Montana Water Center
Annual Technical Report
FY 2004

Introduction

The Montana Water Center addresses needs that can be served by water research and education, and mobilizes the resources of our state's university system to fill those needs. We work daily with scientists, water managers and decision makers.

The annual USGS Water Research base grant intitiated vital research in Montana this year on beaver wetlands, post-fire erosion control, riparian system evaluation methodologies, microbial ecology in coalbeds, and surface-water/ ground-water interactions. Also this year, the Center accomplished a number of water information transfer actities, reported below.

Research Program

About 60 percent of the annual 104(b) base funding is channeled directly to water research that addresses specific problems in Montana. With direction from an advisory committee of water scientists, research priorities change from year to year and are keyed to critical regional issues like groundwater contamination, post-fire soil erosion, and runoff dynamics. Investigators and their students from campuses throughout the state are involved.

During this reporting period, the Montana Water Center initiated five new 104b research projects. The following narratives report the outcomes of seven research projects completed this year, and three ongoing projects, for a total of ten active projects.

Also this year, the Montana Water Center initiated a student research fellowship program offered to worthy undergraduate or graduate student researchers at a Montana institution who are addressing critical water resource issues. From a pool of 14 competitive applications, the Center awarded $5,000 to its first Student Water Research Fellow, Megan McBride, University of Montana graduate student. She conducted research during 2004-2005 entitled "Recreation on the Upper Yellowstone River: Use and Place Attachment," the thesis for which appears in this report.

Amphibian habitat distribution and the population structure of Columbia spotted frogs, Rana luteiventris, in western Montana watersheds

Basic Information

Title:	Amphibian habitat distribution and the population structure of Columbia spotted frogs, Rana luteiventris, in western Montana watersheds
Project Number:	2004MT27B
Start Date:	3/1/2004
End Date:	8/28/2005
Funding Source:	104B
Congressional District:	At large
Research Category:	Biological Sciences
Focus Category:	Wetlands, Ecology, Conservation
Descriptors:	beaver wetlands, Columbia spotted frog, Rana luteiventris, population ecology, conservation genetics, landscape ecology
Principal Investigators:	Lisa Eby

Publication

1. Project was extended. Publications forthcoming.

Abstract

Wetlands are known for their valuable role in providing flood and erosion control, enhancing water quality and providing wildlife and fish habitat. Given the rate of loss and degradation of wetlands, it is important for us to understand the role that these systems play in the functioning of biotic communities. Given the large magnitude of wetland loss across Montana and the importance of wetland ecosystems to biota, it is not surprising that 60% of the threatened and endangered species in the state rely on wetlands to meet all or part of their seasonal needs (Montana Natural Resource Conservation 2001). Amphibian populations are one such species that are dependent upon small lentic habitat such as wetlands. One-third of all amphibian species in Montana are listed as species of special concern by the Montana National Heritage Foundation and Montana Department of Fish, Wildlife, and Parks. It is likely that changes in the availability of lentic habitat at the landscape and watershed scales are important to Montana's amphibian species. Historic loss of habitat has been demonstrated to have impacts on amphibian populations and communities in other systems. We are examining a large database of watersheds in western Montana to examine the quantity and distribution of amphibian habitat across western Montana. In addition, we are examining the genetic structure of amphibian populations within several watersheds to determine whether amphibians are functioning as panmictic populations, metapopulations, or isolated populations. An understanding of both the quantity and distribution of critical amphibian habitat as well as the functioning of these populations at the watershed and landscape scales are needed for the development of conservation and management plans.

Introduction

Over the past decade amphibians have been the focus of increasing concern because of potential population declines. Although amphibian populations naturally undergo wide fluctuations in number (e.g., Pechmann et al. 1991) and there are many factors that are negatively affecting amphibian populations; habitat loss and fragmentation are often cited as key factors in imperiled amphibian populations (e.g., Blaustein et al. 1994). Although the question of whether current land use practices have been a major factor behind current losses is equivocal, impacts from the historic loss of habitat through both changing land use and management activities have been demonstrated (Hecnar and M'Closkey 1996, Knapp and Matthews 2000). Loss of amphibian diversity in temperate regions of North America have been tied to the historic draining of wetlands and clearing of forests (Hecnar and M'Closkey 1996), while the introduction of non-native fish in historically fishless lakes led to population declines of the mountain yellow-legged frog (Knapp and Matthews 2000).

Historically much of the lentic habitat in North America was created by beaver activity. More than 70% of the original riparian areas in the continental U.S. have been substantially altered by either removal of beaver or human developments (Megahan and King 1985). These areas play an important role in maintaining biodiversity; riparian areas occupy 1% of North America's landscapes but host 80% of our threatened and endangered species. Their role may be critical in the intermountain west, where alterations to the hydrology and nutrient flow of subalpine and mid-elevation valleys by beaver have been shown to be important for maintaining the characteristics of aquatic and riparian systems (e.g., Dahm and Sedell 1986). For many species of lentic breeding amphibians, beaver wetlands act as overwintering and breeding habitat. Disruption of the temporal and spatial distribution of these critical habitats may fragment amphibian populations.

Although the changes in aquatic habitat on aquatic fauna have been examined in terms of species diversity, no one has examined how these changes alter the functioning of the populations. One group of organisms that historically may have relied heavily on beaver-created habitat is amphibians. Habitat destruction and fragmentation is thought to be one of the leading causes behind the current rash of amphibian extinctions (Blaustein et al. 1994). Beaver created wetlands (or more recently human stock ponds) and could be a major source of water bodies between the valley bottom and high elevation ponds in several regions in western Montana (Maxwell pers. comm.).

Although amphibians are frequently characterized as having limited dispersal, strong site fidelity, and spatially disconnected breeding habitats, very few tests of these metapopulation structure assumptions are tested in the literature (Smith and Green 2005). The few places where it has been examined, researchers find that frogs may function as metapopulations (Sjogren 1991, Hitching & Beebee 1997, Vos et al. 2001). If populations function as metapopulations, then factors that decrease dispersal rates and distances can result in population declines and increase susceptibility to loss (Soule 1987). However, the applicability of the metapopulation model to other species in the *Rana* genus and to the West may be problematic because of differences in the vagility and life history of the species, as well as the heterogeneity of the western landscape (Blaustein et al. 1994). Thus, understanding population structure and the relationship between population connectivity and landscape characteristics is an important component of management for this species.

Objectives of the project

The purpose of this proposal is to (1) examine the quantity and distribution of critical amphibian habitat across western Montana, (2) examine the genetic structure of amphibian populations within several watersheds, and (3) examine whether either of the above are likely influenced by the presence of beaver activities. Specifically, we are assessing amphibian habitat by examining the number and distribution of potential breeding sites and the occurrence of beaver activity from an existing database for western Montana. Then using 6 focal watersheds, we will examine rates of change in quantity and distribution of this critical habitat in aerial photos over the last 70 years in watersheds with and without beaver. Finally, we plan to examine whether these amphibian populations are functioning as panmictic populations, metapopulations, or isolated populations and whether the landscape changes associated with beaver activity (through altering the distance between breeding populations) may affect the genetic structure of amphibian populations.

Study Sites

This study is being conducted in southwestern Montana. To examine the quantity and distribution of amphibian habitat, we have focused on selecting watersheds from two strata (4 and 6) in southwestern Montana from the statewide amphibian monitoring database (Maxell unpublished data). In addition, we collected samples of three paired watersheds (6 total watersheds) for aerial photo and genetics work. These three paired watersheds were relatively closed systems with similar geomorphology and hydrology but with different distances among lentic sites and therefore different potential connectivity. One pair of watersheds were sampled in 2003 (Lower Wise River - HUC 995 and Pintler Creek – HUC 35). In 2004, all six watersheds were sampled (1) in the Pioneers (Alder Creek – HUC 24, Squaw Creek – HUC 57), (2) Lower Wise River - HUC 995 and Pintler Creek – HUC 35 and (3) in the Bitterroot Mountains (Cache Creek – HUC 11, and N. Fork of Fish Creek – HUC 19).

Methods

Quantity and distribution of amphibian breeding habitat in western Montana today

We have queried an existing database, developed for monitoring lentic amphibian presence/absence that consists of approximately 170 6^{th} code hydrological unit code (HUC) watersheds that were randomly selected in southwestern Montana (Maxell unpubl. data). Bryce Maxell created the database collaboratively with multiple state and federal agencies (DEQ, NHP, MFWP, USFS). Western Montana was stratified by ecoregion, and within each stratum watersheds (6^{th} code HUCs) containing at least 25% federal or state land were randomly selected. The number and total area of the watersheds chosen within each stratum was proportional to the area of each individual stratum relative to the total area for all strata. Within each 6^{th} code HUC, field crews surveyed all standing water bodies on public lands (and some private lands) identified from topographic maps or aerial photos. Amphibians were surveyed using timed visual encounter and dip net sampling. In addition, habitat characteristics, associated with area, depth, type and area of emergent vegetation, substrate, fish presence, and water permanence were all recorded. Standing water bodies are classified as active breeding sites for frogs, possible breeding sites (no breeding observed but physical characteristics of sites would support breeding activity), and overwintering sites (physical characteristics of sites would support overwintering). Breeding was defined as the presence of amplexed pairs, egg masses or tadpoles. The physical characteristics for breeding include shallow water and emergent vegetation. The physical characteristics generally believed to be required for over wintering include permanent flowing water or depths greater than 2 m (Pilliod 2002; Turner 1960).

All surveyed watersheds within strata four and six, were extracted from the database. For any watershed with Columbia spotted frog breeding, we examined the type of site (glacial created, human created, beaver created, off river backwater). If more than one breeding site was present we collected elevation, type of site, and over land distance measures. In addition, we projected the data into ARCGIS and measured riparian distance among sites.

Changes in the quantity and distribution of amphibian breeding habitat in watersheds with and without beaver over the last 70 years

Each of the six focal watersheds have historic aerial photos periodically from the 1940s through the present. Using aerial photos and maps from the last ~70 years we are assessing the number and location of lentic sites for at least 3 different time periods (~1940, ~1970, and ~1990). GIS layers are being created using scanned and ortho-rectified photos for each watershed. Using similar methods as Johnston and Naiman (1990) and Snodgrass (1997), we are able to determine the rate of creation and loss of lentic sites within watersheds with and without beaver activity over the last 70 years. Georectified orthophotos have been downloaded from the Montana Natural Resource Information System (NRIS). The historical photos and maps of each watershed are being examined, water bodies are located, scanned, ortho rectified, and compared with previous years. The final result of this effort will allow us to examine the creation and loss of lentic sites over time and the distance among sites over time within our focal watersheds.

Population structure of amphibian populations in western Montana

To understand the functioning of these populations, we have chosen genetic tools. Dispersal rates of these species are difficult to measure as the majority of dispersers are likely metamorphs that are too small to tag with a transmitter, but have very high mortality rates (very low mark-recapture rates) to estimate dispersal. Genetics provides a tool that allows us to estimate the amount of gene flow between breeding populations. Within our six focal watersheds, approximately 30 Columbia spotted frog tadpoles at each breeding site were collected. We sampled all breeding sites within each watershed. Techniques for DNA extraction, PCR amplification, and allele separation and scoring have followed the procedures outlined in Funk et al. (2005). Pure Gene ® kites (Gentra) were used to extract the DNA according to manufacturer's instructions. Samples are being run using standard PCR and gel electrophoresis techniques for 6 microsatellites previously established for Columbia spotted frogs (Funk et al. 2005). F_{ST}, allelic richness, and a Mantel's test of genetic and geographic distance will allow us to determine the amount of gene flow between breeding populations and whether the frogs within beaver and non-beaver watersheds are functioning as one mixed (panmictic) population, as metapopulations, or as isolated populations.

Results and Continuing Work

Quantity and distribution of amphibian breeding habitat in western Montana today

We first ran a nonparametric multivariate test (Non metric multidimensional scaling) with the dataset (# lentic sites, # breeding sites, site type, distance among sites, elevation) to examine whether there are patterns or biases in the data set associated with sampling regime (% of private, unsampled land in the watershed) and geographic location (stratum and range). There were no patterns in the data set (strata four and six) associated with these factors so we analyzed the entire data set as one. Overall there were 109 watersheds (6^{th} code HUCs) in strata four and six where Columbia spotted frogs were detected. Of those watersheds where Columbia spotted frogs were present, 83.5% were watersheds with beaver present. Beaver watersheds had significantly higher numbers of lentic sites and breeding sites. In addition, most of the breeding sites were beaver created sites. Our expectations that beaver sites would make up the majority of mid-elevation sites and that watersheds with beaver would have shorter distances among breeding sites was not demonstrated in our data. Contrary to our expectations, watersheds with beaver had on average longer distances among breeding sites.

Changes in the quantity and distribution of amphibian breeding habitat in watersheds with and without beaver over the last 70 years

The paired focal watersheds for each site have historic aerial photos from either the 1960s or early 1970s. We have begun to evaluate the historic photos to determine the rates of creation and loss of potential breeding sites in watersheds with and without beaver activity over the last 70 years. GIS layers are being created using scanned and ortho-rectified photos for each watershed. Historic photos have been evaluated for two of the sites and photos are being collected for the other four sites. In the one pair of watersheds in the Bitterroot Mountains that have been examined, the beaver watershed had a higher turnover rate with 16 different sites created over the 62 years. Once other paired watersheds have been completed we will determine whether the increase in turnover rate of lentic sites in beaver watersheds is the same across our different study areas.

Population structure of amphibian populations in western Montana

All 2003 samples have been run using standard PCR and gel electrophoresis techniques for 6 microsatellites previously established for Columbia spotted frogs (Chris Funk, pers. comm.). We have checked our allelic diversity and heterozygosity to ensure we have reasonable estimates using tadpole samples (versus adults as other studies have done (Funk 2005)). Overall, we see high F_{ST} values among sites, similar to the values that have been seen among mountain ranges in previous work (Funk 2005). As expected closer sites appear to have greater genetic connectivity. We are currently running 2004 samples. Once the 2004 genetic samples are complete we will run analyses to examine how genetic connectivity is related to riparian and overland distance, as well as, continue our formal analyses to look at isolation by distance, and the scale of population in these contrasting watersheds through assignment tests.

Literature cited

Blaustein, A.R., D.B. Wake, and W.P. Sousa. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. Conservation Biology 8:60-71.

Dahm, C.N. and J.R. Sedell. 1986. The role of beaver on nutrient cycling in streams. Journal of the Colorado-Wyoming Academy of Science. 18(1): 32

Funk, W.C. et al. 2005. Population structure of Columbia spotted frogs (*Ranaluteiventris*) is strongly affected by the landscape. Molecular Ecology 14:483-496.

Hecnar, S.J. and R.T. M'Closkey. 1996. Regional dynamics and the status of amphibians. Ecology 77:2091-2097.

Hitchings, S.B., and T.J.C. Beebee. 1997. Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: implications for biodiversity conservation. Heredity 79:117-127.

