Introduction

The Montana University System Water Center is 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 statewide 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 to help set research priorities. During the 2007/2008 research year, the Montana Water Research Advisory Council members were:

Gretchen Rupp, Director and Steve Guettermann, 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

Mark Aagenes, Montana Trout Unlimited, Conservation Director

Sarah Carlson, Montana Association of Conservation Districts, Executive Director

Art Compton, Montana Department of Environmental Quality, Administrator

Julie Dalsoglio, EPA Region 8 – Media Manager

Larry Dolan, Montana Department of Natural Resources and Conservation, Hydrologist

Krista Lee Evans, Environmental Quality Council, Legislative Staff

Hal Harper, Montana Governor's Office, Chief Policy Advisor

John Kilpatrick, USGS – Helena, Assistant Director

Ron Nadwornick, USDA, Montana State Resource Conservationist

Glenn Phillips, Montana Department of Fish, Wildlife and Parks, Future Fisheries

J. P. Pomnichowski, Montana State Legislator

Daniel Sullivan, Montana Department of Agriculture, Technical Services Bureau Chief

Tyler Trevor, Montana University System, Associate Commissioner

Mike Volesky, Montana Governor's Office, Natural Resources Policy Advisor
Research Program Introduction

Research Program Through its USGS funding, the Montana Water Center partially funded five water research projects for faculty at three of Montana's state university campuses. It also provided research fellowships to five students involved with water science and aquatic habitat research. Here is an introduction to their work.

Dr. Chris Gammons, of Montana Tech, is studying “Temporal and spatial changes in the concentration and isotopic composition of nitrate in the Upper Silver bow Creek drainage, Montana: Year 2.” The project received $6,800 this year and final results are being analyzed.

Dr. Joel Harper of the University of Montana received $8,941 for his study of “Historical and future streamflow related to small mountain glaciers in the Glacier Park Region, Montana.” Considerable progress has been made on reduction of existing data garnered through field work and incorporated into computer modeling.

Dr. Clayton Marlow and his research student Richard Labbe, received $2,000 for their work titled “Sediment and heavy metal source determination and reduction at a reclaimed abandoned mine site, Alta Mine, Jefferson County, Montana.” Labbe presented the final results in his dissertation at Montana State University during Spring 2008.

Dr. Lucy Marshall of Montana State University, received $17,000 for her study of “Predictive modeling of snowmelt dynamics: thresholds and the hydrologic regime of the Tenderfoot Creek Experimental Forest, Montana.” Work has focused on the development of conceptual snowmelt/hydrologic models.

Dr. Steve Parker, Montana Tech, received $8,585 for his research project: “Carbon cycling and the temporal variability in the concentration and stable carbon isotope composition of dissolved inorganic and organic carbon in streams.” This work has been key with getting a team of student field researchers involved in data collection and analysis of specific diel cycles.

Student Research Fellows The Water Center's Student Water Research Fellowship Program awarded research grants to five students from three Montana institutions of higher education. Each showed competence in studying a water resource problem that is impacting water quality or an aquatic species. They are:

Magnus McCaffery, Ph.D. student in Wildlife Biology at the University of Montana, for his study of the influence of beaver on brook trout invasion and cutthroat trout displacement

Michael Meeuwig, Ph.D. student in Biological Sciences at Montana State University, for his investigation on populations of bull trout in Glacier National Park.

Eric Boyd, Ph.D. student in Microbiology at Montana State University, to study natural mercury in aquatic environments.

Lisa Bithell Kirk, Ph.D. student in Microbial Geochemistry at Montana State University, to investigate microbial transformation of selenium in phosphate mine wastes.

Keri Petritz, Master's student in Environmental Engineering at Montana Tech, for her study of resource recovery from flooded underground mine workings.
**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**
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

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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

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 where 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 where 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.
Seepage along the W Gallatin River near Four Corners Nov 2005

*Net change in discharge is positive 41 cfs*

**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.
Seepage along Gallatin River
August 2006
Net flux for the entire reach is negative 4 CFS

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 (Figures 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

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Publication
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

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Publication
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

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Publication
2006 Water Center Fellowship: Progress Report

Effects of Road Culverts on Eastern Montana Prairie Fish Assemblages

Leo R. Rosenthal
M. S. Candidate
Department of Ecology
Montana State University
(406) 994-1823

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*.

  $\Rightarrow$ 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 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.

  $\Rightarrow$ 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**

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### Publication

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

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 (\(\Psi_{\text{predawn}}\)) coupled with soil water potentials (\(\Psi_{\text{soil}}\)) extrapolated from soil water content (\(\theta\)) 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 
avoveground 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 ($\Psi_{soil}$) 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 ($\theta$) 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 ($\Psi_{soil}$) 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 menzesii*) 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 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 overlaying 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 2/3 rds 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 areencased 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.

![Percentage of Aspen compared to all trees](image)
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.

References:


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

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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/m$^2$ 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 (NH$_3$ or NH$_4^+$, 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.

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

![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.](image)
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.
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.

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

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Publication
Problem statement:

The cycling of carbon in aquatic environments is a critical component of the ecology of these systems. Daily changes in the concentration of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) can have a significant influence on the health of aquatic organisms as well as on the mobility of metals and other toxic contaminants in lotic environments.

Large, daily changes in the concentration of contaminant and toxic materials in flowing waters have been documented in a significant number of streams. These diel concentration changes can impact human and aquatic health significantly as well as posing problems for effective water sampling and monitoring activities. The results of this project will provide valuable information for water managers, geochemists and other hydrological professionals investigating aquatic systems.

The USGS Toxic Substances Hydrology Program recently headlined the importance and implications for gaining more insight into the causes of diel concentration changes in metals and other chemical species in streams (USGS Toxic Substance Hydrology, 2005); the PI for this proposal was a collaborator in two of the publications referenced on this site (Gammons et al., 2005; Parker et al., 2005). This emphasizes the importance of understanding the mechanisms underlying the biogeochemical processes that cause these daily changes in stream chemistry.

Results and benefits statement:

This project will yield valuable insight into the degree of interchange and connection between the organic and inorganic carbon pools in streams. It is critical to have a better and more detailed understanding of the underlying mechanisms and quantitative relationships involved in the daily cycles and movement of carbon. 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.

Secondly, DOC is known to be involved in complexation and transport of metals in streams. Daily changes in concentrations of DOC may be important in the daily changes in loads of metals being transported. This project would produce valuable insight into the correlation between the changes in concentration of dissolved organics and concentrations of metals in solution.

Project description:

Background: Diel (24-h) concentration fluctuations of metals and other chemical species have been well documented in the literature (e.g. Fuller and Davis 1989; Bourg 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). Daily cycles of temperature, oxidation-reduction, photosynthesis and respiration force cyclic chemical and physical changes in parameters such as pH, alkalinity, specific conductivity, dissolved oxygen, redox speciation, dissolved carbon dioxide, and dissolved and particulate trace and major element concentrations. Transport within rivers of metals and metalloids and the impacts from development and climate change can have a
significant effect on aquatic and human health. Consequently, a better understanding of the chemistry of river systems is needed, and will lead to improved understanding of the mechanisms affecting diel concentration changes of a variety of chemical species.

The timing, during the day, of sample collection can have an impact on the observed concentrations of analytes of concern. For example, dissolved copper in a near neutral reach of Fisher Creek, MT, a heavily mine impacted stream, has been shown to undergo 140% concentration changes during a diel cycle (Gammons et al., 2005). Figure 1 shows the dissolved Cu concentration in Fisher Creek in two separate years indicating that this is reproducible geochemical process. Dissolved zinc concentrations in Prickly Pear Creek, MT underwent a 500% change in one twenty-four hour sampling period (Nimick et al., 2005). In a “healthy” river system the diel pH cycle is largely driven by aquatic plants and microbes that consume or produce CO₂ (Pogue and Anderson 1994; Parker et al., 2005). A number of physical, biological and chemical factors influence the major and trace element concentrations on a diel basis. Temperature and pH dependent sorption to substrate and suspended inorganic and organic surfaces are thought to play an integral role in influencing the cation and anion concentration cycles (Fuller and Davis, 1989; Machesky, 1990; Stumm, 1992; Rhodda et al., 1996; Nimick et al., 1998; Trivedi and Axe, 2000; Jones et al., 2004). Additionally, there may be an increase in groundwater flow and exchange with hyporheic water at night due to a decrease in evapo-transpiration. Bed sediments and hyporheic water in the upper reaches of the Clark Fork River drainage have been shown to contain significant concentrations of As, Cu, Fe, Cd, Mn and Zn (Benner et al. 1995; Nagorski and Moore 1999) and these contaminants in the sediment region may move into the water column.

Rivers operate within a complex interface between chemistry, biology and geology and there is a critical need to better understand this interrelationship. Gaining insight into the underlying mechanisms controlling concentration changes of a variety of chemical species will help us achieve a better fundamental understanding of how streams function.

First objective: DOC represents the single largest pool of reduced organic carbon in most aquatic systems that is available to heterotrophic microorganisms as an energy source (Volk et al., 1997). The types and concentration of the DOC can have a significant influence on the chemical composition of surface waters (McKnight et al., 1997).
Temporal changes in $\delta^{13}$C -DOC on a diel scale have not been previously demonstrated. The PI has recently published results demonstrating that there is a significant and reproducible diel cycle in $\delta^{13}$C –DIC in both the Clark Fork (CFR) and Big Hole Rivers (BHR) in Montana as well as a substantial cycle in $\delta^{18}$O-DO in the BHR (Parker et al., 2005; Parker, 2005). These changes are affected by in situ biological processes and Figure 2 shows that the isotope composition of DIC and dissolved oxygen (DO) in the BHR vary significantly over a diel period. These daily changes in the isotope composition of the DIC and DO are caused by the combined effects of photosynthesis and respiration of aquatic plants and microbes as well as contributions from gas-exchange and groundwater (GW). Since community respiration is using the DOC as a carbon source and other aquatic species are producing organic molecules as a by-product of their daily productivity it is reasonable to expect changes in the isotopic composition of the DOC as it is influenced by the daily and seasonal changes in the sources of the organic carbon (Barth and Veizer, 1999; Ziegler and Fogel, 2003; Zeigler and Brisco, 2004). The proposed project would include a diel field experiment at the Dickie Bridge site on the BHR for which a substantial body of data is already available (Gammons et al., 2001; Ridenour, 2002; Wenz, 2003, Parker et al., 2005). [This site was used as the field site for the PI’s recent publication dealing with cycling of the stable isotopes of DIC and DO.] A second field experiment would be conducted on the CFR near Deer Lodge for which a substantial body of data is also available (Brick and Moore, 1996; Parker, 2005). Metals in the bed and flood plain sediments of the CFR due to the mining history of the in Butte and Anaconda areas (Moore and Louma, 1990) make this a good site for investigating the interaction of dissolved and particulate phase metals with diel changes in the concentration of DOC. This investigation would be similar to the one on the BHR but include samples for the determination of dissolved and total metals concentrations.

Second objective: Both DOC and DIC have important roles in the natural processes that take place in streams. DIC can accumulate in surface waters from air-water gas exchange, community respiration, weathering of minerals or from GW contributions. Sources of DOC can include decomposition of organic matter and production by aquatic plants and microbes. However, little is known about the connection between these general classes of carbon compounds. Microbes using DOC as a carbon source will produce CO$_2$ from respiration with a carbon isotope signature characteristic of the organic carbon substrate (Clark and Fritz, 1997). It should be possible to quantify the contribution of carbon to the DIC pool that originates from
respiration versus that from other sources by examining the daily changes in the $^{13}$C-isotope composition. In temperate regions, plant organic mater, that serves as the carbon source for microbial respiration has a $\delta^{13}$C of $\sim -28$ per mil and respiration using this organic matter will preserve this isotope value (Clark and Fritz, 1997). At the same time, atmospheric carbon is about $-7$ to $-8$ per mil and consequently DIC produced by gas exchange will be considerably heavier. It should be possible to determine the contributions of carbon from DOC to the DIC pool using a mass-balance method. Therefore the movement of carbon from the DOC pool to the DIC pool would be quantified.

Third objective: Generally, the major source of DOC in natural waters falls in the category of naturally occurring matter (NOM), and most of the NOM fits into the operationally defined subcategories of fulvic acids (FA) and humic acids (HA) (Macalady, 1998). The fulvic and humic acids are well known for their abilities at complexing heavy metal ions in solution (Saar et al., 1982; Clapp et al., 1998). (Many types of dissolved organics are known to be strong ligands for binding heavy metals in solution.) It is known that the fulvic and humic acids can contribute to daily variations in surface water iron concentrations by affecting the iron redox cycling by affecting the photoreactivity of the aqueous system (Voelker, et al. 1997; Hrncir and McKnight, 1998). Changes in the concentration of dissolved metals in relation to the concentration of DOC and DIC should yield valuable insight into daily mobilization and transport of metals from the contaminated bed sediments.

The Co-PI on this proposal and his students have been studying the concentration of DOC in the acid mine, metal-rich, waters of the Berkeley Pit (Cameron et al., 1999; Cameron et al., 2005). This work has demonstrated the positive correlation that exists between the concentrations of the DOC and the dissolved iron with depth in this complex aqueous system. And, the on-going work is examining the distribution of types of organic matter within the DOC umbrella (Cameron and Johnson, 2004).

### Timeline:

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### Methods procedures and facilities:

The PI (in conjunction with collaborators) has conducted 12 diel sampling experiments on seven different streams in the past four years including the BHR and the CFR. Methods for collecting water samples to examine concentrations of dissolved inorganic carbon (0.1 μm filtered), $\delta^{13}$C-DIC as well as dissolved and total metals concentrations have been detailed in Gammons et al. (2005), Parker et al. (2005) and Parker (2005). Samples for $\delta^{13}$C-DOC analysis will be prepared and analyzed after Gandhi et al. (2004). All stable isotope analysis 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.

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 δ^{13}C –DIC and δ^{13}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. A portable Hach spectrophotometer will be used to measure dissolved (filtered) concentrations of nitrate, phosphate and Fe(II). Regular stream flow measurements would be taken and a staff gage would be read hourly in order to establish a stream-flow rating curve for the site. 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 (see timeline).

- **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, WTW portable multi-probe system, Marsh-McBirney current flow meter.
Related Research (References):


Training potential:

This project would involve two undergraduate students from Montana Tech for assistance with both field and laboratory work. These students would receive background and training in field techniques such as sample collection, measurement of field parameters (pH, DO, SC, T, ORP etc) and stream flow measurements. Additionally, the students would be trained in the operation of the Total Carbon Analyzer (TOC), Inductively Coupled Plasma Spectrometer (ICP-AES) and Atomic Absorption Spectrometer (AAS). These students would be involved in the analysis of the results and data. They would present these results at the undergraduate research symposium held at Montana Tech each year and at another appropriate state or regional conference. The students would also be involved in manuscript preparation for eventual publication.

PI-Parker has recently 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 begin establishing an independent research program dealing with scientific issues surrounding Montana’s rivers.
Biographical Sketch: (PI) Stephen R. Parker:

(i) Professional preparation:
  - Indiana University, M.S., Biochemistry, August 1972.
  - University of Montana, Ph.D., Environmental Chemistry, July 2005

(ii) Appointments:
  - 2001 to present, Assistant Professor, Dept. of Chemistry and Geochemistry, Montana Tech of the University of Montana, Butte, MT.
  - 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

(iv) Recent meetings and presentations:
2) American Chemical Society (Montana section), Butte, MT, April 2005, Diel cycles in stable isotopic composition of dissolved O₂ and CO₂ in a river due to biogeochemical processes.
4) American Geophysical Union, San Francisco, Dec. 2004, Diel changes in stable carbon isotope ratios and trace element concentrations in the Clark Fork River, MT.
6) American Water Resources Association (Montana Section), Helena, MT, October 2004, Fisher Creek, MT and Rio Agrio, Argentina: A geochemical comparison of a mining impacted stream in Montana with a geogenically derived acidic river in Patagonia.
7) Geogenically acidic water systems: volcanic waters, mining lakes and rivers, Caviahue, Argentina, March 2004, Diel (24-hour) changes in metal concentration in a mountain stream impacted by acid mine drainage: Possible similarities to Rio Agrio, Argentina.
Biographical Sketch: (Co-PI) Douglas Cameron

(i) Professional Preparation:
- Montana State University, B.S., Chemistry, June 1975.
- Purdue University, M.S., Analytical Chemistry, August 1978.
- Purdue University, Ph.D., Analytical Chemistry, December 1979.

(ii) Appointments:
- 1999 to present, Professor in the Chemistry and Geochemistry Department at Montana Tech of the University of Montana.
- 1993-1999, Associate Professor in the Chemistry and Geochemistry Department at Montana Tech of the University of Montana.
- 1990-1993, Assistant Professor in the Chemistry and Geochemistry Department at Montana College of Mineral Science and Technology.
- 1989-1990, Staff Member in the Analytical Chemistry Group of the Chemical and Laser Sciences Division at Los Alamos National Laboratory.
- 1986-1989, Senior Research Chemist with Western Research Institute, a division of the University of Wyoming Research Corporation.
- 1986-1989, Adjunct Assistant Professor of Chemistry at the University of Wyoming.
- 1980-1986, Senior Research Chemist with Unocal Corporation, Science and Technology Division, Brea, CA.

(iii) Recent Publications:
Impacts of beaver on invasion ecology of brook trout (Salvelinus fontinalis)

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

Project type: Research
Focus categories: Conservation (COV), Ecology (ECL), Invasive Species (INV)
Research category: Biological sciences
Keywords: beaver, brook trout, Castor canadensis, cutthroat trout, invasion ecology, Oncorhynchus clarki, Salvelinus fontinalis.
Start date: March 1, 2006
Principal Investigator: Dr. Lisa Eby (Assistant Professor), Department of Ecosystem and Conservation Sciences, College of Forestry and Conservation, University of Montana, Missoula, MT 59812. Email: lisa.eby@umontana.edu. Phone: (406) 243-5984.
Co-PI: Magnus McCaffery (Ph.D. Student), Wildlife Biology Program, College of Forestry and Conservation, University of Montana, Missoula, MT 59812. Email: magnus.mccaffery@umontana.edu. Phone: (406) 370-5242.
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 westslope 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 assemblages. Increases in water storage capacity through beaver impoundments improve riparian habitat, and potentially augment water supply and late season flows. These aspects of beaver impoundments have resulted in the active transplantation of beaver as restoration tools into degraded wetlands of the Pacific northwest. 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. Transplantation 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 construction.

Promoting beaver on the landscape, either through natural population expansion or active transplantation 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 systems, there is also the possibility of negative effects on native fish species, such as barrier creation and warming of coldwater streams. 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. 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*), as well as providing important winter habitat for many stream fishes including cutthroat trout (*Oncorhynchus clarki*) and bull trout (*Salvelinus confluentus*).

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 survival. Thus, understanding both what limits the spread of the 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 systems. Any factors that affect demographic parameters such as growth rates, age-0 recruitment, and dispersal can influence the spread of an exotic species. 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 range.
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°C. 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).

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<td>Facilitate brook trout spread</td>
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<td>Distribution of BT</td>
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<td>Abundance</td>
<td>↑ BT in beaver streams</td>
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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.
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.

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<th>RESPONSE VARIABLES</th>
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<td>Enhance negative interactions</td>
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<td>Juvenile BT growth</td>
<td>↑ juvenile BT growth rates &amp; survival in beaver streams</td>
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<tr>
<td>Juvenile WSC growth</td>
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<td>Composition (BT:WSC abundance)</td>
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**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 equation that characterizes the potential growth at a given temperature. This equation was originally formulated for bull trout, but performs well when applied to cutthroat trout.

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°C. 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 cutthroat.

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 temperature is considerably higher than realized temperatures.

![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.](image-url)
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 Thomas 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).

Since we expect negative competitive interactions between brook trout and westslope cutthroat to adversely affect juvenile cutthroat most, 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 rates 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 in each stream are shown in bold, and were calculated with Sloat’s equation. The dashed lines indicate the temperature range in which brook trout are thought to outcompete cutthroat trout.
References cited

2. Thomas, H. M. 1996. Competitive interactions between a native and exotic trout species in high mountain streams. Page 44. Utah State University, Logan, UT.
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

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

By
Kenneth Bates
Montana Tech of the University of Montana
Department of Chemistry and Geochemistry

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

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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. Similarly, dissolved zinc concentration changes of 500% have been observed in the Prickly Pear Creek, Montana.

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 microorganisms, 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.

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. 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.

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.\textsuperscript{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 calcite\textsuperscript{14}.

