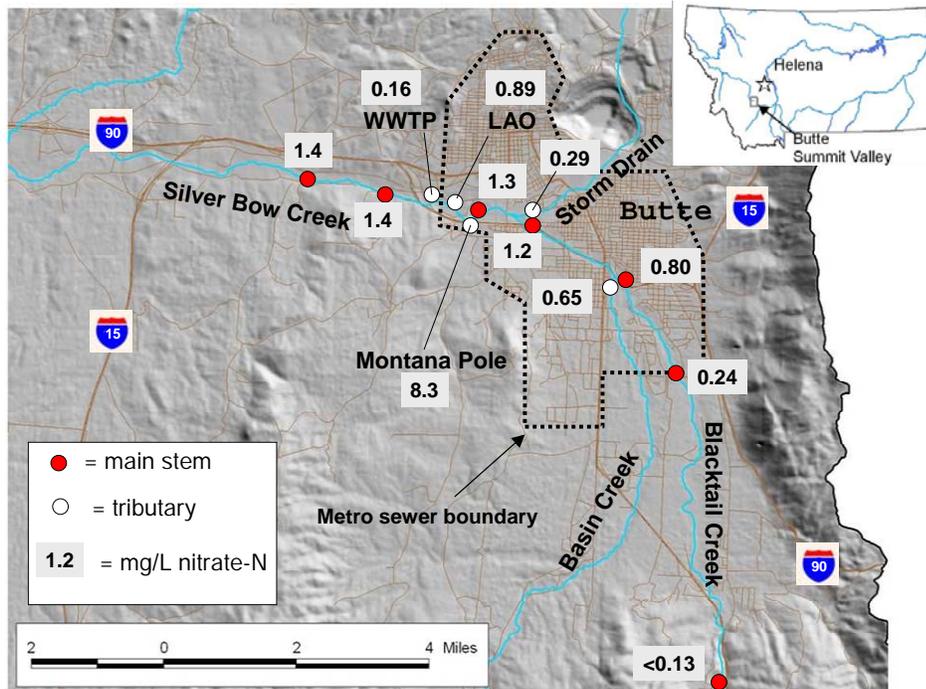


Fig. 1. Map of the Butte Summit Valley, showing sampling locations used in this study. Data next to each sample site are dissolved nitrate results (mg/L as N) obtained by our group in October, 2006. Abbreviations: WWTP = Waste Water Treatment Plant discharge; LAO = Lower Area One discharge.

The flow of Silver Bow Creek immediately below the WWTP discharge point is continuously monitored by a USGS gaging station. Another USGS gaging station is located near the mouth of Blacktail Creek, just upstream of the confluence with the Metro Storm Drain. Streamflows in Silver Bow Creek typically fall in the 20 to 30 cfs range under baseflow conditions, but can show sharp increases to several hundred cfs after heavy rain or snowmelt events. Because of the aforementioned diurnal pattern in the WWTP discharge, the flow of Silver Bow Creek below the WWTP also shows a diurnal cycle under baseflow conditions.

Land use varies from urban/mining/residential in the north part of the valley to lower density residential sub-divisions in the southern part of the valley, with the usual recreational amenities such as golf courses and horses. All residences outside of the municipal water and sewer district (Fig. 1) rely on individual well and septic systems. Like many intermontane valleys in the West, the Summit Valley is expanding, with dozens of new homes constructed each year, mostly in areas outside of the Butte sewer district. The Montana Bureau of Mines and Geology (MBMG) has documented chronically high levels of nitrate in groundwater wells throughout the Butte Summit Valley (Carstarphen et al., 2004), and high nitrate levels in the shallow aquifer were recently responsible for stalling the permitting process for a new sub-division in the southern part of the valley (see “Septic Shock”, The Montana Standard, Sept. 17, 2006). However, prior to the current study, little published information existed on nutrient concentrations in surface water. A large amount of information on nutrient concentrations in Silver Bow Creek does exist in the

“gray literature” of government and consultant reports, and one of the activities during Year 1 of this study has been to assemble this information. Although not reported here, the results of this synthesis will be included in the final deliverable of the project, which will constitute the MS thesis of Beverly Plumb.

Summary of Progress from Year 1 Funding

Field sampling in Year 1 of this project began in May 2006, and continued through April of 2007. Because this project was recently granted a 2nd year of funding from the Water Center, what follows is a brief summary of Year 1 activities and results only. A complete interpretation of the data will be given at a later date. Some of the results that follow were presented by MS student Beverly Plumb at the 2006 Montana AWRA conference in Polson, MT (Plumb and Gammons, 2006).

1. Synoptic sampling was performed along the course of upper Silver Bow Creek and its tributaries in May, June, August, October, December 2006, and January, March, April 2007. These data show a moderate increase in nitrate and phosphate concentrations and loads from non-point source pollution as surface water of Blacktail and Basin Creeks makes its way through the Butte Summit Valley (Fig. 1, Fig. 2a). Concentrations of nitrate at the mouth of Blacktail Creek (USGS gaging station 12323240) average near 1 mg/L (as N) during normal flows, which is quite high for a Montana stream, showing clear evidence of nutrient impairment. Blacktail Creek is a gaining stream through its lower reaches, and the majority of the nitrate in lower Blacktail Creek most likely comes from contaminated shallow groundwater in the Butte valley. The source of the nitrate in the shallow aquifer is believed to be a combination of septic tank leachate from non-sewered homes and subdivisions, animal waste, and organic or chemical fertilizers. This hypothesis is consistent with preliminary stable isotope results discussed below.

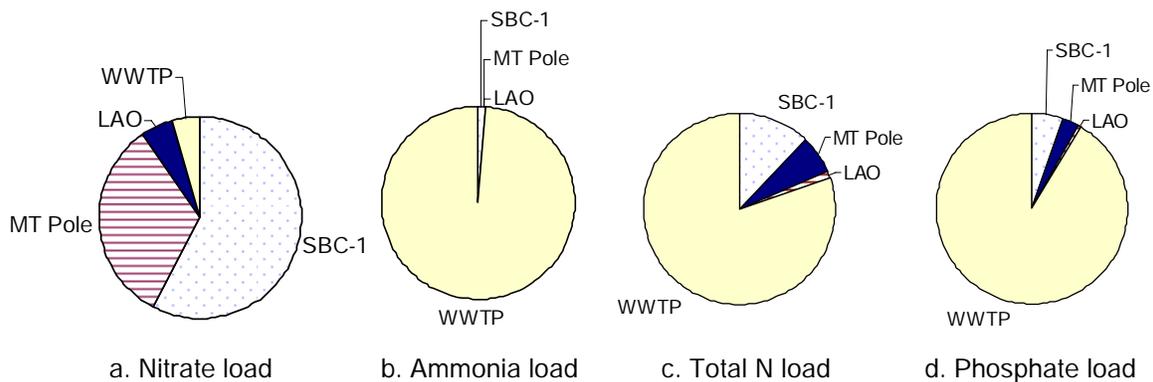


Figure 2. Comparison of nutrient loads in October, 2006. SBC-1 represents the total load in Silver Bow Creek upstream of the three identified point sources.

2. In addition to the chronically elevated nitrate levels in the tributaries to Silver Bow Creek (represented by SBC-1 in Fig. 2), significant increases in nitrate load come from treated groundwater from the Montana Pole Superfund Site (MT Pole), with lesser contributions from treated groundwater from the Lower Area One lime treatment plant (LAO), and effluent water from the Butte Waste Water Treatment Plant (WWTP). It is important to stress that no nutrients

are added to the groundwater that is treated at MT Pole and LAO. The MT Pole site uses a combination of physical and microbial processes to degrade chlorinated hydrocarbons in a highly contaminated groundwater plume on the south side of Silver Bow Creek, whereas LAO uses lime addition to treat metal-contaminated groundwater from the north side of Silver Bow Creek. The WWTP effluent has little nitrate, but very high concentrations and loads of dissolved phosphate and ammonia (Fig. 2b), which together severely degrade the water quality of Silver Bow Creek for many miles downstream (see below).

3. In August of 2006, a 4 mile long “dead zone” was documented below the confluence of the WWTP effluent and Silver Bow Creek. In this reach, the concentration of dissolved oxygen was observed to drop below 5 mg/L for an extended period (> 6 hours) during the night (Fig. 3). Such low levels are lethal to trout, especially if combined with other stresses (such as high ammonia, high nitrite, high pH, or high temperature). In the middle of the dead zone, the concentration of DO dropped below 1 mg/L for over 12 hours between 6 PM and 8 AM (Fig. 4). The unusual DO consumption is believed to be due to addition of nutrients, as well as biological and chemical oxygen demand, from the WWTP. From early July to late August, the streambed through this reach of Silver Bow Creek was choked with a 2 foot thick standing crop of algae and aquatic macrophytes (Fig. 4). This biomass was not apparent in April and May, and also had sloughed out of the stream bed by late September. Monitoring of DO levels in the dead zone in October 2006 showed that DO concentrations did not drop below 5 mg/L at any time during the day or night. This suggests a much lower level of biological and/or chemical oxygen consumption in the colder seasons. Whereas this is good news from the point of view of trout, on the flip side we also observed a further downstream persistence of elevated ammonia concentrations in October. Ammonia at levels we have measured in Silver Bow Creek below the WWTP (up to 5-6 mg/L as N) is toxic to trout (ammonia toxicity depends on water temperature and pH).

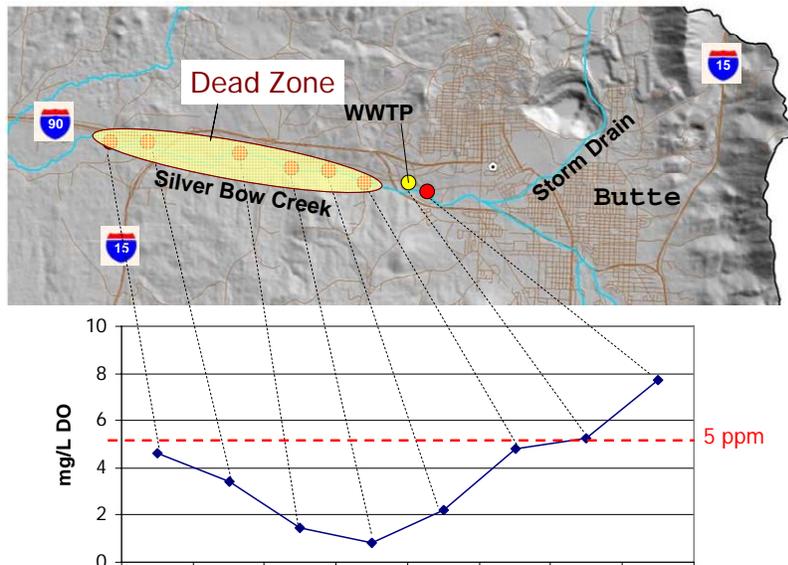


Fig. 3. Dissolved oxygen in Silver Bow Creek measured manually between 4 AM and 6 AM on August 30, 2006 by undergraduate students Stacy Wilcox and Ericka Sholey. The circled area has been termed a “dead zone”, by analogy with similar hyper-eutrophic zones found in lakes or oceans.

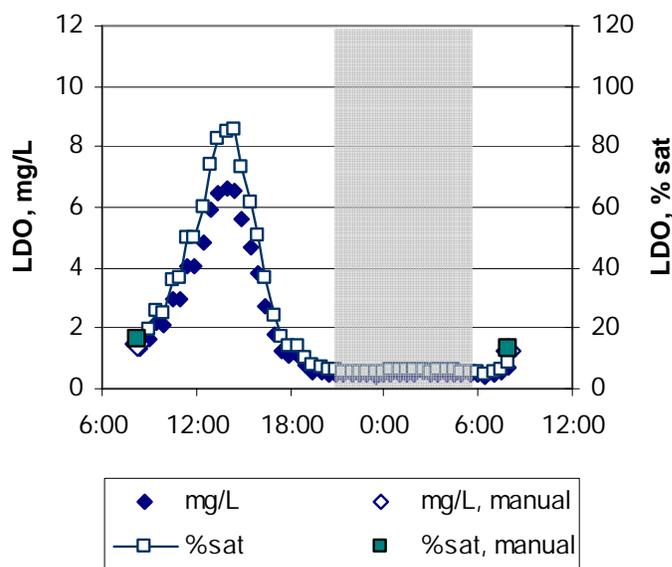


Fig. 4. Biomass (mainly vascular aquatic plants) in upper Silver Bow Creek about 2 miles below the WWTP discharge point (August, 2006). The diagram on the right shows the 24-hour cycle in dissolved oxygen concentration at this site. Continuous data were collected by a Hydrolab Minisonde with luminescent DO probe (LDO), whereas the green squares at either end were collected using a hand-held instrument. The shaded area denotes night-time. DO levels dropped below 1 mg/L for roughly 12 hours on this date.

4. Concentrations and loads of total dissolved ammonia (NH_4^+ and organic- NH_3) from the WWTP are very high, and overwhelm all other inputs of bio-available nitrogen in the rest of the watershed (Fig. 2b, 2c). Detailed synoptic investigations in August showed that oxidation of ammonia – most likely catalyzed by microbes – resulted in an increase in dissolved nitrite and nitrate concentration with distance below the WWTP confluence (Fig. 5). Once ammonia levels reached background (roughly 1.5 miles below the WWTP), nitrite levels also dropped to background (near or below detection limit). This is explained by the fact that nitrite is most often formed as an intermediate step in the conversion of ammonia to nitrate. Because the most bio-available form of nitrogen for plants is ammonia, it is the addition of ammonia (and also phosphate) from the WWTP that is believed to be primarily responsible for the incredible build-up of plants and algae in the “dead zone” during the summer.

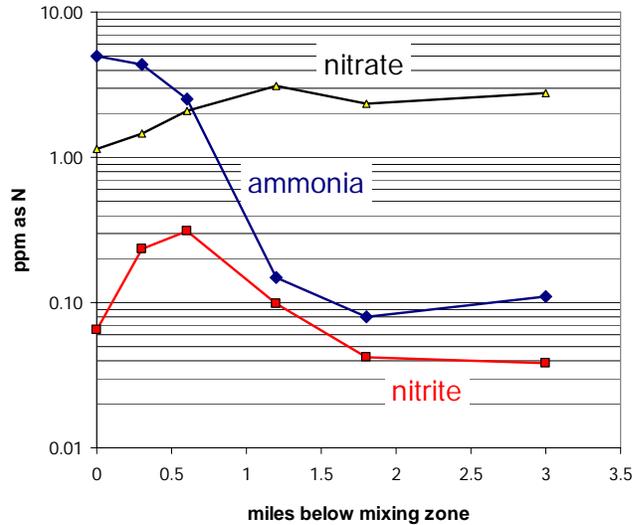


Fig. 5. Changes in concentration and speciation of dissolved N below the WWTP effluent, measured on August 9, 2006. The increase in nitrate concentration in the first 1.5 miles below the WWTP is believed to be due to oxidation of ammonia, first to nitrite, and then to nitrate.