Johnston, C.A. and R.J. Naiman. 1990. Aquatic patch creation in relation to beaver population trends. Ecology 71:1617-1621.

Knapp, R.A. and K.R.Matthews. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. Conservation Biology 14:428-438.

Megahan. W.F., and P.N. King. 1985. Identification of critical areas on forest lands for control of nonpoint source pollution. Environmental Management 9:7-18.

Montana Natural Resource Conservation Service. 2001 The State of the Land Report, Natural Resource Assessment of Montana.. USDA.

Pechman, J.H.K., D.E. Scott, R.D. Semlitsch, J.R. Caldwell, L.J. Vitt, and J.W. Gibbons. 1991. Declining amphibian populations: the problems of separating human impacts from natural fluctuations. Science 253:892-895.

Pilliod, D.S., C.R. Petereson, and P.I. Ritson. 2002. Seasonal migration of Columbia spotted frogs (*Rana luteiventris*) among complementary resources in high mountain basin. Canadian Journal of Zoology 80:892-895.

Sjogren, P. 1991. Extinction and isolation gradients in metapopulations: the case of the pool frog (*Rana lessonae*). Biological Journal of the Linnean Society 42:135-147.

Smith, M.A., and D.M. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography 28:110-128.

Snodgrass, J.W. 1997. Temporal and spatial dynamics of beaver-created patches as influenced by management practices in a south-eastern North American landscape. The Journal of Applied Ecology 34:1043-1056.

Soule, M.E. (editor) 1987. Viable populations for conservation. Cambridge University Press, Cambridge, United Kingdom.

Turner, F.B. 1960. Population structure and dynamics of the western spotted frog, *Rana pretiosa*, in Yellowstone National Park Wyoming. Ecological Monographs 30:251-278.

Vos, C.C., A.G. Antonisse-De Jong, P.W. Goedhart, and M.J.M. Smulders. 2001. Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). Heredity 86: 598-608.

Evaluation of various methods to assess condition of perennial stream ecosystems

Basic Information

Title:	Evaluation of various methods to assess condition of perennial stream ecosystems
Project Number:	2004MT30B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	at large
Research Category:	Biological Sciences
Focus Category:	Management and Planning, Conservation, Ecology
Descriptors:	proper functioning condition, riparian health, stream biodiversity
Principal Investigators:	Clayton Marlow

Publication

1. Miller, T.J. and C.B. Marlow. Submitted 2005. Evaluating riparian health assessment methods for perennial streams in Montana. Journal of Range Ecology and Management.

Abstract

The purpose of this project is to determine the relationship between several riparian inventory methodologies and in-stream biological conditions. Many state and Federal land management agencies infer water quality and fish habitat conditions from land-based evaluation of riparian vegetation and channel morphology. However, there is minimal documentation that the metrics used in the riparian condition inventories are correlated with water quality or in-stream habitat conditions. We are requesting support to sample additional streams in central and eastern Montana to expand the data base already developed for the western part of the state.

Introduction

The evaluation of streamside or riparian health has become a major focus of government agencies, private land owners, and the general public when formulating land management decisions (Fleming et al. 2001). These interests coincide with the broad societal desire to maintain or restore stream ecosystem stability and biotic integrity (Magurran 1987; Resh et al. 1995). Throughout the past decade numerous stream and riparian health assessment protocols have been developed by different federal agencies interested in characterizing the health of these systems. Federal agencies that currently use riparian assessment protocols include the Natural Resource Conservation Service (NRCS), Bureau of Land Management (BLM), U.S. Forest Service (FS), and Environmental Protection Agency (EPA). Similar assessment protocols are also being used by more than 85% of state water quality programs (Southerland and Stribling 1995). All these protocols were developed to provide a qualitative rating of a riparian system's condition in relation to its site potential (Prichard 1998; NRCS 2004a).

Because these assessment protocols are ocular (indirect) estimates or subjective classification of physical parameters of stream systems (Poole et al. 1997), their use may lead to inaccurate assessments of riparian health and biotic integrity. Not only may different assessment protocols differ in stream health ratings, but it has also been found that different observers using the same protocol differ in their evaluation of stream health within the same stream reach (Roper et al. 2002). Furthermore, assessment protocols are applied statewide, regionally and nationally, which may not take into consideration the potential differences in riparian ecosystems due to climate and physiographic province (Ward et al. 2003; Resh et al. 1995). The question then arises: do current assessment protocols reflect ecosystem function and/or water quality across these large spatial scales and are the results congruent with the assessment of in-stream or habitat conditions?

In an effort to address this question we focused on the on the application of different riparian assessment protocols on the same perennial stream systems, and then to evaluate their ability to reflect both ecosystem function and aquatic macroinvertebrate distribution across different climatic and physiographic provinces. To accomplish this goal we identified potential stream sites on private lands in western and eastern Montana during spring 2004; negotiated cooperative agreements with landowners in April and May 2004 and began collecting field data in July 2004.

Background

Because riparian health or status is weighed heavily in management plans for Federal lands and on private property when landowners are involved in Federal conservation assistance projects we chose two of the most commonly used riparian assessment protocols in Idaho, Montana and Wyoming. Two other assessment methods that used a similar approach and appeared to have promise were included in the project evaluation. The two riparian assessment protocols most often used by Federal land management agencies in Montana and neighboring states are a modified version of the assessment protocol first described by Prichard (1998) in *Lotic Wetland Health Assessment for Streams and Small Rivers* (Bureau of Land Management 2003) and *Riparian Assessment for Lotic Systems* (Montana Natural Resource Conservation Service, 2004). Similar assessment methods added to this study were the *Stream Visual Assessment Protocol* developed by the National Aquatic Assessment Workgroup, Natural Resource Conservation Service, in 1998 and the riparian vegetation based, *Monitoring Vegetation Resources in Riparian Areas* (Winward 2000). Testing four similar methodologies reduced the likelihood of bias for or against a specific assessment method.

Objectives

A. Assess riparian health/status on the same stream reaches in western and eastern Montana using protocols described in *Lotic Wetland Health Assessment for Streams and Small Rivers* (Bureau of Land Management 2003), *Riparian Assessment for Lotic Systems* (Montana Natural Resource Conservation Service, 2004), *Stream Visual Assessment Protocol* (Natural Resource Conservation Service (1998) and *Monitoring Vegetation Resources in Riparian Areas* (Winward 2000).

B. Measure and describe stream habitat conditions, i.e. pool-riffle ratios, streambed embeddedness, stream velocity, and macroinvertebrate populations in reaches were riparian assessments were conducted.

C. Compare and contrast riparian health/status scores from each of the assessment protocols with the presence/absence of pollution intolerant macro-invertebrate taxa and taxa diversity for the same stream reaches. The null hypothesis assumes that measures of stream physical conditions, stream health assessments, and aquatic macroinvertebrate assemblages will not differ across the state of Montana.

Methods and Materials

Site Descriptions:

Streams were selected based on four criteria: 1) streams are located in Montana, 2) streams must be low gradient (< 0.02%) and perennial, 3) streams located in western Montana must derive their source from the Rocky Mts., and 4) streams located in the east must derive their source from the prairie. A total of ten streams were selected for this study.

Western stream sections were chosen on the basis of Rosgen C type morphologies that are typical of open meadows (Rosgen 1996). The five western streams selected were located on private property and subject to annual livestock use and hay production. The names of these streams are Cottonwood Cr., Lower and Upper Nevada Cr., South Boulder Cr., and South Willow Cr. South Boulder and South Willow streams have similar average annual precipitation (46-cm) and elevations (1 615-m – 1 737-m) (WRCC 1999). Both of these streams have deep to very deep well drained soils with a texture that is predominantly sandy to course sandy loam. Parent material consists of granite, limestone, and igneous rock (NRCS 2004b). The other three western stream sections have similar elevations (1 250-m – 1 433-m), and deep to very deep, poor to well drained soils (NRCS 2004b). Cottonwood Cr. has the highest average annual precipitation (53-cm) of the western streams, and a soil texture that is predominately a gravelly loam (NRCS 2004b; WRCC 2004f). Cottonwood Cr. along with the 2 Nevada Cr. sections consists of parent material dominated by glacial till and drift. The two stream sections of Nevada Cr. have an average annual precipitation of 47-cm, and a loam to a silty clay loam soil texture (NRCS 2004b; WRCC 2004c). The two Nevada Cr. sections are divided by the Nevada Cr. Reservoir. Upper Nevada Cr. is located three miles above the reservoir, while Lower Nevada Cr. is located four miles below the reservoir.

Eastern streams were more difficult to locate due to lack of abundance and the intermittent nature found in prairie environments. The five stream sections selected were located on private property, and the Rosgen stream classification ranged from E type to G type morphologies (Rosgen 1996). The five eastern streams were subject to both annual livestock use and hay production. The names of these streams are Little Spring Cr., Louse Cr., Mission Spring Cr., Rosebud Cr., and Otter Cr. Louse Cr. the northern most eastern stream has an elevation of 1 192-m and an average annual precipitation of 39-cm (WRCC 2004e). Soils are very deep well drained loam to silty clay loams with parent materials that consist of limestone and marly shale (NRCS 2004b). Little Spring Cr. is located at an elevation of 1 341-m and an average annual precipitation of 39-cm (WRCC 2004a). Soils are moderate to deep well drained loam and clay loams, and parent materials consist of mudstone, siltstone, and sedimentary beds (NRCS 2004b). Mission Spring Cr. derives its source from the Yellowstone R. through hyporeic flow and resurfaces in hay meadows at an elevation of 1 323-m, and receives an average annual precipitation of 42-cm (WRCC 2004d). Soils are very deep, poor to well drained loam and silty clay loams, and parent material is predominantly derived from alluvium deposition (Soil Data Mart 2004). Otter and Rosebud streams are located in the south eastern corner of Montana with elevations at 884-m – 975-m, and an average annual precipitation of 33-cm – 36-cm (WRCC 2001, 2004b). Rosebud Cr. soils are very deep, well to moderately drained loams, and parent material predominantly composed of sedimentary rock (NRCS 2004b). Otter Cr. soils are very deep, well drained loams, and parent materials consist of scoria and sandstone (NRCS 2004b).

Stream and Riparian Assessment Protocols and Bank Stability ratings:

1. Proper Functioning Condition (PFC)**.** This assessment protocol is a modified version of the original PFC (Prichard 1998), and was developed by the USDI Bureau of Land Management (BLM) in the state of Montana and Idaho. The protocol is described in the US Lotic Wetland Health Assessment for Streams and Small Rivers (*Survey*) (BLM 2003). It is a first approximation designed to provide a visual rapid assessment of overall health and condition of lotic sites and systems. PFC assessment is based primarily on physical, hydrologic, and vegetative factors. These factors address a reach's ability to perform certain functions such as: sediment trapping, bank building and maintenance, water storage, aquifer recharge, flow energy

dissipation, maintenance of biotic diversity, and primary production. The condition of a reach is ranked by scores totaled for all factors (11) evaluated and that total divided by the possible perfect score and multiplied by 100. The resulting score is used to arrive at a rating category; proper functioning (80% – 100%), functioning at risk (60% – 79%), or nonfunctioning (< 60%).

2. Riparian Assessment for Lotic Systems (NRCS)**.** This assessment protocol was developed by the Natural Resource Conservation Service in 2004, to provide a rapid assessment of sustainability and function of lotic riparian systems (NRCS 2004a). The NRCS protocol is similar to PFC protocol, and is designed as a "first cut" visual evaluation of a lotic riparian system health and condition. Scores are based on reach similarity to the highest ecological status or potential natural community of that system. This assessment protocol is based primarily on the evaluation of factors that support critical riparian functions such as: sediment trapping, bank building and maintenance, water storage, aquifer recharge, flow energy dissipation, maintenance of biotic diversity, and primary production. The NRCS protocol rates sites or reaches by dividing the summed scores of all factors (10) by the potential score and multiplying it by 100. The rating is then categorized as sustainable (80% – 100%), at risk (50% – 80%), or not sustainable (< 50%).

3. Stream Visual Assessment Protocol (SVAP)**.** This assessment protocol was developed by the Aquatic Assessment Workgroup (NRCS), to evaluate condition of aquatic ecosystems associated with lotic systems (NRCS 1998). The SVAP is based primarily on physical conditions that relate riparian and instream attributes to ecological health criteria. The SVAP assesses ecosystem complexity and diversity of habitat for organisms and related functional hydrologic properties. This protocol was designed to be an easy to use visual assessment for landowners to evaluate lotic conditions on their land and through continued monitoring. The SVAP rates sites or reaches by dividing the summed scores of all factors (15) by the number of actual factors scored. The rating is then categorized as excellent (> 9), good (7.5 – 8.9), fair (6.1 – 7.4), or poor (< 6). For the purpose of this study the PFC, SVAP, and NRCS assessment protocols will be used as riparian and stream indicators of ecological function and health of lotic systems. Identification teams consisting of local NRCS and BLM personal conducted the assessments to avoid researcher bias.

4. US Forest Service Greenline Bank Stability (GL**)**. This measure evaluates the first vegetative community types on or near the water's edge and their ability to buffer against forces of moving water (Winward 2000). Riparian vegetative communities measured adjacent to the stream channel were based on this methodology developed by Winward (2000). Vegetative community types adjacent to the stream are indicators of channel and bank stability. Assessment of individual reaches were then categorized by a stability rating excellent (9 – 10), good (7 – 8), moderate (5 – 6), poor (3 – 4), and very poor (0 – 2) (Winward 2000).

*Study Design***:**

Once stream sections were selected by the researcher they were divided into 4 individual reaches, each approximately 110-m in thalweg length. Individual reaches were separated by a distance of 6 times bank full width or if a reach could not fit within management boundaries such (i.e. fences) it was placed on the other side of the boundary so that it would not be divided. On some streams all reaches were exposed to the same management practice, while others were separated by fenced boundaries and exposed to different management livestock and irrigation practices. Streams where reaches differed in management were Cottonwood Cr., South Willow

Cr., Louse Cr., Little Spring Cr., and Mission Spring Cr. Each reach was assigned as the sampling unit.

1. Riparian and Instream measures: Riparian and instream ecological parameter measurements consisted of channel and floodplain cross-section morphologic characteristics, substrate composition, discharge, instream habitat, riparian vegetative composition, and aquatic macroinvertebrate assemblages. All measures were taken during base flow to reduce variability.

Permanent cross-sections were established as the starting point for each reach. Methods used to measure channel and floodplain cross-section morphology are based on Rosgen (1996). Parameters measured were entrenchment ratio, gradient, Wolman pebble count, and discharge measured in cubic ft per second (CFS). Entrenchment ratio measures the streams ability to access the floodplain during high flow events, which enables the stream to dissipate energy and trap sediment. Gradient was measured using a survey transit by taking stream water surface elevation measures 30-m upstream and 30-m downstream from the permanent reach cross-section and dividing the difference in elevation by 60-m. The Wolman pebble count was developed to characterize substrate composition of percent fines and course material (Wolman 1954). A grid was also used to calculate percent fines, which counted particle sizes less than 2-mm in size (Overton et al. 1997). Grid measurements were measured in the tail-outs of 3 different pools within a reach to calculate a mean for percent fines.