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.\textsuperscript{15} The half-life of Mn\textsuperscript{2+} in well-aerated waters at pH 8 is approximately 100 days, while the half-life of Fe\textsuperscript{2+} in a few minutes.\textsuperscript{16} This rapid reduction of iron and the slower reduction of manganese to the less soluble Fe\textsuperscript{3+} and Mn\textsuperscript{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\textsuperscript{2+} to Mn\textsuperscript{4+} and the subsequent formation of HMO is an autocatalytic reaction.\textsuperscript{17} The autocatalytic formation of

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 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 in situ. 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 20µm 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.

**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. 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
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

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Publication
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.

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)

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

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<tr>
<th>Strain Type</th>
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<th>- for marker</th>
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<tr>
<td><strong>Glacier human isolates</strong></td>
<td>6 (67%)</td>
<td>3 (33%)</td>
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<tr>
<td><strong>Glacier animal isolates</strong></td>
<td>3 (5%)</td>
<td>61 (95%)</td>
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<td><strong>ECOR human isolates/lab strains</strong></td>
<td>33 (85%)</td>
<td>6 (15%)</td>
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<td><strong>ECOR animal isolates</strong></td>
<td>8 (24%)</td>
<td>25 (76%)</td>
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<tr>
<td><strong>Minnesota human isolates</strong></td>
<td>20 (42%)</td>
<td>28 (58%)</td>
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<tr>
<td><strong>Minnesota animal isolates</strong></td>
<td>43 (21%)</td>
<td>161 (79%)</td>
</tr>
<tr>
<td><strong>Clinical human isolates</strong></td>
<td>3 (19%)</td>
<td>13 (81%)</td>
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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](image.png)

**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. col*. 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.
Temporal and spatial changes in the concentration and isotopic composition of nutrients in the upper Silver Bow Creek drainage, Montana: Year 2

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Publication

Temporal and spatial changes in the concentration and isotopic composition of nutrients in the upper Silver Bow Creek drainage, Montana: Year 2

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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
December 17, 2007

Chris Gammons
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Dept. of Geological Engineering
cgammons@mtech.edu
406 496-4763
**Brief summary:**

In the Fall of 2007 we decided to discontinue the monthly synoptic nutrient samplings of Silver Bow Creek and tributaries. This was mainly because the project is running out of money, but also because M.S. student Bev Plumb has plenty of data in hand, and needs to begin work compiling and interpreting these data, and writing her thesis. We anticipate an April 2008 date for her thesis defense. The thesis will constitute the final deliverable to the Water Center for this project.

Other project news:

- We received data from the stable isotope lab in Waterloo, Ontario on the N and O isotopic composition of nitrate from a second set of water samples collected in July, 2007. These data compliment the data collected in 2006, and will be discussed in the thesis of Bev Plumb. Unfortunately, due to an oversight from the Waterloo lab, the samples that were to be preserved for ammonia analysis degraded, and so no new results were obtained on the isotopic composition of ammonia in the creek.

- With funding from the Montana DEQ, we conducted a very detailed diel sampling of Silver Bow Creek in July of 2007, which included monitoring for nutrients. This work was assisted by Bev Plumb and several undergraduate students. The results are very interesting, but are somewhat out of the scope of the current project, as they include new information on diel cycling of heavy metals and stable isotopes of dissolved oxygen. A new MS student – John Babcock – has taken over this project and will be summarizing the data – along with some new field work planned for the summer of 2008 – in his MS thesis, most likely to be defended in November of 2008.

- Bev Plumb gave a poster at the 2007 AWRA meeting in Lewistown, MT (Plumb and Gammons, 2007).

**Budget status**

There is still a couple thousand dollars left in the budget for this project. I intend to use most of this to help fund some more diel field work for John Babcock’s thesis project, most likely in July or August of 2008. This is more or less a continuation of Bev Plumb’s work, but with more emphasis on diel phenomenon. The funding we received in 2007 for diel sampling from the DEQ was a “once-off” opportunity, and so we currently have no external funds for John, other than what is left in the 104b USGS grant.

**Reference cited:**

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

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<td>Stephen Parker, Douglas Cameron</td>
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Publication
Identifying and characterizing sources of dissolved organic carbon in the Big Hole and Clark Fork Rivers, a continued investigation

A report to the Montana Water Center summarizing a project supported by USGS 104(b) Water Resources Research Program (grant #06HQGR0096, MSU sub contract #G221-06-W0973).

Prepared by:
Stephen Parker
and
Douglas Cameron
Department of Chemistry and Geochemistry
Montana Tech of the University of Montana
Butte, MT.

Student researchers involved in this project:
Garrett Smith, undergraduate, Montana Tech
Ken Bates, undergraduate, Montana Tech
Charmaine Weyer, undergraduate, Montana Tech

Note: A preliminary summary of these results were presented by students Ken Bates and Garrett Smith at the Montana Section of AWRA in Polson, MT in October of 2006. A more complete summary of this investigation will be presented at the 2008 meeting.

May 21, 2008
Abstract:

This report summarizes investigations into the diel (24-h) processes that control the concentration of dissolved inorganic (DIC) and dissolved organic carbon (DOC) in two rivers in southwest Montana, USA. The objectives were: 1) to measure $^{13}$C stable isotope composition of DIC and DOC over a 24-h period, 2) to investigate the connection between the diel cycles of DIC and DOC in these streams, and 3) to investigate the connection between concentrations of DOC and diel concentration cycles of dissolved metals.

The study sites were on the Clark Fork (CFR) and Big Hole (BHR) Rivers of southwestern Montana, USA. The headwaters of these streams are in the same geographic area but they flow to opposite sides of the continental divide and they have very different geochemical characteristics. The two rivers showed substantial differences in the temporal concentration patterns of both DIC and DOC suggesting that different mechanisms are having a major influence on the carbon cycles.

In the CFR the concentration of DIC decreased during the daytime as a result of photosynthetic removal of CO$_2$ and increased over night when respiration (in the absence of photosynthesis) returns CO$_2$ to the water column. DOC showed the inverse relationship; increasing in the daytime and decreasing overnight. The $^{13}$C stable isotope composition of DIC ($\delta^{13}$C-DIC) became isotopically enriched during the day and lighter over night. Results similar to these for $\delta^{13}$C-DIC have been reported previously and have been shown to be primarily controlled by photosynthesis and respiration. The $\delta^{13}$C-DOC displayed the inverse temporal pattern to that of DIC and was attributed to mechanisms controlled by photosynthesis and respiration.

In the BHR the DIC displayed little diel concentration change which is unusual for highly productive aquatic systems that have large diel changes in pH. However, the $\delta^{13}$C-DIC did show a more typical diel pattern characteristic of the influences of photosynthesis and respiration. This is interesting since it indicates that the composition of DIC can change while the concentration stays relatively constant. DOC during the 2006 BHR sampling increased dramatically during the night which is opposite to the result observed in the CFR. This result suggests that different processes are controlling the organic carbon in the two rivers.
1 - Introduction:

Diel 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 reproducible processes that play an integral role in the health and water quality of river systems. The diel variations are driven by the normal photoperiod, which influence: photosynthesis and respiration of aquatic organisms; daily instream temperature cycles; daily changes in dissolved gas gradients between air and water; and affect 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. 2005a; Parker et al. 2007). Healthy river systems can exhibit large diel temperature, pH, O\textsubscript{2} and CO\textsubscript{2} cycles that are largely driven by aquatic plants and microbes that alternately consume or produce CO\textsubscript{2} depending on whether photosynthesis or respiration is the dominant process (Odum 1956; Pogue and Anderson 1994; Nagorski et al. 2003, Parker et al. 2005).

Many studies have detailed diel concentration fluctuations of metals and arsenic in streams receiving mining related contamination (McKnight and Bencala 1988; McKnight et al. 1988; Fuller and Davis 1989; Bourg and Bertin 1996; Brick and Moore 1996; Sullivan et al. 1998; Nimick et al. 1998, 2003, 2005; Scott et al. 2002; Jones et al. 2004; Gammons et al. 2005a, 2005b; Parker et al. 2007a, b). These and subsequent studies have contributed to the knowledge and understanding of diel processes, however there is much that is not well understood. Rivers operate within a complex interface between chemistry, biology and geology and there is a critical need to better understand this interrelationship. Transport within rivers of metals and metalloids
and the impacts from development and climate change can have a significant effect on aquatic and human health. Gaining insight into the underlying mechanisms controlling concentration changes of important chemical species will help us achieve a better fundamental understanding of how streams function.

The timing, during the day, of sample collection can have an impact on the reported concentrations of analytes of concern (Nimick et al., 2003). For example, dissolved copper in a near neutral reach of Fisher Creek, MT, a heavily mine impacted stream, has been shown to undergo as much as 140% concentration changes during a diel cycle (Gammons et al., 2005; Parker, 2005). Dissolved zinc concentrations in Prickly Pear Creek, MT underwent a 500% change in one twenty-four hour sampling period (Nimick et al., 2005). A number of physical, biological and chemical factors influence the major and trace element concentrations on a diel basis. Temperature and pH dependent sorption to substrate and suspended inorganic and organic surfaces are thought to play an integral role in influencing the cation and anion concentration cycles (Fuller and Davis, 1989; Machesky, 1990; Rhodda et al., 1996; Nimick et al., 1998; Trivedi and Axe, 2000; Jones et al., 2004). Additionally, there may be an increase in groundwater flow and exchange with hyporheic water at night due to a decrease in evapo-transpiration. Bed sediments and hyporheic water in the upper reaches of the Clark Fork River drainage have been shown to contain significant concentrations of As, Cu, Fe, Cd, Mn and Zn (Benner et al. 1995; Nagorski and Moore 1999) and these contaminants in the sediment region may move into the water column.

Previous work has demonstrated that there is a significant and reproducible diel cycle in $\delta^{13}$C –DIC in both CFR and BHR in Montana as well as a substantial cycle in the $^{18}$O composition of dissolved oxygen (DO, $\delta^{18}$O-DO) in the BHR and Mill-Willow Bypass (Parker et
al., 2005, 2007; unpublished data). These daily changes in the isotope composition of the DIC
and DO are caused by the combined effects of photosynthesis and respiration of aquatic plants
and microbes as well as contributions from gas-exchange and groundwater (GW).

Dissolved organic carbon (DOC) represents the single largest pool of reduced carbon in
most aquatic systems that is available to heterotrophic microorganisms as an energy source
(Volk et al., 1997). The types and concentration of the DOC can have a significant influence on
the chemical composition of surface waters (McKnight et al., 1997). There is little literature that
reports investigations of temporal changes in DOC and $\delta^{13}$C -DOC on a diel scale. Since
community respiration is using the DOC as a carbon source and other aquatic species are
producing organic molecules as a by-product of their daily productivity it is reasonable to expect
changes in the isotopic composition of the DOC as it is influenced by the daily and seasonal
changes in the sources of the organic carbon (Barth and Veizer, 1999; Ziegler and Fogel, 2003;
Zeigler and Brisco, 2004).

DIC is defined as the total of inorganic carbon and can accumulate in surface waters
from air-water gas exchange, community respiration, weathering of minerals or from GW
contributions. DOC can include water soluble forms of amino acids, carbohydrates, organic
acids, alcohols as well as fulvic and humic acids. Sources of DOC can include decomposition of
organic matter and production by aquatic plants and microbes. Microbes using DOC as a carbon
source will produce CO$_2$ from respiration with a carbon isotope signature characteristic of the
organic carbon substrate (Clark and Fritz, 1997). In temperate regions, plant organic matter, that
serves as the carbon source for microbial respiration has a $\delta^{13}$C of ~ -28‰ (Clark and Fritz,
1997). At the same time, atmospheric carbon is about -7 to -8‰ (NOAA, 2006) and
consequently DIC produced by gas exchange will be considerably heavier than that produced by respiration.

A significant portion of DOC in natural waters falls in the category of naturally occurring matter (NOM), and most of the NOM fits into the operationally defined subcategories of fulvic acids (FA) and humic acids (HA) (Macalady, 1998). The fulvic and humic acids as well as other organic acids are well known for their ability to complex heavy metal ions in solution (Saar et al., 1982; Clapp et al., 1998). It is known that the fulvic and humic acids can contribute to daily variations in surface water iron concentrations by affecting the iron redox cycling by affecting the photoreactivity of the aqueous system (Voelker, et al. 1997; Hrncir and McKnight, 1998). Changes in the concentration of dissolved metals in relation to the concentration of DOC and DIC should yield valuable insight into daily mobilization and transport of metals from the contaminated bed sediments.

1.1 – CFR site:

The field site on the Clark Fork River (46° 23’ 51” N; 112° 44’ 33” W) was within the city limits of Deer Lodge, MT and directly across the river from the USGS gaging station (Fig. 1, 1375 m elevation).
The Clark Fork here is a second order stream with a discharge of roughly 0.6 to 60 m$^3$ s$^{-1}$ depending on the time of year. The mining and smelting centers of Butte and Anaconda are situated at the headwaters of the upper Clark Fork River along Silver Bow and Warm Springs Creeks, respectively (Fig. 1). The floodplain and streambed of Silver Bow Creek and the upper Clark Fork River contain highly elevated quantities of metals and metalloids (arsenic, iron, copper, zinc, lead, cadmium) deposited by the mining, milling, and smelting activities (Moore and Luoma, 1990). Currently, most of the heavy metal load in Silver Bow Creek is removed by
a lime treatment facility at Warm Springs (Fig. 1). However, concentrations of dissolved arsenic in water exiting the treatment ponds are elevated (> 20 µg L\(^{-1}\)) during summer base-flow periods (Duff, 2001; Gammons et al. 2007). Diel changes in the concentration of metals and arsenic have been previously characterized (Brick and Moore, 1996; Parker, 2005; Gammons et al. 2007).

1.2 – BHR site:

The field site on the Big Hole River (45° 48’ 28” N, 113° 18’ 51” W) was about 50 m upstream from the USGS gaging station (Mudd Creek, Fig. 2) at about 1795 m elevation. The BHR is a headwater tributary to the Missouri River. It is undammed and drains a sparsely-populated, high elevation basin (~1900 m above sea level) of approximately 7200 km\(^2\) in extent. This river is relatively pristine and there is little historical impact from mining or industrial sources. The principal activities in the basin are farming, ranching and recreation.

Previous work (Gammons et al., 2001; Ridenour, 2002; Wenz, 2003) has summarized the general geochemical characteristics of the Big Hole River. Overall, the Big Hole River at Mudd

Figure 2: Site map showing the Mudd Creek Bridge sampling area of the Big Hole River located in southwestern Montana.
Creek Bridge can be classified as a Na-Ca-bicarbonate water, with alkaline pH and low to moderate alkalinity.

2 – Methods
2.1- Field methods
2.1.1 – Clark Fork River

Diel sample collection at CFR began on 27-July, 2006 at 1115-h and continued until 1315-h on 28-July. All times are reported as local time (MDT, GMT -0600).

Streamflow measurements were taken from the USGS gaging station at Deer Lodge which was within 30 m of the sampling site. In situ temperature, pH, specific conductivity (SC), DO concentration and percent O$_2$ saturation were measured at each sampling time with a datasonde and a hand-held meter. The instruments were calibrated using water-saturated air for the DO probe and standard buffers (pH 7 & 10) for the pH probe. Post deployment calibration checks showed that the DO probes were within 2% of pre-deployment calibration. A post-deployment calibration check of the pH probe indicated that the reproducibility was within ±0.1 pH units. Two samples were collected for laboratory analysis of DO using the Winkler method (Wetzel and Likens 1991). Unfiltered water was collected in 500 mL glass bottles with no head space and stored on ice until analysis in the laboratory within 48 hours. These analyses were compared to the DO measurements using hand and in situ instruments (discussed in results).

Water was sampled from the main stem of the river in a well mixed, rapidly flowing reach approximately 3 m from shore and at a depth approximately half way between surface and bottom. Raw and filtered water samples were collected for metals analysis in HDPE bottles that were rinsed with 5% HNO$_3$ and triple rinsed with deionized water prior to sampling. Filtration was done using a peristaltic pump and disposable 142 mm diameter 0.1-μm cellulose-ester filter membranes (for further details see Gammons et al. 2005a). Raw and filtered samples were
acidified onsite to 1% v/v using concentrated trace metal grade HNO₃. Filtered unacidified samples were collected for anion analysis by IC. Samples for δ¹³C isotope analysis of dissolved inorganic carbon (δ¹³C-DIC) were collected in 125 or 250 mL acid-washed, oven dried glass bottles with no head-space and the DIC was precipitated in the laboratory as SrCO₃ after Usdowski et al. (1979).

A detector was used to measure photosynthetically active radiation (PAR) flux values (400-700 nm, μE m⁻² s⁻¹) which were based on the manufacturer’s calibration of the sensor. The PAR went to zero at 2100 hours in the evening and rose above zero at 0630 in the morning. This dark period is represented by the shaded region on all diel graphs.

All samples collected in the field were stored on ice, in sealed plastic bags and returned to the laboratory immediately following the field work.

2.1.2 – Big Hole River:
Two separate diel samplings occurred on the BHR approximately one year apart: 7 to 8-August, 2006 and 31-July to 1-Aug, 2007. Sampling was performed similar to that described on the previous section on the Clark Fork site. Alkalinity was measured in the field using a Hach titrator and standardized H₂SO₄.

2.2 – Analytical methods
Samples (SrCO₃) for δ¹³C-DIC and δ¹³C-DOC were analyzed using a Eurovector elemental analyzer interfaced to a Micromass Isoprime stable isotope ratio mass spectrometer after Harris et al. (1997) and Gandhi et al. (2004), respectively. Replicate analyses indicated an RSD of 0.95% for δ¹³C-DOC and 0.55% for δ¹³C-DIC.

Major and trace element concentrations of acid-preserved filtered samples were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using EPA method 200.15 and a Thermo Jarrell Ash model IRIS ICP in the Environmental Biogeochemistry
Laboratory at the University of Montana. Field and laboratory duplicate samples for the elements reported in this study agreed within 10%. Spike recovery for all elements was within 85 to 115%. Values obtained by ICP analysis of the field blanks were all below the practical quantifiable limits (PQL) for Al, As, Cu, Fe, S and Zn. The Ca and Mn concentrations of field blanks were above the PQL, but were 0.3% and 2.7%, respectively, of the average measured values of all samples.

All analysis for DIC and DIC was performed at Montana Tech using an Ionics (Model 1505) Total Carbon Analyzer. All samples for DIC analysis used filtered, unacidified water. Samples for DOC analysis used filtered water that was acidified to 1% (v/v) with concentrated H₃PO₄ and sparged for 5 minutes with N₂. These samples were then sparged for an additional three minutes with CO₂-free air and analyzed. Standards were prepared from a stock solution of 1000 mg L⁻¹ potassium hydrogen phthalate (KHP) prior to each analysis. All glassware used for carbon analyses were acid-washed and oven dried prior to use. Replicate analyses indicated an RSD of 5% for DOC and DIC.

3 – Results and discussion:

3.1 - Clark Fork River

3.1.1 – River parameters

Temperature, flow, pH and specific conductivity from the diel sampling on the CFR in 2006 are show in Figure 3. A diel pH change of approximately 0.6 units was observed which is attributed to daytime net consumption of CO₂ by photosynthesis (increase in pH) and night time production of CO₂ by community respiration. The temperature reached a daytime maximum of 24.6 °C and night time low of 16.5 °C. A small diel change in flow of approximately 13% was observed most likely due to evapo-transpiration in the streamside riparian zones. The dissolved oxygen reached a daytime high of 158% of saturation and a night time low of 65%.
Figure 3: Temperature and pH (a), specific conductivity (SC) and flow (b) and dissolved oxygen (c) at the Clark Fork River sampling site in Deer Lodge. Shaded bars in all diel graphs represent nighttime as determined by PAR signal of zero.

3.1.2 – DOC and DIC

The dissolved organic carbon (DOC) showed an 86% diel change in concentration from a minimum of 124 to a maximum of 231 μmol C L⁻¹ (Fig. 4). At the same time the average concentration of dissolved inorganic carbon (DIC) was ~52-times higher than that of the DOC.
The DIC showed a 5-fold diel change in concentration from a minimum of 3.4 to a maximum of 17.0 mmol C L$^{-1}$ (Fig. 4).