5. A diel (24-h) study was conducted in July 2006 at monitoring stations above and below the WWTP effluent. At the downgradient station along Silver Bow Creek, concentrations and loads of ammonia increased at night and decreased during the day (data not shown). However, it is not known with confidence whether this cycle represents changes in the rate of ammonia breakdown (e.g., due to daytime uptake by photosynthetic plants or increased rate of ammonia oxidation in warm temperatures), or is due to diel changes in ammonia loading from the WWTP point source. The WWTP effluent decreased in flow during the night, as did the loading of total dissolved ammonia. Additional diel work is planned for the summer of 2007. An important objective of this work will be to confirm whether or not the diel changes in ammonia concentration are due to in-stream phenomena or to mixing of up-gradient waters.

6. Another objective of the Year 1 project was to use stable isotopes as tracers of sources of nutrients into upper Silver Bow Creek. Filtered, one-gallon water samples were collected in early October for isotopic analysis of $\delta^{15}\text{N}$ -ammonia, as well as $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate. The results (Fig. 6) show a range in $\delta^{15}\text{N}$ -nitrate from +5.0 to +12.5 per mil, with a possible trend of decreasing $\delta^{15}\text{N}$ with distance downstream, and a range in $\delta^{15}\text{N}$ -ammonia of +8.9 to +15.7 per mil, with a possible trend of increasing $\delta^{15}\text{N}$ with distance downstream. In general, the N and O isotopic composition of nitrate for the surface water samples are very similar to results obtained by the Montana Bureau of Mines and Geology (MBMG) and HKM labs for shallow groundwater in the Butte Summit Valley. The LAO sample had an unusually high $\delta^{18}\text{O}$ -nitrate value, which will be tested by follow-up sampling in 2007. We plan to collect a more detailed synoptic set of samples in the summer of 2007 to see if the inferred trends in $\delta^{15}\text{N}$ -nitrate and $\delta^{15}\text{N}$ -ammonia are real. Our hypothesis is that ammonia-oxidizing bacteria in Silver Bow Creek selectively metabolize isotopically light NH_4^+ , which then becomes isotopically light NO_3^- . The result is a lowering of the average $\delta^{15}\text{N}$ -nitrate of the stream, while enriching the residual ammonia in heavy N. This hypothesis explains the contrasting trends in isotopes of nitrate and ammonia, but needs to be tested by follow-up sampling in 2007.

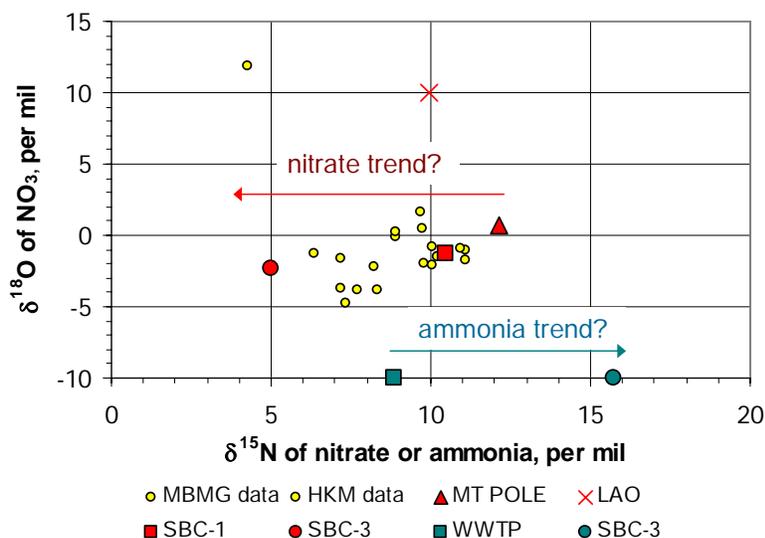


Figure 6. Stable isotope results for surface water samples collected in this study. Data for nitrate are shown in red. Data for ammonia are shown in green (arbitrarily plotted at $y = -10$). Also shown are isotopic analyses of shallow groundwater wells from the Butte Summit Valley (small open circles).

7. We had also planned to investigate diurnal (24-h) changes in the stable isotopic composition of nitrate and ammonia, as well as dissolved N_2 and O_2 gas. However, because our colleague Simon Poulson – who performs the specialized isotopic analyses of dissolved gas – was on sabbatical in Japan, this activity was not performed in 2006. We intend to do this in the summer of 2007.

Budget

As of this writing the project is well within budget, and the project end date was recently extended through the end of 2007. Much of the budgeted analytical money is set aside for stable isotopic analysis, and we still haven't received the invoice from Waterloo Lab for our preliminary set of isotopic analyses. We anticipate spending quite a bit more money in 2007 for analysis of conventional nitrate and ammonia isotopes, as well as isotopes of dissolved O_2 and N_2 gas. Additional analytical expenses are associated with quantification of dissolved nitrate. Although we had originally planned to use a HACH spectrophotometer for nitrate analysis, we decided during the summer of 2006 that this method gave unreliable results. As a result, all samples are now analyzed for nitrate – along with a complete suite of major anions - by ion chromatography at the Murdock Laboratory, Univ. of Montana (Missoula, MT). This adds to the analytical costs, but the project is still anticipated to be well within budget through 2007 into 2008.

Presentation of Results

Preliminary results were presented by graduate student Beverly Plumb at the 2006 AWRA conference in Polson, MT (Plumb, 2006), and by undergraduate students Ericka Sholey and Stacey Wilcox at the 2007 Montana Tech Undergraduate Research Symposium (Wilcox and Sholey, 2007). Bev Plumb is expected to write her thesis in the Fall of 2007, and defend either in December 2007 or the following semester. This thesis will contain all of our data, and will be

the final deliverable for the project. We also anticipate submitting one or more papers for publication in a scientific journal.

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Carbon cycling and the temporal variability in the concentration and stable carbon isotope composition of dissolved inorganic and organic carbon in streams

Basic Information

Title:	Carbon cycling and the temporal variability in the concentration and stable carbon isotope composition of dissolved inorganic and organic carbon in streams
Project Number:	2006MT89B
Start Date:	3/1/2006
End Date:	12/31/2007
Funding Source:	104B
Congressional District:	At large
Research Category:	Climate and Hydrologic Processes
Focus Category:	Geochemical Processes, Hydrogeochemistry, Water Quality
Descriptors:	None
Principal Investigators:	Stephen Parker, Douglas Cameron

Publication

Title: Identifying and characterizing sources of dissolved organic carbon in the Big Hole and Clark Fork Rivers, a continued investigation

PI: Stephen Parker Ph.D., Associate Professor, Department of Chemistry and Geochemistry, Montana Tech of The University of Montana, Butte, MT

Co-PI: Douglas Cameron, Ph.D., Professor, Department of Chemistry and Geochemistry, Montana Tech of The University of Montana, Butte, MT

Project Type: Biogeochemical research into the cycling of carbon between DOC and DIC pools using carbon stable isotopes.

Focus Category: Geoche, Hydgeo, WQL.

Research category: Hydrological processes

Keywords: Dissolved organic carbon, dissolved inorganic carbon, carbon isotopes, diel, biogeochemistry

Start date: Mar. 1, 2007

End date: Feb. 28, 2008

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Congressional district: at large

Abstract:

Recent work has identified daily concentration cycles in dissolved organic (DOC) and inorganic carbon (DIC) in the Clark Fork (CFR) and Big Hole (BHR) Rivers in Montana¹. The DOC in the two rivers showed inverse temporal patterns suggesting that very different mechanisms control the processes in these two rivers. Preliminary study using an isolation chamber on the BHR suggested that the large nighttime increase in DOC observed may have been due to groundwater influx through benthic sediments associated with the daily evapotranspiration cycle. The stable C-isotope composition of the DIC was also investigated. The $\delta^{13}\text{C}$ -DIC in both the CFR and BHR showed the patterns expected for DIC that is influenced primarily by photosynthesis and respiration.

¹ Smith, M. G., Parker, S. R., Cameron, D. C., Abstract, Montana AWRA meeting, Oct. 2006.

This proposal outlines work to further test the reproducibility of the observed diel cycles in DIC and DOC. Also, the work described will help define the sources and causes of these daily DOC changes using additional laboratory and field experiments. Furthermore, the project will attempt to identify the types and molecular-weight distributions of organic compounds that make-up the DOC using Liquid Chromatography-Mass Spectrometry (LC-MS) analysis.

Title: Identifying and characterizing sources of dissolved organic carbon in the Big Hole and Clark Fork Rivers, a continued investigation.

Problem statement:

Recent investigations of this research group, which have been funded by the Water Resources Research Program (USGS, 104(b)), have identified diel concentration cycles in dissolved organic and inorganic carbon (DOC & DIC) in the Clark Fork (CFR, Fig. 1) and Big Hole (BHR, Fig. 2) Rivers in Montana. In the CFR the diel changes in DIC are well correlated with the daytime removal of CO₂ by photosynthesis and the nighttime increase from respiration (Parker et al., 2005). The DOC concentrations were low and showed an increase during the day and a decrease at night (Fig. 1).

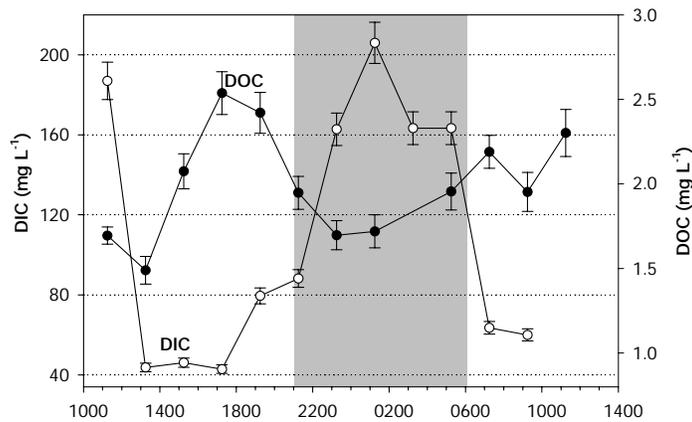


Figure 1: DIC & DOC in the Clark Fork River, July, 2006. Time is shown on the horizontal axis and the shaded area represents night time.

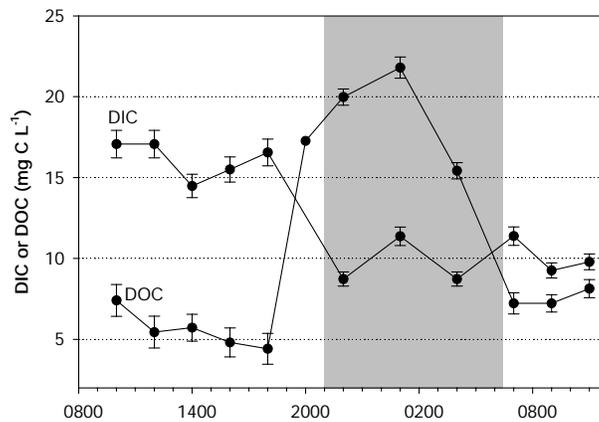


Figure 2: DIC & DOC in the Big Hole River, Aug. 2006.

During the day plants and algae are known to exude (“leak”) carbon compounds being produced during photosynthesis (Falkowski & Raven, 1997; Ziegler and Fogel, 2003). These reduced organics and others produced by microbial degradation of detritus are known to be the

single most important forms of reduced carbon in streams available to heterotrophic microorganisms as an energy source (Volk et al., 1997).

DOC in the BHR showed the inverse temporal pattern (to that of the CFR) with a significant concentration increase at night while the DIC did not follow the “typical” relationship normally associated with highly productive rivers and streams (as seen in Fig. 1). These two dramatically different outcomes emphasize the need for a more thorough understanding of how carbon compounds are produced and consumed within aquatic systems. Additionally, the types and concentration of the DOC can have a significant influence on the chemical composition of surface waters (McKnight et al., 1997).

This proposal adds two new components to the on-going study. 1) Shallow groundwater and hyporheic water in the vicinity of the rivers will be sampled using piezometers to attempt to identify the groundwater contribution of carbon to the river. 2) Liquid chromatography-mass spectrometry will be used to identify the types and mass distributions of DOC found in the CFR and BHR.

In summary, this proposal outlines work to better define the sources and causes of these daily DOC and DIC changes as well as attaining a better understanding mechanistically of the differences between the CFR and BHR carbon dynamics. Additionally, the project will attempt to identify the types of organic compounds that make-up the DOC, the molecular weight distributions of the material that make up the DOC, and how type and distribution vary with time. Knowing the types of organics will help to better determine the sources within the river system.

The distinctly different dynamics detailed above between the BHR and CFR underscore the need for a better understanding of how different river systems function. The outcomes of this project will be important to researchers and water managers investigating energetics and dynamics of hydrological systems.

Results and benefits statement:

This proposal will expand the previous investigations by looking for a connection between organic and inorganic carbon in the local shallow groundwater with respect to those compounds found in the rivers. Rivers are dynamic, “living” systems that are an integral component of the global hydrological network and there is a need for a better fundamental understanding of the science of how rivers and other hydrological systems function.

The details from the first parts of this investigation outlined above (problem statement) have shown that two rivers in close proximity to each other have very different behaviors in terms of the diel concentration cycles of carbon compounds that are critical to the ecological function of the lotic system as a whole. By gaining a better understanding of the factors contributing to these differences in diel carbon cycles, an improved insight will be provided into the subtlety of the biogeochemical cycling of carbon compounds in streams.

Nature, scope and objectives of project:

Background: Diel (aka: diurnal) processes in surface waters are regular, dynamic, changes in physical and biogeochemical parameters that occur over 24-h periods. Investigations over the past 16 years have shown that diel changes in the concentration of chemical species in flowing systems are robust, reproducible processes that play an integral role in the health and water quality of river systems (e.g., Fuller and Davis 1989; Bourq and Bertin 1996; Brick and Moore

1996; Sullivan et al. 1998; Nimick et al. 2003, 2005; Jones et al. 2004; Gammons et al. 2005; Parker et al., 2005, in press). The diel variations are driven by the normal photoperiod, which influences: photosynthesis and respiration of aquatic organisms; daily instream temperature cycles; daily changes in dissolved gas gradients between air and water; and affects either directly or indirectly a variety of other photo-catalytic processes (e.g., photo-reduction of metals; Mn, Sunda et al. 1983; Sunda and Huntsman 1994; Fe, McKnight et al., 1988; McKnight and Bencala 1988; Sullivan et al., 1998; Gammons et al., 2005). Healthy river systems can exhibit large diel pH, O₂ and CO₂ cycles that are largely driven by photosynthetic aquatic plants and algae. Typically, this results in the net production of O₂ during the daytime while at night the reverse process happens (net production of CO₂) when respiration is operating in the absence of photosynthesis (Odum 1956; Pogue and Anderson, 1994; Nagorski et al., 2003).