Instream habitat measures were based on Overton et al. (1997). Habitats were identified and measured as pools, riffles, and glides. Width depth ratios, surface area and volume were measured for each habitat within the entire length of each reach. Habitat measures for cover were based on undercut banks, vegetative overhang, and large wood and boulders along and within the stream channel throughout the length of the reach. Bank stability (GL) measurements were made on each side of the stream for the length of each reach (110-m).

Aquatic macroinvertebrate assemblages have become a common tool used as indicators of stream health and water quality (Wiggins 1996; Barbour et al. 1999; Bollman 2002). Federal agencies such as the EPA use aquatic macroinvertebrates as key assessment methods to characterize stream condition and water quality. Aquatic macros were sampled in 3 different riffle habitat types or glides when riffle habitats were not available in each reach. This produced 12 samples per stream. Samples were collected during the month of September in 2003 and 2004. Insects were collected using a D-frame dip net, and kicking the streambed material for one minute per sample per habitat. Samples were then stored in whirl packs with 2 x Kahles solution, and were taken to a lab for sorting and identification. Samples were picked and sorted to approximately 500 organisms. Taxa were then identified to family except for Ephemeroptera-Plecoptera-Trichoptera (EPT), which were identified to genus using Merritt and Cummins 3rd edition (1996).

Once the aquatic macros were identified they were then placed into functional feeding groups and regional tolerance values (T) to organic pollutants (Barbour et al. 1999). Regional tolerance values were used to calculate the field biotic index (FBI) to distinguish water quality for each reach (Hilsenhoff 1988). The aquatic macro assessment was also used to determine diversity measures. Family and EPT diversity were measured by Shannon's H', which is an index of equitability among rare and common taxa (Peet 1974; Gurevitch et al. 2002).

Data Analysis**:**

Individual reaches were set as the sample unit (sample size $n = 40$ units), and a significance level ≤ 0.05. The program Minitab was used to conduct a two sample *t*-test of population means between western ($n = 20$) and eastern ($n = 20$) stream reach assessment scores. Assessment protocols and GL were left in their numerical scores for this analysis. A simple kappa coefficient was used to measure interrator agreement between assessment protocols and GL. Assessment protocols and GL were placed into their functional rating categories of sustainability/good-excellent, at risk/fair, and non-sustainable/poor. The functional rating categories were set at 3 for good condition, 2 for fair condition, and 1 for poor condition. When kappa is positive the observed agreement exceeds chance agreement, and its magnitude reflects the strength of the agreement (SAS/STAT 1999). If kappa is negative the observed agreement is less than the chance agreement. The test of symmetry ($Pr > S$) specifies if the agreement is similar between protocols. If $Pr > S$ are greater than α 0.05 then the agreement is considered to be similar. Simple linear regression models (SLRM) were used to distinguish between assessment protocols that best reflect aquatic macroinvertebrate diversity, richness, and tolerance/intolerance measures (R program). Classification and regression tree models (CART) were used to create a visual model to explain correlations between taxonomic presence/absence with environmental parameters and assessment protocols. CART was used to characterize abiotic relationships with aquatic macroinvertebrate taxa presence/absence. Two genera of Trichoptera were characterized, *Glossosoma sp*. (T = 0) and *Helicopsyche sp*. (T = 4). One genera of Ephemeroptera was characterized, *Callibaetis sp*. (T = 9); and the other taxa was the family Grammaridae (T = 4). These aquatic macroinvertebrates represent low, moderate, high water quality conditions.

Results

The results from the two sample t-test indicate that the SVAP was the only assessment protocol that differentiated between eastern and western stream reaches in the state of Montana (Table 1). All other protocols including the GL could not significantly distinguish between western and eastern provinces. Greenline did not significantly differ ($P = 0.07$) between eastern and western stream reaches, but eastern stream reach health scores were typically higher.

The simple kappa coefficients agreements between protocol ratings of reach condition are represented in Table 2. The PFC and NRCS assessment protocols had the only significant agreement for the health of stream reaches. However, the relationship between PFC and NRCS is not strong (*kappa* = 0.52), and there were some differences between functional ratings of 1 and 2. All other kappa coefficients resulted in non-similar agreements between protocol functional ratings of stream reach conditions.

Table 1. Two sample *t* test of assessment and Greenline scores between western and eastern stream reaches (n = 40)

Two Sample *t* test			
Protocol	Location	Mean Score ± SE	*P*
[1]PFC	West	68.4± 1.7	0.40
	East	75.0 ± 3.9	
[2]NRCS	West	76.1 ± 15.5	0.20
	East	72.5 ± 13.6	
[3]SVAP	West	7.11 ± 0.2	< 0.01
	East	4.99 ± 0.2	
[4]GL	West	6.85 ± 0.1	0.07
	East	7.30 ± 0.2	

[1]PFC = U.S. Lotic Wetland Health Assessment for Streams and Small Rivers (*Survey*); [2]NRCS = Riparian Assessment for Lotic Systems; [3]SVAP = Stream Visual Assessment Protocol; [4]GL = Greenline

The SVAP was the best predictor of aquatic biotic integrity and water quality across stream reaches (Table 3). The simple linear regression models for SVAP had the best fit with the highest R^2 and lowest residual variance throughout the data for EPT diversity (Fig. 1), richness, and FBI scores. The PFC assessment protocol did indicate a significant linear relationship ($P \leq 0.05$) with the response variables; however, the R^2 was low indicating a higher degree of unexplained variance within the models (Fig. 2). The NRCS assessment protocol and GL did not signify significant relationships and adequate R^2 with aquatic biotic integrity and water quality across stream reaches (Figs. 3–4).

Table 2. Kappa Coefficients agreement between NRCS, PFC, SVAP and GL ratings of stream reach condition

Kappa Coefficients						
Comparisons	Kappa	[1]95% CL (L)	[2]95% CL (U)	[3]O-S Pr > Z	[4]T-S Pr > ∣ Z ∣	[5]Pr > S
NRCS x PFC	0.52	0.31	0.73	< 0.01	< 0.01	0.15
NRCS x SVAP	- 0.11	- 0.29	0.07	NS	NS	0.01
NRCS x GL	- 0.15	- 0.35	0.06	NS	NS	< 0.01
PFC x SVAP	0.21	0.002	0.41	0.02	0.03	< 0.01
PFC x GL	- 0.21	- 0.44	0.02	0.03	NS	0.03
SVAP x GL	- 0.11	- 0.3	0.05	NS	NS	< 0.01

[1] 95% CL (L) = Lower Confidence Limit; [2] 95% CL (U) = Upper Confidence Limit; [3] O–S Pr > Z = One-Sided Probability > Z-test; [4] T–S Pr > Z = Two-Sided Probability > Z-test; [5] Pr > S = Probability > Statistic

Table 3. SLRM of assessment protocols and GL scores for all streams in correlation with EPT diversity, richness, and FBI

	Simple Linear Regression Relationships					
	[1]EPT Diversity		[2]EPT Richness		[3]FBI	
Protocol	*P*	R^2	*P*	R^2	*P*	R^2
[4]PFC	< 0.01	0.34	< 0.01	0.22	< 0.01	0.29
[5]NRCS	NS	0.02	NS	0.01	NS	0.02
[6]SVAP	< 0.01	0.75	< 0.01	0.87	< 0.01	0.80
[7]GL	NS	0.02	NS	0.04	NS	0.03

[1]EPT Diversity = Ephemeroptera–Plecoptera–Trichoptera Diversity; [2]EPT Richness = Ephemeroptera–Plecoptera–Trichoptera Richness; [3]FBI = Field Biotic Assessment; [4]PFC = U.S. Lotic Wetland Health Assessment for Streams and Small Rivers (*Survey*); [5]NRCS = Riparian Assessment for Lotic Systems; [6]SVAP = Stream Visual Assessment Protocol; [7]GL = Greenline

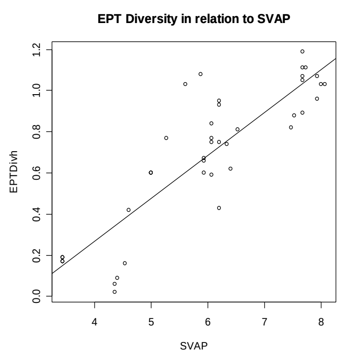


Figure 1. SLRM of EPT diversity relationship with SVAP scores.

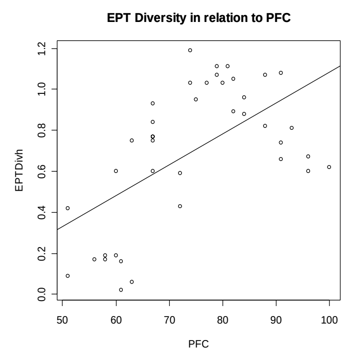


Figure 2. SLRM of EPT diversity relationship with PFC scores.

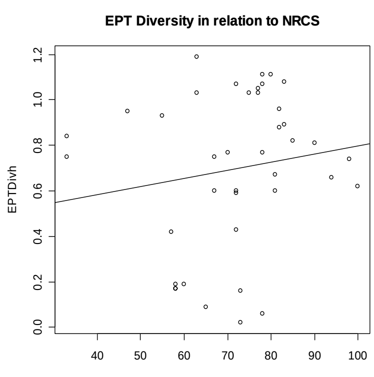


Figure 3. SLRM of EPT diversity relationship with NRCS scores.

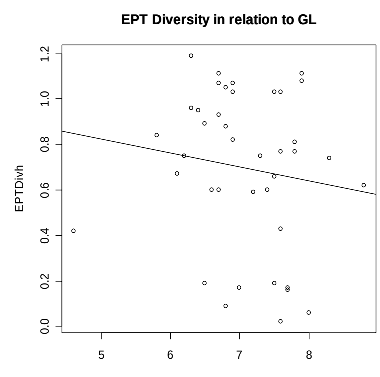
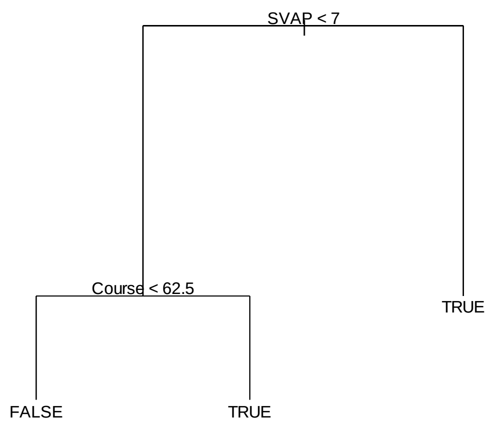


Figure 4. SLRM of EPT diversity relationship with GL scores.

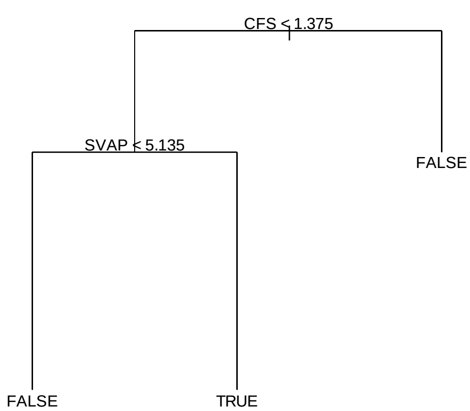
Results from the CART models suggest that substrate composition, CFS, habitat units, and SVAP reflected correlations with presence/absence of the selected taxa. The genus *Glossosoma sp*. has a strong correlation with SVAP and percent course material (Fig. 5). This genus was present in all stream reaches where SVAP was > 7. In reaches where SVAP was < 7 the proportion of course substrate material > 62.5% determined the presence of *Glossosoma sp*. This genus correlates with streams that have higher water quality, riparian health, and a high proportion of course substrate material.



Number of terminal nodes: 3
Residual mean deviance: 0.2997 = 11.09 / 37
Misclassification error rate: 0.1 = 4 / 40
1) root 40 53.84 FALSE (0.6000 0.4000)
  2) SVAP < 7 28 22.97 FALSE (0.8571 0.1429)
    4) Course < 62.5 20 0.00 FALSE (1.0000 0.0000) *
    5) Course > 62.5 8 11.09 TRUE (0.5000 0.5000) *
  3) SVAP > 7 12 0.00 TRUE (0.0000 1.0000) *

Figure 5. Richness of the genus *Glossosoma sp*. correlated with SVAP and percent course stream bed material.

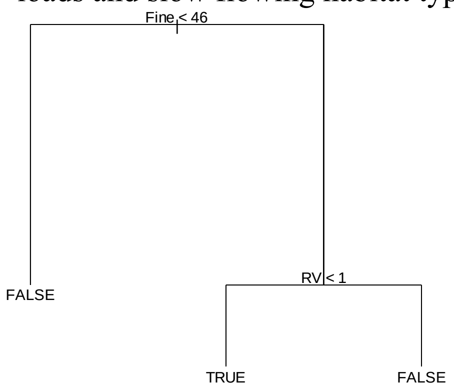
The genus *Helicopsyche sp*. has a strong correlation with CFS and SVAP in its distribution patterns (Fig 6). CFS > 1.375 *Helicopsyche sp*. was absent, which correlated with all western streams except Lower Nevada Cr. The next determining factor for presence/absence was the SVAP rating. Reach ratings < 5.135 indicated this genus's absence. Presence and absence of *Helicopsyche sp*. correlates with streams that may be considered as moderate to fair water quality and habitat conditions.



Number of terminal nodes: 3
Residual mean deviance: 0.2056 = 7.606 / 37
Misclassification error rate: 0.025 = 1 / 40
1) root 40 53.840 FALSE (0.60000 0.40000)
  2) CFS < 1.375 28 38.240 TRUE (0.42857 0.57143)
    4) SVAP < 5.135 11 0.000 FALSE (1.00000 0.00000) *
    5) SVAP > 5.135 17 7.606 TRUE (0.05882 0.94118) *
  3) CFS > 1.375 12 0.000 FALSE (1.00000 0.00000) *

Figure 6. Richness of the genus *Helicopsyche sp*. correlated with CFS and SVAP.

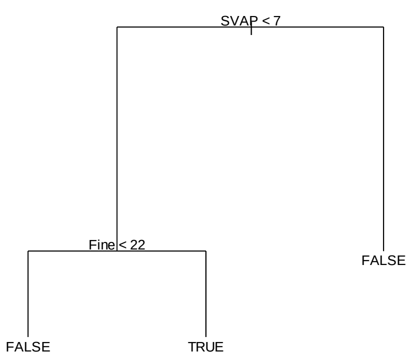
The genus *Callibaetis sp*. has a strong correlation with percent fines and riffle habitat types (Fig. 7). This genus was absent from reaches with percent fines < 46%. The next environmental parameter that best explains presence and absence of this genus was riffle volume (RV < 1 m^3 per reach). The presence of this genus indicates streams that have high sediment loads and slow flowing habitat types such as pools and glides.