The diel change in DOC is most likely produced by “leakage” of soluble organic carbon compounds during photosynthesis (i.e., amino acids, sugars, organic acids) followed by consumption of those soluble organics by heterotrophic microbes during the night (Refs). The increase in DOC early in the morning (~01:00 to 06:30) may be due to the small increase in flow observed during the same time which may have contributed organic carbon from streamside and benthic sediment sources. However, benthic and streamside pore waters were not sampled for DOC in this study. The DIC concentration decreased during the daytime due to removal of CO$_2$ by photosynthesis and increased at night due to respiration. The decrease in DIC in the early morning before daylight is most likely due to degassing of CO$_2$ since the partial pressure will be high and the pH is at its lowest point.

The molar ratio of DIC/DOC reaches a minimum of 16 at ~17:00 and a maximum of 104 at ~06:00 (Fig. 4). This large increase in DIC relative to DOC at night suggests that oxidation of
DOC contributed only a small part of the increase in DIC. Further analysis is in progress to characterize the dissolved organics which will give more insight into the composition of these compounds and the potential amount of CO$_2$ that could be produced by oxidation.

![Graph: Molar ratio of DIC to DOC at CFRG in 2006.](image)

**Figure 4:** Molar ratio of DIC to DOC at CFRG in 2006.

**3.1.3 – Isotope composition of DOC and DIC**

![Graph: δ$^{13}$C of DIC and DOC during the CFR diel sampling.](image)

**Figure 5:** δ$^{13}$C of DIC and DOC during the CFR diel sampling. Error bars represent 5% RSD.

The $^{13}$C composition of DOC and DIC also showed diel changes (δ$^{13}$C-DIC, δ$^{13}$C-DOC; Fig. 5). Diel changes in δ$^{13}$C-DIC have been observed previously in the CFR and BHR (Parker et al., 2005, 2007). Photosynthesis removes CO$_2$ during the day with a reported enrichment of
about -29‰ (Falkowski and Raven, 1997); such that the remaining DIC becomes isotopically enriched. During the night community respiration returns isotopically light biogenic CO$_2$ to the water such that the DIC becomes isotopically depleted. The $\delta^{13}$C-DOC showed the inverse diel trend to that of the DIC. The concentration of DOC increased during the day and was interpreted as soluble organics that were “leaking” from photosynthetic organisms (Fig. 4) and since photosynthesis discriminates against the heavier carbon isotope it follows that the DOC produced during this time will be isotopically lighter. As photosynthesis decreases in the late afternoon (~17:00) community respiration depletes this pool of soluble organics, discriminating against the heavier isotope (kinetically) such that the remaining DOC pool becomes isotopically enriched. After ~23:00 the isotopic composition of the pool stabilizes at approximately -26.9‰. The $\delta^{13}$C of the local aquatic and streamside vegetation was not measured, but temperate region C$_3$ plants should have an isotope composition in the range of -27 to -28‰ (Clark and Fritz, 1997). Consequently, this $\delta^{13}$C-DOC plateau from 01:00 to 09:00 may reflect the DOC being produced by microbial degradation of detritus with an isotope signature typical of temperate region vegetation. As community respiration consumes this isotopically light DOC it produces light CO$_2$ which causes the $\delta^{13}$C-DIC to continue dropping until photosynthesis reverses the trend.

### 3.1.4 – Dissolved metals in the CFR

The concentration of dissolved Mn and Zn were examined over the 24-h period to determine if a correlation with the observed diel cycle in DOC was evident. Both Mn and Zn concentrations decreased during the daytime and increased at night (Fig. 6) which is consistent with results previously reported in this reach of the Clark Fork River (Brick and Moore, 1996; Parker et al., 2007). Since the concentration of DOC increased during the daytime while both Mn and Zn decreased (reverse at night) it is unlikely that complexation of these metals by organic carbon had a significant influence as a control on the dissolved concentrations of Mn and
Zn. The dissolved Cu concentration during this sampling was near the instrument detection limit (0.05 μmol L\(^{-1}\)) and did not display a diel pattern.

![Graph showing Mn and Zn concentrations](image)

Figure 6: Dissolved (0.1 μm filtered) Mn and Zn concentrations in the CFR during the 2006 sampling. Error bars represent 5% RPD based on analysis of duplicate field samples.

### 3.2 – Big Hole River

#### 3.2.1 – River parameters

Two separate diel samplings within eight days of the same time of year were conducted on the BHR in 2006 and 2007. The average flow in 2007 was about 1.7-times higher than in 2006 (Fig. 7). The flow during the diel sampling period in 2006 showed an unusual pattern with a sharp drop during the late afternoon (~17:00, Fig. 7b) and then a gradual increase with a maximum at about 01:00. The flow in 2007 showed a gradual decrease throughout the afternoon with a minimum about 21:00 through 02:00 followed by a gradual increase through the morning. The 2007 pattern is more typical of one expected to be produced by evapo-transpiration from lush riparian zones while the pattern observed during the lower flow period in 2006 is not well understood. The pattern observed in the pH in 2006 is very unusual with a sharp pH drop in late afternoon (~17:00) at approximately the same time that the flow dropped (Fig. 7a). This was
followed by a gradual increase in pH with a maximum at approximately 03:00; the same time that the flow peaked in 2006.

The late afternoon decrease in flow in 2006 described above was also accompanied by a sharp increase in SC at the same time (~18:00, Fig. 7b). The SC dropped after this time with a minimum at ~03:00, the same time that the flow reached a maximum. This behavior in flow is unusual and will be discussed further in conjunction with the DOC results. The behavior of DO
in 2006 was normal with the exception of a small shoulder at approximately 19:00 which corresponds to the sharp flow decrease in the late afternoon.

In 2007 the changes in flow, SC and pH were more typical of “normal” stream behavior. The diel change in DO was not as large in 2007 as in 2006 and is possibly related to the larger flow (greater volume of water). Maximum stream temperature was higher in 2007 than 2006 with a larger diel change than in 2006.

3.2.2 – DOC and DIC

The behavior of DOC during the 2006 and 2007 diel samplings was dramatically different (Fig. 8).

![Figure 8: Concentrations of dissolved inorganic (DIC) and dissolved organic (DOC) carbon in the Big Hole River near Mudd Creek Bridge in 2006 (a) and 2007 (b). Error bars represent 3% RSD.](image)

A 4.6-fold minimum to maximum change in DOC in the BHR was observed in 2006 while no diel change in DOC concentration was observed in 2007. The night time peak in DOC occurred at ~01:00 which coincides with the timing of the maximum values reached by both flow and pH (Fig. 7a & b). One possible explanation for this significant increase in DOC may be linked to the increase in flow. After the late afternoon decrease in flow (~17:00) the following increase may have include a groundwater component from bank-storage and/or benthic sediments. This water
may have carried additional concentrations of DOC from decaying organic matter in the sediments that produced the DOC increase in the river. This increase in flow would have been higher in pH to produce the simultaneous nighttime increase in that parameter (Fig. 7a).

Another theory that has been considered to explain the large diel changes in flow that have been observed periodically during late summer base flow in the BHR has been the presence of large stretches of algae and macrophytes that “dam” the river during the day when photosynthesis is operating and sink towards the bottom at night allowing the trapped water to flow down the river as a wave. This might explain the decrease in flow in the late afternoon during 2006 followed by the upstream water flow increasing as the damming actions ceases. This pulse of water that was retarded by the plants and algae during the day may be higher in pH due to photosynthesis and contain higher amounts of DOC due to leakage of photosynthates as discussed above in section 3.1.2.

These explanations for the abnormal flow, pH and DOC behavior observed in 2006 are currently the focus of a continued investigation to better understand the hydrology and geochemistry of the upper Big Hole.

**3.2.3 - Isotope composition of DIC**

The stable isotopes of DIC were examined from both the 2006 and 2007 samplings (Fig. 9). The pattern is typical of $^{13}$C changes in DIC reported previously (Parker et al., 2005) and similar to those observed in 2006 on the CFR (Fig. 5). There was no discernible diel pattern in DIC concentration in the BHR during both 2006 and 2007. At the same time the isotope composition changed ~3.3 and 2.7‰ in 2006 and 2007, respectively. Additionally, the $\delta^{13}$C-DIC in 2006 did not show any influence of the aberrant pH and flow cycle which appears to be have been associated with the diel change in DOC (Fig. 8). This emphasizes the dynamic nature of the inorganic carbon pool. While the concentration is not changing significantly the isotopic
composition of the pool was constantly changing and this was reflected by the diel pattern of $\delta^{13}$C-DIC.

![Graph showing diel pattern of $\delta^{13}$C-DIC](image)

**Figure 9:** $\delta^{13}$C-DIC at the BHRG-1 site during the 2006 and 2007 diel samplings. Error bars represent 3% RSD based on duplicate determinations

### 4 – Conclusions:

Diel concentration cycles in DOC were observed in both the CFR and BHR in 2006. However, no change in DOC concentration was observed in the BHR in 2007 at approximately the same time of year. The flow in the BHR was ~1.7 times higher in 2007 suggesting the presence of diel changes in DOC may be linked to circumstances present during low, base flow conditions. These dynamics are not well understood and are the subject of further study.

The timing of the DOC cycles in the CFR and BHR in 2006 were different at both sites (Figs. 4 & 8). In the CFR the DOC reached a maximum concentration at approximately 16:00 in the afternoon which is approximately the same time that dissolved Mn and Zn reached minimum concentrations (Fig 6). This suggest that while these metals may be complexed by DOC in solution it doesn’t appear to be a significant contributor to the diel metals concentration changes since they display the inverse temporal pattern. A model recently proposed by Parker et al.
2007) suggests that diel metals concentration changes in the CFR are controlled by oxidation-reduction conditions at the biofilms surfaces. During the daytime the biofilms becomes highly oxidizing while photosynthesis is operating (high pH, high O$_2$) and Mn(IV)- and Fe(III)-oxides are formed which act as strong sorbents for other metals such as Zn and Cu. At night the redox conditions at the biofilms surfaces reverse and some of the oxidized Mn and Fe may be reduced which allows the dissolved concentrations of Mn and Zn to increase.

The DOC cycle in the BHR in 2006 reached a maximum concentration at ~01:00 which is approximately the time that the flow reached its maximum (Figs. 7b & 8). The pH during the 2006 sampling showed an unusual bi-phasic pattern with a maximum around 14:00 and then again at about 02:00. Between these two maximums there was a steep drop in pH with a minimum at ~17:00. Normally pH will reach a minimum in the early morning (~07:00 in 2007, Fig. 7d). This pH increase during the night could not have been caused directly by photosynthesis removing CO$_2$ from the water. However, since the flow increased at the same time this pattern is consistent with a mass of water moving into this reach that was chemically modified (higher pH, lower conductivity). Two theories suggested to explain these data are: 1) increased flow from streamside regions and/or benthic sediments at night as evapo-transpiration decreases, and 2) the possibility that algae and macrophytes in upstream reaches float up and partially dam the river during the day due to photosynthesis and then sink at night, releasing the water.

The first explanation is supported by preliminary results of samples collected from a number of seeps along the BHR above the sampling site in 2007. The water from these seeps had DOC concentrations 2-3 times higher than the river (Parker, unpublished results). At the same time water extracted from shallow benthic sediments had DOC concentrations similar to
that of the river. The pH of the water from the seeps was in general lower than that of the river. This makes it seem unlikely that the increase in pH in conjunction with the increase in DOC was due to higher infiltration of water from streamside areas.

This latter explanation predicts a “slug” of water moving downstream that would be of higher pH due to photosynthetic removal of CO$_2$ and have a higher concentration of DOC due to leakage of soluble organic photosynthates during the day. It is interesting to note that the $\delta^{13}$C-DIC during both 2006 and 2007 had the same pattern and in 2006 appeared to show no influence from the observed flow and pH increase during the night. This suggests that the biological processing of inorganic carbon is acting at a time scale resulting in a “normal” $\delta^{13}$C-DIC pattern. The night time decrease in $\delta^{13}$C-DIC is explained by production of biogenically light CO$_2$ by community respiration, but this is inconsistent with the increase in pH that was observed at night in 2006. An alternate explanation is that the night time increase in pH is not connected to decreasing CO$_2$ but is being buffered by the increased amounts of DOC. If the observed increase in DOC and pH was largely made up of weak organic acids that are largely deprotonated at high pH then it might be possible that the pH could remain elevated while the CO$_2$ concentration increased resulting in the normal $\delta^{13}$C-DIC profile observed. Additionally, protons produced by the increasing CO$_2$ concentration would be titrated by the conjugate base form of organic acids present in this high pH wave. This explanation is speculative at this point but investigations are in progress to attempt to characterize the DOC produced in the BHR and CFR during 2006. A more thorough understanding of the type and size of the organics is necessary to predict the effect they might have on river chemistry.
Acknowledgements:
We thank Heiko Langer of the Geological Sciences Department of the University of Montana and Simon Poulson of the University of Nevada-Reno for help with analytical work. This work was funded by the Montana Water Center under the U. S. Geological Survey, Water Resources Research Program (104(b)) and the Montana Tech Undergraduate Research Program.

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Historical and Future Streamflow Related to Small Mountain Glaciers in the Glacier Park Region, Montana

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Publication

Interim report: *Historical and Future Streamflow Related to Small Mountain Glaciers in the Glacier Park Region, Montana*

Funded by U.S.G.S 104b program, Montana Water Center

Joel Harper  
Department of Geosciences  
University of Montana

1. RESEARCH ACTIVITIES

Work to date has been split between field data collection and office data reduction and computer modeling. One of two field campaigns has been successfully completed, and considerable progress has been made on reduction of existing data and computer modeling.

1A. Field campaign on Sperry Glacier, Glacier National Park, May-September 2007.

During summer 2007 we installed:

- Two meteorological stations, one on the glacier margin and on a tower drilled into the ice. The ice station includes a sonic ranger for continuous measurement of snow and ice melt.

- Two logging GPS receivers on the ice and one GPS base station on the margin. The survey grade GPS units (Trimble R7) and base station corrections allow for sub millimeter precision measurements of ice motion.

- 12 ablation stakes for ongoing measurements of the summer melt portion of the glacier’s mass balance.

- Two time lapse cameras for photogrammetric measurement of snow melt.

We also made measurements of the glacier’s surface mass balance. Winter accumulation was measured through snow pit measurements of snow density, and numerous snow depth measurements across the glacier. Summer ablation was measured through repeat measurement of an ablation stake network.

Finally, we collected ice penetrating radar measurements of the glacier’s ice thickness.
1B. Laboratory work

**Numerical simulations.**
- We have put a considerable work effort into modifying our cellular automata model which simulates glaciers on a large landscape. We have automated the parameter adjustment processes with feedback from spatially based convergence criteria (i.e., the model continually adjusts model parameters until it properly grows glaciers where map data indicate glaciers exist).

- We have begun the process of simulating present and past ice coverage in the regions of Sperry, Blackfoot and Jackson Glaciers. The modeling work is finding surface mass balance, ice thickness and velocity values which result in known glacier geometries. The surface mass balance yields an estimate of water runoff from the ice covered landscape.

**Data Analysis**
- GPS data have been processed for kinematic corrections to our local base station

- We have completed a DEM of the glacier surface based on data collected in August of 2007.

- We have begun work on historical data analysis of nearby SNOTEL and weather station data for a study of the long term trends in glacier mass balance.

- We have completed photogrammetric analysis of time lapse images to map transient snow line retreat.

2. INITIAL FINDINGS

Currently 37 glaciers exist in Glacier National Park (GNP), Montana, USA. These glaciers are small cirque glaciers with a maximum size of approximately 1 km$^2$. A century ago the glaciers in GNP were larger, up to 8 km$^2$ and more numerous. The runoff from glaciers in this region is an important and sometimes the only source of discharge in the typically dry summer months of July, August, and September. We present a case study of Sperry Glacier in order to investigate the changes in runoff between 1850 and present. Sperry Glacier is located on the west side of the Continental Divide and the headwall of the glacier lies at about 2800 m. The snout has retreated from an elevation of 1900 m in 1850 to about 2300 m in 2005. The glacier area has been reduced over the same time period from 3.76 km$^2$ to only 0.84 km$^2$.

Direct measurements of runoff from Sperry Glacier (or any other GNP glacier) are not available. We employ two methods to compute present day summer ablation from the glacier and make the assumption that all ablation results in runoff. First, we employed a distributed energy balance model, adjusted from Brock and Arnold (2000), using data from an on-site meteorological station. This modeling supports the notion that little ablation results from sublimation or evaporation and that shortwave energy is the dominant source for melting the ice. Second, we made direct measurement of surface melt using an ablation stake network and continuous measurements of melt at a single point with a sonic ranger. Ablation measurements showed daily melt rates averaged 56 to 59 mm w.e. during summer and revealed little to no elevation gradient along the glacier. The distributed energy balance model slightly
underestimated the daily melt, and had a tendency to produce a stronger elevation gradient in the melt rate than indicated by direct observations.

We examined scenarios for runoff from Sperry Glacier in 1850 by employing a numerical reconstruction of the glacier. We used a cellular automata technique which includes rules for surface accumulation and ablation, including snow accumulation from avalanching off the cirque walls, and down-valley mass transfer by glacier motion (Harper and Humphrey, 2003). Here we focus on one end-member scenario that considers no change in the glacier’s accumulation gradient (only increased ablation due to warming) between 1850 and present. This assumption along with the modeled annual mass balance gradient yields a solution for the 1850 summer ablation along the glacier.

Total melt runoff from Sperry Glacier in 1850 is estimated to be \(17 \times 10^6\) m\(^3\). Our methods and assumptions suggest summer runoff from Sperry Glacier has decreased on the order of 75%, from approximately \(17 \times 10^6\) m\(^3\) in 1850 to approximately \(4.3 \times 10^6\) m\(^3\) in 2005. Such a reduction has likely had a significant impact on basin ecological systems and represents an important water resource to local downstream users in this dry summer climate regime.

3. FUTURE WORK OBJECTIVES
We will conduct a second field campaign during May-September 2008. This work will focus on the surface mass balance and surface energy balance of the glacier. We plan to install two meteorological stations on the ice for more detailed energy balance modeling work. Office work will focus on refining our mass balance calculations from field data. Additional work will focus on extending the mass balance record through analysis of historical weather and snow data. Numerical simulations of the 1850 glaciers will continue. Finally, we are digitizing historical topographic maps of Sperry Glacier to use with the 2007 DEM we created in order to computer long term volume changes of the glacier.

4. PUBLICATIONS/PRESENTATIONS
We made a presentation with published abstract at the conference “Glaciers in Watershed and Global Hydrology” August 27-31, 2007, Obergurgl Austria. This is an international meeting sponsored by the International Association for Hydrological Sciences (IAHS) which is focused on glacier runoff and water resources. The meeting brought together glaciologists and water managers working on this topic.


Results were highlighted in a keynote presentation by PI Harper at the NOAA Great Divide Weather Workshop, October 3, 2007, Great Falls, Montana.
Predictive Modeling of Snowmelt Dynamics: Thresholds and the Hydrologic Regime of the Tenderfoot Creek Experimental Forest, Montana

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Publication
Predictive Modeling of Snowmelt Dynamics, Thresholds and the Hydrologic Regime of the Tenderfoot Creek Experimental Forest, Montana

PI Lucy Marshall, Assistant Professor of Watershed Analysis, Montana State University, 406-994-4796, lmarshall@montana.edu. PI Brian McGlynn, Assistant Professor of Watershed Hydrology, Montana State University, 406-994-7690, bmcglynn@montana.edu.

General progress to date
We began this project in March of this year and focused on:

- Snowmelt and hydrologic model development
- Uncertainty framework implementation and algorithmic comparison
- Snowmelt monitoring and sample collection with an intensive Spring field campaign.

Specific activities and findings are summarized according to the project objectives below.

Objective 1: To synthesize extensive existing field observations at the Tenderfoot Creek Experimental Forest into a conceptual predictive modeling framework evaluating the first order controls on spatio-temporal runoff dynamics, new snowmelt/old groundwater runoff partitioning and the impact of terrain and forest practices.

Model development
We have undertaken initial data collation for implementing model approaches. Data has been provided by Ward McCaughey of the Rocky Mountain Research Station and the US Forest Service, and by co-PI Brian McGlynn. Initial model testing and development has used SNOTEL snow water equivalent and precipitation data, and streamflow data. 4 years of data have been used for model development and calibration, and a 5th year of data is used for model assessment/validation.

Progress has focused on development of initial conceptual snowmelt/hydrologic models. We have coded and tested several parsimonious hydrologic models, including a classic soil moisture accounting “bucket” style hydrologic model, a probability distributed model to characterize spatial storage heterogeneities, a temperature index snowmelt model, and a combined temperature/radiation index snowmelt model.