Recent work by this research group has investigated the diel changes in the concentration of DOC and DIC in two rivers as discussed in the problem statement. This work is currently in progress and includes the analysis of the carbon stable isotope composition of inorganic ($\delta^{13}\text{C}$ -DIC) and organic carbon ($\delta^{13}\text{C}$ -DOC) compounds. Additionally, the concentration of dissolved and particulate phase metals is being determined. DOC is known to complex dissolved metals and the relationship between these organic compounds and transport needs to be better understood.

First objective: The first component of this project continuation would be to: 1) conduct additional diel samplings on both the CFR and BHR to determine if the observed changes in DOC and DIC are reproducible, 2) use an isolation (flux) chamber (Fig. 3) to determine if the DOC and DIC changes are produced by instream processes or from groundwater influx, and 3) use stream-side piezometers as sampling sites to measure groundwater levels, chemical composition, isotope composition and types of DOC and DIC.

One important part of investigating and measuring diel changes in the concentration of a variety of analytes has been to show that these processes are robust and reproducible on a year to year scale. This question is important with respect to DOC since there is little published literature that discusses short term (diel) temporal changes in organic carbon.



Figure 3: One of the isolation (flux) chambers used in the BHR. Tubing is connected to a streamside peristaltic pump. Conductivity, dissolved oxygen and pH probes are inserted into the chamber and it is filled with small rocks and periphyton.

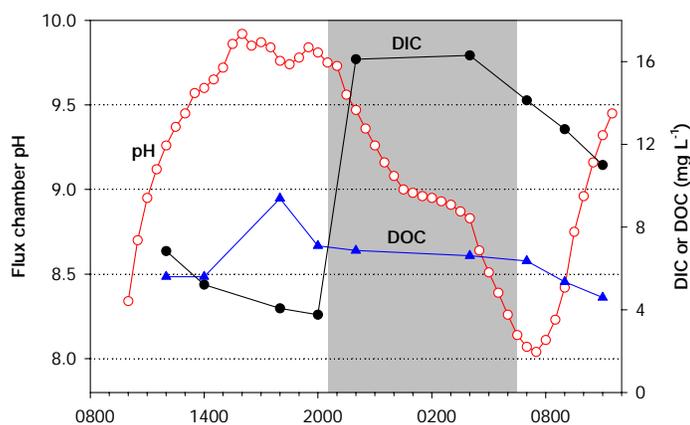


Figure 4: pH, DIC and DOC in the isolation chamber in the Big Hole River, Aug. 2006.

Secondly, the origin of the DOC is important to understand. Groundwater influx through benthic sediments may be an important source of carbon to streams. So, a component of this study will be to use isolation chambers to better separate the effects of instream process versus those produced by groundwater contributions. The isolation chambers (Fig. 3) that we have been using are constructed out of clear acrylic plastic and are filled with small river cobbles with attached periphyton. The chambers (and connecting tubing) are submerged in the river so that they are temperature equilibrated and receive approximately the same light as the river bottom. During the sampling experiments the chambers are continuously mixed with a small peristaltic pump that circulates the enclosed water. This method was used at the BHR site in Aug. 2006. A normal diel cycle of pH and DIC was observed in the chamber (Fig. 4). At the same time little change in DOC was observed when compared to river (Fig. 2). This suggests that the large increase overnight observed in the BHR may be due to groundwater influx not instream processes. An isolation chamber was not used in the CFR sampling for organic carbon and would be included in the continued investigation.

In this proposed continuation of the current study we want to better assess the question of groundwater versus instream processes in terms of organic carbon contributions by using streamside piezometers² at both the CFR and BHR sites. Water level in the piezometers versus the stream would be monitored over the diel sampling period. This would provide a measure of the direction of flow between surface and groundwater. Additionally, the piezometers would provide a sampling point to shallow groundwater in the vicinity of the river. Both groundwater and river water would be sampled for the same parameters which would allow a better comparison of the relative contributions of DOC and DIC from both sources. Another field technique that will be used to help determine the DOC concentrations, and whether there are changes in the types of organics present is UV-VIS spectrometry. A portable, fiber optic spectrometer would be used to take hourly scans across the UV-VIS spectrum and note any changes in total absorption and also the wave lengths of absorption. Many naturally occurring soluble organic species such as fulvic acids have characteristic absorption spectra which could be monitored over the sampling period. Purchase of this spectrometer is included in the project budget.

² A piezometer is a small diameter water well used to measure the hydraulic head of groundwater in aquifers.

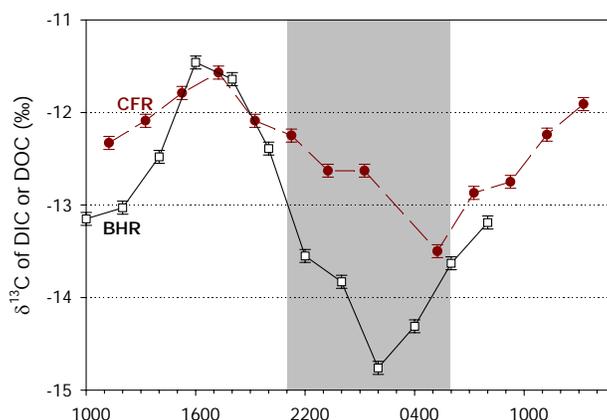


Figure 5: $\delta^{13}\text{C}$ -DIC from the CFR and BHR, summer 2006. [Note: the samples for analysis of $\delta^{13}\text{C}$ -DOC have been sent to the lab but the results were not available at the time of submission of this proposal (11/20/06).]

Second objective: Laboratory work using Liquid Chromatography coupled with a Mass Spectrometer (LC-MS) would be performed to identify, where possible, individual compounds as well as the molecular weight distribution and classes of organics present throughout the diel cycle. For uncontaminated natural waters, the bulk of the DOC is typically natural organic matter. Low molecular weight and hydrophilic organic compounds such as free amino acids, simple carboxylic acids, carbohydrates and some hydrocarbons are common components of natural organic matter (NOM) in natural waters (Macalady, 1998) and can be identified as individual compounds. Operationally defined classes of NOM; fulvic, humic, and hydrophilic acids typically make up the bulk of aquatic NOM. Individual compounds within these classes are difficult to identify; however, molecular weight profiles can be used to study changes in composition and concentration. The Bruker LC/MS instrument at Montana Tech has the capability for MS/MS analyses. Consequently, it will be possible to look for changes in some functional group composition of the NOM in the molecular weight profiles. Questions that would be addressed include: 1) can individual organic compounds be identified? 2) Does the composition of DOC change throughout the diel period? 3) Is there a change over the diel period in terms of the classes of organics based on the mass? And, 4) what is the relationship of the ^{13}C composition of the organics to the types and masses found? The first stages of this project include investigating the C-stable isotope composition of the DIC and DOC in samples already collected (Fig. 5). These results are particularly interesting since Fig. 2 showed that the total concentration of DIC in the BHR did not change significantly over the diel period but the $\delta^{13}\text{C}$ -DIC shows the normal isotope composition change expected as a result of the influence of photosynthesis and respiration.

Timeline:

	2007			2008	
	May-June	July-Aug	Sep-Dec.	Jan-Apr.	Oct
LC-MS analysis (2006 samples)					
Piezometer installation					
Field measurements, sample collection					
LC-MS analysis (2007 samples)					
Analysis of results					
Presentation of results					

Methods, procedures and facilities:

The PI (in conjunction with collaborators) has conducted 17 diel sampling experiments on eleven different streams (on three continents) in the past five years including the BHR and the CFR. Methods for collecting water samples to examine concentrations of dissolved inorganic carbon as well as dissolved and total metals concentrations have been detailed in Gammons et al. (2005) and Parker et al. (2005).

Samples for DIC, DOC, $\delta^{13}\text{C}$ -DIC and $\delta^{13}\text{C}$ -DOC analysis will be collected in the field in acid washed, oven dried, glass bottles. Preparation and analysis for $\delta^{13}\text{C}$ -DOC will follow the methods detailed by Gandhi et al. (2004). All stable isotope analyses will be performed by Dr. Simon Poulson at the University of Nevada-Reno. The PI on this proposal has worked collaboratively on several projects previously with Dr. Poulson.

All DIC and DOC analysis will be performed at Montana Tech by the student supported through this project. The Department of Chemistry and Geochemistry has an Ionics (Model 1505) Total Carbon Analyzer that will be used for all samples collected for this project.

Diel field experiments will include hourly samples collected over a 24-h period for the determination of the concentration of DOC and DIC as well as $\delta^{13}\text{C}$ -DIC, $\delta\text{D-H}_2\text{O}$, $\delta^{18}\text{O-H}_2\text{O}$, and $\delta^{13}\text{C}$ -DOC. All field sites will also have *in situ* datasondes for measurement of pH, temperature, specific conductivity, photosynthetically active radiation and other pertinent parameters. The datasondes will be deployed for at least 24-hours before and after the diel sampling experiment to monitor the reproducibility of the observed chemical cycles. The CFR and BHR sampling sites are adjacent to USGS gaging stations which provide accurate streamflow data at 15 minute intervals. Additionally, seasonal sampling over the funding period covered by this proposal would be conducted to establish a range of values for the measured parameters over a year-long time period.

Piezometer transects would be used to determine the groundwater levels in the sampling area with respect to river level. The piezometers are also needed to characterize the chemistry of shallow groundwater, as well as hybrid waters in the hyporheic zone. Nested piezometers may be used to evaluate vertical head gradients in the vicinity of creek beds, and therefore the gaining or losing characteristics of the stream. At the end of the project, the piezometers can be left in place for long-term monitoring, or removed.

All piezometer waters and surface waters will be analyzed in the field for temperature (T), pH, Eh, dissolved oxygen (DO), specific conductivity (SC) and alkalinity. Analyses of groundwater samples will employ a peristaltic pump connected to a low-volume flow cell, to prevent contamination from air. The sample will be pumped until key parameters measured in the flow cell stabilize (e.g., pH, DO, Eh, T). [These data will be used to map spatial and temporal

gradients.] Once the water chemistry in the flow cell stabilizes as described above, samples will be collected for $\delta\text{D-H}_2\text{O}$, $\delta^{18}\text{O-H}_2\text{O}$, $\delta^{13}\text{C-DIC}$, $\delta^{13}\text{C-DOC}$, $\delta^{18}\text{O-DO}$ and ICP analysis for metals. The use of stable isotopes is an important tool for investigating the dynamic connection of the ground water-surface water system.

All LC-MS analysis will be performed at Montana Tech using the Bruker Datonics, Esquire 4000 Liquid Chromatograph-Mass Spectrometer. The co-PI (Cameron) is an experienced analytical chemist with background in using the LC-MS for identification of organic compounds. Extraction of the dissolved organic matter and separation into various compound classes will be accomplished using standard column separation procedures for DOC (Macalady, 1998). LC/MS analyses will be done using both atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). Where possible individual compounds will be identified using reference compounds and the corresponding retention times and mass spectra. When reference compounds are not available, comparisons to literature spectra or interpretation of the MS and MS/MS spectra will be used for tentative identifications.

- **Laboratory:** The PI shares a well equipped laboratory at Montana Tech with Dr. Christopher Gammons (Dept. of Geological Engineering, Montana Tech). The space is approximately 1000 sq. ft. and has facilities for preparative work and wet chemical analysis. The co-PI also has similar laboratory space at Montana Tech. The Montana Tech Chemistry & Geochemistry Dept. also has an analytical facility that has the following instrumentation: ICP-AES, AAS, GF-AAS, IR and FTIR spectrometer, UV-VIS spectrometer, Raman spectrometer, 300 MHz NMR, LC-MS, TOC analyzer, Ion Chromatograph.
- **Computer:** The PI has an office computer for writing and data analysis as well as a laptop computer for field work.
- **Office:** The PI has adequate office space with access to printers, photocopiers and scanners.
- **Other:** Field equipment includes (shared with C. Gammons): Peristaltic pump and in-line filtration system, Hach portable spectrometer, portable fluorimeter, Troll 9000 DataSonde, Hydrolab 3 DataSonde, Hydrolab mini-sonde, WTW portable multi-probe system, Marsh-McBirney current flow meter.

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Training potential:

This project would provide continued support for an undergraduate student from Montana Tech for assistance with both field and laboratory work. The student that has been working on the previously funded parts of this project is currently a sophomore who is interested in continuing into this next phase. He recently presented his results at the AWRA meeting in Polson, MT (Smith, M.G., Parker, S.R., Cameron, D.: Preliminary investigations of temporal variability in the concentration and composition of DIC and DOC in streams, MT-AWRA, Oct. 13, 2006). This student would continue to receive background and training in field techniques. Additionally, he would be trained to run the LC-MS for analysis of the types of DOC in river samples. He would continue to be involved in the analysis of the results and data as well as presenting these results at the undergraduate research symposium held at Montana Tech each year and at another appropriate state or regional conference. The student would also be involved in manuscript preparation for eventual publication.

PI-Parker has recently (2005) completed a Ph.D. in Environmental Chemistry with The University of Montana-Missoula working in the area of riverine biogeochemistry. The doctoral work included studies to better understand the diel processes involved in both carbon cycling and the mobilization and transport of metals in streams. Funds provided for the project described in this proposal will allow the PI to continue establishing an independent research program dealing with scientific issues surrounding Montana's rivers

Biographical Sketch: (PI) Stephen R. Parker:

(i) Professional preparation:

Hope College, B.A., Chemistry, June 1970.

Indiana University, M.S., Biochemistry, August 1972.

University of Montana, Ph.D., Environmental Chemistry, July 2005.

(ii) Appointments:

1) 2006-present, Associate Professor, Dept. of Chemistry and Geochemistry, Montana Tech of The University of Montana, Butte, MT.

2) 2001 to 2005, Assistant Professor, Dept. of Chemistry and Geochemistry, Montana Tech of The University of Montana, Butte, MT.

3) 1988 to 2001, Laboratory Director and Adjunct Instructor, Dept. of Chemistry and Geochemistry, Montana Tech of The University of Montana, Butte, MT.