Number of terminal nodes: 3
Residual mean deviance: 0.2861 = 10.59 / 37
Misclassification error rate: 0.075 = 3 / 40
1) root 40 42.65 FALSE (0.7750 0.2250)
  2) Fine < 46 26 0.00 FALSE (1.0000 0.0000) *
  3) Fine > 46 14 18.25 TRUE (0.3571 0.6429)
    6) RV < 1 6 0.00 TRUE (0.0000 1.0000) *
    7) RV > 1 8 10.59 FALSE (0.6250 0.3750) *

Figure 7. Richness of the genus *Callibaetis sp*. correlated with percent fines and riffle volume (RV)

The family Grammaridae has a strong correlation with SVAP and percent fines (Fig. 8). This family was not present on reaches with SVAP ratings < 7, which accounts for three of the western streams, South Boulder, South Willow, and Cottonwood. For reaches with SVAP < 7 this family was present only on reaches where percent fines were > 22%, which excluded all western streams except Lower Nevada Cr. and all eastern streams. Grammaridae presence correlates with fair to poor water conditions in the reaches measured across the state of Montana.



Number of terminal nodes: 3
Residual mean deviance: 0.2997 = 11.09 / 37
Misclassification error rate: 0.1 = 4 / 40
1) root 40 53.84 TRUE (0.4000 0.6000)
  2) SVAP < 7 28 22.97 TRUE (0.1429 0.8571)
  3) SVAP > 7 12 0.00 FALSE (1.0000 0.0000) *
  4) Fine < 22 8 11.09 FALSE (0.5000 0.5000) *
  5) Fine > 22 20 0.00 TRUE (0.0000 1.0000) *

Figure 8. Richness of the family Grammaridae correlated with SVAP and percent fines.

Discussion

The SVAP was the only assessment protocol that distinguished between streams in western and eastern geological provinces. SVAP reach scores were higher on western streams indicating substantial physical differences among instream characteristics between the provinces. Dissimilarities amongst protocols and GL agreement of reach ratings were evident in the results, and only PFC and NRCS protocols had a significant relationship with a moderate kappa coefficient. It would make sense that PFC and NRCS are similar because both methods emphasize and evaluate similar characteristics within a stream system. A similar relationship was also found by Ward et al. (2003). They found that the SVAP and Habitat Assessment Field Data Sheet (HAFDS), which target similar parameters resulted in a strong positive correlation (r = 0.81). The original version of PFC which focuses more on hydrologic functions had a weak correlation with both the SVAP and HAFDS (r = 0.58 and 0.54). However, Whitacre (2004) found means among protocols for 8 of the 10 physical attributes evaluated differed ($P<0.05$) across three Oregon and three Idaho streams when comparing results from three other riparian assessment methods; the Aquatic and Riparian Effectiveness Monitoring Program (AREMP), the Environmental Monitoring and Assessment Program (EMAP) and PACFISH/INFISH Effectiveness Monitoring Program (PIBO). Not only is there a weak agreement between protocols, but potential variability amongst observer evaluations of stream and riparian condition exists (Roper et al. 2004). Coles-Ritchie et al. (2004) found high variability among observers when conducting greenline (Winward 2000) surveys on different reaches with different community types and stability conditions. They found that the mean agreement for all observers was 38%, and the maximum and minimum 49% and 29%. Hannaford and Resh (1995) found that individual riparian site assessments varied considerably among college student groups. Thus is must be assumed that differences may have occurred between the different ID teams that evaluated stream reaches in this study. Observations by Miller suggest that differences in familiarity with the various assessment methods and riparian monitoring experience among the various BLM and NRCS teams could have contributed to the variation in stream reach scores across Montana.

The differences found in assessment protocols are not only reflective of the variability in stream reach condition and observer experience, but also in the various methods' ability to reflect aquatic macroinvertebrate diversity, richness, and water quality. The data collected in this study suggests that SVAP was the only assessment protocol that had a significant and strong linear relation with these three instream parameters. SVAP best exemplifies instream conditions because it takes into consideration not only vegetative and hydrologic characteristics, but also substrate composition, instream habitat types, water clarity, and aquatic macrophyte production. In other words SVAP evaluates parameters suggested by Resh et al. (1995) that capture the stream's ability to influence aquatic biotic integrity. Furthermore assessment protocols that reflect aquatic macroinvertebrate assemblages would indicate direct responses to changes in water quality, chemistry, and geological regions (Resh et al. 1995).

Environmental parameters that have been found to have some of most significant relationships to aquatic macroinvertebrate assemblages are substrate composition and annual stream flow (Allan 1995; Scarsbrook 2002). A study by Beisel et al. (1998) measured seven environmental parameters in northeastern France, and found substrate to be the dominant factor that influenced the community structure of aquatic taxa, and found that current velocity and water depth were secondary factors. Substrate composed of medium particle sizes such as gravel and cobble generally increases the abundance and richness of benthic invertebrates while excessive sediment is considered a pollutant in streams and can have negative affects on aquatic biota (Waters 1995). Nerbonne and Vondracek (2001) found that percent fines and embeddedness of the substratum were negatively correlated with aquatic macroinvertebrate assemblages. These findings related to substratum composition and aquatic macroinvertebrates are similar to the stream characteristics identified as being important in this study suggesting that western stream reaches typically had fewer fines, greater CFS, presence of taxa with a low tolerance to pollution and higher EPT diversity and richness measures. Eastern stream reaches typically had greater proportions of fines, lower CFS, presence of taxa that are moderate to highly tolerant of pollution and have lower EPT diversity and richness measures.

While PFC and NRCS may give valuable information on proper functioning condition and sustainability of flood plain communities throughout Montana they appear weak in their ability to reflect water quality and aquatic biotic integrity because they do not include information on substrate composition. The SVAP, which includes a more detailed evaluation of instream characteristics such as substrate composition, provides a stronger indirect measure of water quality and instream habitat conditions than does PFC, NRCS or GL. The components within the SVAP and the results of this study correspond with relationships reported from other studies in the United States and France.

Implications

The SVAP is an example of an assessment protocol that produces a floodplain/riparian vegetation status score that is more indicative of instream habitat conditions and water quality than the other developed protocols such as PFC and NRCS. However, our results also suggest that the PFC and NRCS protocols are robust enough to be used to assess riparian status across a broad range of physiographic conditions without introducing too much bias into the outcome. Nonetheless, it is clear that high PFC or NRCS scores do not automatically imply high water quality nor diverse macroinvertebrate populations. Management goals for high water quality and enhanced cold-water fisheries would be best supported by assessment and monitoring efforts using SVAP or other protocols that assess stream substrate characteristics. Use of the PFC, NRCS, or GL with the SVAP would provide a more in-depth evaluation of riparian function and processes than can be achieved with a single methodology. The integration of both SVAP and NRCS for example would result in little additional effort and cost when applied to a stream reach, and would provide a better understanding of the aquatic and terrestrial conditions within that stream system.

Acknowledgments

Dave and Peggy Mannix (lower Nevada Cr.), Melanie Mannix (upper Nevada Cr.), Bill and Rhoda Powell (South Boulder Cr.), Tim and Renee Stephens (South Willow Cr.), Doug Ensign(Mission Spring Cr.), Roger Indreland (Little Spring Cr.), Tom Andres (Otter Cr.), and Basin Angus Ranch (Louse Cr.) provided access to private lands. Pete Husby, Joe Fidel, Tim Griffiths, and Kate Cooper (NRCS Bozeman State Office); Glen Green, Michelle Gorder, John Bowe (Deer Lodge NRCS); Chuck Roloff (Big Timber NRCS); Stephanie Sever (Forsyth NRCS), Kathy Knobloch (Lame Deer NRCS), and Chad Crause (Lewistown NRCS) provided assistants for stream reach assessments. Funding was provided by the Bozeman Montana Water Center, Montana BLM Butte Area Office, and the Montana State University Agriculture Experiment Station.

Literature Cited

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, 2nd edition. *EPA 841-B-99-002*. US Environment Protection Agency; Office of Water; Washington, D.C.

Beisel, J.N., P. Usseglio-Polatera, S. Thomas, and J. C. Moreteau. 1998. Stream community structure in relation to spatial variation: the influence of mesohabitat characteristics. *Hydrobiologia* 389:73-88.

Bollman, Wease. 2002. An Assessment of Ward Creek: Habitat and aquatic invertebrate assemblages. Rhithron Associates, Inc. Missoula, Montana.

Coles-Ritchie, M. C., R. C. Henderson, E. K. Archer, C. Kennedy, and J. L. Kershner. 2004. Repeatability of riparian vegetation sampling methods: how useful are these techniques for broad-scale, long-term monitoring? *Gen. Tech. Rep. RMRS-GTR-138*. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. 18 p.

Fleming, W., D. Galt, and J Holechek. 2001. 10 Steps to evaluate rangeland riparian health. *Rangelands* 23(6):22-27.

Gurevitch, J., S. M. Scheiner, and G. A. Fox. 2000. The ecology of plants. Sinauer Associates, Inc., Publishers: Sunderland, Massachusetts United States.

Hannaford, M. J. and V. H. Resh. 1995. Variability in macroinvertebrate rapid-bioassessment surveys and habitat assessments in northern California stream. *Journal of the North American Benthological Society* 14(3):430-439.

Hilsenhoff, W.L. 1988. Rapid field assessment of organic pollution with a family-level biotic index. *Journal of the North American Benthological Society* 7:65-68.

Merritt, R.W. and K.W. Cummuns. 1996. An introduction to the aquatic insects of north america: 3rd edition. Kendall/Hunt Publishing Company.

Naiman, R. J., R. E. Bilby, and P. A. Bisson. 2003. Riparian ecology and management in the pacific coastal rain forest. *BioScience* 50(11):996-1011.

Nerbonne, B. A. and B. Vondracek. 2001. Effects of Local Land Use on Physical Habitat, Benthic Macroinvertebrates, and Fish in the Whitewater River, Minnesota, USA. *Environmental Management* 28(1):87-99.

Overton, C.K., S.P. Wollrab, B.C. Roberts, and M.A. Radko. 1997. R1/R4 (Northern/Intermountain Regions) Fish and Fish Habitat Standard Inventory Procedures. *Gen. Tech. Rep. INT-GTR-346*. Intermountain Research Station, Ogden, UT.

Peet, R. K. 1974. The Measurement of Species Diversity. *Ann. Rev. Ecol. System*, 5:285-307.
Prichard, D. 1998. Riparian Area Management: A User Guide to Assessing Proper Functioning Condition and the Supporting Science for Lotic Areas. *Tech. Ref. 1737-15*. National Applied Resource Sciences Center, Denver, CO.

Resh, V. H., R. H. Norris, and M. T. Barbour. 1995. Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. *Australian Journal of Ecology* 20:108-121.

Rosgen, D. 1996. Applied River Morphology: 2nd edition. Hilton Lee Silvery. Pagosa Springs, Colorado.

Scarsbrook, M. R. 2002. Persistence and stability of lotic invertebrate communities in New Zealand. *Freshwater Biology* 47:417-431.

SAS Institute Inc., SAS/STAT® *User's Guide, Version 8,* Cary, NC: SAS Institute Inc., 1999. 3884 p.1255-1312.

Southerland, M. T. and J. B. Stribling. 1995. Status of biological criteria development and implementation. *In:* W.S. Davis & T. P. Simon [EDS.]. Biological Assessment and Criteria: Tools for Water Resource Planning and Decision-making. Lewis Publishers, Chelsea, MI.

USDI, BLM. 2003. *U.S. Lotic Wetland Health Assessment for Streams and Small Rivers (Survey) User Manual. Available at:* www.bitterrootrestoration.com. *Accessed 22 February 2005.*

USDA, NRCS. 1998. Stream visual assessment protocol. *NWCC-TN-99-1*. National Water and Climate Center, Portland, Oregon.

USDA, NRCS. 2004a. ECS-environmental-riparian assessment. *TN-MT-24, 190-VI*.

USDA, NRCS. 2004b. *Soil Data Mart. Available at:* http://soildatamart.nrcs.usda.gov. *Accessed 16 March 2005.*

Ward, T. A., K. W. Tate, E. R. Atwill, D. F. Lile, D. L. Lancaster, N. McDougald, S. Barry, R. S. Ingram, H. A. George, W. Jensen, W. E. Frost, R. Phillips, G. G. Markegard, and S. Larson. 2003. A comparison of three visual assessments for riparian and stream health. *Journal of Soil and Water Conservation* 58: 83-88.

Wiggins, G. B. 1996. Larvae of the North American Caddisfly Genera (Trichoptera): 2nd Edition. University of Toronto Press.

Winward, Alma H. 2000. Monitoring the vegetation resources in riparian areas. *Gen. Tech. Rep. RMRS-GTR-47*. Ogden, UT: U.S. Department of Agriculture, Forest Service, and Rocky Mountain Research Station.

Wolman, M.G. 1954. A method of sampling coarse river-bed material. *Eos. Trans. AGU* 35: 951-956.

WRCC (Western Regional Climate Center). 1999. *Pony, Montana climate summary. Available at:* http://www.wrcc.dri.edu/summary/climsmmt.html. *Accessed 18 March 2005.*

WRCC (Western Regional Climate Center). 2001. *Birney, Montana climate summary. Available at:* http://www.wrcc.dri.edu/summary/climsmmt.html. *Accessed 18 March 2005.*

WRCC (Western Regional Climate Center). 2004a. *Bigtimber, Montana climate summary. Available at:* http://www.wrcc.dri.edu/summary/climsmmt.html. *Accessed 18 March 2005.*

WRCC (Western Regional Climate Center). 2004b. *Busby, Montana climate summary. Available at:* http://www.wrcc.dri.edu/summary/climsmmt.html. *Accessed 18 March 2005.*

WRCC (Western Regional Climate Center). 2004c. *Lincoln Ranger STN, Montana climate summary. Available at:* http://www.wrcc.dri.edu/summary/climsmmt.html. *Accessed 18 March 2005.*

WRCC (Western Regional Climate Center). 2004d. *Livingston, Montana climate summary. Available at:* http://www.wrcc.dri.edu/summary/climsmmt.html. *Accessed 18 March 2005.*

WRCC (Western Regional Climate Center). 2004e. *Moccasin, Montana climate summary. Available at:* http://www.wrcc.dri.edu/summary/climsmmt.html. *Accessed 18 March 2005.*

WRCC (Western Regional Climate Center). 2004f. *Seeley Lake, Montana climate summary. Available at:* http://www.wrcc.dri.edu/summary/climsmmt.html. *Accessed 18 March 2005.*

Defining river recharge and three-dimensional areas of contribution to production wells adjacent to a losing river, Western Montana

Basic Information

Title:	Defining river recharge and three-dimensional areas of contribution to production wells adjacent to a losing river, Western Montana
Project Number:	2004MT31B
Start Date:	3/1/2004
End Date:	11/30/2005
Funding Source:	104B
Congressional District:	At large
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Hydrology, Water Use
Descriptors:	river recharge, groundwater aquifer
Principal Investigators:	William W. Woessner

Publication

1. Cook, R.C.. A.A. Tallman and W.W. Woessner. 2004. Preliminary results for defining river recharge and the fate of arsenic in the shallow groundwater system adjacent to a losing river, western Montana. Center for Riverine Science and Stream Renaturalization Conference Proceedings. Univeristy of Montana, Missoula.
2. Tallman, A.A., R.C. Cook and W.W. Woessner. 2004. Preliminary results of a capture zone study on bank-side production wells and their interaction with a perched river in an alluvial aquifer, western Montana. Center for Riverine Studies and Stream Renaturalization Conference Proceedings. University of Montana, Missoula.
3. Tallman, A.A., R.C. Cook and W.W. Woessner. 2005. Identifying the factors controlling the sources and quantity of water captured by municipal supply wells in the highly conductive Missoula aquifer, western Montana. National Ground Water Association Ground Water Summit.