We have also developed a Matlab based GUI for delivery of these models. The GUI allows the models to be accessed by less experienced users and can be compiled into a Matlab-independent platform to enhance the models’ portability. The GUI will be refined and modified as more hydrologic models are added to our existing suite.

Progress has additionally been made in developing the model uncertainty framework in which the models are calibrated and specified. We have implemented Markov chain Monte Carlo
(MCMC) algorithms for parameter estimation and uncertainty assessment of the initial developed models. Two adaptive algorithms have been compared, the Adaptive Metropolis (AM) algorithm, developed by Haario et al [2001], and the Delayed Rejection Adaptive Metropolis (DRAM) algorithm, developed by Haario et al [2006]. Both algorithms were implemented as the means for performing uncertainty and parameter estimation for a conceptual precipitation-runoff model based on the Probability Distributed Model (PDM), developed by Moore [1985], and including a temperature- and radiation-index snowmelt routine. The two algorithms were compared using 4 years of 12-hourly meteorological and runoff data from the Stringer Creek watershed in the Tenderfoot Creek Experimental Forest. A manuscript describing these algorithms has been submitted to the journal Water Resources Research.

**Objective 2: To initiate watershed tracer experiments quantifying new snowmelt runoff direct contributions to streamflow and resident (old) groundwater contributions to spring runoff across sub-watersheds to explicate the residence time of new water within in each of 7 watersheds and better inform conceptual model underpinnings.**

**Hydrologic data**
Continuing data collection and data gathered since the project inception includes:

**Stream flow**
- Monitored discharge at the outlets of 7 nested watersheds
- Monitored real time specific conductance and temperature at 8 locations

**Wells and piezometers**
- Installed (many existing) and monitored (real-time water level data collection)
  - 100 wells
  - 20 piezometers

**Lysimeters**
- Installed 4 recording snowmelt lysimeters

**Rainfall**
- Built and installed an incremental rainfall sampler
- Monitored 5 existing recording rain gauges

**Soil water**
- Monitored 8 soil suction lysimeters
- Monitored 13 recording soil water content nests

**Water sample collection**
Collected 800 water samples for isotopic, dissolved organic carbon (DOC), and major ion analysis from stream flumes, groundwater wells, piezometers, snow and soil lysimeters, and rainfall sampler. 2-3 day interval for all flumes during snowmelt shifting to weekly to bi-weekly during late summer baseflow. Rainfall and snowmelt lysimeters sampled during events (at least daily). GW wells, piezometers, and soil lysimeters sampled weekly during snowmelt and bi-weekly during summer baseflow.
Sample analysis
   Approximately 300 samples analyzed for DOC and major ions to date. Isotope analyses awaiting further sample collection before lab analysis.

Communication of Results and Future Work Plan
We are on track with our proposed objectives, data collection, and analyses, and have taken into account the reviewer comments in developing our research plan. This work will result in multiple presentations at national meetings (to date posters have been presented at the AWRA Montana Annual meeting, and the American Geophysical Union Fall Meeting), a MS thesis in progress, and a manuscript recently submitted to the journal Water Resources Research. We are planning to expand our initial selection of models to look at models of increasing complexity and spatio-temporal scales.

Plan for the next 12 months
1. Expand conceptual model development to address residence time findings and models of increasing complexity and spatial representation.
2. Expand model assessment to include other watershed data
3. Develop and undertake model validation exercises
4. Continue sample and data collection
5. Analyze remaining samples for ions, DOC, and isotopes
6. Begin residence time modeling
Sediment and Heavy Metals Source Determination and Reduction at a Reclaimed Abandoned Mine Site, Alta Mine, Jefferson County, MT

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Publication
INTRODUCTION

The intentions of this interim report are to update the Montana Water Center and U.S. Geological Survey Water Resources Research program on the progress of the research project: “Sediment and Heavy Metals Source Determination and Reduction at a Reclaimed Abandoned Mine Site, Alta Mine, Jefferson County, MT.” The report includes a brief project background, problem statement, and list of objectives targeted at the problem. The methods, procedures, and facilities used to accomplish each objective are reviewed, with emphasis on methods that have been modified from the original project proposal. Finally, the progress of each objective is outlined and preliminary results are included.

Recall that the Alta Mine is an abandoned lead and silver mine located in the Lake Helena Watershed. Specifically, the Alta tributary converges with Corbin Creek, a small stream near Jefferson City, MT, which has recently been subject to the establishment of Total Maximum Daily Loads (TMDL) for sediment, heavy metals, and arsenic. The Alta tributary is fed by the discharge of acid sulfate waters from a deep mine shaft, (known as the #8 shaft), on site. A reclamation project was completed by the Montana Department of Environmental Quality (MT-DEQ) in 1999 in an attempt to alleviate human health risk posed by waste rock piles laden with heavy metals and arsenic. During reclamation, the Alta Mine was divided into an upper and lower site; however, only the lower Alta was reclaimed. The project included: removal of 154,000 yd$^3$ of waste rock; construction of terraces on steep slopes adjacent to the Alta tributary; an attempt to seal the #8 shaft, and an effort to re-vegetate the reclaimed slopes. At the inception of the current project, both the upper and lower Alta appeared to be inhospitable to vegetation; and both are thought to persist as sources of heavy metals, arsenic, and sediment discharge to the stream.

The problem addressed by the current research is the lack of baseline information necessary to successfully revegetate the Lower Alta or to determine the tributary’s contribution to surface water impairments in Corbin Creek. The objectives of the project include: assessing the quality of soil and growth media on site; determining the state of vegetative cover; testing soil amendments and applicable seed mixes; quantifying the amount of sediment and heavy metal contamination that Alta contributes to Corbin Creek impairments; and comparing contaminant loads in Corbin Creek above and below the Alta tributary to recently established TMDLs. Through our objectives, limitations in mined land rehabilitation will effectively be linked to problems in water quality restoration.
PROGRESSION OF METHODS, PROCEDURES, AND FACILITIES

Soil and Vegetation Baseline
Post reclamation soil and vegetation characteristics were measured in summer of 2006. The characterization included excavating 30 soil pits on an approximate 75 foot grid across the Lower Alta site. Soil pits were sampled at 2 intervals (0 – 6 inches and 24 – 30 inches) for organic matter (OM), nutrients (P,N), pH, EC, water extractable heavy metals, total heavy metals, acid-base potential (ABP), and texture. Concurrent with soil pit excavation, vegetative cover (Daubenmire 1959) was measured across 2 perpendicular 164-foot transects centered at each soil pit. The regression approach to ANOVA was applied to link soil parameters to patterns of sparse vegetative cover. All work planned for establishing the soil and vegetation baseline was completed; and a brief summation of outcome appears in the results section of this report.

Soil Amendment and Revegetation Treatments
Administrative influence and logistical complications required that the work plan to amend and revegetate the south-facing slope of the Lower Alta reclaimed area be modified from the project proposal. The original extent of the revegetation effort included the entire south facing slope of Lower Alta; however, reclamation was reduced to a series of small treatment plots at the request of MT DEQ. With limited space, it became necessary to reduce the number of treatments. The erosion control fabric treatment was eliminated because it presented the highest economic cost. Lime and compost amendments were tested for their efficacy at increasing soil productivity and supporting vegetative cover during the summer of 2007. Vegetation plots, planted in late April and early May 2007, were treated with either lime alone, compost alone, a combination of both, or seeded with no amendment. Each treatment was replicated 4 times on 20 ft by 30 ft plots. Plots were prepared for seeding and amendments were incorporated with a Link-Belt 2700 excavator. A seed mix consisting of both grasses and forbs already present on site and additional species that are adapted to the site for drought, acidity, or metals tolerance was broadcast on the plots.

Silt fences were installed below treatments as a best management practice and to quantify the amount of soil loss from each plot. The collection area behind the silt fence was painted yellow to discern soil loss from fence anchor material. In an attempt to preclude run-on to the treated area, the excavator was also used to dig a trench approximately 70 feet upslope of the plots. The trench did not preclude run on from upslope and the fence was breached by late summer rainfall events. No results will be produced from the upslope sediment catches. All other work, in terms of soil amendment and revegetation, has proceeded as planned. Results are included in the following section.

Water Quality
This section reviews the methods and procedures used to quantify the Alta Mine’s contribution to water quality impairments in Corbin Creek. Extensive sampling for lead (Pb), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), and total suspended solids (TSS) was performed during summer 2007 at 6 locations in the study area (Figure 1).
The 6 sampling locations were: below the Upper Alta waste rock pile; at the #8 shaft; below the Lower Alta reclaimed area; above the confluence of the Alta tributary and Corbin Creek (in the tributary); above the confluence of the Alta tributary and Corbin Creek (in the creek); and below the confluence of the 2 streams.

The original project proposal outlined our intent to sample suspended sediment with USGS standard sampling equipment, namely US DH-48 and US U-59 samplers. However, the height of the water column in both the Alta tributary and Corbin Creek was insufficiently high to accommodate these pieces of equipment. Instead, suspended sediment samples were collect frequently in an open bottle (US DH-48 variety), as suggested by Edwards and Glysson (1970). Samples were analyzed according to standard methods (American Public Health Association 1989) for TSS.

Heavy metals and As samples were collected as planned at each of the 6 sampling locations. Measures of total recoverable (TR) metals and total soluble (TS) metals were made. TR and TS samples were prepared by acidifying to pH<2 or by filtering (0.2 um) and acidifying to pH<2, respectively. Samples were analyzed by Energy Laboratories, Inc. of Billings, MT with inductively coupled plasma mass spectroscopy (ICPMS).

Four (4) of the 6 sample locations, excluding the #8 shaft and Corbin Creek above the Alta tributary, were gauged with Ecotone WM water level monitors. The water level monitors recorded gauge height on a 15-minute interval. Gauge heights were then related to stream flow by power function rating curves. Stream flow was measured with a Swoffer 3000 velocity meter (velocity x area method) or with a calibrated bucket and stopwatch. The reconstructed channel of the Lower Alta reclaimed area was sufficiently confined and contained engineered drop structures that allowed the most accurate estimates of flow by calibrated bucket and stop watch.
PRELIMINARY RESULTS

Soil and Vegetation Baseline
The survey of site conditions conducted in summer 2006 revealed that the Lower Alta reclaimed site only averaged 11.7% vegetative cover. Cover was slightly better on slopes of north aspect (17.9% cover) than on south-facing slopes (6.1% cover). Soil quality was highly variable and constituents were heterogeneously distributed around the site (Table I).

Table I. Selected Soil Parameters on Lower Alta Reclaimed Site, Summer 2006.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Range</th>
<th>Analyte</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic (As)</td>
<td>24 – 2,100 mg/kg</td>
<td>OM</td>
<td>0.64 - 4.21 %</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>51 - 1,800 mg/kg</td>
<td>Nitrate (N)</td>
<td>1 - 5 mg/kg</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>198 - 718 mg/kg</td>
<td>K-extractable</td>
<td>40 - 160 mg/kg</td>
</tr>
<tr>
<td>paste pH</td>
<td>2.68 - 7.23</td>
<td>ABP</td>
<td>-45 - 12 t-lime/Kt</td>
</tr>
</tbody>
</table>

Lead (Pb) and Arsenic (As) concentrations as high as 1,800 mg/kg and 2,100 mg/kg, respectively, were found on site. Pb and As concentrations used as soil screening levels during the 1999 reclamation project were 235 mg/kg and 368 mg/kg, respectively.

It was thought that low vegetative success would correspond with poor soil quality. Vegetative cover was regressed against all of the sampled soil parameters and slope aspect to determine which soil characteristics, or combination of characteristics, were significant inhibitors of vegetative succession. Those models that were significant (p<0.1) appear in Table II.

Table II. Models of Vegetative Cover Based on Soil Parameters.

<table>
<thead>
<tr>
<th>Model</th>
<th>R^2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.23</td>
<td>0.059</td>
</tr>
<tr>
<td>Aspect</td>
<td>0.24</td>
<td>0.054</td>
</tr>
<tr>
<td>pH + Aspect</td>
<td>0.39</td>
<td>0.041</td>
</tr>
<tr>
<td>pH + ABP</td>
<td>0.49</td>
<td>0.013</td>
</tr>
<tr>
<td>pH + ABP + Aspect</td>
<td>0.57</td>
<td>0.014</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>Model</th>
<th>F*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH, (pH + ABP)</td>
<td>6.54</td>
<td>0.024</td>
</tr>
<tr>
<td>pH, (pH + Aspect)</td>
<td>3.36</td>
<td>0.090</td>
</tr>
<tr>
<td>pH, (pH + ABP + Aspect)</td>
<td>4.78</td>
<td>0.030</td>
</tr>
<tr>
<td>(pH + ABP), (pH + ABP + Aspect)</td>
<td>2.35</td>
<td>0.150</td>
</tr>
</tbody>
</table>

Significance level is p< 0.1

Aspect, ABP, and pH were the only 3 factors found to be significant at limiting vegetation. Sparse vegetation appears to be largely a function of pH and ABP combined. Heavy metals, both total and total water extractable, were surprisingly not found to limit vegetative cover.

Soil Amendment and Revegetation Treatments
Soil and vegetation in the treated plots were examined at the end of the growing season to determine if any significant changes had been made in the quality of growth media on site (Table II).
Table II. Results of Wilcoxon Rank-Sum Test for Vegetation Treatments.

<table>
<thead>
<tr>
<th>Plot</th>
<th>No. of treatments</th>
<th>Mean pH</th>
<th>Mean Organic Matter (%)</th>
<th>Mean Canopy Cover (%)</th>
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<tr>
<td>Control</td>
<td>4</td>
<td>3.68b</td>
<td>2.15b</td>
<td>1.48b</td>
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<tr>
<td>Lime</td>
<td>4</td>
<td>5.73b</td>
<td>2.38b</td>
<td>3.38a,b</td>
</tr>
<tr>
<td>Compost</td>
<td>4</td>
<td>7.08a</td>
<td>4.28a,b</td>
<td>5.53a</td>
</tr>
<tr>
<td>Compost &amp; Lime</td>
<td>4</td>
<td>7.27a</td>
<td>5.1a</td>
<td>8.65a</td>
</tr>
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</table>

Values followed by same letter within columns are not significantly different. (p<0.1)

A Wilcoxon Rank-Sum test was applied to compare pH, OM, and canopy cover between treatments. Even the most successful treatments were disappointing in terms of renewed vegetative succession; however, compost and compost and lime treatments were found to offer significantly better growth media than control plots.

**Water Quality**

Much of the work yet to be done for the duration of this project involves further analyzing preserved samples for heavy metals and arsenic and shrinking the wealth of data collected in summer of 2007. However, we are confident that acid sulfate waters discharged by the Alta mine are the substantial contributor of heavy metals and As to Corbin Creek. Zn loads in the Alta tributary immediately above Corbin Creek were as high as 93 lb/day; while, Zn loads in Corbin Creek above Alta peaked at 3 lb/day (Figure 2).

![Zinc Load in Alta Tributary and Corbin Creek](image)

Figure 2. Zinc load in Alta tributary and Corbin Creek, Summer 2007.

TMDL allocations for Corbin Creek were set at 2.35 lb/day. Cu, As, Cd, and Pb exhibit similar patterns in their distribution between Alta and Corbin Creek. A clear pattern in suspended sediment was harder to establish. At present, it appears that the Alta tributary and other sources in the upper Corbin Creek watershed may contribute equally to sediment impairments. For the sake of brevity, those results will only be included in the final report.
DISCUSSION

Research at the Alta Mine to date has revealed that land reclamation practices, such as those applied in 1999 and in summer 2007, were limited by a harsh soil environment. Further, land reclamation and re-vegetation attempts have done little to improve water quality that is impaired by acid generation in deep underground mine workings. The Alta tributary carried metal loads up to 30 times Corbin Creek’s background level; and surface flows in Corbin Creek above the Alta tributary ceased completely in June 2007 for the duration of the study period.

REFERENCE


Student Fellowship: The influence of beaver on brook trout invasion and subsequent native westslope cutthroat trout displacement in southwestern Montana

Basic Information

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<tr>
<td>Principal Investigators:</td>
<td>Magnus McCaffery, Lisa Eby</td>
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Publication

Student Fellowship: The influence of beaver on brook trout invasion and subsequent native westslope cutthroat trout displacement in southwestern Montana
Title: Impacts of beaver activity on invasive brook trout and native westslope cutthroat trout in southwestern Montana

Abstract

Invasion of ecosystems by nonnative species is often responsible for reshaping natural biological communities. In the Rocky Mountains, brook trout (Salvelinus fontinalis) invasion has been implicated in the decline of westslope cutthroat trout (Oncorhynchus clarkii lewisi), a native species of special concern in Montana. Although research has established that negative interactions between these species likely occur at the juvenile stage, there remain gaps in our understanding of the landscape factors that influence the extent of invasion, and resulting cutthroat declines. For example, beaver (Castor canadensis) are capable of altering stream habitat characteristics considerably, but it is not known how beaver disturbance influences brook trout invasion success, and the consequences for native cutthroat trout populations. To address this, I used temperature loggers, mark-recapture, and habitat surveys to establish how beaver affect (i) stream temperatures, (ii) brook and cutthroat trout distributions within watersheds, and (iii) species interactions between cutthroat and brook trout. Distribution and temperature data show that beaver-induced stream warming sustains brook trout invasion at higher elevations, while brook trout presence acts to reduce cutthroat trout growth rates. Ongoing analyses of growth rates from scales, and examination of demographic rates of both species will lend greater insight into how beaver impact this system.

Introduction

Beaver (Castor canadensis) play a keystone role on the landscape, driving a significant watershed disturbance regime through their feeding and damming behaviors. 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 assemblages. Increases in water storage capacity through beaver impoundments improve riparian habitat, and potentially augment water supply and late season flows. These aspects of beaver impoundments have resulted in the active transplantation of beaver as restoration tools into degraded wetlands of the Pacific northwest. 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. Transplantation of beaver into tributary streams of the Big Hole River was one approach considered by the BHWC as an alternative to increasing landscape water storage through human dam construction.

Promoting beaver on the landscape, either through natural population expansion or active transplantation 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 systems, there is also the possibility of negative effects on native fish species, such as barrier creation and warming of coldwater streams. 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. 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), as well as providing important winter habitat for many stream fishes including cutthroat trout (Oncorhynchus clarkii) and bull trout (Salvelinus confluentus).

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 survival. 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 systems. Any factors that affect demographic parameters such as growth rates, age-0 recruitment, and dispersal can influence the spread of an exotic species. 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 range. 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°C. 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.

The objective of this research is to (1) identify causal mechanisms that may implicate beaver in facilitating brook trout invasions of 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*: I 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 hypotheses regarding how streams with beaver ponds may affect brook trout (BRK) spread compared with non-beaver control streams. For example, if beaver ponds facilitate BRK spread I predict that there will be an increased distribution, abundance, and population growth rate exhibited by BRK in beaver systems relative to controls.

<table>
<thead>
<tr>
<th>RESPONSE VARIABLES</th>
<th>Streams with beaver ponds compared with non-beaver controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Facilitate brook trout spread</td>
</tr>
<tr>
<td>Distribution of BRK</td>
<td>† BRK distribution, especially at higher elevations above ponds</td>
</tr>
<tr>
<td>Abundance</td>
<td>† BRK in beaver streams</td>
</tr>
<tr>
<td>BRK population growth rate</td>
<td>† BRK population growth</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>RESPONSE VARIABLES</th>
<th>Streams with beaver ponds compared with non-beaver controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enhance negative interactions</td>
</tr>
<tr>
<td>Juvenile BRK growth</td>
<td>† juvenile BRK growth rates &amp; survival in beaver streams</td>
</tr>
<tr>
<td>Juvenile WSC growth</td>
<td>† juvenile WSC growth rates &amp; survival in beaver streams</td>
</tr>
<tr>
<td>Composition (BRK:WSC abundance)</td>
<td>† BRK:WSC ratio in beaver streams</td>
</tr>
<tr>
<td>WSC population growth rate</td>
<td>† WSC population growth</td>
</tr>
</tbody>
</table>
Study Sites

Five study streams were chosen in southwestern Montana to investigate how beaver influence stream temperatures, and the ecology of brook trout and westslope cutthroat trout. These were located in the Beaverhead-Deerlodge National Forest, adjacent BLM and private lands around the Big Hole River drainage in southwest Montana (Map 1). Study sites incorporated three 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).

Methods

Influence of beaver on stream temperatures:

To evaluate impacts of beaver on stream temperatures, temperature loggers were deployed longitudinally along each stream (controlling for elevation) 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 were collected and replaced for the duration of summer 2007, thus yielding over a year of relatively continuous temperature data.