(iii) Recent publications

1) Parker, S. R., Gammons, C. H., Jones, C.A., (in press). Role of hydrous iron oxide formation in attenuation and diel cycling of dissolved trace metals in a stream affected by acid rock drainage, *Water, Air, Soil Poll.*

2) Nimick, D.A., McCleskey, R. B., Gammons, C.H., Cleasby, T.H., & Parker, S.R., (in press) Diel Mercury-Concentration Cycles in Streams Affected by Mining and Geothermal Discharge. *Sci. of the Total Env.*

3) Wood S. A., Gammons C. H., Parker S. R., The behavior of REE in naturally and anthropogenically acidified waters. *Journal of Alloys and Compounds* 418, 161-165 (2006), DOI:10.1016/j.jallcom.2005.07.082.

4) Parker, S. R., Poulson, S. R., Gammons, C. H., Biogeochemical Controls on Diel Cycling of Stable Isotopes of Dissolved O₂ and Dissolved Inorganic Carbon in the Big Hole River, Montana, *Environmental Science and Technology*, 39(18), 7134-7140, (2005), DOI: 10.1021/es0505595

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6) Parker, S.R., Gammons, C. H., Pedrozo, F.L., Wood S. A., (in review) Diel changes in metal concentrations in a geogenically acidic river: Rio Agrio, Argentina. [submitted to *J. Volcanology & Geothermal Res.*]

7) Parker, S. R., Gammons, C. H., Poulson, S. R., DeGrandpre, M. D., (in review). Diel changes in stable carbon isotope ratios and trace element concentrations in the Clark Fork River, MT. [submitted to *App. Geochem.*]

(iv) Recent meetings and presentations:

1) American Water Resources Association (Montana Section), Polson, MT, October 2006, Diel iron behavior in the hyper-acidic waters of the Rio Tinto and Rio Odiel, Andalusia, Spain., Parker, S.R., Gammons, C.H., Nimick, D.A., Snyder, D.

2) Goldschmidt Conference, Moscow, ID., May 2005. Diel cycles in stable isotopic composition of dissolved O₂ and DIC in a river due to biogeochemical processes, Stephen R. Parker, Simon R. Poulson, Christopher H. Gammons, Michael D. DeGrandpre.

3) American Geophysical Union, San Francisco, Dec. 2004, Diel changes in stable carbon isotope ratios and trace element concentrations in the Clark Fork River, MT.

(v) Recent research funding sources:

1) Mine Waste Technology Program (EPA), \$70,000, The role of biogeochemical processes affecting the flux of metals to and from sediments in mining impacted streams, awarded Sep. 2006.

2) USGS 104(b) (Montana Water Center), \$14,646, Carbon cycling and the temporal variability in the concentration and stable carbon isotope composition of dissolved inorganic and organic carbon in streams, awarded Jan. 2006.

Biographical Sketch: (co-PI) Douglas Cameron**Academic Rank:** Professor**Degrees:** Ph.D. Analytical Chemistry, Purdue University, 1979
M.S. Analytical Chemistry, Purdue University, 1978
B.S. Chemistry, Montana State University, 1975**Related Experience:**

- 8/2005 to present. **Department Head** in the Chemistry and Geochemistry Department and Pre-Professional Health Studies at Montana Tech of the University of Montana.
- 8/1999 to present. **Professor** in the Chemistry and Geochemistry Department at Montana Tech of the University of Montana.
- 8/1993 – 8/1999. **Associate Professor** in the Chemistry and Geochemistry Department at Montana Tech of the University of Montana.
- 8/1990 – 8/1993. **Assistant Professor** in the Chemistry and Geochemistry Department at Montana College of Mineral Science and Technology.

Recent Publications:

- 1) Cameron, D.; Willett, M.; Hammer, L., "Distribution of Organic Carbon in the Berkeley Pit Lake, Butte, Montana," *Mine Water and the Environment*, 2006, 25(2), 93-99.
- 2) Cameron, D.; Willett, M.; Hieb, A., "Berkeley Pit Lake - Organic Carbon Remediation Potential," *Mine Waste Technology Program Activity IV, Project 16, Interagency Agreement between the Department of Energy and the U. S. Environmental Protection Agency, DE-AC22-88ID12735, Butte, MT, accepted.*
- 3) Cameron, D.; Willett, M., "Organic Carbon in Berkeley Pit Sediments," *Mine Waste Technology Program Activity IV, Project 11, Interagency Agreement between the Department of Energy and the U. S. Environmental Protection Agency, DE-AC22-88ID12735, Butte, MT, April 2005.*
- 4) Cameron, D., "Characterization, Remediation, and Resource Recovery: the Berkeley Pit and the Mine Waste Technology Program," *Interagency Agreement between the Department of Energy and the U. S. Environmental Protection Agency, DE-AC22-88ID12735, Butte, MT, October 2003.*

Recent Meetings and Presentations:

- 1) Cameron, D.; (Johnson) Brown, N., "Characterization of Dissolved Organic Carbon in Berkeley Pit Lake Water Using Stirred-Cell Ultrafiltration and Spectroscopic Analysis," *Pit Lakes 2004, Reno, NV, November 16-18, 2004.*
- 2) Cameron, D.; "Teaching Problem Solving in Chemistry - An Example to Stimulate Discussion," *Confchem On-Line Conference "Problem Solving in Chemistry", <http://www.ched-ccce.org/confchem/>, June 2002.*
- 3) Cameron, D.; Willett, M., "An Investigation into the Effects of Organic Amendments on Berkeley Pit Lake Water Quality Under Various Environmental Conditions," *2000 Conference on Hazardous Waste Research, Denver, Colorado, May 23-25, 2000.*
- 4) Cameron, D.; Willett, M.; Hammer, L. T.; Jonas, J. P.; Syverson, M. T., "Determination and Characterization of Organic Carbon in the Water and Sediment of the Berkeley Pit Lake, Butte Montana," *2000 Conference on Hazardous Waste Research, Denver, Colorado, May 23-25, 2000.*
- 5) Cameron, D.; Willett, M.; Hammer, L. T.; Jonas, J. P.; Syverson, M. T., "Determination and Characterization of Organic Carbon in the Water and Sediment of the Berkeley Pit Lake, Butte Montana," *Workshop on the Characterization, Modeling, Remediation, and Monitoring of Mining-Impacted Pit Lakes, Reno, NV, April 4-6, 2000.*

Impacts of beaver on invasion ecology of brook trout (*Salvelinus fontinalis*)

Basic Information

Title:	Impacts of beaver on invasion ecology of brook trout (<i>Salvelinus fontinalis</i>)
Project Number:	2006MT92B
Start Date:	3/1/2006
End Date:	12/31/2007
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Research Category:	Biological Sciences
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Descriptors:	
Principal Investigators:	Lisa Eby, Magnus McCaffery

Publication

Title: Impacts of beaver on invasion ecology of brook trout (*Salvelinus fontinalis*)

Water Resources Research Program – Interim Report

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Research category: Biological sciences

Keywords: beaver, brook trout, *Castor canadensis*, cutthroat trout, invasion ecology, *Oncorhynchus clarki*, *Salvelinus fontinalis*.

Start date: March 1, 2006

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Congressional District: at-large

Abstract:

As a keystone species, beaver promote the creation and maintenance of wetland areas, provide complex habitat for wildlife and fish, improve water quality, and augment late season flows. Beaver ponds create excellent juvenile rearing and overwintering fish habitat resulting in substantial benefits to native fish species. Promoting beaver through either natural population expansion or active transplantation for watershed restoration purposes is gaining favor with some landowners and managers, but is a very controversial strategy. Aside from direct human-beaver conflicts such as flooding of agricultural lands and damming of irrigation systems, there is also the possibility of negative effects on native fish such as, barrier creation and the potential of beaver ponds to facilitate invasion by exotic fish species. In Montanan streams, brook trout are an exotic species whose invasion often displaces native cutthroat trout through competitive interactions. Even though many of Montana's native species often benefit from beaver ponds, it has also been suggested that the more pool-adapted and temperature tolerant brook trout have a competitive edge in beaver ponds over more riffle-adapted colder water species. Use of these habitats as "source" populations may then enable their colonization of colder "sink" habitats, thus sustaining invasions across a larger range. Beaver ponds may therefore (i) be detrimental to natives through the creation of warmer, pool habitat that gives brook trout a competitive advantage, or (ii) act as a buffer, facilitating coexistence of both species by adding habitat size and complexity. Analyses of data collected in the summer and autumn of 2006 show that beaver do have observable effects on stream temperature regimes, and that distributions and growth rates of brook trout and westlope cutthroat could be tied to this habitat modification. Completion of fieldwork, scheduled for summer 2007, will allow definitive conclusions regarding the influence of beaver disturbance on brook trout invasions and the implications for westslope cutthroat trout.

Introduction

Beaver (*Castor canadensis*) play a keystone role on the landscape, driving a significant watershed disturbance regime through their feeding and damming behaviors. Their impoundments create lentic habitat in otherwise lotic systems, leading to fundamental changes in channel geomorphology, hydrology and nutrient cycling. Consequently, beaver have been shown to promote changes in succession dynamics, increase biotic productivity, and enhance diversity of floral and faunal assemblages³⁻⁷. Increases in water storage capacity through beaver impoundments improve riparian habitat, and potentially augment water supply and late season flows⁸. These aspects of beaver impoundments have resulted in the active translocation of beaver as restoration tools into degraded wetlands of the Pacific northwest⁹. This restoration strategy is of increasing interest to landowners and managers in Montana, especially in light of prolonged drought conditions. For example the Big Hole Watershed Committee (BHWC), a group that acts as a liaison between land management agencies and the public, is currently evaluating proposals to remedy water shortage problems in the upper Big Hole River watershed of western Montana. This area, like much of the western U.S., is experiencing an extended drought period linked to gradual climatic change, exacerbated by a shift to more water intensive land-use practices. Translocation of beaver into tributary streams of the Big Hole River was one considered by the BHWC as an alternative approach to increasing landscape water storage through human dam construction¹⁰.

Promoting beaver on the landscape, either through natural population expansion or active translocation of beaver, is a controversial strategy. Aside from direct human-beaver conflicts such as timber damage, flooding of agricultural, grazing, and developed lands, and damming of culverts and irrigation systems³, there is also the possibility of negative effects on native fish species, such as barrier creation and warming of coldwater streams¹¹. Relatively little is known about the effects of beaver impoundments on stream fish assemblages in North America. Fish community shifts have been demonstrated to be highly variable among and within regions, affected by beaver pond age, position in the watershed, and dependent on the original (pre-beaver) conditions and species present^{3,12}. The patterns and mechanisms behind how beaver may influence fish community structure, abundance and distribution is a contested issue in the western U.S. and in Montana in particular. The formation of pool habitat may increase water temperatures, prey availability to fish, and juvenile rearing habitat for species such as Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*)¹³, as well as providing important winter habitat for many stream fishes including cutthroat trout (*Oncorhynchus clarki*) and bull trout (*Salvelinus confluentus*)¹⁴.

In mountain streams of western North America brook trout are an exotic species, and their invasion of pristine ecosystems often results in displacement of native cutthroat trout through age-specific biotic interactions that reduce juvenile cutthroat trout survival¹⁵. Thus, understanding both what limits the spread of the distribution of brook trout within a system and what factors influence the outcome of cutthroat and brook trout species interactions is critical for the conservation and management of cutthroat trout in mountain ecosystems of the western U.S. Gradual upstream declines in growth rates associated with declining water temperatures may explain the upstream limit for brook trout in some mountain stream systems¹⁶. Any factors that affect demographic parameters such as growth rates, age-0 recruitment, and dispersal can influence the spread of an exotic species¹⁷. Furthermore, it has been posited that brook trout, which are more pool adapted and temperature tolerant, may have an advantage in beaver ponds, and can use these habitats as “source” populations, enabling them to colonize colder “sink” sections of the stream, thus sustaining invasions across a larger range¹⁵. In addition, beaver

ponds may alter the outcome of species interactions between westslope cutthroat and brook trout. If beaver ponds provide habitat that preferentially increases abundances of brook trout in a stream, then their impact on westslope cutthroat may be larger. Also, elevation of stream temperature has been implicated in an increased ability of brook trout to outcompete westslope cutthroat trout, with research suggesting enhanced brook trout competitive ability between 13°C and 17°C². Therefore, if beaver ponds increase overall stream temperatures, brook trout may have a greater competitive advantage over cutthroat trout.

The presence of beaver on the landscape is a controversial issue. Our discussions with various federal and state fisheries biologists in Montana reveal that different managers, often working in the same drainages often have polarized views on the subject. This sometimes culminates in some managers transplanting beaver into watersheds as restoration tools, whilst others remove them as a nuisance species. Management efforts to improve landscape water retention must work in synchrony with efforts to curtail brook trout spread and maintain native cutthroat trout populations. To be effective, such efforts should be based on a sound scientific understanding of the ecological mechanisms operating within the system. It is therefore imperative that we enhance our knowledge as to how beaver activity influences processes related to exotic species invasion in western Montana.

Objectives of the project

The objective of this research is to (1) evaluate potential causal mechanisms associated with beaver facilitation of brook trout invasions in pristine mountain ecosystems, and (2) assess potential consequences of this relationship on cutthroat trout populations. The three main themes of this research, and the predictions associated with each, are:

- (i) *The influence of beaver activity on stream temperatures:* We predict that beaver impoundments will increase temperatures in the created pool as well as downstream of the impoundment, thus affecting a large portion of the watershed’s thermal regime.
- (ii) *The influence of beaver activity on exotic and native salmonid species distribution and abundance:* This theme includes multiple predictions (Table 1).
- (iii) *The influence of beaver activity on exotic/native species interactions:* This theme includes multiple predictions (Table 2).

Table 1. *Predictions* associated with our *hypotheses* regarding how streams with beaver ponds may affect brook trout (BT) spread compared with non-beaver control streams.