ABSTRACT:

The Clark Fork River in Missoula MT has been reported to provide 50 to 80 % of the recharge to the Sole Source Missoula Aquifer that serves over 60,000 Missoula area residents. If this principle recharge source to the unconfined aquifer becomes contaminated, water from high yield municipal wells may be at risk. This work is addressing the zones of contribution and source water quality. This includes characterization of stream stage, streambed temperature gradients, streambed vertical gradients, and streambed hydraulic conductivity as well as water level trends, the distribution of aquifer hydraulic conductivity, and both vertical and horizontal gradients. These parameters along with sets of geochemical data are being used to produce and calibrate a three-dimensional transient ground water flow model that examines the timing, quantities and sources of water to riverside production wells. The preliminary conceptual model suggests the river is perched 5 to 16 ft above the aquifer and is losing water to the aquifer. The wells derive about 90.5% of their water from river recharge and approximately 7% from underflow originating from an up gradient canyon. These values are supported by vertical leakage rates computed from head and in-stream temperature profiles and discharge estimates computed using general aquifer properties. The results are also supported by general chemistry and stable isotope data. Numerical modeling will be used to evaluate the current conceptual model and test additional representations as new data are generated.

OBJECTIVES:

The purpose of this study is two-fold; 1) to determine the capture zone for municipal wells near the Clark Fork River based on transient modeling of the aquifer system and 2) to investigate the fate and transport of arsenic in the Clark Fork River – Missoula Aquifer system.

METHODS:

Thirty five groundwater and surface water sites are monitored. Water chemistry is monitored at 22 sites including 4 production wells 15 monitoring wells, 3 surface water locations, and one streambed peizometer. Water levels are monitored at 35 sites, 3 production wells, 28 monitoring wells and 4 surface water sites (Figure 1.).

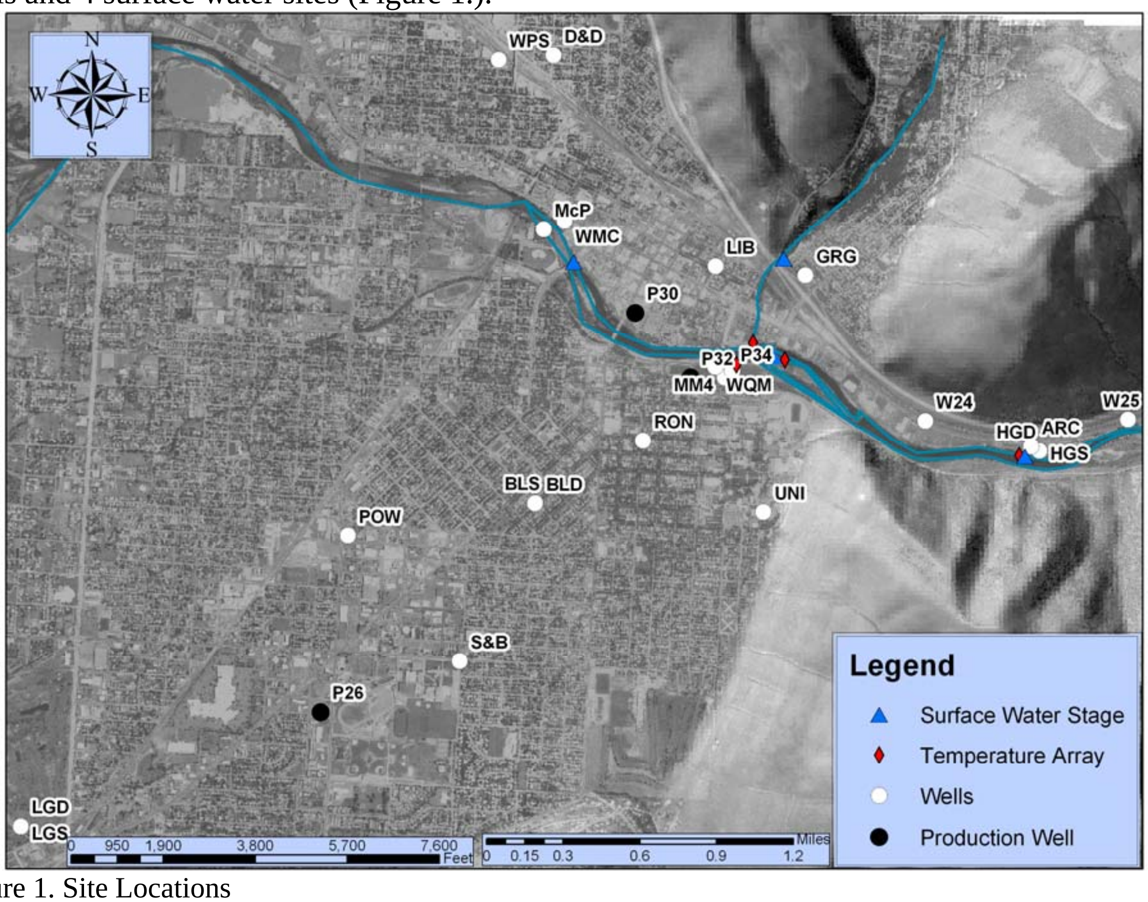


Figure 1. Site Locations

CHEMISTRY METHODS

Sample Sites: Nineteen wells (four deep production wells and 15 monitoring wells), and three surface water sites (Clark Fork River in two sites, Rattlesnake Creek), were sampled weekly from May the sites will be sampled three times a month.   to August, biweekly until October, then monthly through February. From March through June

Field Methods: Field geochemical parameters (pH, temperature and electric conductivity) were measured at each sampling site. Monitoring wells were pumped (using a Grundflos pump) until three bore-hole volumes had been removed. A new disposable polyethylene bailer was then used to collect enough water to rinse the bottle and obtain a sample. Production wells were sampled from a spigot before any treatment. The Clark Fork River and Rattlesnake Creek were sampled by wading out into moving water and sampling at a range of depths to obtain a representative sample. During each round of sampling field duplicates and blanks were taken for quality assurance and quality control.

All samples were collected using ultra clean techniques to reduce the possibility of contamination (after Mickey, 1998 and MBEL Sampling Method #2). This process involved using ultra cleaned 120 mL nalgene bottles that were doubled-bagged and handled by one set of “clean hands” and one set of “dirty hands.” “Dirty hands” only touched the external bag, while “clean hands” only touched the internal bag and sample bottles. Each bottle was rinsed with sample water three times. Surface water samples were collected underwater, and samples from wells were filled to overflowing and capped so that there was minimal head space. “Clean hands” placed the sample in an individual zip-lock bag which was then placed in an open external bag. “Dirty hands” sealed the external bag. Additional samples for anions were collected in non-acid washed bottles. These samples were collected after the ultra clean samples since detection levels for anions are much higher and the same precautions do not need to be taken. All samples were stored on ice.

Lab Methods: Samples were filtered in lab as soon as possible (and no longer then 48 hours after sampling) using ultra clean syringes and <0.45 μm syringe filters (MBEL SOP 2004_06_21 Tallman) All filtering took place in a hood (MBEL McKinnon and Nagorski, 2000). New ultra clean 30 mL bottles were rinsed and filled, then acidified with 2% HNO_3 for preservation. Anion samples were filtered after the respective ultra clean samples, using the same filter but first rinsing the syringe with the anion sample. Isotope samples were not filtered; instead a 30 mL bottle was filled to an inverted meniscus and capped to incorporate as little air as possible. Isotopes were analyzed at The University of Alaska Fairbanks by the Water and Environmental Research Center’s Stable Isotopes Facility. All other analyses were performed at The University of Montana. The ultra clean samples were analyzed for As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, and Zn using MEBL EPA6020mod method ICP-MS analysis for metals. Alkalinity was determined in mg/L of $CaCO_3$ using titration, and F, Cl, NO_3, NO_2 and SO_4 were determined using Ion Chromatography.

Quality control/quality assurance: Field duplicates and blanks, along with lab splits are used with internal and external standards to ensure precision and accuracy. From the field duplicates and lab splits a 95% confidence level was calculated for each result from MS, IC and Isotope analyses.

MODELING METHODS

Field and Analytical Methods:

The geologic and hydrogeologic setting was characterized with a detailed review of the literature, interpretation of well logs, core data, geologic studies, drillers' data, consultant reports, and the drilling of four additional monitoring wells. Well logs were utilized to create lithologic cross sections of the Missoula Aquifer.

The water table was monitored through a network of 29 wells established along the river near production wells and extending down groundwater gradient. Two production wells and five monitoring wells were instrumented with Solinst Leveloggers, recording water level and temperature data on an hourly basis. Twenty-nine wells located along the CFR and down gradient were measured for water level every two weeks through the peak of the hydro period and then on a monthly basis, using electric tape measurements to the top of casing.

Surface water stage measurements were collected at three sites along the CFR and at one site on Rattlesnake Creek. Stage monitoring sites along the CFR included a staff gauge in Hellgate Canyon, a mini-stilling well fitted with a Solinst Levelogger below the walking bridge and a bridge-to-water measurement site at Orange St. Bridge. The Rattlesnake Creek stage was monitored via a bridge-to-water measurement site on Railroad St. Bridge. Supplementary river flow measurements were collected from the upper and lower USGS gauging sites.

Fluxes through the streambed were obtained using temperature trends and from stream discharge measurements. The river, Rattlesnake Creek, and the irrigation ditch were instrumented with multilevel temperature recording sandpoints. Temperatures were recorded on an hourly basis by a series of temperature i-buttons at one foot intervals to a depth of three feet below the river surface (Johnson et. al., 2005). Hydraulic conductivities and fluxes were estimated for the streambed by calibrating a one-dimensional heat transport model using VS2DHI, to observed temperature trends (Bartilino and Niswonger, 1999, Constantz 1996 &1998, Constantz et al., 2003, Hsieh et. al., 2000, Ronan et al., 1998). Streambed fluxes were also calculated from Stream discharge measurements performed at four transects along the CFR. The gaging was done in March utilizing a SonTek Acoustic Doppler Profiler RiverCAT (Sloat, 2003). Two additional stream discharge measurements were taken along Rattlesnake Creek utilizing a SonTek Acoustic Doppler Flowmeter.

Further, aquifer characterization to derive hydrogeologic properties and hydrogeologic boundaries included pumping and slug tests. Three pump tests were executed, pumping the Arthur St. well *(P32)* and monitoring both the Madison Street well *(P34)* and well MM4. Each test was run for 6 hours, pumping at a rate of 3500 gpm, water levels were recorded at 10 second intervals. The pump tests were analyzed with the Neuman method (Fetter, 1994). Hydraulic conductivity of the aquifer was estimated at four additional sites based on pneumatic slug tests utilizing a Geoprobe Pneumatic Slug Test Kit, with adaptations to fit various well casing sizes (Geoprobe Systems, 2002). The pneumatic slug tests were analyzed with a high K Bouwer and Rice model (Butler and Garnett, 2000). The hydraulic conductivity of the streambed was also estimated at four sites via falling head tests. A minimum of three falling head tests were performed at each site. A steel peizometer was installed in the stream bed and fitted with a 2.5 ft falling head cell, marked at 4 inch intervals. Time was recorded as the water level dropped to each successive interval. The streambed falling head tests were analyzed with the Bouwer and Rice equation (Bouwer, 1989 & Fetter, 1994).

The water budget, geology, and hydrogeology of the system were compiled into a conceptual model of the aquifer. A three-dimensional transient numerical model was designed with Ground Water Vistas modeling program. The model encompasses approximately five square miles, discretized in 150 X 150 ft grid spacing. It is unconfined with an approximate thickness of 250 ft. The model is three dimensional, with seven layers, the depth and thickness of these layers are based on well screen locations and on the silty sand layer lying at approximately 100 ft below the surface (Figure 2). Hydraulic conductivities have been set based on my conductivity tests and the tests of other local studies. The model was calibrated to observed head data and river flux determined from stream gaging and temperature trends.

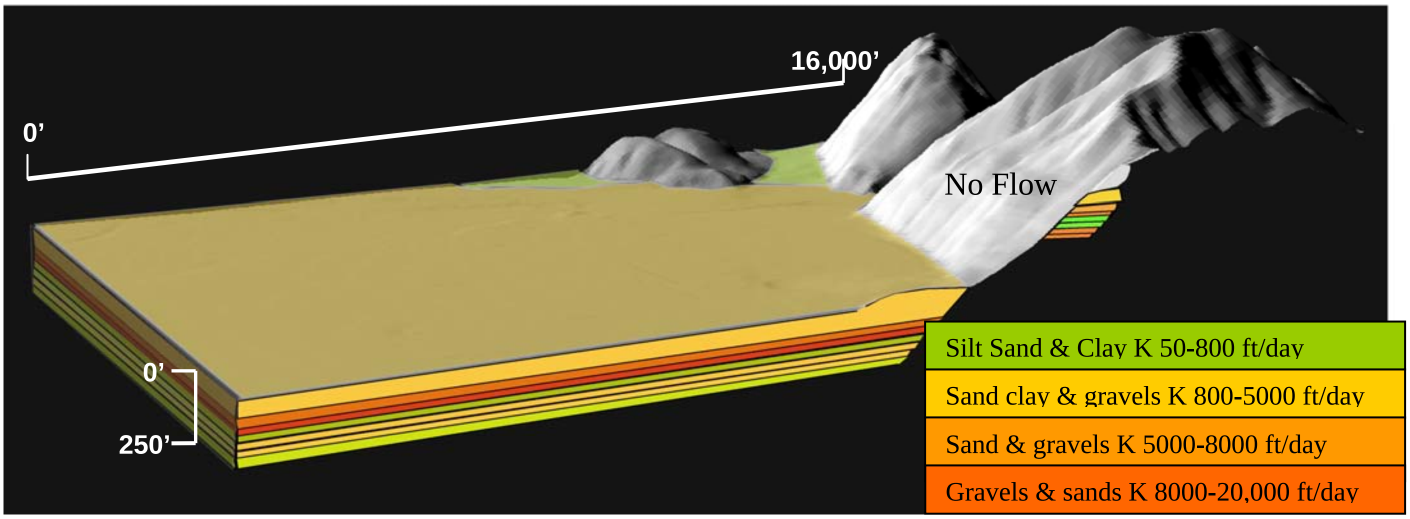


Figure 2. Generalized 3-D model setup

PRELIMINARY RESULTS:

CHEMISTRY RESULTS

Isotopes: The isotope data to date shows that there is a strong connection between the Clark Fork River and groundwater (Figure 3). The slight differences between the groundwater and the river water suggest that some other sources (regional precipitation, springs, and/or regional groundwater) are also contributing water to the Missoula aquifer. However, the isotopic signal of the groundwater appears to be driven primarily by the chemistry of the Clark Fork. Sampling will continue through June in order to catch a runoff event with a distinct isotopic signal.