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

Six 200 m sections within the mid- and high-elevations of each stream were randomly selected, and sampled twice in summer 2006 and twice in summer 2007. Each section was
temporarily block-netted, and electrofished using a Smith-Root model 15-D backpack electrofisher. All brook trout and cutthroat trout were identified, measured, and weighed. Trout greater than 55 mm were individually tagged with a Passive Integrated Transponder (PIT) tag and scales were taken for aging and additional growth analysis. The methods used for scale analyses are outlined in Appendix A. During the second, recapture session of each year, each stream section was re-sampled. New fish were processed as above, while recaptures were measured, weighed and re-released.

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

**Results-to-date and discussion**

*Influence of beaver on stream temperatures:*

Temperature was 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°C. Therefore, by examining detailed stream temperature profiles in beaver and non-beaver systems, it was 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 for beaver and non-beaver streams within the study area shows distinctive differences, with the non-beaver systems exhibiting a predictable increase in stream temperature with a reduction in elevation, whereas beaver streams are highly variable, with higher overall rates of temperature increase with decreased elevation (Figure 1).

![Figure 1: Average summer 2006 (1st July – 31st August) temperatures in beaver (BV) (n=4) and non-beaver (Non-BV) (n=3) streams.](image)

Streams influenced by beaver are therefore generally warmer for a given elevation, with large increases in temperature closely correlated with the location of beaver ponds (Figure 2). Thus, beaver were found to elevate stream temperatures to levels at which westslope cutthroat trout and brook trout could realize optimal growth rates.
Influence of beaver on brook trout/cutthroat trout distributions, abundances, and growth rates:

Observed increases in stream temperature, manifested in beaver systems, suggests that beaver may play a role in brook trout invasions and their interactions with westslope cutthroat trout. In the summers of 2006 and 2007, I completed two mark and subsequent recapture sessions (median recapture rate approximately 20%). A total of 1200 brook trout and 902 westslope cutthroat were PIT tagged during this study. This allowed the calculation of species distributions, relative composition, and individual growth rates.

Beaver were found to increase fish abundance, and influence species distribution and composition, whereby the non-beaver system exhibits an increase in the relative proportion of cutthroat relative to brook trout with increasing elevation (Figure 3a). While the beaver system shows the same gradual increase in the proportion of cutthroat trout in upstream reaches, brook trout were found to dominate the community throughout the entire stream (Figure 3b). Beaver therefore enhance the ability of brook trout to invade into higher reaches of a watershed. This supports the hypothesis that beaver facilitate brook trout spread (Table 1), although I have not yet ascertained how brook trout population growth rates are affected by beaver presence.

Figure 2: Summer 2006 stream temperature profiles for typical beaver and non-beaver systems. Dashed lines indicate optimal growth temperatures for each species.

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Figure 3: Average summer temperature and relative composition of WSC and BRK in (a) non-beaver, and (b) beaver treatments. Rectangles denote location of beaver ponds. Dashed lines indicate temperature zone at which BRK gain a competitive advantage over WSC in interspecific interactions.
Influence of beaver on exotic/native species interactions:

Since it was expected that negative competitive interactions between brook trout and westslope cutthroat would be most important at the juvenile stage\(^6\), the distribution of fish in the size range 55-150 mm were examined in relation to available temperatures within the stream. Distributions of this smallest size class caught during the first capture session were related to temperature data for the three treatments (Figure 4).

All study streams exhibit a broad range of average summer temperatures from high to low elevations (~5°C to ~19°C). Where brook trout are absent, westslope cutthroat juveniles preferentially select temperatures between 14 and 16°C. In streams with both brook trout and beaver, there is a relatively high degree of overlap in brook and cutthroat trout distributions, with cutthroat occupying slightly lower temperatures (10 - 12°C). The least overlap between brook trout and cutthroat occurs where beaver are missing from the stream, with most brook trout juveniles found in warmer areas, and most cutthroat occupying colder stream sections. Calculation of potential growth rates\(^3\) for cutthroat trout using stream temperatures experienced by the juvenile cutthroat in each of these treatments indicate that highest growth rates are expected in the absence of brook trout, while lowest potential growth rates are expected where
brook trout are present and beaver are absent. Hence, the presence of beaver in a watershed may buffer the negative effects of brook trout competition on westslope cutthroat populations.

As predicted from the above temperature and distribution analysis, growth rates calculated from recapture data show that in the absence of brook trout, westslope cutthroat realize maximum rates of growth (Figure 5). This growth rate is significantly higher than that of the other treatments, as would be expected from the differences in cutthroat trout distribution with temperature. However, cutthroat growth rates in the presence of brook trout are statistically the same in both the presence and absence of beaver, and are much lower than would be expected based on temperature alone. Therefore, it is likely that interspecific competition with brook trout is involved in depressing cutthroat growth rates.

It is unclear from the recapture data how beaver affect brook trout individual growth rates (Figure 5). The observed decrease in growth in the beaver system could be an artifact of low sample size in the non-beaver system or the result of density-dependence in the beaver treatment, given the large increase in fish abundance in this system. Further analyses using fish scale techniques are underway to resolve this uncertainty.

In conclusion, my data indicate that beaver facilitate the spread of brook trout through increased distribution and abundance of this species (Table 1; Figure 3; Figure 4), although I have yet to establish how brook trout population growth is affected. Using the analyses completed to date, the presence of beaver appears to weakly buffer negative interactions between brook trout and cutthroat (Table 2) by slightly reducing individual brook trout growth rates. However, this does not appear to significantly benefit westslope cutthroat trout individual growth (Figure 5). Given this lack of difference in individual cutthroat growth rates, and that the ratio of brook trout to cutthroat are comparable in both non-beaver and beaver treatments, it can be concluded that beaver have no effect on interactions between brook trout and westslope cutthroat trout. This conclusion should be treated as speculative until additional analyses that will increase sample size, and estimate survival rates, and population growth rates have been completed.
Continuing work

Scale analysis to further investigate individual growth rates:

Scale samples taken from every captured fish are being analyzed to relate circuli spacing to somatic growth rate (see Appendix A). This technique will augment the growth rate estimates from recapture data by increasing sample size. It is clear from recapture data that brook trout are having a large negative effects on cutthroat growth rates, but using scale analysis should allow me to separate out confounding factors such as differences in elevation, and gain a better understanding of how the presence of beaver influence this species interaction. In addition, increasing sample size should allow the error around my growth estimates to be calculated with more precision, thereby giving a clearer picture of how brook trout are influenced by beaver presence.

Population modeling – estimating survival and population trajectories:

Through altering stream temperature regimes and influencing individual fish growth rates, the presence of beaver in a system has the potential to change demographic components of brook trout and westslope cutthroat trout, such as survival rates. To ascertain how beaver may be influencing brook and cutthroat trout population growth rates, I will use methods adapted from Hilderbrand\textsuperscript{19} to simulate cutthroat and brook trout populations using stochastic, stage-structured projection matrix models. Since cutthroat trout exhibit length-specific maturity and fecundity, each stage will represent a demographically relevant life history phase: stage 1 = juvenile (age 0), stage 2 = non-reproductively mature fish aged 1 or older (<150mm), stage 3 = mature fish (150-200mm), and stage 4 = fish greater than 200mm (Table 3; Figure 6). The same matrix stage transitions will be used to model both brook trout and cutthroat populations, based on the assumption that these species are approximately comparable in terms of size-at-age and maturity\textsuperscript{16}.

Table 3: Stage transition matrix that will be used for brook trout and westslope cutthroat trout population projections

<table>
<thead>
<tr>
<th>Stage class</th>
<th>Juvenile</th>
<th>Subadults (&lt;150mm)</th>
<th>Adults (150-200mm)</th>
<th>Adults (&gt;200mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile</td>
<td>0</td>
<td>0</td>
<td>$F_3$</td>
<td>$F_4$</td>
</tr>
<tr>
<td>Subadults (&lt;150mm)</td>
<td>$P_{21}$</td>
<td>$P_{22}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adults (150-200mm)</td>
<td>0</td>
<td>$P_{32}$</td>
<td>$P_{33}$</td>
<td>0</td>
</tr>
<tr>
<td>Adults (&gt;200mm)</td>
<td>0</td>
<td>0</td>
<td>$P_{43}$</td>
<td>$P_{44}$</td>
</tr>
</tbody>
</table>

Figure 6: Stage transition loop diagram for cutthroat trout in the presence of brook trout. The fat black arrows represent the juvenile stage transitions most likely to be affected by interspecific competition with brook trout.
To build the matrix, vital rates collected in the field (survival, growth rate, and hence stage-specific survivorship) will be used in conjunction with values of size-specific fecundity in the literature for both species\textsuperscript{e.g.20}. I am currently estimating these parameters from field data using the demographic computer program TMSURVIV. Environmental stochasticity will be incorporated at each time step, and a ceiling function will be used to prohibit the sum of stages 2, 3, and 4 from exceeding the carrying capacity (the maximum population size of fish aged 1 or older) within a simulation run\textsuperscript{19}.

I will then calculate elasticities and use life stage simulation analysis (LSA) to ascertain the relative importance of individual vital rates on the growth of brook trout and westslope cutthroat trout populations. Specific questions that I will be able to answer using these methods are: (i) how does the presence of beaver influence population growth rates of brook trout and cutthroat trout in a stream? (ii) How does interspecific competition with brook trout influence different cutthroat trout life stages in beaver and non-beaver streams?
References cited

1. Thomas, H. M. 1996. Competitive interactions between a native and exotic trout species in high mountain streams. Page 44. Utah State University, Logan, UT.


Appendix A

Scales are often used to estimate age and somatic growth of fish. Circulus spacing is positively correlated with somatic growth\(^1\).

The growth rate of each fish sampled will be ascertained by marking the position of each circulus, from the focus to the scale’s growing edge (along the longest axis), then measuring the inter-circuli distance.

Example of a westslope cutthroat trout scale. Circuli are marked along the longest axis from the focus to the growing edge.
# Basic Information

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## Publication

- Student Fellowship: Population Genetics and Distribution of Bull Trout Salvelinus confluentus Inhabiting Lakes within Glacier National Park, Montana

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[Student Fellowship: Population Genetics and Distribution of Bull Trout Salvelinus confluentus Inhabiting Lakes within Glacier National Park, Montana](#)
Population Genetics and Distribution of Bull Trout *Salvelinus confluentus* Inhabiting Lakes within Glacier National Park, Montana

**Michael H. Meeuwig**

US Geological Survey, Montana Cooperative Fishery Research Unit, Montana State University – Ecology, PO Box 173460, Bozeman, MT 59717-3460

**Abstract.** – Connectivity among populations may increase the probability of persistence for species that are characterized by small local populations. Populations of adfluvial bull trout in Glacier National Park, Montana, occupy discrete habitats (i.e., lakes) connected through a stream network, and dispersal among these habitats may be important for the life history of this species. Genetic data have been useful for identifying population genetic structure and connectivity among local populations for a variety of taxa. Microsatellite DNA and landscape structure were used to examine the relationship between landscape heterogeneity and genetic structure of bull trout in 16 lakes within Glacier National Park, Montana. Genetic diversity of bull trout was lower for lakes located upstream of dispersal barriers compared to lakes not isolated by dispersal barriers. The presence of dispersal barriers had a strong influence on the genetic similarity among bull trout from different lakes. Distance between lake pairs had a moderate influence on genetic similarity among bull trout from different lakes, and distance composed of first through fourth order streams had a stronger influence that distance composed of fifth order streams. Similarity of lake habitat had a relatively small influence on the degree of genetic similarity among lakes. These data indicate that population genetic structure of bull trout in Glacier National Park is influenced by landscape heterogeneity and that connectivity among local populations has been present over evolutionary time scales; therefore, resource managers must address this importance when considering future habitat impacts and management decisions.

The bull trout *Salvelinus confluentus* is a species of char endemic to western North America. Declining trends in bull trout populations throughout their native range has prompted increased interest in, and designation of this species as threatened under the US Endangered Species Act in 1998. Glacier National Park, Montana, contains some of the last, relatively unperturbed lake habitat available to adfluvial bull trout in their native range. This area has great potential for maintaining source populations important to the Columbia River Basin. However, recent research (Fredenberg 2002; Meeuwig and Guy 2007) has identified dramatic declines of bull trout over the last 30 years in the four largest lakes within Glacier National Park (Lake McDonald, Kintla Lake, Bowman Lake, and Logging Lake). These declines are associated with corresponding increases in numbers of invasive lake trout *Salvelinus namaycush* (Fredenberg 2002) that have colonized these waters from downstream sources since their introduction into the Flathead River system in 1905 (Spencer et al. 1991).

The declining trend of bull trout following the introduction of lake trout is indicative of a population-level response to the addition of a potential resource competitor (Mathews 1998). Additionally, best available science indicates that conversion of native bull trout ecosystems to lake trout-dominated systems is a common result once lake trout become established (Donald and Alger 1993), and extirpation of bull trout from some of these lakes may occur in the near future (Fredenberg 2002).

Bull trout often occur in relatively small local populations that may be susceptible to extinction as a result of environmental, demographic, or genetic stochasticity. However, stochastic events effecting one local population may be mitigated by immigration from surrounding populations (e.g., metapopulation dynamics and rescue effects). For example, dispersal of bull trout among lakes within Glacier National Park may supplement populations depressed by invasive lake trout, as well as mediate problems associated with small populations (e.g., random genetic drift; Hallerman 2003). Therefore, conservation of bull trout may require the maintenance of dispersal and gene flow among interconnected local populations (Rieman and Allendorf 2001).
Neutral genetic markers provide a useful tool for evaluating genetic diversity and similarity among local populations associated with population connectivity or gene flow (Gharrett and Zhivotovsky 2003). Additionally, recent research has expressed the importance of incorporating landscape structure into the traditional population genetics framework (see Manel et al. 2003). Population genetic studies often incorporate spatial structure into analyses, but this is normally limited to simple patterns of isolation by distance (Wright 1943). However, populations in natural environments often inhabit areas of great landscape heterogeneity. Therefore, the goal of this research is to examine genetic diversity and similarity among local populations of bull trout in Glacier National Park in relation to landscape heterogeneity as measured by isolation by distance (i.e., stream distance among local populations), barrier structure (i.e., the presence of barriers to dispersal among local populations), and habitat similarity among local populations.

Methods

Field Methods

Bull trout were sampled from 16 lakes in Glacier National Park during the summers of 2004, 2005, and 2006 (Figure 1). Glacier National Park lakes were sampled using gill nets, backpack electrofishing, and hook-and-line. A small tissue sample (25 mm$^2$) was removed from the anal fin of bull trout using surgical scissors. Tissue samples were stored in 95% ethanol and transported to Montana State University for analysis (laboratory of Steven T. Kalinowski). Additional tissue samples were obtained from previous sampling in Glacier National Park (Dux, unpublished data; Fredenberg, unpublished data) in order to increase sample sizes for sites that were represented by few individuals.

Landscape and lake characteristics were determined either from available map and geographic information systems (GIS) data or on-site during the summers of 2004, 2005, and 2006. Stream distance between all pairs of lakes and stream order were determined from a GIS stream layer (simple polyline; NAD 1983 UTM projected coordinate system). Dispersal barriers were located by walking stream reaches from each study lake to the stream's confluence with either the North Fork Flathead River or the Middle Fork Flathead River (Figure 1). Waterfalls with a vertical drop of 1.8 m or greater were classified as barriers (Evans and Johnston 1980) and their location was recorded. Lake surface area, maximum length, and elevation were determined from a GIS lake layer (simple polygon; NAD 1983 UTM projected coordinate system). Lake maximum depth was determined from available bathymetric maps (USFWS 1977) or on-site using a handheld depth finder (model LPS-1, VEXILAR, Inc., Minneapolis, Minnesota).

Lake maximum depth was not measured at Upper Lake Isabel due to logistical constraints; therefore, maximum depth for Upper Lake Isabel was estimated using a linear regression with no intercept (PROC NLIN; SAS Institute 1989). The predictor variable for this regression was lake surface area (Log$\log_{10}$ transformed to normalize data) for Glacier National Park study lakes less than 100 m deep.

Laboratory Methods

DNA was extracted from bull trout tissue samples using a QIAGEN DNeasy Tissue Extraction Kit (QIAGEN Inc., Valencia, CA). Eleven fluorescently labeled microsatellite loci (DeHann and Ardren 2005) were amplified using the polymerase chain reaction (PCR) method in a DNA Engine DYAD thermal cycler (Bio-Rad Laboratories, Hercules, CA). For each sample, four multiplex PCR reactions and one single reaction were carried out. The number of loci per multiplex reaction varied from two to four and reaction conditions varied to optimize PCR products.

PCR products were placed into one of two groups based on fluorescent label and allele length to minimize overlap among loci with similar fluorescent labels. Allele lengths were determined using an ABI 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA) and allele calls were made using GeneMapper software (GeneMapper version 3.7, Applied Biosystems, Foster City, CA).

Data Analysis

Genetic diversity for each lake was calculated as expected heterozygosity (averaged across loci), allelic diversity, and private allelic diversity using the software package HP-Rare (Kalinowski 2005; http://www.montana.edu/kalinowski/HPRare/HP-Rare_Home.htm). Allelic diversity and private allelic diversity were adjusted for sample size and averaged across loci (Kalinowski 2004, 2005);
additionally, allelic diversity and private allelic diversity were examined using a hierarchical structure based on whether lakes were isolated by migratory barriers or not. Pairwise $F_{st}$ values were used as a measure of genetic distance (Frankham et al. 2002) to compare the degree of genetic dissimilarity between lakes (larger values are associated with greater dissimilarity). Pairwise $F_{st}$ values were calculated between each lake pair using the software package Genepop (http://genepop.curtin.edu.au/).

A correlation matrix approach (Smouse et al. 1986) was used to relate landscape characteristics to genetic dissimilarity among lakes. For this analysis the dependent matrix was a matrix of $F_{st}$ values between lake pairs. This matrix was related to independent matrices defining stream distance between lake pairs, barrier structure between lake pairs, and dissimilarity in lake morphometry between lake pairs. Stream distance between lake pairs was defined by two matrices: 1) stream distance...
between lake pairs composed of first through fourth order streams (i.e., tributaries to the North Fork and Middle Fork Flathead rivers; Figure 1) and 2) stream distance between lake pairs composed of fifth order streams (i.e., the North Fork and Middle Fork Flathead rivers; Figure 1).

Barrier structure was categorized in one of three ways depending on the presence and location of barriers with respect to the lake pair: 1) potential for two-way dispersal between the lake pair (Figure 2), 2) potential for one-way dispersal between the lake pair (Figure 3), and 3) no potential for dispersal between the lake pair (Figure 4).

Morphometric dissimilarity between lake pairs was estimated based on a principal components analysis (PROC PRINCOMP; SAS Institute 1989) of lake surface area, maximum length, elevation, and maximum depth. The first two principal components accounted for 93.61% of the variation in the data. A biplot was constructed using principal component scores for the first two principal components, the Euclidean distance between each pair of lakes was calculated from this biplot, and this distance was used as a measure of lake dissimilarity.

Multiple linear regression (PROC REG; SAS Institute 1989) was used to examine the variability in genetic dissimilarity explained by stream distance between lakes, barrier structure between lakes, and lake dissimilarity. Model significance ($\alpha = 0.05$) was determined using a partial Mantel test using the software package multi_mantel (http://anolis.oeb.harvard.edu/~liam/programs/multi_mantel/mm_manual.html). Additionally, the marginal contributions of stream distance, barrier structure, and lake dissimilarity were assessed using coefficients of partial determination (Neter et al. 1996).

**Results**

Multiple barriers were located in Kintla Creek downstream of Upper Kintla Lake; the most substantial barriers were a waterfall measuring 2.8 m high and a waterfall measuring 6.7 m high (Figure 1). One barrier was located in Camas Creek downstream of both Trout Lake and Arrow Lake measuring 7.2 m high (Figure 1). Three barriers were located in Park Creek downstream of both Lake Isabel and Upper Lake Isabel; measuring 1.8 m, 2.4 m and 2.7 m high (Figure 1). Lake surface area varied from 5.3 ha to 2780.9 ha.

**FIGURE 2.** – Schematic representation of two lakes (filled ovals) with no barriers within the stream network (solid line) connecting the lakes. In this situation dispersal (dashed line) may occur from Lake A to Lake B without obstruction [upper panel (A)], and dispersal may occur from Lake B to Lake A without obstruction [lower panel (B)].
**FIGURE 3.** – Schematic representation of two lakes (filled ovals) with one barrier (solid bar) within the stream network (solid line) connecting the lakes. In this situation dispersal (dashed line) may occur from Lake A downstream over the barrier to Lake B without obstruction [upper panel (A)], but dispersal may not occur from Lake B upstream over the barrier to Lake A [lower panel (B)].