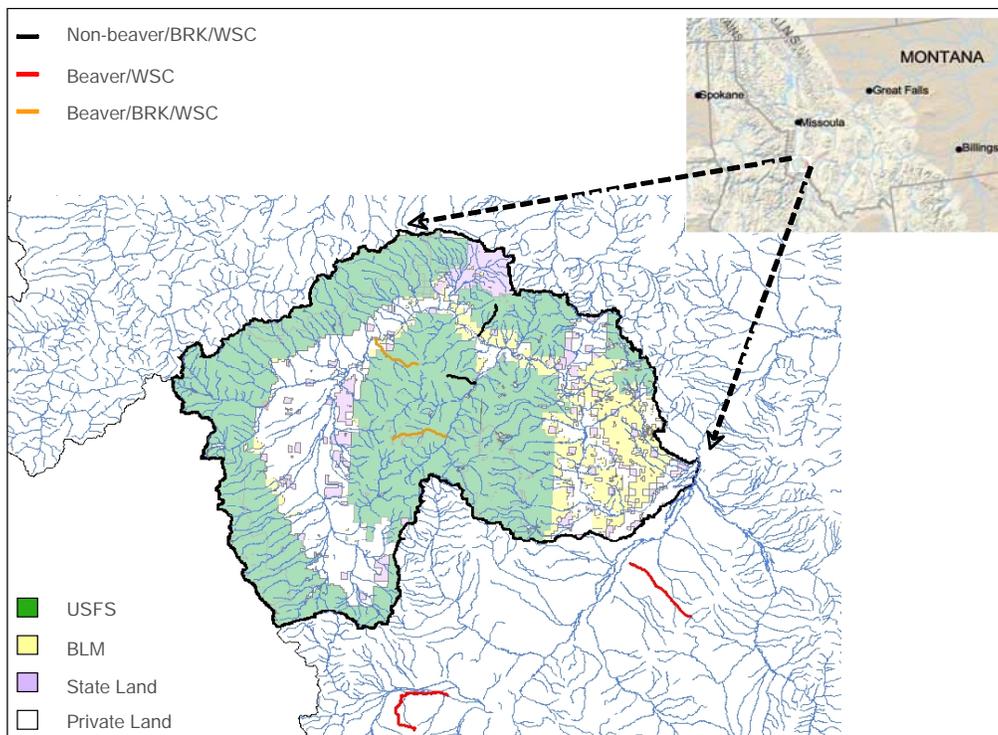
RESPONSE VARIABLES	<i>Streams with beaver ponds compared with non-beaver controls</i>		
	<i>Facilitate brook trout spread</i>	<i>Reduced brook trout spread</i>	<i>Have no effect on brook trout spread</i>
Distribution of BT	<i>↑ BT distribution, especially at higher elevations above ponds</i>	<i>↓ BT distribution with elevation</i>	<i>No difference in distribution</i>
Abundance	<i>↑ BT in beaver streams</i>	<i>↓ BT in beaver streams than control streams</i>	<i>No difference in BT abundance</i>

Table 2. *Predictions* associated with our *hypotheses* regarding how streams with beaver ponds may influence the outcome of species interactions between brook trout (BT) and westslope cutthroat trout (WSC) compared with non-beaver control streams.

RESPONSE VARIABLES	<i>Streams with beaver ponds compared with non-beaver controls</i>		
	<i>Enhance negative interactions</i>	<i>Buffer negative interactions</i>	<i>Have no effect</i>
Juvenile BT growth	↑ juvenile BT growth rates & survival in beaver streams	No difference or ↓ juvenile BT growth rates and survival in beaver streams	No difference in BT growth rates and survival
Juvenile WSC growth	↓ juvenile WSC growth rates & survival in beaver streams	↑ WSC growth rates and survival in beaver streams	No difference in WSC growth rates and survival
Composition (BT:WSC abundance)	↑ BT:WSC ratio in beaver streams	↓ BT:WSC ratio in streams with beaver ponds than control streams	No difference in ratio of BT:WSC

Study Sites

To investigate the influence of beaver on stream temperatures and brook trout and westslope cutthroat trout species interactions, we chose six study streams. These were located in the Beaverhead-Deerlodge National Forest and adjacent BLM and private lands in or near the Big Hole River drainage in southwest Montana (Map 1). Study sites incorporate three replicated treatment types: (i) beaver, westslope cutthroat, and brook trout (ii) beaver and westslope cutthroat (no brook trout), and (iii) westslope cutthroat and brook trout (no beaver).



Map 1: Map of study area showing the locations of treatment streams

Methods

Influence of beaver on stream temperatures:

To evaluate impacts of beaver on stream temperatures, temperature loggers were deployed longitudinally along each stream and set to record data every 30 minutes. Within beaver ponds, loggers were placed along a depth gradient to evaluate if there was summertime stratification and maintenance of deeper cool water. Temperature loggers were deployed in spring 2006, retrieved and data downloaded in autumn 2006, then reinstalled in the stream to collect data every 2 hours during the winter months. In summer 2007, these loggers will be collected and replaced for the duration of summer 2007, giving us over a year of relatively continuous temperature data for these streams.

Influence of beaver on brook trout/cutthroat trout distributions, abundances, and growth rates:

In early summer 2006, we block-netted and electrofished (using a Smith-Root model 15-D backpack electrofisher) six 200 m sections within mid- and high-elevations of each stream. All brook trout and cutthroat trout were identified, measured, and weighed. Additionally, trout greater than 55 mm were individually marked with a Passive Integrated Transponder (PIT) tag and scales were taken for aging and growth rate calculations. In late summer/early autumn 2006, we re-sampled each stream section. New fish were processed as above, whilst recaptures were measured, weighed and re-released.

Potential growth of cutthroat trout was calculated using average seasonal temperature data for each stream and a growth equation¹ that characterizes the potential growth at a given temperature. This equation was originally formulated for bull trout, but performs well when applied to cutthroat trout¹.

Results and continuing work

Influence of beaver on stream temperatures:

Temperature is considered an important factor in determining how westslope cutthroat trout and brook trout interact. Indeed, research has shown that brook trout are able to outcompete cutthroat at temperatures of between 13°C and 17°C². Therefore, by examining detailed stream temperature profiles in beaver and non-beaver systems, it is possible to ascertain how beaver influence stream habitat characteristics, and determine how this may impact competitive interactions of our focal fish species. Examination of temperature profiles of Johnson Creek (a non-beaver stream) and Squaw Creek (a beaver stream) shows distinctive differences, with the non-beaver system showing a gradual increase in stream temperature with a reduction in elevation (Figure 1a). This is contrasted by Squaw Creek, which exhibits a much more dynamic temperature profile, with areas of rapid warming observed at known beaver pond locations (Figure 1b).

The vertical temperature profile of a beaver pond on Squaw Creek shows distinct temperature stratification with depth. Beaver ponds elevate surface temperatures at the site and a short distance downstream of the site. Some beaver ponds may provide cooler refuge habitat through the summer depending on their depth. Temperature profiles for a series of other beaver and non-beaver streams display a similar pattern and are shown in Appendix A.

Influence of beaver on brook trout/cutthroat trout distributions, abundances, and growth rates:

The observed increase in stream temperature in beaver systems suggests that beaver may play a role in brook trout invasion of a system and their interaction with westslope cutthroat trout. This is likely due to the temperature pattern observed in Squaw Creek, whereby the stream

appears to be warmed to temperatures that favor brook trout in competitive interactions with westslope cutthroat¹⁸.

In summer 2006, we completed a mark and subsequent recapture session (median recapture rate = 20%). A total of 909 brook trout were captured (498 PIT tagged) and 591 westslope cutthroat (524 PIT tagged). This allowed us to calculate species distributions, relative composition, and within-season growth rates.

Initial analysis of data from a Squaw Creek (beaver) and Johnson Creek (non-beaver) suggests that beaver may influence species distribution and composition. In the non-beaver stream there is an increase in the relative proportion of cutthroat relative to brook trout with increasing elevation (Figure 1a). The site at 2137 m represents the upper distributional limit for both species due to the presence of an impassable barrier immediately upstream. In Squaw Creek however, the pattern of fish species composition is subtly different from that observed in the non-beaver watershed (Figure 1b). There are gradual increases in the proportion of cutthroat upstream, but brook trout continue to dominate the community through the entire stream reach.

This suggests that beaver may be influencing the ability of brook trout to invade into higher reaches of the watershed, relative to non-beaver streams. These differences exist across our focal streams, but are based only on one field season and as such, are speculative.

During the 2006 mark-recapture sessions over 1000 fish were individually tagged. Recapture of known individuals provides an estimate of growth rates for brook and cutthroat trout in each treatment. Analysis of recapture data from these streams suggests that brook trout and cutthroat trout grow faster at higher temperatures (Figures 2a & 2b). The average potential growth rate of cutthroat trout based only on temperature¹ is considerably higher than realized

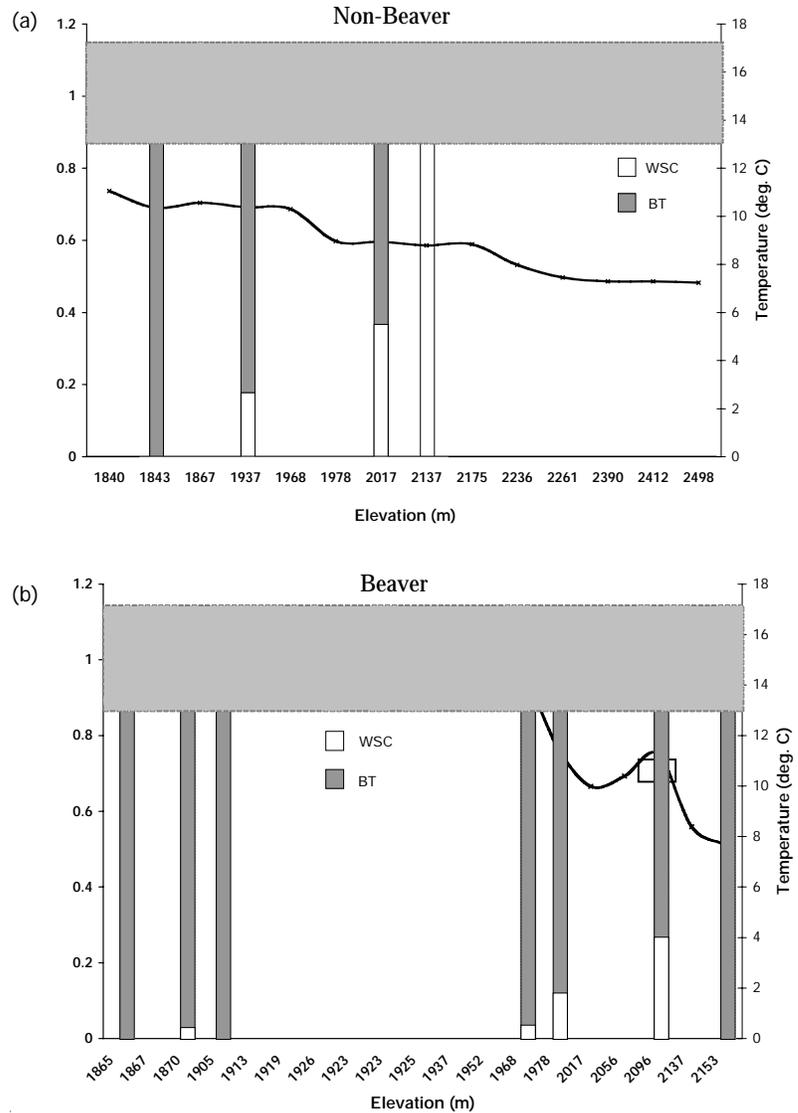


Figure 1: Average summer temperature profile and relative composition of westslope cutthroat (WSC) and brook trout (BT) in (a) Johnson Cr. (non-beaver), and (b) Squaw Cr. (Beaver). Rectangles on Squaw Cr. temperature profile denote areas on known beaver activity

values, and suggests that factors other than temperature (such as interspecific competition or prey availability) is influencing the growth rate of westslope cutthroat trout. In the beaver system, despite a consistently high potential growth rate for cutthroat, the average summer temperatures consistently lie within the temperature range defined by Thomas² as conferring competitive advantage to brook trout in interspecific interaction with cutthroat trout. This corresponds with the observation that few cutthroat trout are found at elevations within this temperature range (Figure 1a and 1b).

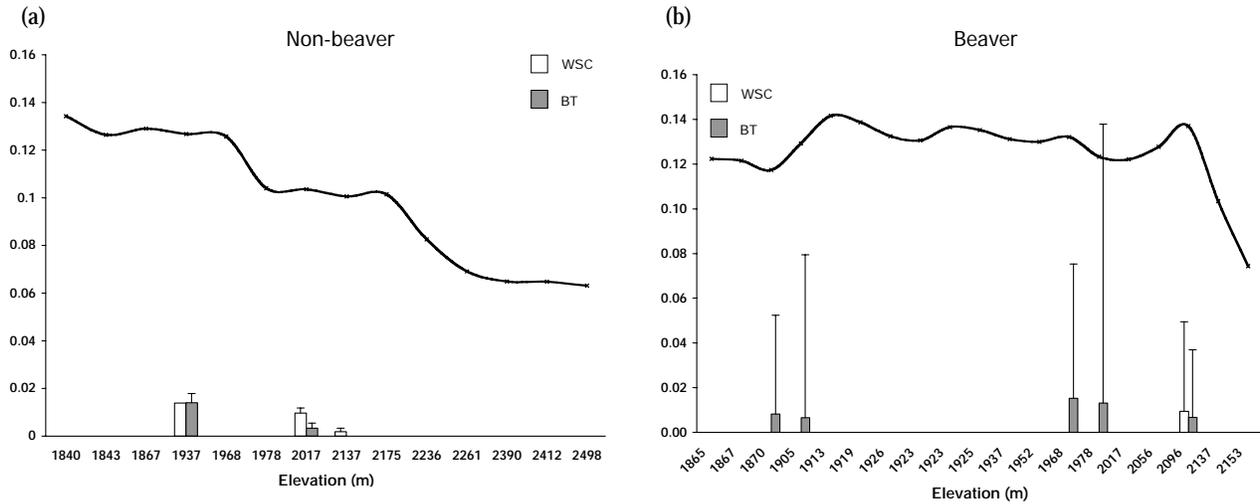


Figure 2: Average potential WSC growth based on temperature, and within-season growth rates (+/- 1 SE) for westslope cutthroat (WSC) and brook trout (BT) in (a) Johnson Cr. (non-beaver/WSC/BT), and (b) Squaw Cr. (Beaver/WSC/BT).

Since we expect negative competitive interactions between brook trout and westslope cutthroat to adversely affect juvenile cutthroat most¹⁵, we have begun to examine how the distribution of fish in the size range 55-150 mm corresponds to available temperature within the stream. Distributions of juvenile fish caught during the first capture session were related to temperature data for three streams (Figure 3), representing each treatment type. Where brook trout are not present, westslope cutthroat juveniles select temperatures between 14 and 16°C. Where both brook trout and beaver are present there is a relatively high degree of overlap in brook and cutthroat distributions, and where beaver are absent there appears to be less overlap between the two species. Where brook trout occur with cutthroat, brook trout appear to dominate areas with higher temperatures. Calculation of potential growth rates¹ for cutthroat trout at the actual temperatures experienced by the juvenile cutthroat in each of these stream indicate that highest growth rates are expected in the absence of brook trout, while the lowest growth rates are most likely where brook trout are present and beaver are absent (Figure 3). Hence, the presence of beaver in a watershed may buffer the negative effects of brook trout competition on westslope cutthroat populations.