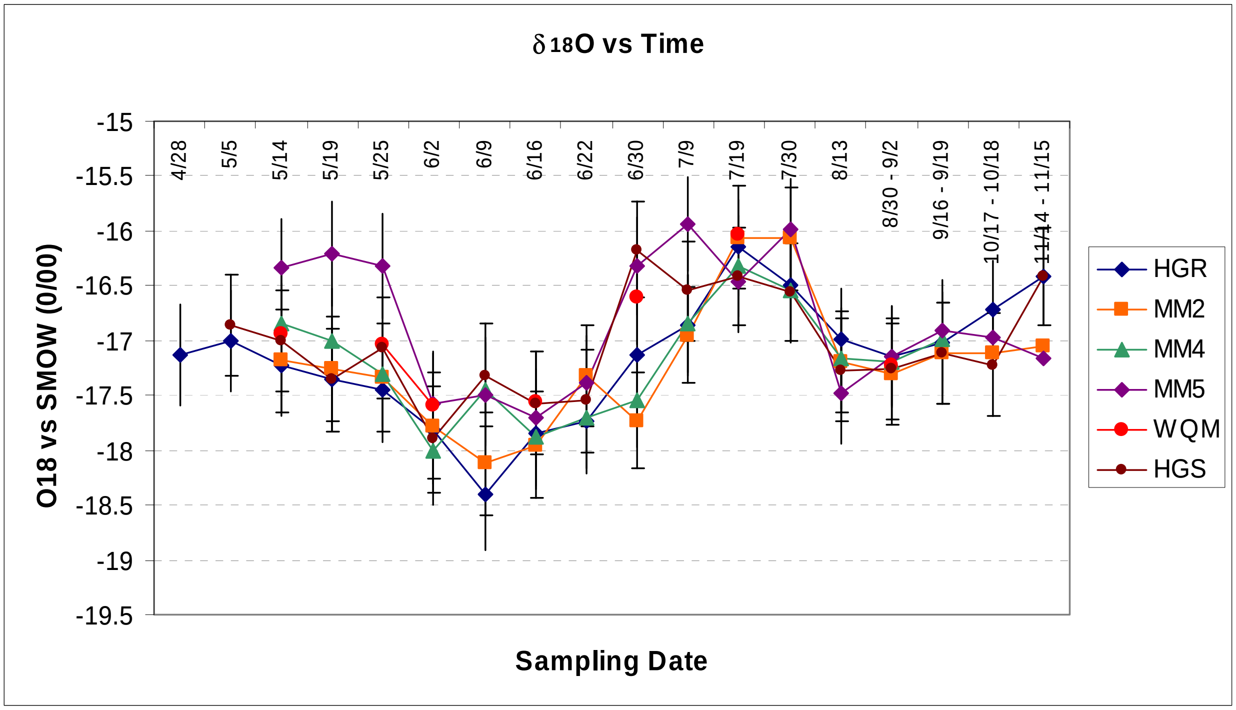


Figure 3. The Clark Fork River (HGR) controls the isotopic signal of groundwater. MM2, MM4, MM5, WQM and HGS are all monitoring wells near the river. Similar trends are found for distal wells.

General Chemistry: Most elements (with the exception of Cu) behave conservatively in the river, and concentrations are controlled by discharge. River discharge does not appear to control the concentrations found in groundwater samples. This is most likely due to chemical and physical reactions taking place in the vadose zone and/or in the aquifer. Plotting all of the sample sites on a piper diagram (Figure 4) illustrates the similarity among water types.

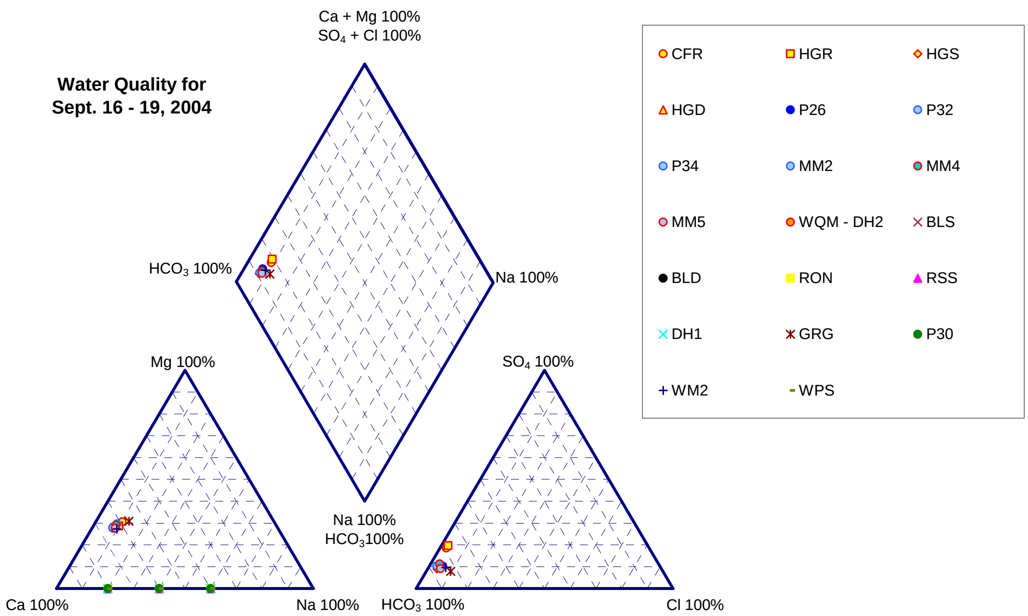


Figure 4. All water types plot in the same general area, making distinction between the river and groundwater difficult.

Arsenic: The fate of arsenic in the system is very interesting. Most of the year, it appears as though As is lost to the aquifer or vadose zone. This happens when there is a higher concentration of As in the river than in the groundwater (Figure 5). There are other times, when As values are higher in the groundwater than the river, when excess As is released from the aquifer system to the groundwater (Figure 5).

Milltown Reservoir was lowered approximately 8 feet during July, and was held at a low stand until the middle of August. Samples taken every three days during that period at both a shallow monitoring well and a production well show an immediate response to the increased concentration of arsenic in the river (Figure 6). While the level of arsenic in the river has remained high, concentrations in the wells declined to levels similar to pre-drawdown conditions (Figure 7).

In general, the wells farther from the river have lower As concentrations, and the lowest concentrations are in wells on the north side of the river (Figure8). Rattlesnake creek has very low concentrations of arsenic and is therefore probably diluting the groundwater on the north side of the river.

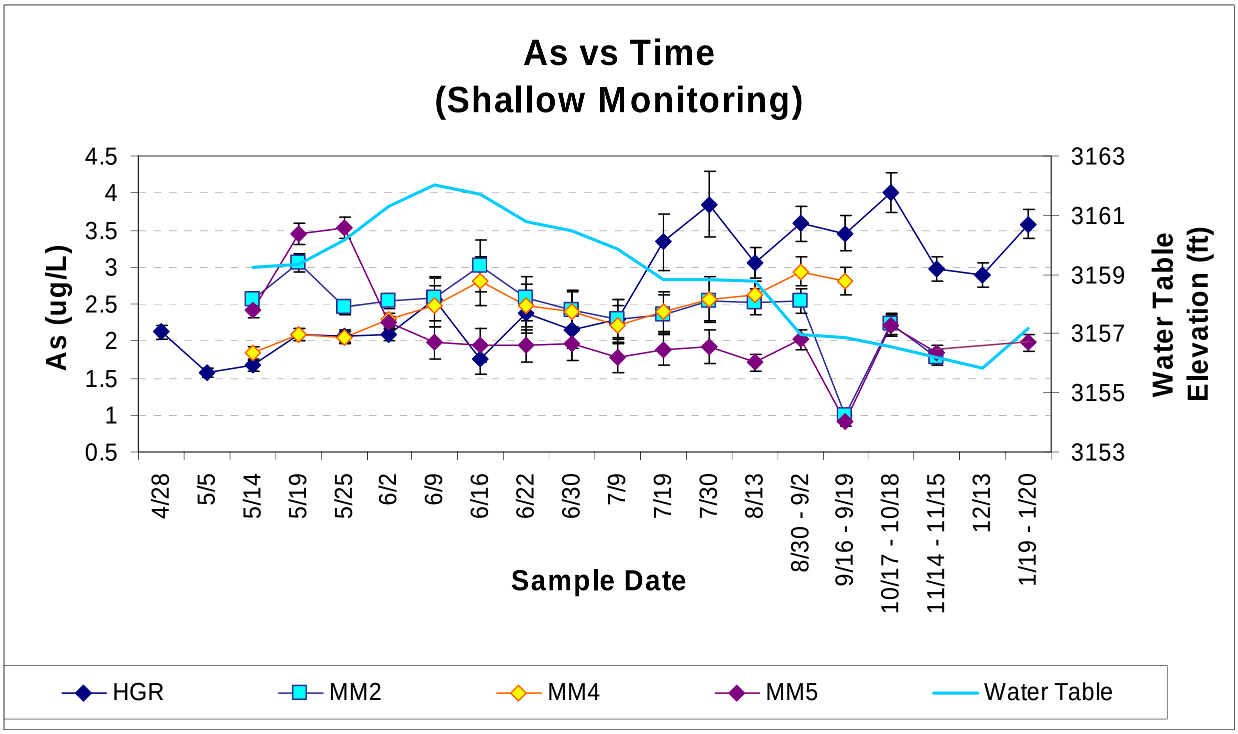


Figure 5. Note that when the water table is high (May through July), As in the monitoring wells (MM2 and MM4) is higher than in the river (HGR). After the Milltown drawdown (7/19) the river had higher arsenic values than any of the monitoring wells.

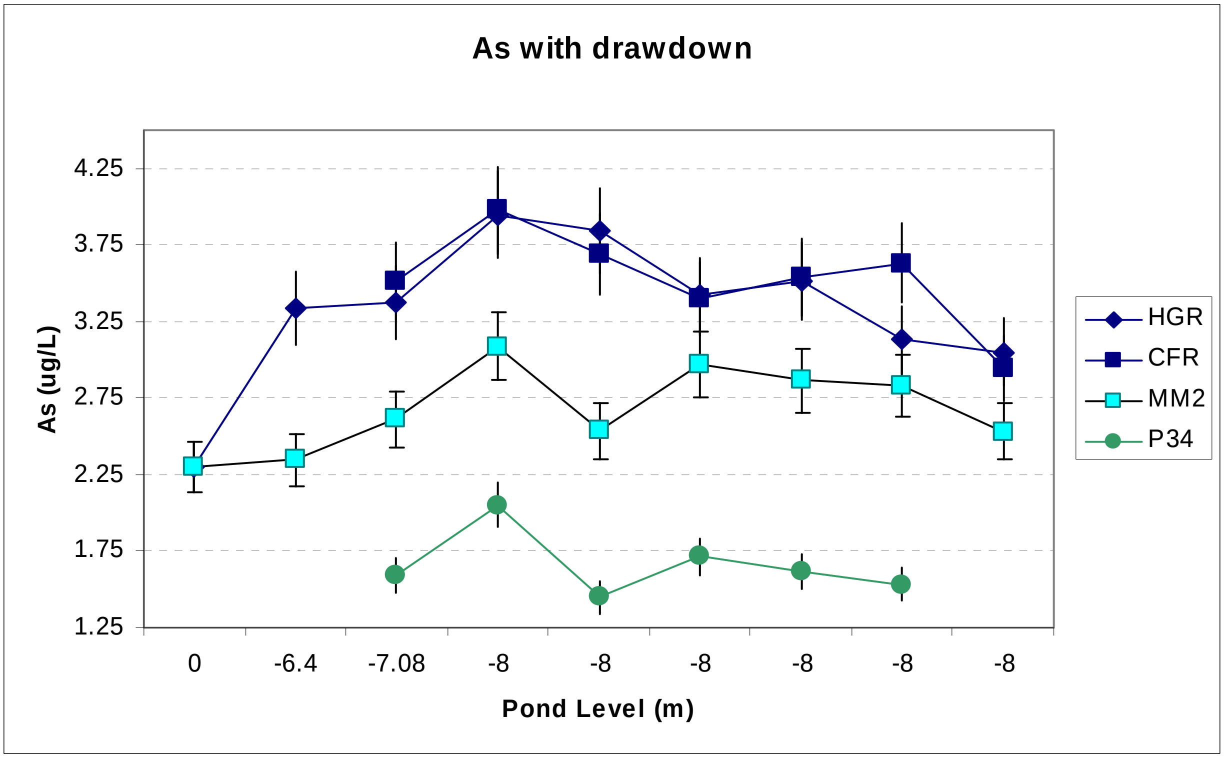


Figure 6. Arsenic concentrations in the river (HGR and CFR), a shallow monitoring well (MM2) and a production well (P34) during the drawdown of Milltown reservoir.