**FIGURE 4.** – Schematic representation of two lakes (filled ovals) with two barriers (solid bar) within the stream network (solid line) connecting the lakes. In this situation dispersal (dashed line) may not occur from Lake A upstream over the barrier to Lake B [upper panel (A)] and dispersal may not occur from Lake B upstream over the barrier to Lake A [lower panel (B)].
TABLE 1. Presence of barrier downstream of study lakes and lake surface area, maximum length, elevation, and maximum depth for lakes sampled in Glacier National Park, Montana.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Barrier present</th>
<th>Surface area (ha)</th>
<th>Maximum length (km)</th>
<th>Elevation (m)</th>
<th>Maximum depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akokala Lake</td>
<td>No</td>
<td>9.5</td>
<td>0.7</td>
<td>1443</td>
<td>6.9</td>
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<tr>
<td>Arrow Lake</td>
<td>Yes</td>
<td>23.9</td>
<td>0.8</td>
<td>1241</td>
<td>16.5</td>
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<tr>
<td>Bowman Lake</td>
<td>No</td>
<td>697.5</td>
<td>10.5</td>
<td>1228</td>
<td>77.1</td>
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<tr>
<td>Cerulean Lake</td>
<td>No</td>
<td>20.3</td>
<td>0.7</td>
<td>1423</td>
<td>35.9</td>
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<tr>
<td>Harrison Lake</td>
<td>No</td>
<td>162.6</td>
<td>2.3</td>
<td>1126</td>
<td>41.1</td>
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<tr>
<td>Kintla Lake</td>
<td>No</td>
<td>694.1</td>
<td>6.8</td>
<td>1222</td>
<td>118.9</td>
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<tr>
<td>Lake Isabel</td>
<td>Yes</td>
<td>18.3</td>
<td>0.6</td>
<td>1742</td>
<td>16.0</td>
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<tr>
<td>Lake McDonald</td>
<td>No</td>
<td>2780.9</td>
<td>15.2</td>
<td>961</td>
<td>141.4</td>
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<tr>
<td>Lincoln Lake</td>
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<td>Logging Lake</td>
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<td>450.6</td>
<td>7.9</td>
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<td>67.5</td>
<td>2.0</td>
<td>1277</td>
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<td>351.8</td>
<td>4.8</td>
<td>1346</td>
<td>83.2</td>
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<td>Trout Lake</td>
<td>Yes</td>
<td>87.4</td>
<td>2.8</td>
<td>1190</td>
<td>49.8</td>
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<tr>
<td>Upper Kintla Lake</td>
<td>Yes</td>
<td>189.5</td>
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<td>1332</td>
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<tr>
<td>Upper Lake Isabel</td>
<td>Yes</td>
<td>5.3</td>
<td>0.3</td>
<td>1826</td>
<td>16.3*</td>
</tr>
</tbody>
</table>

*Maximum depth for Upper Lake Isabel was estimated using linear regression without an intercept using surface area and maximum depth data from lakes less than 100 m deep.

ha, maximum length varied from 0.3 km to 15.2 km, elevation varied from 961 m to 1826 m, and maximum depth varied from 6.9 m to 141.4 m (Table 1).

The number of bull trout sampled varied from 7 to 20 individuals per lake with most lakes represented by 20 individuals (total \( N = 279 \); Table 2). Expected heterozygosity varied from 0.2227 to 0.7379, allelic richness varied from 1.6910 to 4.2685, and private allelic richness varied from 0.0061 to 0.6902 among lakes (Table 2). Allelic richness and private allelic richness were greater for lakes not isolated by migratory barriers compared to lakes that were isolated (upstream) by migratory barriers (Figure 5).

Pairwise \( F_{st} \) values varied from < 0.01 to 0.66 (Appendix 1). There was a significant linear relationship between genetic dissimilarity (pairwise \( F_{st} \) values) and stream distance, barrier structure, and morphometric dissimilarity (partial Mantel test \( P < 0.01 \)) and the landscape variables accounted for a relatively high proportion of the variability in genetic dissimilarity (adjusted \( R^2 = 0.6756 \)). Inclusion of fifth order stream distance in the model reduced error sums of squares by 8.13%. Inclusion of barrier structure in the model reduced error sums of squares by 46.10%. Inclusion of lake dissimilarity in the model reduced error sums of squares by 6.56%.

Visual inspection of a plot of model predicted \( F_{st} \) versus observed \( F_{st} \) (Figure 6) indicated that the model generally underestimated genetic dissimilarity associated with Harrison Lake; additionally, expected heterozygosity and allelic richness were lower in Harrison Lake than in other lakes not isolated by barriers. Based on these observations, the regression model was re-run with Harrison Lake removed. For this model, there was a significant linear relationship (Figure 7) between genetic dissimilarity (pairwise \( F_{st} \) values) and stream distance, barrier structure, and morphometric dissimilarity (partial Mantel test \( P < 0.01 \)) and the landscape variables accounted for a greater proportion of the variability in genetic dissimilarity (adjusted \( R^2 = 0.8637 \)). Inclusion of fifth order stream distance in the model reduced error sums of squares by 17.44%. Inclusion of first through fourth order streams in the model reduced error sums of squares by 31.40%. Inclusion of barrier structure in the model reduced error sums of squares by 72.48%. Inclusion of lake dissimilarity in the model reduced error sums of squares by 1.95%.
TABLE 2. – Sample size (N), expected heterozygosity ($H_e$), allelic richness ($A$), and private allelic richness ($A_p$) for bull trout from Glacier National Park, Montana, lakes that are isolated or not isolated by barriers. Table sorted according to whether lake is isolated and then by expected heterozygosity.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Isolated</th>
<th>N</th>
<th>$H_e$</th>
<th>$A$</th>
<th>$A_p$</th>
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<tr>
<td>Arrow Lake</td>
<td>Yes</td>
<td>20</td>
<td>0.2227</td>
<td>1.6910</td>
<td>0.0061</td>
</tr>
<tr>
<td>Trout Lake</td>
<td>Yes</td>
<td>20</td>
<td>0.2544</td>
<td>1.8912</td>
<td>0.0132</td>
</tr>
<tr>
<td>Upper Kintla Lake</td>
<td>Yes</td>
<td>20</td>
<td>0.2928</td>
<td>2.0051</td>
<td>0.0376</td>
</tr>
<tr>
<td>Upper Lake Isabel</td>
<td>Yes</td>
<td>7</td>
<td>0.3057</td>
<td>1.9430</td>
<td>0.1995</td>
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<tr>
<td>Lake Isabel</td>
<td>Yes</td>
<td>20</td>
<td>0.4517</td>
<td>2.8427</td>
<td>0.3028</td>
</tr>
<tr>
<td>Harrison Lake</td>
<td>No</td>
<td>20</td>
<td>0.3667</td>
<td>2.3817</td>
<td>0.5428</td>
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<tr>
<td>Middle Quartz Lake</td>
<td>No</td>
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<tr>
<td>Cerulean Lake</td>
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<td>0.5315</td>
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<td>3.2830</td>
<td>0.2084</td>
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<tr>
<td>Lower Quartz Lake</td>
<td>No</td>
<td>20</td>
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<td>Bowman Lake</td>
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<td>20</td>
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<td>0.6902</td>
</tr>
<tr>
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<td>No</td>
<td>17</td>
<td>0.7379</td>
<td>4.1469</td>
<td>0.4465</td>
</tr>
</tbody>
</table>

Discussion

Genetic structure of bull trout from 16 lakes in Glacier National Park was influenced by landscape characteristics. The most dominant landscape characteristic influencing genetic structure was the presence of dispersal barriers. Within this system waterfalls appear to act as a genetic discontinuity (Manel et al. 2003) resulting in a sharp gradient in both genetic diversity and genetic similarity when comparing lakes not isolated and lakes isolated by barriers. These data, and patterns of fish species diversity within Glacier National Park (Meeuwig and Guy 2007; Meeuwig et al. in review), provide evidence that the barriers present in this system have been effective at limiting dispersal over a long period of time.

The potential for dispersal barriers within this system to isolate headwater populations of adfluvial bull trout may have important biological significance. First, isolated populations are often more susceptible to the negative influences of environmental, demographic, and genetic stochasticity. Indeed, lakes isolated by barriers within this system showed reduced genetic diversity, which is often associated with a population’s evolutionary potential and which is predicted for isolated populations (Frankham et al. 2002). However, relative abundance of bull trout is generally high in lakes located upstream of dispersal barriers in Glacier National Park.
Second, dispersal barriers in this system may have a positive effect of buffering isolated bull trout populations from invasion and establishment of nonnative fish species. Currently, lake trout have been documented in nine of the sixteen lakes examined in this study (Meeuwig and Guy 2007; Meeuwig et al. in review). Of the seven lakes examined in this study where lake trout have not been documented, five are located upstream of barriers. Evidence suggests that population level declines of adfluvial bull trout are often associated with the invasion and establishment of lake trout (Donald and Alger 1993; Fredenberg 2002). Therefore, although isolated populations of bull trout may be susceptible to stochastic events, dispersal barriers may limit the potential for negative impacts associated with lake trout invasion.

Model predictions generally underestimated genetic dissimilarity between Harrison Lake and other lakes examined in this analysis. It is not clear why Harrison Lake should not fit model predictions; however, within the confines of this analysis there are possible speculative reasons. Using these data it was possible to simulate the presence of a barrier downstream of Harrison Lake and re-run the model. This simulation resulted in similar results as compared to the model where Harrison Lake was removed (data no shown). Therefore, if a barrier did exist downstream of Harrison Lake it would greatly increase the model fit.

It is possible that a barrier to dispersal does exist downstream of Harrison Lake that was not observed during surveys. However, the entire stream reach was surveyed following methodologies used in other streams and no structures within Harrison Lake were classified as barriers. It is also possible that a barrier (as defined in this study) once existed in Harrison Creek downstream of Harrison Lake, but no longer is present. The structures classified as barriers in this study are contemporary barriers that have either not always been there or were at one time ineffective as barriers to dispersal as evidenced by the presence of fish in lakes located upstream of these structures. Another possible reason for the observed trend for Harrison Lake is that some other barrier (other than a waterfall) exists in this system. We used waterfalls with a vertical drop of 1.8 m or greater as a criteria for barriers to dispersal; however, other physical or chemical factors may act as barriers to dispersal (e.g., thermal barriers, chemical barriers, high gradient cascades, shallow stream reaches, etc.). Therefore, the lack of a barrier, as defined in this study, does not necessarily mean that no dispersal impediment exists.

![Figure 6](image1.png)

**Figure 6.** Predicted versus observed $F_{st}$ (genetic dissimilarity) based on a multiple linear regression model including the influence of stream distance, barrier structure, and lake dissimilarity between lake pairs for 16 lakes in Glacier National Park, Montana. Open circles represent comparisons that include Harrison Lake as one of the lake pairs. Dashed line is a one-to-one line.

![Figure 7](image2.png)

**Figure 7.** Predicted versus observed $F_{st}$ (genetic dissimilarity) based on a multiple linear regression model including the influence of stream distance, barrier structure, and lake dissimilarity between lake pairs for 15 lakes in Glacier National Park, Montana. Harrison Lake has been removed from this analysis due to its potential influence as an outlier. Dashed line is a one-to-one line.
With the influence of Harrison Lake removed from the model, stream distance had a strong influence on genetic dissimilarity among lakes, and a greater influence was observed for first through forth order streams than for fifth order streams. This influence may be observed for many pairs of lakes within Glacier National Park. For example, Kintla Lake and Lake McDonald are geographically well separated (Figure 1); however, bull trout from these two lakes are genetically very similar ($F_{st} < 0.01$; Appendix 1). Under a more ‘typical’ isolation by distance scenario this pattern would not seem to fit well. However, the overwhelming majority of the distance between these two lakes is composed of fifth order stream, with only a small portion of the stream distance between these lakes composted of first through forth order streams. Therefore, by considering these stream types separately it was possible to gain a better understanding of how distance combines with coarse-scale habitat type to influence genetic connectivity in this system.

Dispersal conditions may differ in different types of streams. Many studies examine isolation by distance (Wright 1943) and consider just the distance between sites. However, it may be important to consider the type of habitat present within the distance metric (e.g., high gradient stream, shallow stream, low velocity stream, etc.). By doing so it may be possible to partition the effects of different habitat types within the distance between sites and provide a better understanding of how the landscape is influencing genetic structure.

These data have illustrated the importance and benefits of considering landscape characteristics when examining population genetic structure in heterogeneous landscapes. Of importance are the potentially dramatic influences of genetic discontinuities (e.g., dispersal barriers) as well as variability within a seemingly homogeneous habitat type (e.g., streams). These data also provide important information to resource managers. Genetic data examined in this study indicate a fairly complex pattern of genetic connectivity and discontinuity among adfluvial bull trout in Glacier National Park. An understanding of this type of pattern is essential for understanding the potential future impacts, such as habitat fragmentations that may act in a similar manner to the natural barriers observed in this study. Additionally, relatively well connected local populations (e.g., lakes) may be important to the overall persistence of bull trout in portions of this system (e.g., Kintla Lake and Lake McDonald).

Acknowledgments

Partial funding for this project was provided by the Montana Water Center, US Fish and Wildlife Service, US Geological Survey, and US National Park Service. Field assistance was provided by H. Hodges, C. Penne, D. Pewitt, L. Rose, and S. Townsend. Additional assistance was provided by W. Fredenberg, C. Guy, S. Kalinowski, W. Michels, and N. Vu. Use of trade or firm names in this document is for reader information only and does not constitute endorsement of a product or service by the US government.

References


applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.


### APPENDIX 1.

Pairwise $F_{st}$ values based on analysis of 11 microsatellite loci for bull trout from 16 lakes in Glacier National Park, Montana.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Akokala Lake</th>
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<th>Bowman Lake</th>
<th>Cerulean Lake</th>
<th>Harrison Lake</th>
<th>Kintla Lake</th>
<th>Lake Isabel</th>
<th>Lake McDonald</th>
<th>Lincoln Lake</th>
<th>Logging Lake</th>
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Student Fellowship: Natural Mercury Bioaccumulation in Aquatic Environments

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Publication
Selective grazing of microbial mats by dipteran larvae leads to biomagnification of methylmercury in a geothermal food web

Eric S. Boyd, Department of Microbiology, Montana State University

Complex networks of species interacting on different trophic levels underlie the flow of energy in all natural ecosystems, yet our understanding of such processes involving microbial producer species and higher trophic structure is limited, in particular in geothermal systems. Molecular techniques were employed in the present study to identify the organisms involved in material transfer between primary producers and secondary invertebrate consumers in geothermal-based food webs in the Norris Geyser Basin of Yellowstone National Park (YNP).

Stratiomyid larvae phylogenetically related to *Odontomyia occidentalis* (98.6% 28S rRNA gene sequence homology) were observed in high densities in the phototrophic mats of geothermal springs, suggesting these mats to be a food source for larvae. Analysis of ribulose-1,5-bisphosphate carboxylase (*rbcL*) genes recovered from the microbial mat and the foregut of larvae inhabiting the mats suggest that stratiomyid larvae selectively graze algal populations within the mat. Phylotypes that were abundant in the microbial mat were absent in the foregut of larvae, suggesting population-specific grazing. The *rbcL* genes shared between the microbial mat and larval foregut communities were most closely related to the chlorophytes *Chlamydomonas reinhardtii* and *Chlorella* sp.

Previous studies indicate mercury (Hg) bioaccumulation in chlorophytes such as *Chlamydomonas reinhardtii* (4). In addition, elevated levels of total Hg (THg) have been reported in the water of thermal springs in areas of YNP with Hg-enriched soil (1-3) (Fig. 1), and mono-methylated Hg (MeHg) has been detected in the mat microbial biomass (1). To determine if THg and/or MeHg bioaccumulate in the phototrophic mat biomass, samples of mat were collected from springs with low (<10 ng L⁻¹) and high (>10 ng L⁻¹) concentrations of THg in the aqueous phase. Microbial mat biomass contained THg at concentrations ranging from 150-18000 ng g⁻¹ dry weight (dw) and MeHg at concentrations ranging from 2.9-7.3 ng g⁻¹ dw. When expressed as a ratio of MeHg/THg, phototrophic mat biomass sampled from springs with >10 ng L⁻¹ THg in the aqueous phase contained ratios which were 2-3 orders of magnitude lower than biomass collected from springs with aqueous phase THg concentrations <10 ng L⁻¹. The induction of genes involved in the demethylation of MeHg occurs when THg exceeds 10 ng L⁻¹ (5). Thus, the difference in ratios from springs with high versus low THg concentrations may be attributable to induction of genes involved in demethylation, thereby decreasing the MeHg concentration in the aqueous phase. MeHg demethylation would decrease the concentration of MeHg available for bioaccumulation and thus, may lead to a lower MeHg/THg ratio.

To determine if MeHg bioaccumulates in the tissues of larvae as a consequence of grazing mats containing elevated MeHg, the tracheal tissue from larvae were removed and subjected to MeHg analysis. Analysis of tracheal tissue from larvae revealed MeHg bioaccumulation 2.7- to 5.5-times greater than the concentrations bioaccumulated in mat biomass. We have observed *Charadrius* sp. (Killdeer) preying upon larvae in geothermal springs in the Norris Geyser Basin. To determine if MeHg bioaccumulation extends to Killdeer as a result of consuming larval biomass, a feather molted from a bird observed preying upon larvae was collected and analyzed. The feather contained MeHg at a concentration 6-times greater than determined in larval tissues. Together these findings suggest that invertebrate grazing of microbial mat populations serves as a mechanism for MeHg biomagnification in food webs emanating from these geothermal ecosystems.
References


Student Fellowship: A Proposal for Research on In–situ Subsurface Microbial Transformation of Selenium as Source Control in Backfilled Phosphate Overburden, SE Idaho

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Publication
Microbial Transformation of Selenium in Phosphate Mine Wastes, SE Idaho

Report on Research Progress

Lisa Bithell Kirk
Land Resources and Environmental Science Department
Montana State University
Bozeman MT

Montana Water Center
Bozeman MT

December 26, 2007
This report summarizes ongoing doctoral research into the effect of microbial transformation on selenate mobility from mined phosphate waste in SE Idaho, which has been funded by the MT Water Center during 2007, as well as by the Inland Northwest Research Alliance Subsurface Science Research Initiative and the Idaho Mining Association, and the Montana Water Center. Additional funding, through the EPA STAR Fellowship program, began in fall 2007 and will continue through the end of the project in 2009.

Problem Summary

Mine operators struggle with predicting seepage chemistry and optimizing waste management practices. In-situ selenate reduction offers significant potential for operational source control, which is widely recognized as the best possible means of reducing water quality impacts associated with mining operations (US EPA, 2003). Eight years of monitoring data from the Dry Valley mine suggest that in-situ microbial reduction of Se successfully prevents release to groundwater (Maxim, 2007) and raises questions of how conditions at Dry Valley compare to other SE Idaho phosphate operations and whether facilities can be designed to promote natural attenuation. Thus, this doctoral research directly targets the microbiology that drives selenate sequestration in these backfill environments and the characterization of environmental factors that support (or limit) it. The ultimate goal is to define how backfill environments might be specifically engineered to support optimal levels of selenate reduction, using a reactive biobarrier approach (Fig. 2), based on experiments designed to answer the following questions:

1. How do microbes affect Se release/attenuation in phosphate backfill?
2. Why do we observe different rates of selenium release in backfills at different locations?
3. Can indigenous microbes provide reliable source control? Under what in situ conditions?

Figure 1. Conceptual hydrogeochemical model, SE Idaho phosphate mine showing stratigraphy and overburden (after Maxim, 2007).

Figure 2. Conceptual model of biobarrier approach to incorporating microbial reduction into in situ selenium management.
Research History and Objectives

Lisa Kirk’s doctoral program was initiated in 2004, following identification of potentially significant microbial reduction of selenium to immobile forms in backfill at Dry Valley (Maxim, 2007; Kirk et al, 2002). Research began in earnest in 2006, following receipt of operations funding from the Idaho Mining Association (IMA) in October 2005, and is ongoing. Since that time, significant effort to enumerate, isolate, and identify organisms using culture-dependent and molecular methods of microbiological analysis have been ongoing, with significant success (Kirk et al, 2006).