We are currently analyzing scales taken from both brook trout and cutthroat trout. This, in conjunction with recapture work in summer 2007 will allow us to estimate growth rates with more precision.

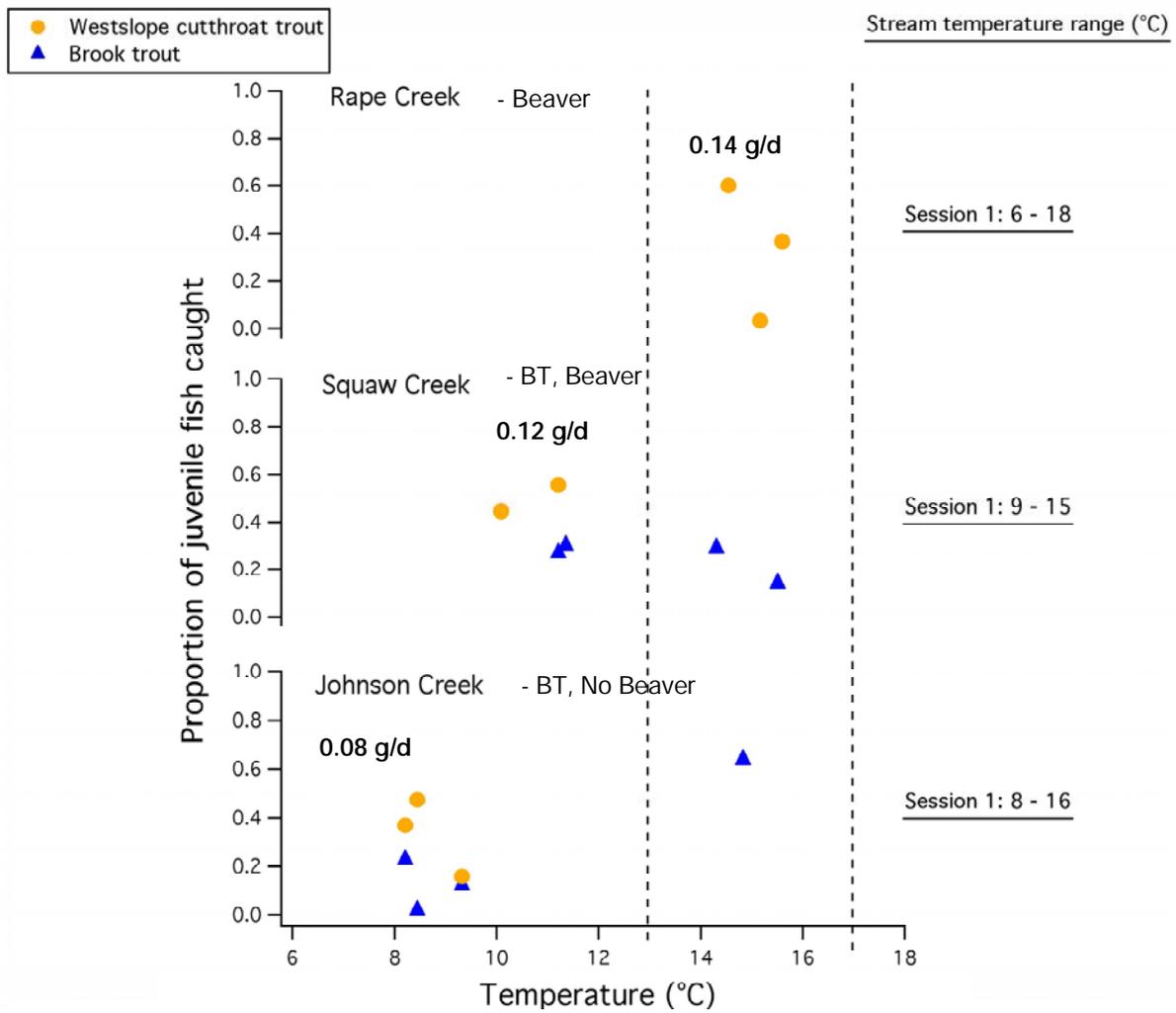


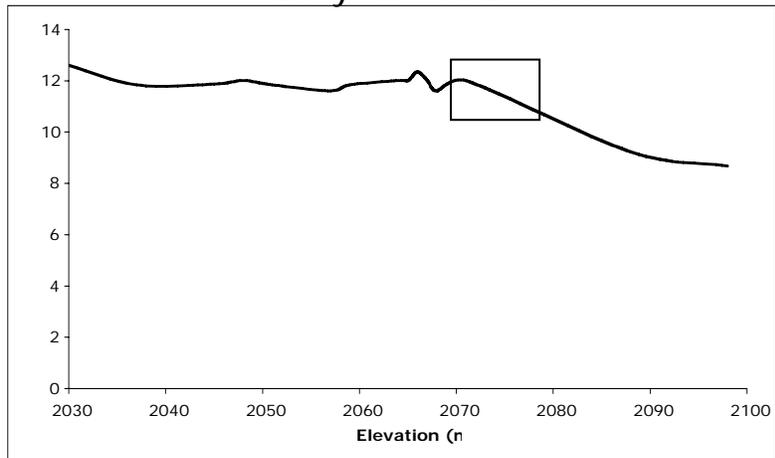
Figure 3: The distribution by temperature of brook trout (triangles), and westslope cutthroat trout (circles) in three treatment types. Potential growth rates of cutthroat trout in each stream are shown in bold, and were calculated with Sloat's¹ equation. The dashed lines indicate the temperature range in which brook trout are thought to outcompete cutthroat trout²

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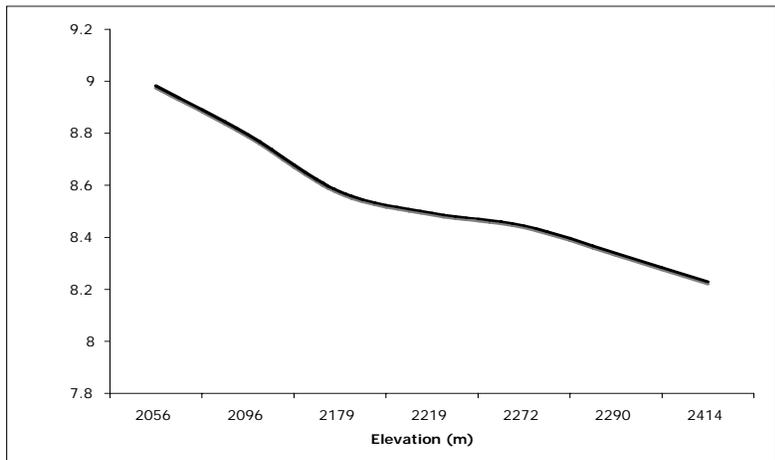
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Appendix A

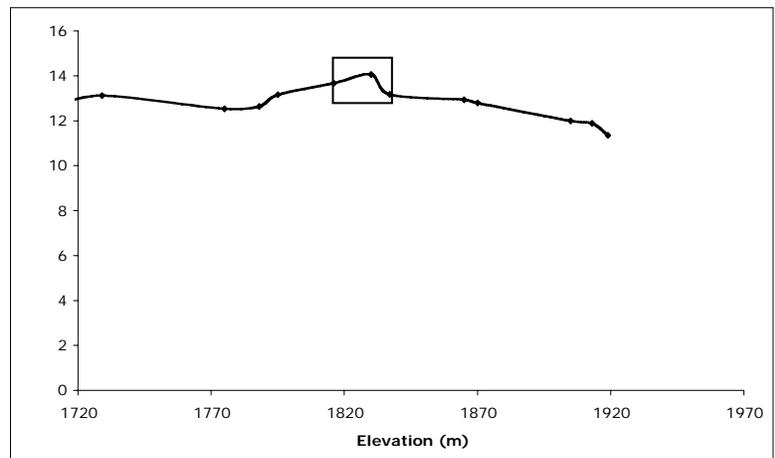
Lacy Creek: Beaver



Stine Creek: Non-beaver



Stone Creek: Beaver



Stream temperature profiles of beaver and non-beaver streams. Rectangles denote areas of known beaver impoundment.

Student fellowship: Further investigation of diel cyclic changes of metals in two Montana rivers.

Basic Information

Title:	Student fellowship: Further investigation of diel cyclic changes of metals in two Montana rivers.
Project Number:	2006MT96B
Start Date:	3/1/2006
End Date:	6/30/2007
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Congressional District:	At large
Research Category:	Water Quality
Focus Category:	Geochemical Processes, Hydrogeochemistry, Solute Transport
Descriptors:	None
Principal Investigators:	Stephen Parker

Publication

Investigations of diel changes in the concentration of
dissolved Mn and Zn and
sediment-water interactions

By
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For
Montana Water Center
January 22nd, 2007

Abstract

Diel concentration changes of metals have been shown to occur in a variety of streams. Photosynthesis and respiration of aquatic plants and microorganisms drive the diel pH cycle in a healthy river. However, temperature-dependent and pH-dependent sorption to surfaces plays a significant role in the concentration cycles of both anions and cations in the river system. Since the transport and fate of chemical species within a river can have a significant impact on the health of the aquatic system and the surrounding environment, an enhanced understanding of the mechanisms affecting diel concentration cycles could lead to a better fundamental understanding of water quality dynamics within natural waters.

In this work, preliminary results are presented for the investigations of diel (24 hour) processes controlling the flux of metals across benthic surfaces across the sediment-water interface.

Previous examinations of the Clark Fork River have demonstrated diel concentration changes in dissolved and particulate forms of Mn, Fe, Zn, Al, and Cu. These concentrations may be affected by daily biogeochemical processes in the benthic biofilm surfaces. It was additionally observed that the concentration of dissolved Mn and Zn cycles were in phase, suggesting that the cycles are linked by a common dependence to temperature, pH, photoperiod, and/or hydrological cycles.

A model has been proposed that links diel concentration changes to the dissolution and precipitation processes in association with biofilm and algal populations through a daily solubility and redox cycle. Initial results of laboratory and in situ experiments are presented providing insight into the role that benthic surfaces have in the diel concentration cycles of metals in streams. Fieldwork included the use of flux-chambers to isolate benthic surfaces from the flowing water column. The concentration changes of Mn and Zn within these isolation chambers are compared to the water column to isolate and identify the origin of the metal diel cycles.

Background

Diel concentration cycling of metals and certain non-metal compounds are well documented.^{1, 2, 3} Daily cycles of temperature, redox, photosynthesis, and respiration predicate cyclic chemical and physical changes in parameters including pH, alkalinity, specific conductivity, dissolved gasses (e.g. oxygen and

1 Parker, S. R., Poulson, S. R., Gammons, C. H., DeGrandpre, M. D., Biogeochemical Controls on Diel Cycling of Stable Isotopes of Dissolved O₂ and Dissolved Inorganic Carbon in the Big Hole River, Montana, *Environ. Sci. & Technol.*, 39(18), 7134-7140, (2005), DOI: 10.1021/es0505595..

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3 Brick C. M. and Moore J. N., 1996. Diel variation of trace metals in the upper Clark Fork River, Montana. *Environ. Sci. Technol.* 30, 1953-1960.

carbon dioxide), redox speciation, and dissolved and particulate element concentrations. The transport and fate of species within a river system can have a significant impact on the health of the aquatic system and the surrounding environment. A better understanding of river system chemistry is necessary, and will lead to improved understanding of the mechanisms affecting diel concentration cycles for a variety of chemical species.

The timing of sampling during the diel cycles can significantly influence the resulting observations of analytes. For example, in the Fisher Creek, Montana, dissolved copper has been observed to undergo a 140% change in concentration during a 24-hour period.⁴ Similarly, dissolved zinc concentration changes of 500% have been observed in the Prickly Pear Creek, Montana.⁵

In a healthy river system, the diel pH cycle is driven by the photosynthesis and respiration cycle of the aquatic plant life and CO₂ consuming micro-organisms⁶, however temperature and pH dependant sorption to substrate, suspended inorganic surfaces, and organic surfaces may also play significant roles in the concentration cycles of both anions and cations in the river system.^{7, 8, 9, 10, 11, 12, 13}

Previous examination of the Clark Fork River, Montana has observed diel concentration changes in dissolved and particulate forms of Mn, Zn, Fe, Al, and Cu.¹⁴ These concentrations may be effected by daily biogeochemical processes in the benthic biofilm surfaces and algal surfaces. It was additionally observed that the concentration of dissolved Mn and Zn cycles were in phase, suggesting that the cycles are linked by a common dependence to temperature, pH, photoperiod, and possibly hydrological cycles.

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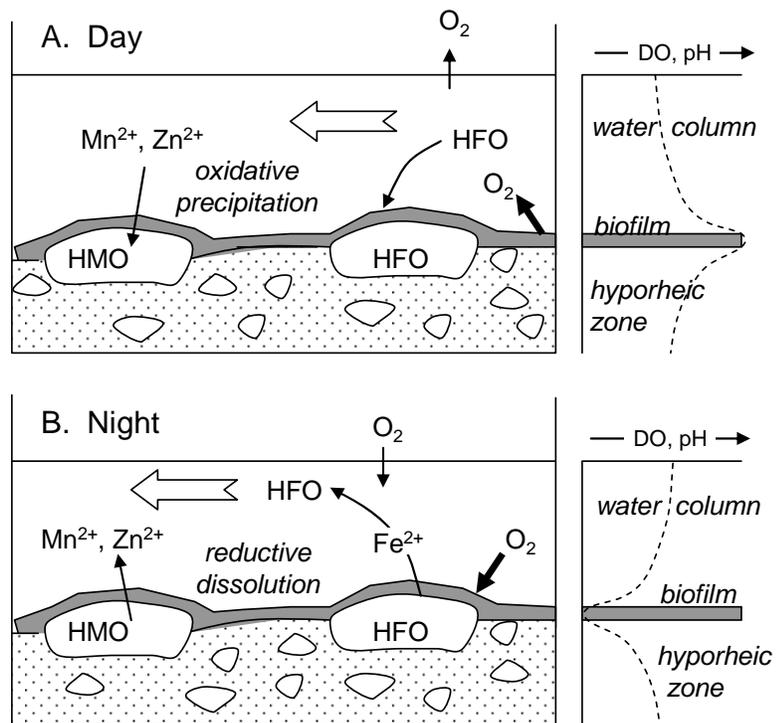
12 Trivedi, P., Axe, L., 2000. Modeling of Cd and Zn sorption to hydrous metal oxides. *Envir. Sci, & Tech.* 34. 2215-2223.

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14 Parker, S. R. Ph. D. Thesis, University of Montana, Missoula, MT, 2005.