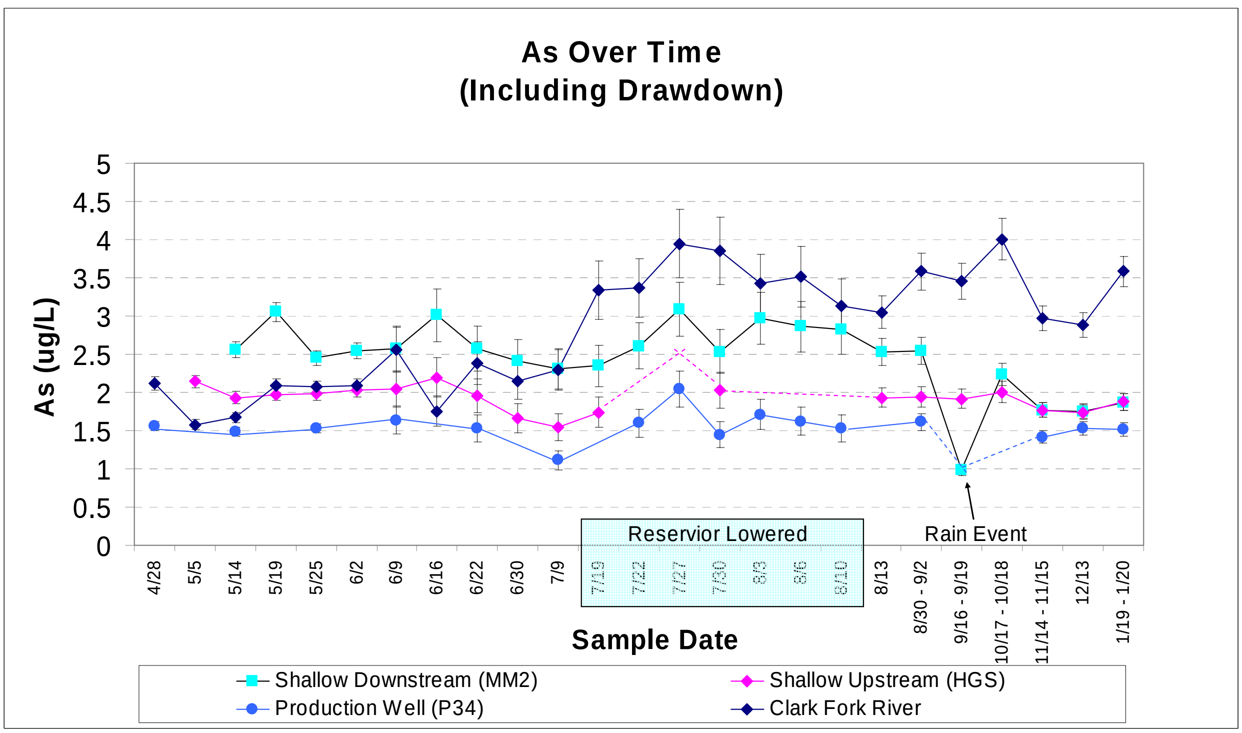


Figure 7. Arsenic data for the study period, including the drawdown event at Milltown (shaded box). The low values at MM2 and P34 are most likely due to a rain event and a pulse of clean water from Rattlesnake creek, since the well upstream from the confluence was not affected.

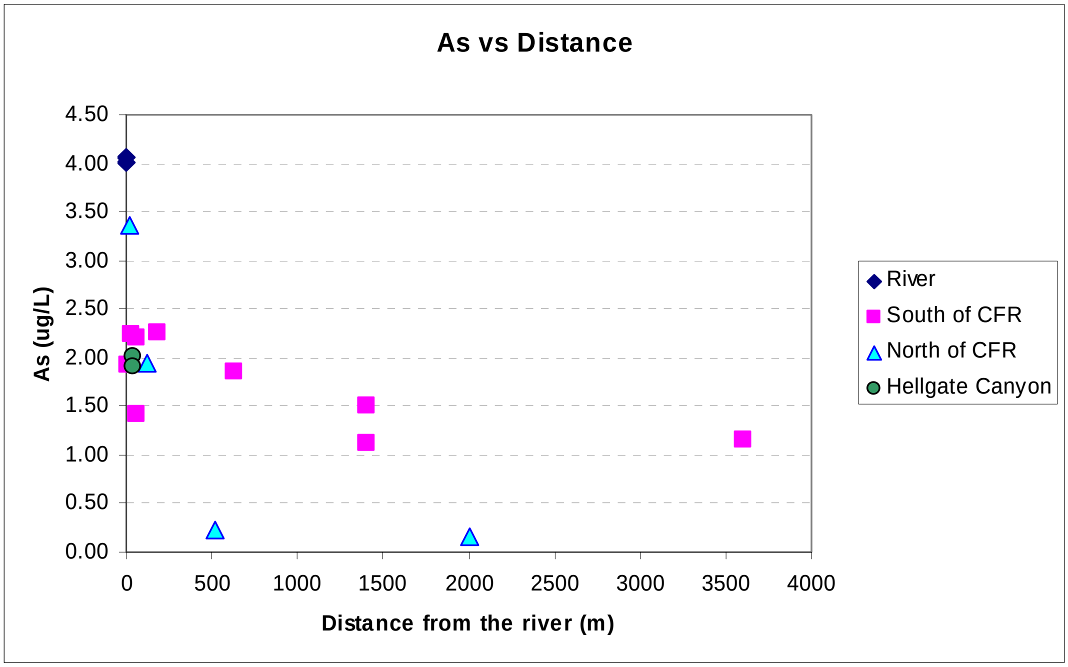


Figure 8. Concentrations of arsenic decrease with distance from the river. Distal wells on the north side have the lowest concentrations.

MODELING RESULTS

Groundwater and surface water monitoring reveal the river is perched above the aquifer. In Hellgate Canyon the river is perched 9 to 10 feet above the water table, and in the Missoula valley the river is perched 16 to 20 feet above the water table. The CFR acts as a hydraulic divide between the northern and southern portions of the valley. However, in the vicinity of Rattlesnake creek this trend is less apparent (Figure 9).

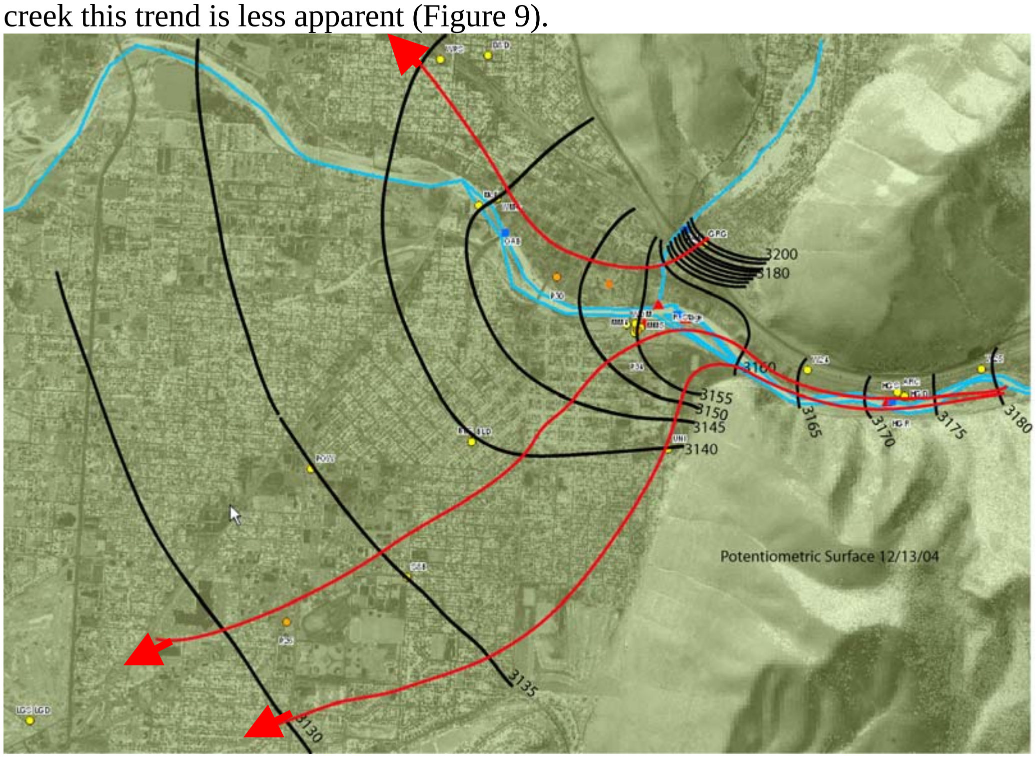


Figure 9. Potentiometric Surface 12/13/2004

Temperature monitoring and modeling indicate the CFR is losing water. Preliminary modeling results suggest the riverbed at the mouth of Hellgate Canyon has a vertical hydraulic conductivity of 5.5 ft/day, a flux of 6.75 ft^3/day per square foot of riverbed. Stream discharge measurements through this area indicate the river is loosing approximately10ft^3/day ranging from 6 to13.4 ft^3/day.

The hydraulic conductivity distribution based on our testing correlates with past conductivity values. Miller, (1991) determined a hydraulic conductivity of 6150 ft/day with his aquifer test at the Maurice St. Well located 500 ft east from the Arthur St. well. Our tests indicate a conductivity of 7030 ft/day at the Arthur St. well (Figure 10).

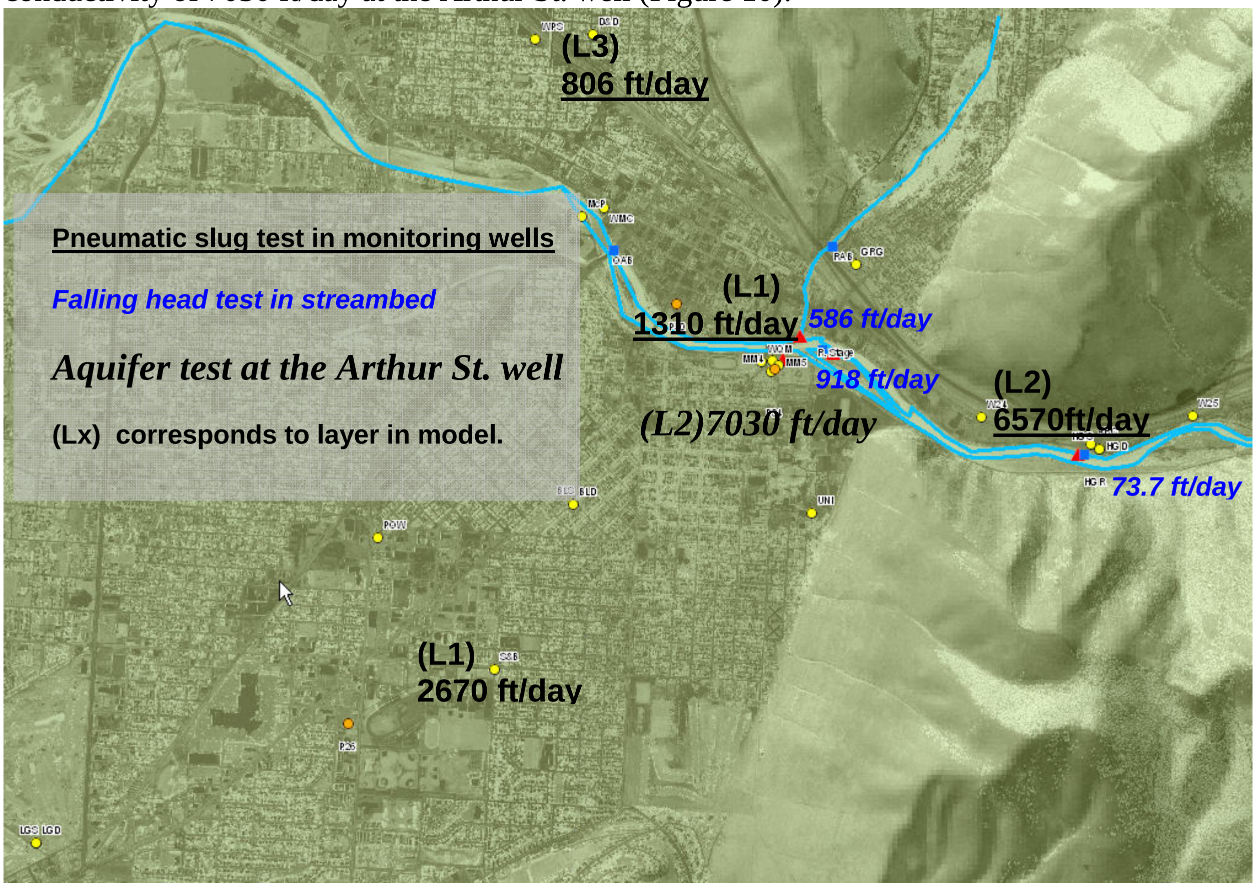


Figure 10. Hydraulic Conductivity Distribution

Preliminary modeling results for steady state conditions of March 2005, indicate the aquifer is primarily recharged by the CFR (Table 1).

Preliminary Modeling Results	
Source	Contribution to Missoula Aquifer
Clark Fork River Leakage	90.5%
Underflow From Up Gradient	7.0%
Rattlesnake Creak Leakage	2.5%

Table 1. Aquifer recharge sources.

PUBLICATIONS ASSOSCIATED WITH THIS PROJECT:

Cook, R.C., Tallman A.A. and Woessner W.W., 2004. Preliminary Results for Defining River Recharge and the Fate of Arsenic in the Shallow Groundwater System Adjacent to a Losing River, Western Montana. Center for Riverine Science and Stream Re-naturalization Conference Proceedings.

Tallman, A.A., Cook, R.C., and Woessner, W.W., 2004 Preliminary Results of a Capture Zone Study on Bank-side Production Wells and Their Interaction With a Perched River in an Alluvial Aquifer, Western Montana. Center for Riverine Science and Stream Re-naturalization Conference Proceedings.

Tallman, A.A., Cook, R.C., and Woessner, W.W. 2005. Identifying the Factors Controlling the Sources and Quantity of Water Captured by Municipal Supply Wells in the Highly Conductive Missoula Aquifer, Western Montana. National Ground Water Association Groundwater Summit, Conference Proceedings.

REFERENCES:

Bartilino J. R. and Niswonger R.G., 1999. Numerical Simulation of Vertical Groundwater Flux of the Rio Grande from Ground-Water Temperature Profiles, Central New Mexico. USGS Water Resources Investigations Report 99-4212, 34p.

Bouwer H., 1989. The Bower and Rice Slug Test- An update. Groundwater, v 27, n 3 p 304 309.

Constantz, J., Thomas, C., 1996. The Use of Streambed Temperature Profiles to Estimate the Depth, Duration, and Rate of Percolation Beneath Arroyos. Water Resources Research, v32,n12, p3597-3602.

Constantz, J. 1998. Interaction Between Stream Temperature, Stream flow, and Groundwater Exchanges in Alpine Streams. Water Resources Research, v34, n7, p1609-1615.

Constantz, J. Cox, M. H., Su, W.G., 2003. Comparison of Heat and Bromide as Groundwater Tracers near Streams. Groundwater v.41, n5, p647-656.

Fetter C.W., 1994. Applied Hydrogeology Ed.3. Macmillan Publishing Company, New York, NY. 691p.

Geoprobe Systems., 2002. Geoprobe Pneumatic Slug Test Kit -Standard Operating Procedure, Technical Bulletin No. 19344. Kejr Inc., Salina, KS. 39 p.

Hsieh, P. A., Wingle, W., Healy, R.W., 2000. VS2DI- A graphical Software Package for Simulating Fluid Flow and Solute or Energy Transport in Variably Saturated Porous Media. USGS Water Resources Investigations Report 9. 16 p.