This project is focused on providing a critical, peer-reviewed research effort to define the factors that promote microbial selenate reduction in backfill, with a goal of improving the ability to evaluate potential impacts of future operations and reduce regulatory uncertainty. The specific goals of this effort included explaining the how, where, and why of microbial transformation of selenium between reduced and oxidized species, and organic complexes, in phosphate backfill (Fig. 3), in comparison to previous work by Knotek-Smith (2003) in surface dumps at Smoky Canyon and to the apparent lack of attenuation in overburden upgradient of existing surface seeps. One initial question included whether apparent differences between internal backfill and external waste rock dumps could be explained by differences in temperature, oxygen, pH, moisture content and geochemistry conditions, such as different mineralogy and trace element content between lithologies. Three objectives are identified, which are described with the methods used to address them below:

1. Identify and enumerate selenate-reducing organisms. Organisms capable of selenate reduction will isolated and identified using both cultivation and non-cultivation dependent (e.g., PCR, DGGE, clone libraries, and genomic analysis) methods. Enumerate organisms based on lithology and aerobic tolerance using most probable number method.

2. Determine which backfill-relevant environmental factors influence microbial selenate reduction, using microcosm experiments to evaluate the influence of key variables such as oxygen, moisture content, available form of organic carbon, and concentrations of potential inhibitors (sulfate, nitrate, and manganese) on magnitude and rate of selenate reduction.

3. Determine selenium reduction rates under environmentally relevant conditions of lithologic variation, moisture, flow rate, and oxygen exposure, using column leach test methods.

This research involves microbial and geochemical analysis of groundwater and overburden samples collected from the Dry Valley, Smoky Canyon, and Enoch Valley mines, located in Caribou County, Idaho. Initial observations in monitoring wells completed in backfill at Dry Valley (GW7D and GW7D2) and Smoky Canyon (GW11) led to the hypothesis of variable microbial transformation of selenium in backfill. This work also relies on additional in situ data characterizing subsurface backfill environments currently being collected by IMA contractors, MFG Environmental Consultants and O’Kane Consultants (MFG, 2006).
Summary of Insitu Conditions for Consideration in Experimental Designs

The insitu conditions described above for the overburden being incorporated in this study of microbial selenium transformation encompass significant variation in lithology, mineralogy, moisture content, and oxygen. In fact, the only constant between the facilities includes the random variation in lithologic sequence of rock mined from the same stratigraphic section of the Meade Peak member of the Phosphoria Fm. and temperatures that are consistently between 7 and 12 °C.

Conditions at Dry Valley reflect prior saturation with subsequent draindown in the upper backfill, with development of an isolated perched zone of saturation at depth in the backfill, and it seems likely that there is flow under unsaturated conditions in the upper backfill leading to saturated flow in the lower backfill. The fine and saturated backfill is separated, however, from the regional aquifer and a capillary break explains the presence of coarse, dry limestone at the base of the drill hole. At Smoky Canyon, conditions appear to be unsaturated throughout the D backfill and A panel dump, with local variation in water content below field capacity. Unsaturated flow cannot occur under these conditions, suggesting that incident precipitation somoves under preferential flow conditions in more saturated zones not intercepted by the drilling. Both the backfill and the dump at Smoky Canyon are fully aerated. At Enoch Valley, conditions seem to lie between the Smoky Canyon and Dry Valley end members; sediments are unsaturated, but oxygen depleted. More limestone appears to be mined at Enoch Valley and Dry Valley than at Smoky Canyon, based on drill intercepts in the study areas. Variation in the massive character of the Rex chert between mine sites, along with varying mounts of limestone production, also affects the extent of explosives use. Experimental conditions should therefore address variation in oxygen, moisture content, differences in sulfate and nitrate concentrations (aqueous) and sulfide content (solid).

It can be seen from the discussion provided above that a number of factors may play a role in fostering, or inhibiting, selenium transformation by microbes, including moisture content, oxygen availability, and lithologic chemistry. Further, the insitu monitoring data suggest coupling of hydrogeological, geochemical, and microbiological processes may significantly influence the extent of reduction or oxidation. For example, the sulfide mineral content and reactivity (both biotic and abiotic) may enhance selenate leachability for a subpopulation of shale and it is possible that sulfide affects the potential for selenate reduction as an inhibitor. Variation in sulfide content between studied materials could explain observed differences in selenate mobility. Apparent differences in selenium leachability under unsaturated and saturated conditions may be related to surface passivation of reactive mineral surfaces, which become less reactive as salts accumulate under dry conditions but which are rinsed and re-exposed under saturated flow conditions. Similarly, the presence of organisms with a high degree of similarity to Dechloromonas denitrificans and aromatica suggest that nitrate, and the form of indigenous hydrocarbon, may directly affect selenate reduction. Altered shales may have different forms of indigenous hydrocarbon present. Conversely, the presence of nitrate in blasted mine waste may facilitate such expression and foster subsequent co-metabolism. The presence of oxygen may inhibit expression of selenate reductase or other enzymes co-factors, such as the fumarate nitrate reductase identified by Yee et al (2007) that allow E. cloacae to completely transform soluble Se(VI) to insoluble Se(0). In spite of the complex setting, and the subtlety of ecological controls on insitu selenium biogeochemistry, the combination of conditions developed at Dry Valley have been sufficient to support this process, and it is logical that potential exists for them to be developed elsewhere through design once they have been thoroughly defined.

The samples collected to represent the range of conditions in backfill during the 2006 drilling program are shown below.
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### MEV Enoch Valley backfill

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Figure 4. Schematic drill log of three holes sampled for microbiological investigation, showing depth, lithology and location. List of samples collected for microbiological analysis provided.
Rationale and Methods by Objective

For regulators and miners to rely on natural attenuation of Se via microbial reduction in subsurface backfills, they must know the identity and distribution of the microorganisms involved, and the environmental factors that influence population levels, activity and rates of transformation. The same is true for microbial oxidation of overburden, whether in backfills or external dumps. These factors include oxygen exposure, moisture content, presence and type of electron donor, and presence of alternative electron acceptors including nitrate and sulfate, along with sulfide, iron, and manganese as potential inhibitors of microbial transformation. The proposed experiments therefore integrate microbiology with geochemistry. They are hypothesis-driven and organized in a hierarchical fashion, with the goal being to characterize microbial selenate reduction in the waste backfill environments that exist at the three mine sites, and thereby establish the fundamental components of a conceptual model of Se fate and transport in backfilled phosphate mine waste under conditions that promote variable selenate reduction. This section provides a brief overview of the rational and methods for each objective defined in Section 1.2.

This section provides an overview of the objectives, hypotheses, and proposed or ongoing experiments. These experiments were preceded by preliminary experiments that sought to define whether observed changes in selenium mobility was microbially mediated at Dry Valley and Smoky Canyon and to make a preliminary comparison of diversity between the two mine sites based on PCR amplification of DNA extracted from the preliminary experiments and original groundwater samples, coupled with DNA separation using DGGE.

**Objective 1: (A) Identify and (B) Enumerate Se-reducing organisms.**

Hypothesis 1.1: Distinct microbial populations will be associated with specific lithologies.

Corollary: Distinct selenate reducers will be associated with specific lithologies.

Hypothesis 1.2: Because of the significant hydrocarbons associated with shale, selenate reducer numbers will be favored by this specific lithology type

Corollary: Variation within shale will occur depending upon form of organic carbon and presence or absence of inhibitors, including sulfide, sulfate, nitrate, iron and manganese.

**Rationale:** Because of the basic differences in mineral composition, weathering-induced surface chemistries of chert, shale, and mudstone differ significantly and thus are expected to promote and host different microbial populations with variable capacity to reduce selenate. Identifying the optimum lithology with respect to selenate reduction will be important to engineering solutions to control Se mobility.

**Objective 1A: Isolation and Identification Methods:** Both cultivation-dependent and molecular methods are being used to characterize microbial communities in groundwater from Dry Valley, Smoky Canyon, and Enoch Valley.

**Cultivation dependent methods.** Enrichment cultures of groundwater from Dry Valley (where Se levels in groundwater are low, presumably due to insitu reduction to elemental selenium), have been incubated under anaerobic conditions with selenate concentrations ranging from 0.2 mM to 20 mM using a range of carbon sources including indigenous carbon extracted from rock, lactate, acetate, pyruvate and mixed H₂-CO₂ gas. Positive cultures with unique morphology from Dry Valley have been isolated on solid media and transferred to pure culture in a synthetic dechloromonas media. Approximately 30 pure cultures from the Dry Valley groundwater are now being screened to eliminate duplicate cultures using polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), clone libraries, restriction fragment length polymorphism (RFLP), and sequencing.
The same approach is being employed to enrich for, and isolate, selenate reducers from the 15 lithology-specific core samples collected at Smoky Canyon and Enoch Valley. Filter-sterilized aqueous extracts of each rock type and groundwater from Dry Valley are amended for use as the basic growth medium in order to use ecologically relevant carbon sources with lithology appropriate ion chemistry. In addition, other enrichments will be spiked with acetate, lactate, pyruvate, benzoate or H₂ as added electron donors. Incubations are conducted at 10°C consistent with subsurface temperatures, until pure cultures are obtained and organisms are identified; subsequent work will be conducted at optimum temperatures to increase culture growth and shorten experiment time. Positive enrichments will be plated on the respective agar media, with different colony morphologies used to identify different selenate reducers.

Transfer of subcultures will generate pure cultures, which will be identified using PCR-cloned and sequenced 16S rDNA. Each pure culture will be characterized for maximum rates of selenate reduction and end product (i.e. selenite or Se⁰), the latter using scanning electron microscopy and X-ray photoelectron spectroscopy. Results of previous mineralogy work focused on speciating Se on mineral surfaces of shale in soil from the Smoky Canyon Mine suggest that it may be necessary to use XAFS and XANES methods (Ryser et al, 2005). These methods will also be used to study field samples and any minerals formed during rate experiments. Isolates will also be used in kinetic experiments described in Objective 3, below. With the exception of the XAFS, all of the analytical instrumentation required for characterization of the reduction end products is available at MSU.

Cultivation-independent molecular methods. PCR will be coupled with DGGE as an initial screen to examine relative differences in total microbial diversity between lithology types. DNA extraction, PCR, and DGGE protocols will be those already developed in the McDermott lab (Norris et al. 2001). Whole community DGGE profiles can be compared to gain a general sense of differences in diversity within and between rock types, and will be compared to the DGGE band(s) of individual isolates to identify which band putatively represents each isolated organism. The same DGGE profile comparison will be used to assess the diversity in the enrichments (above) as a way of determining our cultivation success rate, and can be compared with similar analyses of samples flash frozen at the mine sites to check for any population variability during storage and analysis. Depending on the DGGE profile complexity in each case, bands can be cut out, purified, and sequenced to identify the organisms represented. If DGGE profiles are too complex for band extraction and purification, we may alternatively obtain sequences using 16S rDNA community clone libraries and clone restriction fragment length polymorphism (RFLP) screening.

Enumeration of selenate reducers. The most probable number (MPN) approach has been used to provide an estimate of the number of selenate reducers present in each lithology sample. This very large experiment involved a total of 3600 MPN tubes (with controls). Aqueous rock extracts from chert, mudstone, and shale were serially diluted (10-fold steps) out to 10⁻⁸ dilution, and then used to inoculate 10 ml solutions (5 replicates) of the same rock extract amended with a cocktail of (0.2 mM each) acetate, pyruvate, and lactate, with a field-relevant concentration of 0.1 mM selenate. MPN sets were be incubated aerobically (capped test tubes) and anaerobically (sealed serum bottles with nitrogen, 50% H₂-CO₂ headspace) at 10°C to simulate underground temperatures. ICP-MS was used to detect loss of soluble selenate, which will be scored as a positive. HPLC-ICP-MS will be used to evaluate selenate reduction to soluble selenium species, such as selenite, for a subset of the samples. While this work is complete for the 15 overburden samples collected at Smoky Canyon and Enoch Valley, we plan to set up a similar experiment for sediment collected from groundwater at Dry Valley.

Results of preliminary MPN experiments using these methods to evaluate selenate reducer populations in chert, mudstone, and shale from the drill core samples under anaerobic conditions showed that shale hosted as much as 10⁶ selenate reducers per gram while chert and mudstone hosted much lower populations, between 10¹ and 10² organisms per gram. This has suggested that isolation and enrichment efforts be focused on shale samples.
Objective 2. Determine which backfill-relevant environmental factors influence microbial selenate reduction. Experiments here will focus on determining which environmental factors influence selenate reduction in-situ, including inhibitors, moisture and oxygen.

**Hypothesis 2.1:** Optimum selenate reduction occurs under anaerobic conditions

**Hypothesis 2.2:** Sulfate, nitrate, and manganese are potential inhibitors of selenate reduction.

**Hypothesis 2.3:** Indigenous carbon derived from shales is present in forms and at levels sufficient to support microbial attenuation of mobile selenium.

**Hypothesis 2.3** Sulfide present as pyrite may affect potential for selenate reduction or oxidation.

**Rationale.** Se-reducing organisms are metabolically diverse, and many have an ability to use other oxyanions as electron acceptors for respiration, such as nitrate, nitrite, fumarate, arsenate, thiosulfate, and sulfate (Oremland, 1994; Stolz et al., 1999), along with a variety of electron donors including lactate, acetate, hydrogen, glucose, and aromatic hydrocarbons. Previous work described in the literature notes that there are several potential inhibitors of direct relevance to phosphate mine backfill waste, which include competing electron acceptors such as nitrate, sulfate and manganese. The influence of these potential inhibitors, which can also be co-metabolites in some cases, is complex. For example, as discussed above, selenite is reduced to elemental Se only during denitrification by some organisms (Schroder et. al., 1997), reflecting the involvement of both periplasmic and membrane-associated DMSO reductase enzymes which catalyse reduction of numerous oxyanions including nitrate and arsenic (Santini and Stolz, 2002). The potential importance of co-metabolism of, or inhibition by, nitrate and sulfate in blasted and mined materials therefore cannot be overstated.

Based on published literature, the expectation is that anaerobic conditions will indeed support the greatest rates of selenate reduction. However, my first batch experiments (Section 6) suggest the possibility of a non-respiratory type of reduction, such as that identified by Sarret et. al., 2005. The need for aerobic experiments could be tempered somewhat based on the results obtained in the MPN experiments (e.g. aerobic MPN incubations may demonstrate very low numbers of selenate reducing organisms). Previous geochemical analysis of run-of-mine waste materials have routinely identified significant concentrations of sulfate, nitrate, and manganese that have been shown previously to be inhibitory to selenate reduction (Oremland et. al., 1999). These experiments are designed to assess whether any of these inhibitors are a concern in the backfill environment. Equally important, however, these experiments are also designed to address whether there is adequate indigenous electron donor available for complete attenuation of bioavailable selenium. Though indigenous carbon is abundant in the Meade Peak section, particularly in the shales, the type and complexity of carbon compounds remain unknown. The specific character of native carbon in phosphate waste needs to be determined.

**Methods.** The effects of oxygen, nitrate, sulfate, sulfide and manganese on each of the pure cultures isolated in Objective 1A and on mixed consortia in lithology samples from the above-mentioned cores will be determined. For both types of assays, each inhibitor will be added at levels that span concentrations documented for the waste materials in previous geochemical analyses of backfill materials. To vary oxygen, sealed serum bottles will be used, with the headspace gas mixture reflecting oxygen levels spanning from anaerobic to aerobic by adjusting air and N₂ mixtures. Hydrocarbons will be extracted from rock using a methylene chloride method (EPA Method 3510), followed by gas chromatography (GC-MS) analysis. Quantifying electron donor availability will involve experiments where selenate will be added to samples of the different lithology types (chert, shale, and mudstone) in increments, followed by incubations (10°C) and analysis with ICP-MS to determine whether all of the added selenate has been removed from the aqueous phase. Autoclave-killed controls will be included to monitor for abiotic selenate and selenite sorption.

**Objective 3. Determine Se reduction rates under environmentally relevant conditions.** One of the central questions to be addressed here is whether subsurface temperatures can support sufficient rates of microbial metabolism, such that selenate reduction rates can accommodate potentially short residence times in the backfill environment.
**Rationale.** Changes in rate and extent of microbial transformation of Se in response to oxygen, temperature, moisture content, and lithology are being monitored in situ (by the mining companies) and will be tested *in vitro* (this project) using isolated organisms, mixed consortia, or raw (but sieved) lithology samples. The influence of changes in moisture content, particularly for unsaturated conditions, on rates of Se respiration and/or detoxification represent work not yet documented in the literature. MPN results described above suggest lithologic affect on rate, but temperature, oxygen, and moisture content are also likely to influence kinetics.

**Hypothesis 3.1:** Microbial selenate reduction will be capable of accommodating selenate flows based on infiltration rates predicted for SE Idaho.

**Hypothesis 3.2:** Presence of sulfide minerals will influence the balance of microbial reduction and oxidation.

**Hypothesis 3.3:** Organo-Se mineral compounds will influence rate of selenate production.

**Methods.** Flow columns will be constructed using representative lithology samples from the core. Monolithologic experiments will be designed to address rate under varying conditions of unsaturated moisture content and/or saturated flow, to represent the range of conditions observed in the field, at relevant temperatures under both aerobic and anaerobic conditions. Run-of-mine mixed wastes may also be investigated, if available sample allows. Experiments will be scaled down to allow multiple replicates to be constructed, so that individual columns may sacrificed for analysis of microbial community and mineral products. Inflow solutions will be respective aqueous extracts for each type of rock material being used. Flow rates will be varied to compare unsaturated and saturated flow. Selenate concentrations will also be varied between experiments to test the upper bounds of microbial selenate reduction potential under each flow condition. As above, all experiments will be conducted at 10°C (walk-in cold room directly adjacent to the McDermott lab). In each experiment, flow experiments will be conducted for a minimum of two weeks, with column effluent collected with an automated fraction collector. Fractions will be measured for selenate and selenite using HPLC-ICP-MS. Autoclave killed controls will be used to distinguish biotic and abiotic process influences on the net rate of selenate reduction versus sorption.

This hierarchical structure of experiments will allow systematic evaluation of the variables that have been identified, through literature review and site specific monitoring at three SE Idaho mine sites. As the proposed experiments unfold, results will allow for definition of added experiments (as well as elimination of unnecessary experiments) as key variables are identified which explain the variable ecology of selenate transformation in subsurface phosphate mine waste.
Progress and Preliminary Results

Much progress has been made toward the objective of providing critical, peer-reviewed research into the factors that promote and limit microbial selenate reduction in backfill. Work that has been completed during this time period (or is ongoing), using year one funding of a proposed three year scope of work, is shown in Table 1 and includes:

- Selenium reduction in backfill has been shown to be microbiologically mediated with native (indigenous) carbon in controlled laboratory experiments, at Smoky Canyon, Dry Valley, and Enoch Valley.
- Completion of several sampling visits to Smoky Canyon and Dry Valley in 2005, for collection of overburden samples and groundwater using sterile protocols suitable for collection of microbes.
- Collection of 16 samples of overburden from sonic drill cores under aseptic, gas and temperature relevant conditions, during drilling at Smoky Canyon and Enoch Valley in 2006.
- Sieve analysis of multiple overburden samples from Smoky Canyon and Dry Valley, which indicated significant texture differences between overburden at the two mine sites.
- Most probable number enumeration of selenate reducing microbes in samples of backfilled and dumped overburden has been completed for 3,600 microcosms, which demonstrate that the greatest number of selenate-reducing microbes occur in the center waste shale lithology and that variation exists in the number of selenate reducers within this lithologic group. In a few locations, the number of organisms is quite large. This work included 3,600 analyses of total selenium by ICP-MS, as well as selenium speciation for a much smaller group of samples using an HPLC-ICP-MS method.
- Selenate reduction has been only in anaerobic incubations; no aerobic selenate reduction to elemental selenium has been observed, although it is possible that selenate has been reduced to selenite in some of these experiments.
- Enrichment, cultivation, and isolation of organisms from groundwater collected from monitoring wells GW7D and GW7D2a at Dry Valley has proceeded successfully. Many enrichment cultures are underway for material collected from Smoky Canyon and Enoch Valley in 2006.
- Comparison of diversity in DNA between groundwater samples collected from Smoky Canyon and Dry Valley show distinct differences that may reflect observed differences in selenium mobility. These experiments are currently being repeated to explain these differences.
- Microbes cultured from groundwater at Dry Valley show distinct community patterns that differ between electron donor (carbon) treatments.
- Experimental designs for rate experiments will incorporate in situ relevant conditions documented by MFG and O’Kane consultants.
- Seven grant proposals and three interim progress reports have been written for this project.
- Interim results of this investigation have been presented as a poster at three INRA conferences and as a talk to the NWMA. We plan to present them to the Montana Water Center Annual meeting and at the USEPA STAR conference in 2008. We have also been asked to present our findings to the SE Idaho chapter of SME, which we will happily do this fall or winter.
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REFERENCES


Yee, N., J. Ma, A. Dalia, T. Boongueng, and D.Y. Kobayashi, 2007. *Se (VI) reduction and precipitation of Se(0) by the facultative bacterium enterobacter cloacae SLD1a-1 are regulated by FNR*. Appl. Env. Microbiol., v. 73, no. 6, p.1914-1920.
Resource Recovery from Flooded Underground Mine Workings– Butte, Montana

Basic Information

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<td><strong>Principal Investigators:</strong></td>
<td>Keri Petritz</td>
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Publication
Resource Recovery from Flooded Underground Mine Workings- Butte, Montana
Graduate Student:  Keri Petritz

Introduction

Extensive mining in Butte, Montana has created a system of flooded underground mine workings. The groundwater in the Butte Historic Mining District has excessive concentrations of heavy metals and metalloids due to this past mining activity and the high sulfide mineral content of the area. The US EPA determined that it is technically impracticable to remediate this bedrock aquifer. However, there is still potential for utilizing this water in a beneficial way.