A proposed model links these diel concentration changes to the dissolution and precipitation in association with biofilm and algal populations through a daily solubility and redox cycle.¹⁴ Other mechanisms that may effect the cycles are:

1. Changes in the influx of dissolved metals from the shallow groundwater.
2. pH and temperature dependant sorption to benthic and suspended surfaces
3. Daytime precipitation of Mn and Zn mineral phases of as impurities incorporated into calcite¹⁴.



The basis of this proposed model is that during the photoactive period photosynthesis creates an oxidative zone. The presence of this oxidative zone promotes the oxidative precipitation of hydrous ferric oxides (HFO) and hydrous manganese oxides (HMO). During the respiration period, the consumption of dissolved oxygen creates a reductive zone. This reductive zone promotes the reductive dissolution of HFO and HMO.

The precipitation of Zn has been linked to the formation of HMO.¹⁵ The half-life of Mn²⁺ in well-aerated waters at pH 8 is approximately 100 days, while the half-life of Fe²⁺ in a few minutes.¹⁶ This rapid reduction of iron and the slower reduction of manganese to the less soluble Fe⁺³ and Mn⁺⁴ can account for the nighttime buildup of dissolved Mn that is not accompanied with a corresponding increase in dissolved Fe. The kinetics of HFO formation is well established as a first order reaction, however the reduction of Mn⁺² to Mn⁺⁴ and the subsequent formation of HMO is an autocatalytic reaction.¹⁷ The autocatalytic formation of

¹⁵ Shope, C. L.; Ying X.; Gammons, C.H.; The influence of hydrous Mn-Zn oxides on diel cycling of Zn in an alkaline stream draining abandoned mine lands, *Appl. Geochem.* 21, 476-91, (2006).

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HMO may account for the rapid decrease in dissolved Mn during photoactive periods.

Methods

The investigation was performed in three phases. Phase I consisted of

Figure 1: Second-generation flux-chamber, fitted with WTW Multi-340i.

attempting to reproduce the

dynamic cycles observed in nature in a laboratory environment. Phase II focused on the construction and testing of a viable flux-chamber for use insitu. Phase III utilized the flux-chamber to quantify the biota impact on the diel cycle of dissolved Mn and Zn.

Phase I was performed with the use of a commercial available 20 L aquarium with a side-mounted pump. The filtration system was not installed. The tank bottom was lined with bio-encrusted benthic material and filled to capacity with water obtained at the Arrowhead Recreation Park on the Clark Fork River in Deer Lodge, Montana. A control tank was filled with the water from the same sampling, but the benthic lining was not installed.

Each tank was allowed to rest for five weeks under a simulated photo-cycle to reestablish the photosynthesis and respiration cycles of the biomass. The photo-cycle was simulated by a broad-spectrum fluorescent lamp, controlled by an electro-mechanical timer. A light shield was installed to prevent ambient interference.

After the resting period, the tanks were spiked to 100 ppb Mn and 20 ppb Zn in the form $Mn(NO_3)_2$ and $Zn(NO_3)_2$. The photo-cycle was then extended to a 48-hour period (24 hours light, 24 hours dark) to exaggerate the effect of photosynthesis and respiration on the system. The parameters of the system were recorded with a WTW Multi-340i, recording pH, specific conductivity (SC), dissolved oxygen (DO), and temperature.

60 mL water samples were drawn every 24 hours, immediately prior to changing the photo status of the system. Samples were drawn during each sampling event, filtered (using a 20 μ m syringe filter), and acidified to 1% with Trace Metal Grade (TMG) Nitric Acid (HNO_3). The experiment was preformed for 2 periods (96 hours). Each sample was analyzed by Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS) for Zn and Mn concentrations.

Phase II focused on the development and construction of a flux chamber for in-situ isolation of biomass. The first design model was seriously flawed and failed to isolate the system from the river column . A redesigned model was developed that strong seal from the environment, and did not show positive

pressure or negative pressure leakage in 12-hour tests. The second-generation flux-chamber was approved by the Principle Investigator and used for Phase III.

Phase III integrated the flux-chamber into a diel sampling event at the USGS flow meter located in the Clark Fork River, approximately 20 meters south of the Milwaukee street bridge, in Deer Lodge, Montana. The stream parameters were monitored for 48 hours using a submersible datasonde, collecting pH, DO, SC, and temperature.

Water samples were taken in duplicate hourly from the river for 27 hours. One sample was acidified to 1% TMG HNO₃ without filtering. The other was filtered using a 0.1 μm cellulose filter, and acidified to 1% TMG HNO₃. Each were analyzed for Mn and Zn by GF-AAS and retained for analysis by Inductive Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

In conjunction to the diel sampling of the Clark Fork River, two flux-chambers were installed into the river and filled approximately half full of indigenous biota from the riverbed. One chamber was fitted with a submersible datasonde, the other fitted with a WTW Multi-340i. The latter chamber was used for sampling, while the former was sealed for the entire sampling event. Both chambers were filled with river water, vented to ensure no trapped gasses, and sealed. The chambers were allowed to rest on the riverbed, completely submerged to ensure temperature equilibration with the surrounding river. Chamber circulation was maintained by battery operated peristaltic pump, piped to each end of the chamber.

The sampling chamber was sampled in duplicate every bi-hourly, in the same method as the river. After each sampling, the sampling chamber seal was broken, the chamber flushed, filled, and submerged. This was conducted in conjunction with the sampling of the river so that the metal concentrations of the chamber could be assumed to be the same as the river at the time the chamber was sealed.

The in-situ was repeated on the Big Hole River, at the USGS river flow gage approximately 2 Km east of Wisdom, Montana.



Figure 2: Placing the sealed chamber onto the riverbed

Results

The tank experiment (Phase I) failed to produce a discernable cycle in the system. The concentration of dissolved Mn and Zn decreased to below detectable levels during the first photoactive period, but failed to increase during the respiration periods. pH and DO levels indicate that a viable photosynthesis

and respiration cycle was present within the tank. Figure 3 shows the sample results from Phase I. The shaded areas represent the dark periods.

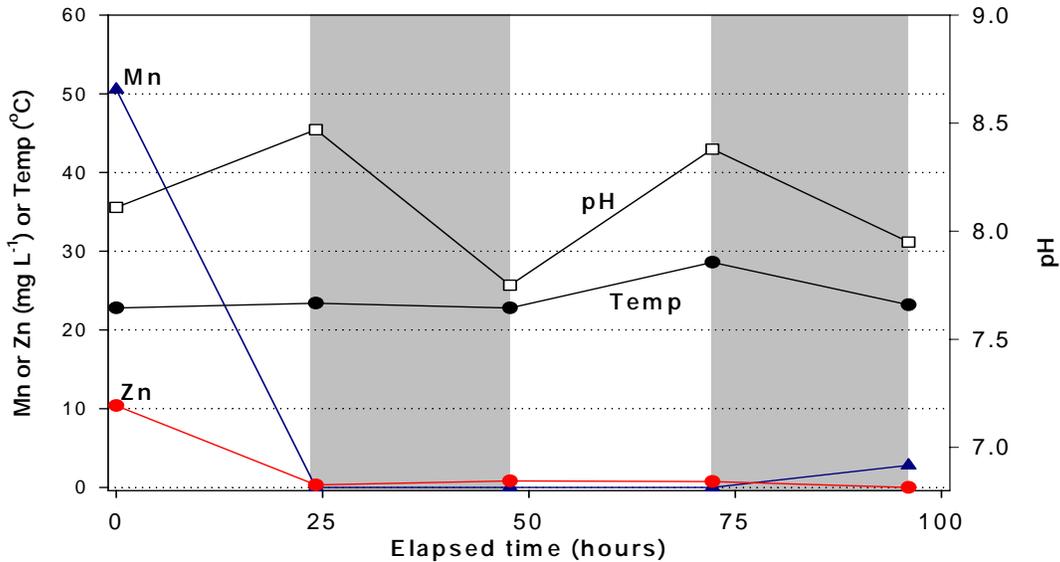


Figure 3: pH, DO, Mn, and Zn results from Phase I.

Preliminary results from Phase III in the Clark Fork River indicate that the chamber photosynthetic cycle increase the amplitude of the diel DO and pH cycles. Figure 4 details the DO and pH cycles in the Clark Fork River and compares the cycles within the chambers.

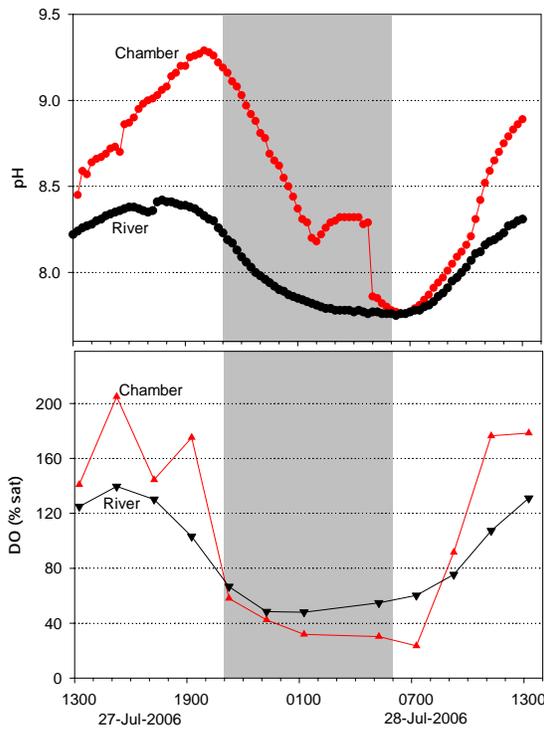


Figure 4: Comparison of pH and DO in the Clark Fork River versus the flux-chambers.

The chamber pH and DO cycles did demonstrate an amplitude increase, however the minimum pH in both the chambers and river were virtually equivalent. The isolation of the chamber from the air column above the river, and diffusion of excess DO to the air column could account for the substantially higher DO in the chamber compared to the river.

Preliminary dissolved metals analysis using a GF-AAS was performed to compare the cycle of dissolved Mn between the Clark Fork River and the chamber.

Figure 5 details the preliminary results. The percent of dissolved Mn change for each two-hour sampling period of the chamber are compared to the river. The chamber appears to increase the rate of dissolved metal sequestration, but does not seem to significantly influence the rate of

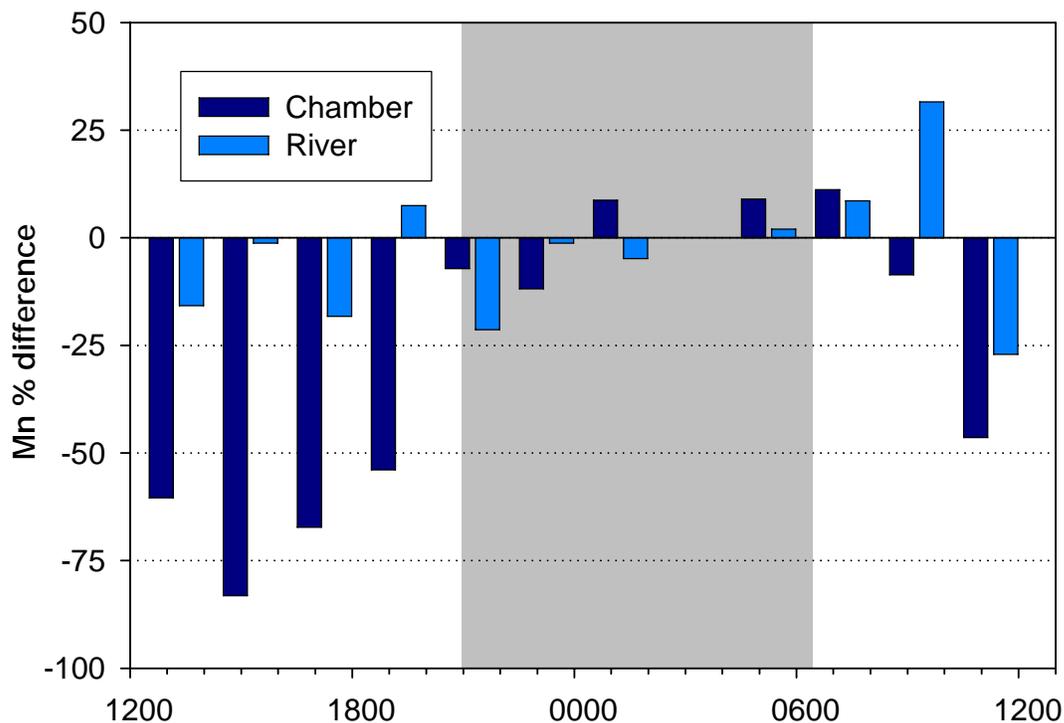


Figure 5: Percentage of dissolved Mn change during a two-hour period in the Clark Fork River and flux-chamber.

nighttime return of the dissolved Mn. A more complete analysis of all samples is currently underway using ICP-AES. Analysis of the replicate sampling of the Big Hole River is planned for early spring 2007, however, the natural dissolved metal concentrations of the Big Hole River may not reveal any further information. Analysis of the Big Hole River samples will be performed by Flame Atomic Absorption Spectroscopy (FAAS) prior to deciding if ICP-AES of the samples is warranted.

Conclusions

Completion of the ICP-AES analysis of the Clark Fork River samples is necessary to make any conclusions on the mechanisms that control the diel cycle of dissolved Mn and Zn. However, the preliminary data does seem to suggest that bioinorganic interactions within the river may have a significant impact on the dissolved Mn concentrations. It is not discernable if the bioinorganic interactions are in any way accountable for the observed increases in the dissolved Mn during the rivers respiration periods.

Additional research into this mechanism is planned, and has been proposed for funding to the United States Environmental Protection Agency and the National Science Foundation. This research will include the development of further flux-chambers, intended to isolate the sediment interactions with the river

and implement microelectrodes to establish the presence of the oxidative and reductive zones that the method suggest are present. If adequate funding is provided, the project will also include Scanning Electron Microscope examination of algal samples taken during a diel sampling event. This data will be used to determine the mineralogical makeup of the HMO and HFO on the algal surface, and analyze the algal samples for absorption and vacuolization of dissolved Mn versus the adsorption to the external cellular wall and associated biofilm colonies on the rivers biota.

Acknowledgments

The author would like to thank the contributions of Stephan Parker, Ph.D. of Montana Tech, Matthew G Smith, Undergraduate Research Assistant, Montana Tech, and USGS, the Montana Water Center, and the Montana Tech URP for the funding provided.

Student fellowship: A genomic and proteomic approach to characterizing natural variation in E. coli: Toward construction of a microbial source tracking database to identify sources of fecal water contamination in the State of Montana

Basic Information

Title:	Student fellowship: A genomic and proteomic approach to characterizing natural variation in E. coli: Toward construction of a microbial source tracking database to identify sources of fecal water contamination in the State of Montana
Project Number:	2006MT98B
Start Date:	3/1/2006
End Date:	6/30/2007
Funding Source:	104B
Congressional District:	At large
Research Category:	Biological Sciences
Focus Category:	Water Quality, Treatment, Methods
Descriptors:	None
Principal Investigators:	William Holben

Publication

Margie Kinnersley
Montana Water Center Student Fellowship Final Report
June 22nd, 2007

Research Project Title: A Genomic and Proteomic Approach to Characterizing Natural Variation in *E. coli*: Toward Construction of a Microbial Source Tracking Database To Identify Sources of Fecal Water Contamination in the State of Montana.

Abstract

The overarching goal of this study is to characterize variation in naturally occurring *E. coli* populations in Western Montana at the genomic, transcriptomic and proteomic levels and to correlate this data with animal host species information to identify animal sources of fecal water contamination. A rep-PCR fingerprint database has been created for the Many Glacier region of Glacier National Park that can be used to roughly classify unknown *E. coli* isolates. Preliminary 2D gel protein profiles for a subset of human, bear and deer *E. coli* show that there are a number of protein composition differences that may be useful for distinguishing human and animal isolates. Microarray-based comparative genome hybridization analysis has also revealed several differences in genome composition that are being investigated as potential biomarkers.

Objectives

The proposed research objectives are as follows:

Objective 1: To collect fecal samples and isolate *E. coli* from humans and several different species of local wild mammal that may be potential nonpoint water contamination contributors.

Objective 2: To characterize genetic and phenotypic differences in *E. coli* populations within individual animals and between host species.

Objective 3: To utilize genotypic and phenotypic information to determine the origin of *E. coli* isolated from contaminated water sources using a relational database.

Project Progress

Objective 1: One hundred and fifty-three *E. coli* strains have been isolated from the feces of ten different species of animal that reside in the Many Glacier area of Glacier National Park, Montana, USA per EPA method 1603. In addition, a reference strain collection (the ECOR collection) consisting of 41 human and 33 animal *E. coli* isolates has been obtained from Michigan State University and 253 human, sheep, goose, cow and pig isolates have been generously donated by Dr. Michael Sadowsky at the University of Minnesota.

Objective 2: Traditional rep-PCR fingerprinting using the BoxA1R primer (genotypic portion of objective 2) has been completed for all Montana isolates. A small database has been constructed for the Many Glacier region of Glacier National Park. Jackknife analysis (a measure of the internal consistency of the database that assesses how often a fingerprint is correctly re-classified into its original group) has been performed. In general, the rate of correct re-

assignment of the fingerprints when each animal species is considered a separate group was somewhat low with an average of 52%. Values ranged from 0% for moose to 100% for coyote. These low values are likely due to the fact that a small number of morphologically distinct colonies were selected from a relatively small number of animals across a wide range of species that all share a common habitat. The overall performance of the database may be improved by the addition of more isolates from each species.

A modified rep-PCR technique in which restriction enzymes were used to create additional fingerprint variation was also applied to a subset of strains from Montana. This new type of fingerprinting has identified a gene region that is widely distributed in human *E. coli* but occurs infrequently in *E. coli* from animal feces. A PCR assay was designed to test for the presence or absence of this marker. Results indicate that 67% of the human isolates from the Many Glacier Hotel raw sewage possess the marker while 95% of the animal strains from the surrounding area do not (Table 1). Eighty-five percent of the human isolates from the ECOR collection are also positive for the marker while only 42% of the Minnesota human isolates and 19% of the Montana clinical isolates score positive. The lower numbers for the clinical and Minnesota strains may be due to differences in how the *E. coli* were initially isolated or may simply reflect geographical variation in genome content. Experiments to determine the effects of isolation technique on this assay are currently underway.

Table 1. Distribution of putative marker identified by rep-PCR in Glacier National Park and ECOR strain collections

	+ for marker	- for marker
Glacier human isolates (9 total)	6 (67%)	3 (33%)
Glacier animal isolates (64 total)	3 (5%)	61 (95%)
ECOR human isolates/lab strains (39 total)	33 (85%)	6 (15%)
ECOR animal isolates (33 total)	8 (24%)	25 (76%)
Minnesota human isolates (48 total)	20 (42%)	28 (58%)
Minnesota animal isolates (204 total)	43 (21%)	161 (79%)
Clinical human isolates (16 total)	3 (19%)	13 (81%)

Twelve isolates with unique fingerprints have been selected for proteomic and microarray analysis. These twelve strains represent three species of host (brown bear, white-tail deer and human) with significantly different digestive system physiology. 2D gel analysis of these strains in triplicate is nearly complete and preliminary analyses have indicated that (1) there exists significant proteomic variation between isolates from different host species and (2)

differences that may be diagnostic for host-species can be identified (Figure 1). Mass spec analysis of all protein spots with host species distribution differences is in progress.

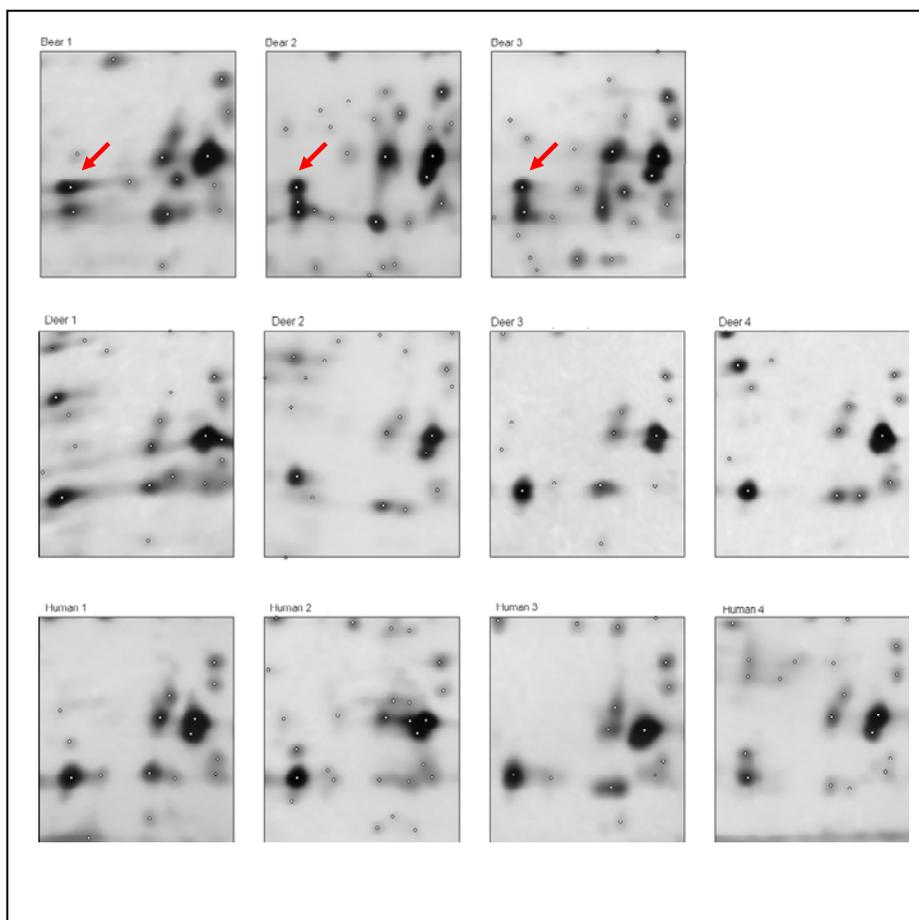


Figure 1. A representative subset of 2D protein expression patterns for three bear, four deer and four human *E. coli* isolates. Each panel represents a small portion of a single 2D gel. The red arrow indicates the position of a protein that may be useful for distinguishing bear samples from human and deer samples.

Microarray-based comparative genome hybridization of these isolates has revealed several gene regions whose presence or absence differs between bear, deer and human *E. coli*. Experiments to determine the distribution of these potential markers in the larger strain collection are in progress.

Objective 3. The rep-PCR fingerprint database can currently be used to roughly classify unknown *E. coli* isolates. The completion of objective 2 will increase the reliability and utility of the database.

Information Transfer Program

During FY 2006 (March 1, 2006 through February 28, 2007), the Montana Water Center developed and sponsored many programs and tools to carry out its mission to investigate and resolve Montana's water problems. It sponsored research, fostered education of future water professionals and water policy decision makers, and provided outreach to water professionals, water users, and communities. With support from the USGS and other partners, the Center was actively involved in the following activities:

Administered the 104b research grants, and promoted interest in the 104g research grant program, all with assistance from and formal communications with the Center's Water Research Advisory Committee (see Introduction for committee member list);

Developed and circulated a Request for Proposals for the competitive student fellowship program, and ultimately offered research awards to eight graduate students from three Montana institutions of higher education;

Trained and mentored two student interns who helped track research findings, disseminate outreach materials and helped write and edit the Montana Water Center's e-newsletter;

On this note, published twelve monthly Montana Water e-newsletters distributed to a database of over 1,500 people. E-news archives are posted at <http://water.montana.edu/newsletter/archives/default.asp>

Launched the Whirling Disease Initiative Data Repository in January 2007, a state-of-the-art resource for archiving and accessing datasets generated by the Whirling Disease Initiative research program. The Data Repository provides unique access online to whirling disease research and management information. It was developed in response to the need for data sharing across disciplines and among researchers, land managers, agencies and the public. The Repository stores data associated with completed research investigations. The archive will also protect against the data loss that could occur over time once investigations are completed. With a newly established data submission and use policy, the Whirling Disease Initiative has established progressive and rigorous standards for data collection and sharing of scientific information.

The Montana Watercourse released its new Volunteer Water Monitoring Guidebook. A practical watershed education and stewardship tool, it covers basic water and watershed science, water monitoring design and methods, and data management and analysis;

Convened the three-day 2006 Northwest Water Policy & Law Symposium in September for water policy and law experts from the public and private sectors. It focused on a) conjunctive management of surface and ground water, b) policy response to new knowledge, and c) water management in fully-adjudicated basins. The Burton K. Wheeler Center at Montana State University and the Inland Northwest Research Alliance were additional symposium partners.

Maintained and expanded MONTANA WATER, the Montana Water Center's web information network at <http://water.montana.edu>. This website includes an events page, news updates, an online library, water-resource forums, a Montana watersheds projects database, an expertise directory, water facts and more;

With input from the Education and Outreach Committee of the Montana Watersheds Coordination Council, completely redesigned and revamped the web site of the Montana Watersheds Coordination Council at <http://water.montana.edu/mwcc/default.php> .The Montana Water Center's Outreach Director served as co-chair for the Council;

The Wild Fish Habitat Initiative, a cooperative effort between the US Fish and Wildlife Service's Partners for Fish and Wildlife Program and the Montana Water Center, continued throughout the year. Research conducted through the Wild Fish Habitat Initiative is carried out by Water Center staff and Montana State University biologists in collaboration with private landowners and private and public-agency biologists. To augment its technology transfer program, the Water Center maintains an Initiative website at <http://water.montana.edu/wildfish/> , of which the case histories database is a prime component.

The Aquatic Sciences Laboratory continues to be a focal point and innovator for aquatic research within the Montana University System. Major research projects conducted in the past year included sentinel cage testing for whirling disease in Yellowstone National Park, a PIT-tag survival and retention study on Arctic grayling, and research on the interactions between New Zealand mudsnails and the threatened Bliss Rapids snail. The lab manager, in his role as aquatic research animal advisor for MSU, has recently undertaken the management of a new leopard frog housing facility for a researcher at the College of Nursing. As part of the outreach program, the lab manager attended the annual American Fisheries Society Meeting, World Aquaculture 2007 and the Midwestern Fish & Wildlife Conference.

With input from the Education and Outreach Committee of the Montana Watersheds Coordination Council, completely redesigned and revamped the web site of the Montana Watersheds Coordination Council at <http://water.montana.edu/mwcc/default.php> .The Montana Water Center's Outreach Director served as co-chair for the Council;

Conducted the state-wide water research meeting on October 12th and 13th in Polson, Montana. The theme of this 23rd annual meeting, held in conjunction with the Montana Section of the American Water Resources Association, was Montana's Lakes and Wetlands: Improving Integrated Water Management. A pre-meeting field trip explored the Flathead regional geology, hydrogeology and wetlands. The meeting attracted over 150 Montana researchers and policy makers who took in plenary presentations by Dr. Richard Hauer, Professor of Limnology, Dr. Michael Suplee, Water Quality Specialist, and Lynda Saul, Montana Department of Environmental Quality Wetlands Manager. Over forty researchers presented information on their latest findings. The web-based archive of this meeting is found at http://awra.org/state/montana/events/conf_archives.htm .

Served as a liaison among the university community, water professionals, and decision makers in local, state, and tribal and federal governments, including attendance at all Montana Legislative Environmental Quality Council meetings and Montana University System research outreach coordination meetings;

Invested time in partnering with other groups with similar goals of translating scientific information for effective problem-solving;

Participated in the 73rd Annual Water School on October 3-6, 2005 at Montana State University. Designed for water and wastewater managers and operators, this training provided information on treatment plant chemical safety, emergency response, developing quality assurance programs, and more. At the close of the training, some operators took the water/wastewater certification exam administered by the Montana Department of Environmental Quality (DEQ). Along with DEQ, this program is conducted

by the Montana Environmental Training Center, the Montana Water Center, and the MSU Department of Civil Engineering;

Created the third annual black-and-white water facts and photos calendar for general circulation titled Montana Water 2007. Each month was dedicated to a different Montana water topic. A copy of this is located at <http://watercenter.montana.edu/publications/other.htm> and

Produced the Montana Water Center's Annual Report, Fiscal Year 2006 covering all of the programs accomplished through the Center's \$2.3M budget, posted at <http://watercenter.montana.edu/publications/reports.htm> .

Student Support

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	1	0	0	0	1
Masters	4	0	0	0	4
Ph.D.	3	0	0	0	3
Post-Doc.	0	0	0	0	0
Total	8	0	0	0	8

Notable Awards and Achievements

Sponsored development of the film "Headwaters to a Continent: Montana's Working Watershed Groups," that received five Telly Awards. The film was produced by Alison Koch, a paleontologist for the National Park Service and student filmmaker, and the Montana Water Center. These prestigious awards, granted by a panel of past Telly winners, honors the best work of video production companies, television stations and corporate video departments from around the world. The film received two 2007 Silver Tellys, the highest Telly award, in the Education and Nature/Wildlife categories. It garnered bronze trophies in the Documentary, Information and Student categories;

Publications from Prior Projects