The Belmont Mine is one of several dozen vertical shafts that were constructed to access the underground mine workings. It is located near the Berkeley Pit Lake viewing stand. A past attempt was made at pumping water from the Belmont shaft for irrigation of a nearby park and a football field. However, after several weeks of continuous pumping, the concentrations of arsenic, manganese, iron, and zinc surpassed the proposed irrigation standards, and using this source of irrigation water was abandoned.

The idea of using Belmont Mine water for irrigation is now being re-visited through a new research grant to MSE-Technology Applications through EPA and DOE's Mine Waste Technology Program. The project has three facets: 1) A new pumping test of the Belmont Mine will be performed in July, 2007, up to a maximum of 45 days in duration. The rate of pumping and length of the test will be set to simulate the water needs of a full irrigation season. Major cations and anions, total recoverable metals, dissolved metals, alkalinity, iron and arsenic speciation, pH, specific conductivity, temperature, dissolved oxygen, oxidation-reduction potential, and stable isotopes of water, dissolved sulfate, and dissolved inorganic carbon will be monitored for the duration of the pumping test; 2) Bench scale and field scale experiments will be performed to test different low-cost technologies for treating the Belmont water to meet irrigation requirements. From prior data, it is expected that water treatment will need to focus on arsenic, iron, zinc, and manganese; and 3) MSE will examine the potential for using the Belmont pumping station for heating and/or air conditioning of nearby buildings.

In summary, this project has the potential to turn an aquifer that was deemed technically impracticable into a valuable resource, and affords an opportunity to learn more about the flooded
underground mine workings of Butte, especially with regards to chemistry, hydrology, and interconnectivity of the mine workings in the southern portion of the district. If successful, using this water in a beneficial way will positively impact the municipal water supply and reduce the stress on the Big Hole River.

Project Objectives

1) To perform a long-term pumping test that adequately stresses the aquifer. Enough water will be pumped in order to simulate two irrigation seasons. The data collected from the long-term test will be analyzed to characterize the hydrogeologic system.

2) To collect water quality samples before, during, and after the pumping tests. These samples will be used to establish the water chemistry of the aquifer in addition to learning the maximum contaminant concentrations.

3) To test water treatment options. Several technologies will be tested to determine the best approach to treatment. Factors include feasibility, effectiveness, and footprint area.

4) To research the potential for heat recovery. The Belmont Mine water has elevated temperatures, and utilizing this resource to heat or cool nearby buildings is a possibility.

5) To collect stable isotope samples. Stable isotope samples will be analyzed in order to determine the sources of water, dissolved sulfate, and dissolved inorganic carbon in the pumped water, and to help track the changes in hydrology or geochemistry with time during the long-term pumping test.

Pumping Test Methods

A 40-horsepower pump was previously installed in a well that intercepts the working of the Belmont Mine at the 600-foot level. Observation well #2 also intercepts the 600-foot mine workings while observation well #1 is collapsed and was used to monitor the hydrologic characteristics of the surrounding bedrock aquifer. Depth, pressure, and temperature were continually monitored in all three wells using pressure transducers throughout the pumping test. E-tape measurements of the observation wells were taken periodically as a backup depth measurement system. A flow cell in conjunction with a
YSI monitor was connected to the system to continually monitor the field parameters of specific conductivity (SC), dissolved oxygen (DO), pH, temperature, and oxidation-reduction potential (ORP).

A step draw down test was performed on July 6, 2008. The primary purpose of the step draw down test was to determine a flow rate that would sufficiently stress the aquifer during the long-term pumping test. After three well volumes were purged, four flow rates (25 gpm, 50 gpm, 100 gpm, and 140 gpm) were performed, each lasting roughly two hours. The results showed that a flow rate of 90 gpm for the long-term test would be appropriate.

The long-term test began on July 10, 2008 at a flow rate of 90 gpm. After 20 days of pumping, it was determined that the aquifer was not being sufficiently stressed, and the flow was increased to approximately 110 gpm. The long-term test continued for 9 weeks, removing approximately 8 million gallons of water from the system; the equivalent of about two irrigation seasons. Effluent water was piped to a storm drain that drains into the Berkeley Pit.

Pumping Tests Results

Observation well #2 and the irrigation well showed almost identical drawdown, suggesting high conductivity and connectivity of the 600-foot workings. There was approximately 60 feet of drawdown.

Sampling

During the step-drawdown test, primary samples were taken after three well volumes were purged, two hours after pumping began, and eight hours after pumping began. During the long-term test, most primary samples were collected every Monday, and secondary samples were collected every Friday. All samples were collected following QA/QC guidelines.

Primary sample parameters:

- major cations and anions (calcium, magnesium, sodium, potassium, sulfate, nitrate, carbonate, and chloride);
- total recoverable metals including aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, nickel, selenium, silver, thallium, vanadium, uranium, and zinc;
• dissolved metals including aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, nickel, selenium, silver, thallium, vanadium, uranium, and alkalinity;
• hardness;
• total suspended solids (TSS)
• stable isotopes of water, sulfate, and carbon ; and
• Iron and arsenic speciation.

Secondary sample parameters:
• total recoverable metals including arsenic, cadmium, iron, lead, manganese, zinc;
• total dissolved metals including arsenic, cadmium, iron, lead, manganese, and zinc.

Lab Results

**Temperature**

The elevated temperature of roughly 19°C would be exceptional for designing a heat pump system. Other heat pump systems have been established in abandoned mines with temperatures lower than this.

**Water chemistry**

Belmont water is slightly acidic and very reduced. Due to a higher net acidity than net alkalinity, the water turns acidic upon oxidation. Iron, arsenic, zinc, and manganese surpassed the proposed irrigation standards after the July 6th sampling event. Since this water is being assessed for use as an irrigation source, the high sulfate concentration is also a concern because vegetation is not tolerant to high-saline water. Using Visual Minteq, it was determined that the water from the Belmont irrigation well is at equilibrium with siderite, rhodocrosite, and gypsum. Siderite and rhodocrosite are carbonate minerals that keep the water buffered at depth. Gypsum is the source for the high sulfate concentration. Table 1 shows values for field parameters and lab analysis that were collected over the duration of the pumping test.
During pumping, most of the metal and metalloid concentrations increased during the first 8 to 10 hours of the pumping test. Figure 2 shows the concentrations of Fe, As, Mn, and Zn versus pumping time.

Table 1: Preliminary data from the pumping test

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<tr>
<td>Gallons approximate</td>
<td>N/A</td>
<td>5,000</td>
<td>390,000</td>
<td>910,000</td>
<td>1,800,000</td>
<td>2,700,000</td>
<td>4,960,000</td>
<td>7,180,000</td>
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<td>pH</td>
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<td>5.8</td>
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<td>5.6</td>
<td>5.7</td>
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<td>Specific Conductance (SC) (mS/cm)</td>
<td>N/A</td>
<td>1,915</td>
<td>2,608</td>
<td>2,644</td>
<td>2,704</td>
<td>2,731</td>
<td>2,604</td>
<td>2,944</td>
<td>3,079</td>
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<td>°C</td>
<td>N/A</td>
<td>16.52</td>
<td>19.14</td>
<td>19.34</td>
<td>19.46</td>
<td>19.46</td>
<td>19.00</td>
<td>19.49</td>
<td>19.53</td>
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<td>ORP [millivolts (mV)]</td>
<td>N/A</td>
<td>-70.1</td>
<td>19.3</td>
<td>58.0</td>
<td>-4.4</td>
<td>-18.7</td>
<td>-18.8</td>
<td>-96.3</td>
<td>-21.0</td>
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<td>Alkalinity [milligrams per liter (mg/L) as CaCO3]</td>
<td>N/A</td>
<td>121</td>
<td>Not analyzed</td>
<td>150</td>
<td>154</td>
<td>158</td>
<td>165</td>
<td>163</td>
<td>148</td>
</tr>
<tr>
<td>As (μg/L)</td>
<td>100 (long-term)</td>
<td>1,190</td>
<td>1,200</td>
<td>1,240</td>
<td>1,320</td>
<td>1,340</td>
<td>1,390</td>
<td>1,560</td>
<td>1,680</td>
</tr>
<tr>
<td>Cadmium (Cd) (μg/L)</td>
<td>10 (long-term)</td>
<td>Not detected</td>
<td>Not analyzed</td>
<td>0.4</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>Not analyzed</td>
<td>1.0</td>
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<tr>
<td>Fe (μg/L)</td>
<td>20,000 (short-term)</td>
<td>28,600</td>
<td>181,000</td>
<td>194,000</td>
<td>182,000</td>
<td>188,000</td>
<td>166,000</td>
<td>194,000</td>
<td>198,000</td>
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<td>Lead (Pb) (μg/L)</td>
<td>5,000 (long-term)</td>
<td>0.8</td>
<td>Not analyzed</td>
<td>Not analyzed</td>
<td>1.7</td>
<td>Not analyzed</td>
<td>0.7</td>
<td>Not analyzed</td>
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<td>Mn (μg/L)</td>
<td>10,000 (short-term)</td>
<td>4,420</td>
<td>24,300</td>
<td>23,700</td>
<td>21,800</td>
<td>21,900</td>
<td>17,600</td>
<td>Not analyzed</td>
<td>20,300</td>
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<td>Zn (μg/L)</td>
<td>10,000 (short-term)</td>
<td>1,990</td>
<td>26,700</td>
<td>25,600</td>
<td>20,900</td>
<td>19,300</td>
<td>10,500</td>
<td>14,200</td>
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<td>SO4²⁻ (mg/L)</td>
<td>N/A</td>
<td>925</td>
<td>Not analyzed</td>
<td>1,670</td>
<td>1,640</td>
<td>Not analyzed</td>
<td>1,800</td>
<td>1,860</td>
<td>2,000</td>
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Figure 2: Concentrations vs. time
The iron is present as ferrous iron and the arsenic is present as both arsenate and arsenite. Stable isotope results suggest that the water was not being pumped from the Berkeley Pit but from the underground system of mine workings.

Treatment studies

About 100 gallons of Belmont water was collected and stored under a constant nitrogen purge for treatment testing. Phase 1 treatment tests were designed to incorporate oxidation and pH adjustment. Oxidation was prompted with either hydrogen peroxide (H₂O₂) or air. Sodium hydroxide (NaOH) was used for pH adjustment. Table 6 describes the steps involved with each test run. Head and final samples were collected; in addition, samples were collected after each step. Samples will be analyzed for Fe, As, Mn, and Zn.

<table>
<thead>
<tr>
<th>Test Run</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; pH Stage</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; pH Stage</th>
<th>Oxidation</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; Step</th>
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<tr>
<td>1</td>
<td>7.5</td>
<td>-</td>
<td>H₂O₂</td>
<td>Oxidation</td>
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<tr>
<td>2</td>
<td>7.5</td>
<td>8.5</td>
<td>Air</td>
<td>NaOH</td>
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<td>3</td>
<td>9.5</td>
<td>-</td>
<td>H₂O₂</td>
<td>NaOH</td>
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<td>4</td>
<td>9.5</td>
<td>-</td>
<td>Air</td>
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<td>8</td>
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<td>8.5</td>
<td>H₂O₂</td>
<td>NaOH</td>
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<td>9</td>
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<td>8.5</td>
<td>H₂O₂</td>
<td>Oxidation</td>
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<td>-</td>
<td>H₂O₂</td>
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<td>12</td>
<td>7.5</td>
<td>-</td>
<td>Air</td>
<td>NaOH</td>
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</table>

A second phase was performed that optimized the most favorable treatment path identified in the first phase. During phase 2, lime instead of NaOH was used for the pH adjustment and peroxide only was used to oxidize the water. A final micro-filtration step and sludge settling rates were added to test runs 17 and 18. The phase 2 samples will be analyzed for As, Zn, Mn, Fe, and total dissolved solids (TDS). Table 7 describes the sampling steps for phase 2.
Table 6: Phase 1 treatment test plan

<table>
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<tr>
<th>Test Run</th>
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<th>2\textsuperscript{nd} pH Stage</th>
<th>Oxidation</th>
<th>1\textsuperscript{st} Step</th>
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<td>13</td>
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<td>H\textsubscript{2}O\textsubscript{2}</td>
<td>Oxidation</td>
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<tr>
<td>14</td>
<td>9.5</td>
<td>-</td>
<td>H\textsubscript{2}O\textsubscript{2}</td>
<td>Ca (OH)\textsubscript{2}</td>
</tr>
<tr>
<td>15</td>
<td>7.5</td>
<td>8.5</td>
<td>H\textsubscript{2}O\textsubscript{2}</td>
<td>Ca (OH)\textsubscript{2}</td>
</tr>
<tr>
<td>16</td>
<td>7.5</td>
<td>8.5</td>
<td>H\textsubscript{2}O\textsubscript{2}</td>
<td>Oxidation</td>
</tr>
<tr>
<td>17</td>
<td>9.5</td>
<td>-</td>
<td>H\textsubscript{2}O\textsubscript{2}</td>
<td>Oxidation</td>
</tr>
<tr>
<td>18</td>
<td>9.5</td>
<td>10.5</td>
<td>H\textsubscript{2}O\textsubscript{2}</td>
<td>Ca (OH)\textsubscript{2}</td>
</tr>
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</table>

Once analyses are received from the lab, they will be evaluated, and the most efficient and most economical treatment will be pursued.
Information Transfer Program Introduction

During the period March 1, 2007 through February 28, 2008, the Montana Water Center drew on its USGS support to conduct these information transfer activities:

* Trained and mentored three student interns who tracked research findings, disseminated outreach materials and helped write the Montana Water Center's e−newsletter;

* Published twelve monthly Montana Water e−newsletters and distributed them to more than 1,600 professionals and decision makers concerned with water resource management. E−news archives are posted at http://water.montana.edu/newsletter/archives/default.asp ;

* Collaborated with the Montana Watersheds Coordination Council to redesign and administer the Council's website http://water.montana.edu/mwcc/default.php ;

* Maintained and expanded the web information network MONTANA WATER, at http://water.montana.edu . This website includes an events page, daily news and announcement updates, an online library, water−resource forums and water source links, a Montana watershed projects database, an expertise directory, water facts and more. The watersheds projects database was greatly expanded, with new projects added from state agency programs, watershed groups, and NGOs;

* Maintained and circulated a small library of paper documents related to Montana water topics;

* Conducted the state−wide water research meeting on October 11th and 12th in Lewistown, Montana. The theme of this 24th annual meeting, held in conjunction with the Montana Section of the American Water Resources Association, was Irrigation Management in Transforming Western Landscapes. A pre−conference titled: Understanding Irrigation Effects on Surface Water and Ground Water was held on October 10th. A field trip led by geologist Dick Berg explored the Lewistown geology and was well attended. The conference attracted nearly 150 Montana researchers and policy makers who took in plenary presentations by John Tubbs of the Department of Natural Resources and Conservation, Dorothy Bradley, 18th Judicial District Court Administrator, and Walt Sales, President of the Association of Gallatin Agricultural Irrigators. 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 ;

* Responded to numerous information requests on water topics ranging from municipal bonding authority to pharmaceuticals in ground water;

* Assisted elected and appointed officials, particularly those serving on the Montana Legislative Environmental Quality Council and Water Policy Interim Committee, the Governor's Drought Advisory Committee and the Water Activities Work Group;

* Convened the “2007 MSU Water Conversation” among administrators and faculty to discuss and coordinate water−related teaching and outreach at Montana State University;

* Sponsored and participated in Montana's 74th Annual Water School September 24th – 27th at Montana State University, for 300 staff members of water and wastewater utilities. This training covered treatment plant operation, chemical safety, emergency response, quality assurance programs, and more;

* Created and distributed 1500 copies of the black−and−white Montana Water 2008 calendar, wherein each month features a different Montana stream restoration project; and
* Produced the Montana Water Center's Annual Report for Fiscal Year 2007 covering all the programs accomplished through the Center's effort. The report is posted at http://watercenter.montana.edu/publications/reports.htm.
Notable Awards and Achievements

Other work undertaken by the Montana Water Center includes:

The Whirling Disease Initiative – Research is concentrated on understanding how the disease affects trout populations, and how management actions may intervene in the process. Dissemination of research and testing results to fishery managers is also a focus this year. Information can be accessed online from http://whirlingdisease.montana.edu. Subjects of current research projects include: – the population impacts of whirling disease in eight Montana watersheds – a risk assessment technique for predicting the spread and severity of the disease – the potential for disease control through manipulation of the alternate host – the emergence of disease resistance in populations of wild trout in Montana – disease effects on isolated populations of cutthroat trout.

An interactive map of disease incidence is being mounted on the internet, showing up-to-date information for eight states. Research data from projects funded over the entire course of the initiative are being assembled into a database that can be used for synoptic studies.

The Small Systems Technical Assistance Center operated by the Water Center is the flagship of a nationwide network that helps small public water utilities provide safe, reliable and affordable drinking water. This year's projects include:

- developing and deploying a distance-learning course on water contamination for operators, managers and board members of water utilities – research on microbial contamination in airliner water tanks – developing training courses on water-system energy efficiency, use of alternative energy sources, water loss testing, and water conservation.

The Water Center operates the web site that provides access to the tools developed by all eight technical assistance centers, and co-sponsors the week-long Montana Water School that draws 250 water treatment operators. Its training courses will be presented at a number of national conferences and workshops this year. To date, more than 50,000 water-utility workers have taken the Center's training courses nationwide.

The purpose of the Wild Fish Habitat Initiative is to evaluate the costs and efficacy of measures to bolster wild fish populations, and publicize the findings among fishery and land managers. This year Initiative investigators are:

- evaluating the biology of fluvial Arctic grayling to aid restoration efforts in the Big Hole River – testing physical and chemical techniques for eliminating invasive fish species from streams – assessing the success of techniques for restoration of cutthroat trout in western watersheds – assembling habitat–restoration case histories to assist land managers and fishery biologists in planning new projects.

The Initiative is a scientific adjunct to the Partners for Fish and Wildlife program operated by the U.S. Fish and Wildlife Service for private landowners, aimed to enhance the effectiveness of that program's habitat restoration projects.

The Montana Watercourse is a statewide program for schools and citizens, providing water information, resources, tools and education. Among this year's projects are:

- developing and leading water-resource education courses for realtors and teachers – piloting a program of certification for volunteer water quality monitors – updating the Landowner's Guide to Montana Wetlands for both eastern and western Montana – circulating water-resource 'teaching trunks' among Montana elementary

The Aquatic Sciences Laboratory, formerly the Wild Trout Research Laboratory, provides aquaculture capabilities to meet the needs of Montana State University and outside investigators. This year two fisheries projects are being completed in the lab, and it is being revamped to host alligator culture, for bone–growth research by MSU dinosaur paleontologists. Projects administered elsewhere on the MSU campus involve the culture and study of freshwater snails and frogs.

Margie Kinnersley is a University of Montana Ph.D. student in the Microbial Ecology program and a recipient of a USGS – Montana Water Center fellowship. Last October, Margie was named the top student presenter at the annual Montana Section of the American Water Resources Association conference for her work titled, "Genomic &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." Margie gave her winning presentation at the annual conference of the American Water Resources Association (AWRA) in Albuquerque in November. All her expenses were paid for by the Montana chapter.