Johnson, A.N., Boer, B. R., Woessner, W.W., Stanford, J.A., Pool, G. C., Thomas, S. A., O'Daniel, S.J., 2005. Evaluation of an Inexpensive Small Diameter Temperature Logger for Documenting Ground Water- River Interactions. Ground Water Monitoring and Remediation. Accepted April 05.

Mickey, J.W., 1998. The Effects of Discharge Variation on Dissolved Element Concentrations Through Milltown Reservoir, MT. Unpublished Masters Thesis, Departmnet of Geology, The University of Montana. 158 p.

Miller, R.D., 1991. Numerical Flow Model of the Missoula Aquifer: Interpretation of Aquifer Properties and River Interaction. Unpublished Masters Thesis, Department of Geology, The University of Montana, 301p.

Murdock Environmental Biogeochemistry Lab: McKinnon, T. and Nagorski, S. 2000. Ultra Clean Filtering Technique.

Murdock Environmental Biogeochemistry Lab: Staff, June 7, 2001. Sampling Method #2.

Murdock Environmental Biogeochemistry Lab: Tallman, A. SOP 2004_06_21, HNO_3 Rinsed Syringes.

Ronan, A.D., Prudic D.E., Thodal C.E., Constantz, J. 1998. Field Study an Simulation of Diurnal Temperature Effects on infiltration and Variably Saturated Flow Beneath an Ephemeral Stream. Water Resources Research, v34, n9, p2137-2153.

Sloat, J.V., 2003. SonTek 3.0-Mhz RiverCAT ADP Demonstration on the Orange River near Aliwal North, Free State, South Africa. SonTek/YSI, Inc. Technical Report. 12 p.

Quantitative assessment of the effectivenes of post-fire erosion control techniques

Basic Information

Title:	Quantitative assessment of the effectivenes of post-fire erosion control techniques
Project Number:	2004MT33B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	At large
Research Category:	Not Applicable
Focus Category:	Geomorphological Processes, Hydrology, None
Descriptors:	erosion, fire, geomorphology
Principal Investigators:	Scott Woods, Thomas DeLuca

Publication

1. Groen A. and S. W. Woods. 2005. Quantitative assessment of the effectiveness of post-fire erosion control techniques. Soil and Water Conservation Society, Montana Chapter, Technical Meeting 1-2 February 2005, Bozeman, Montana.
2. Woods S.W. and A. Groen. 2003. Quantitative assessment of the effectiveness of post-fire erosion control techniques. Wildland Fire Impacts on Watersheds: Understanding, Planning and Response. Denver, Colorado. 21-23 October 2003. Geological Society of America.
3. Groen A. and S. W. Woods. 2003. Quantitative assessment of the effectiveness of post-fire erosion control techniques. American Water Resources Association, Montana State Chapter 2003 Annual Meeting. Butte Montana, 4-5 October 2003.

ABSTRACT

Various methods are available to reduce post fire erosion, but there is relatively little quantitative information on the effectiveness of these techniques. A rainfall simulator was used to compare erosion and runoff rates from 0.5 m^2 plots treated with aerial grass seeding (AG) or straw mulch (SM) to that from untreated control (UC) plots in an area burned by the 2002 Fox Creek Fire in western Montana. The objective was to determine the effectiveness of these treatments for controlling post-fire runoff and erosion. There were ten replicate plots of each treatment and the control, and simulated rainfall was applied to each plot for one hour at an intensity of ~80 mm/hr. In the first year after the fire, the mean total runoff from the AG and SM plots was 30 and 28 mm, respectively, compared to 44 mm for the UC plots. Peak runoff rates from the AG and SM plots had mean values of 41 mm/hr and 40 mm/hr, respectively, compared to 59 mm/hr for the control. Erosion rates from the AG and SM plots were reduced by 25 % and 87 %, respectively, relative to the control. Limited repeat measurements in the second year after the fire indicated a decline of up to 50 % in the peak runoff from the treatments since 2003, presumably due to a decline in fire induced water repellency. Erosion rates could not be measured in 2004 because wind blown silt had accumulated in the plots. While both aerial seeding and straw mulch reduce surface runoff and erosion, straw mulch is more than three times as effective in reducing surface erosion in the first year.

INTRODUCTION AND BACKGROUND

Soil erosion rates in undisturbed forested watersheds are typically very low. However, substantial increases in erosion rates have been observed after forest fires due to the loss of the duff layer, and changes in the soil physical characteristics that increase the surface runoff rate (Helvey, 1980; Morris and Moses, 1987; Robichaud, 2000; DeBano, 2000). Post-fire increases in erosion are a concern due to the loss of soil productivity, and the ecological impacts of increased sedimentation in downstream water bodies (Robichaud et al., 2000). Various erosion control techniques are used to reduce the impact of post-fire erosion on soil and water resources, including: 1) hillslope treatments such as seeding, mulching and straw wattles; 2) in-stream treatments such as straw bales and log check dams; and 3) road rehabilitation treatments such as upgrading of culverts and ditches.

Hillslope treatments are regarded as the most beneficial because they control erosion near the point of origin, thus reducing the probability that eroded soil will reach downstream water bodies (Robichaud et al., 2000).

The costs associated with post-fire erosion control are very high; the U.S. Forest Service spent more than $83 million on its Burn Area Emergency Rehabilitation (BAER) program between 1970 and 2000, of which more than 60% was spent in the 1990s (Robichaud et al., 2000). Public concern over the impacts of forest fires, and the increasing likelihood of large fires near urban interfaces, means that expenditure on post-fire erosion control is likely to remain high. It is therefore essential that erosion control projects employ only the most effective treatments. However, few studies have determined the effectiveness of individual treatments, and most of the studies that have been conducted used only qualitative measures of effectiveness.

A recent review concluded that there is a need for quantitative, statistically defensible data on treatment effectiveness (Robichaud et al., 2000). There is a particular need to assess the effectiveness of hillslope treatments, such as aerial seeding and mulching. The need for research on erosion control treatment effectiveness is particularly great in the northern Rocky Mountain region, where wildfires have burned extensive areas of state and federal land in recent years. An increased understanding of the effectiveness of post-fire erosion control techniques will enable forest managers to achieve more effective post-fire management.

OBJECTIVES

The objective of the study was to measure the effectiveness of aerial seeding and mulching for reducing plot-scale runoff and erosion rates relative to an untreated control.

METHODS AND MATERIALS

Study Site

The study was conducted within the area burned during the 2002 Fox Creek Fire, on the Blackfeet Indian Reservation in northwest Montana (Figure 1). The Fox Creek Fire was a mixed severity fire that burned 2550 ha of mixed spruce-fir forest. Soils in the study area are clayey-skeletal, mixed Typic Cryoboralfs of the Loberg Series (USDA, 1980). These are stony loams that formed out of glacial till and contain 30 to 60 % rock fragments by volume. The mean annual precipitation at St. Mary's, Montana, which lies 4 km west of the study site and 490 m lower in elevation, is 68 cm and the mean monthly temperature ranges from minus 6^{o}C in January to 17 ^{o}C in July.

Erosion Control Treatments

Aerial grass seeding was conducted by the Bureau of Indian Affairs in the early spring of 2003, nine months after the fire, and covered an area of approximately 160 ha. Prior to the seeding operation, ten 9 m^2 plots within the area designated for seeding were covered with tarpaulins to provide comparable unseeded areas in which to establish the straw mulch and control plots. The tarpaulins were removed after seeding was completed, and seed that had blown under the tarpaulin sheets was removed from the plots. Straw mulch was then applied by hand across approximately half of each plot, following the procedures in USDA (1995). The other half of each plot was used as the untreated control.

Erosion measurements

Runoff and erosion measurements were conducted in July 2003 and August 2004 by applying simulated rainfall to 0.5 m^2 plots within the two treatments and the control. Each erosion plot consisted of a 0.71 x 0.71 meter square frame made from 15 cm wide steel sheeting. Plots were installed parallel to the slope with ~5 cm of the plot walls extending below the surface. There were ten replicate plots in each of the two treatments and the control. Rainfall was applied using a Norton type rainfall simulator at an intensity of ~ 80 mm/hr for one hour. Prior to each simulation the rainfall intensity was measured over a 5-minute period using a 0.5 m^2 calibration pan that fitted over the top of the erosion plot. During the simulations, samples were collected every 1 minute for the first 10 minutes and every 2 minutes thereafter in 1-liter Nalgene bottles. Runoff sample volumes were used to calculate the total runoff (mm) and the peak runoff rate (mm/hr). The mass of sediment eroded from the plot (kg/m^2) was determined by filtering the runoff through Whatman 40 Grade (8 μm) filter papers, drying the filter papers in a 105^{o} oven for 24 hours and then weighing them to an accuracy of ± 0.01 g.

Ancillary data

Plot slope was measured using a clinometer laid along the side of the plots. Prior to the start of each rainfall simulation, the antecedent volumetric soil moisture content was calculated from the mean of three measurements conducted within each plot using a Hydrosense soil moisture probe (Campbell Scientific Inc.). Percent vegetation cover was determined by overlaying a grid of 100 points across the plot, and counting the presence or absence of surface vegetation at each point. Soil texture was determined from samples collected adjacent to the plots in accordance with Gee and Bauder (1986) and USDA (1994).

Data analysis

Analysis of variance (ANOVA) was used to compare the following characteristics among the two treatments and the control: slope, antecedent moisture content, vegetation cover, simulated rainfall rate, total runoff, peak runoff and total mass of sediment eroded. Prior to ANOVA the variables were tested for normality using the Kolmogorov-Smirnov test, and transformed where necessary to obtain normality in the dataset. Multiple comparisons (Ott, 1993) were used to determine which means were significantly different, and the Bonferroni adjustment was used to control the experiment-wise error rate at an alpha level of 0.05 (Ott, 1993). All analyses were performed using the SPSS Version 10.0.5 statistical software (SPSS Inc., 1999).

RESULTS

Plot characteristics

The treatment and control plots had similar slope, antecedent moisture content and vegetation cover characteristics (Table 1). Plot slopes ranged from 16 to 36 %, but none of the mean slopes for the two treatments and the control was significantly different ($p = 0.638$). In 2003, the mean antecedent moisture contents ranged from 6.0 % in the mulch plots to 7.3 % in the control, and none of the means was significantly different ($p = 0.197$).

The values for mean vegetation cover in the treatment and control plots were within 1.5 %, and none of the means was significantly different ($p = 0.894$).

Rainfall and runoff rates

In July 2003, the mean rainfall intensities for the simulations performed in the aerial seeded, straw mulch and control plots were 82, 84 and 83 mm/hr, respectively, and these values were not significantly different ($p = 0.720$), indicating that the plots were subjected to similar rainfall inputs.

All of the plots produced a similarly shaped hydrograph, although the runoff rate and timing varied within and between treatments. The early part of the hydrograph consisted of a short period, typically less than 1 minute, with no runoff followed by a steep rising limb with a time to equilibrium (time from start of rainfall to plateau of runoff hydrograph) of between 8 and 30 minutes (Figure 2). The mean time to equilibrium in the control and mulch plots was similar (15.5 and 16.0 minutes, respectively), but it was ~4 minutes longer in the seeded plots (Table 2). Mean values for time to equilibrium were not significantly different ($p = 0.275$). In all but two of the plots, the runoff rate gradually declined after the initial plateau runoff rate had been reached, indicating a hydrophobic response in the soil.

The mean values for total runoff from aerial seeded and straw mulch plots were 30 and 28 mm, respectively, compared to 44 mm for the control plots (Figure 3). Due to high within treatment variability, the means were not significantly different ($p = 0.090$). Peak runoff rates from aerial seeded and straw mulch plots had mean values of 41 mm/hr and 40 mm/hr, respectively, compared to 59 mm/hr for the control (Figure 4). Again, the means were not significantly different ($p = 0.092$).

In 2004, the peak runoff rates from plots were reduced by up to 50 % when compared with 2003 (Figure 5), suggesting that the hydrophobic soil layer was breaking down.

Erosion rates

Total erosion from the plots (kg/m^2) was log normally distributed, so a log transformation was used to normalize the erosion data prior to analysis. In 2003, the mean treatment values were 0.10 kg/m^2, 0.59 kg/m^2, and 0.79 kg/m^2 in the mulched, aerial seeded and control plots, respectively (Table 2, Figure 6). Total erosion from the mulched plots was significantly lower than both the control ($p = 0.001$) and the seeded plots ($p = 0.013$), while total erosion from the seeded plots was not significantly different from the control. In 2004, the accumulation of wind blown silt in the plots prevented measurement of the erosion rates.

DISCUSSION

One of the primary causes of increased erosion from burned areas is the loss of vegetative cover and the protective duff layer, resulting in more rainsplash. Consequently, the effectiveness of aerial seeding and mulching in reducing erosion is largely dependent on the amount of additional ground cover that the treatment produces. In our study area, seeding was not conducted until nine months after the fire because of logistical and climatic limitations, so that there was only a three month period between the application of the grass seed and the rainfall simulations. The mean vegetation cover in the seeded plots was no greater than in the control plots at the time of the 2003 simulations, and the seeding treatment had a correspondingly limited effect in reducing erosion. Application of the seeding treatment prior to snowfall in the same year as the fire may have increased its effectiveness in reducing erosion the following year. The limited additional ground cover created by the aerial grass seeding in 2003 may have also been partly due to the exceptionally dry conditions; total precipitation at Babb, Montana for 1 June – 31 July 2003 was just 42% of average. Greater success might be expected in a wetter year, although the effect of increased ground cover might be offset by the increased rainfall erosivity. Overall the results confirm other studies, which suggest that seeding has only a limited beneficial effect on erosion rates in the first year after a fire (Amaranthus, 1989; Orr, 1970). Since erosion rates are typically at their highest in the first year because of the lack of cover, high rates of overland flow and high sediment availability, this represents a serious limitation in the effectiveness of grass seeding. Grass seeding may not be an appropriate treatment in many burned areas.

In contrast with the grass seeding treatment, straw mulching was highly effective in reducing erosion in the first year after the fire. The effectiveness of straw mulch for reducing erosion has long been recognized in agriculture and the construction industry. The effectiveness of mulching has also been noted in the limited number of studies conducted in burned areas. Wheat straw mulch applied to fill slopes adjacent to perennial streams, firelines and areas of high erosion hazard reduced erosion rates by 11 to 19 m^3 ac^{-1} compared to untreated sites (Miles et al., 1989). Edwards et al., (1995) noted significant reductions in soil loss between sites where mulch was applied at rates of 0.9 and 1.8 t ac^{-1} on slopes ranging from 5 to 9 percent. Erosion rates from the mulched plots were reduced by 87 % relative to the control, a result that is consistent with the limited number of previous studies conducted in burned areas. The effectiveness of the mulching treatment can be attributed primarily to the immediate increase in ground cover that it provides, and the consequent decrease in rainsplash erosion. However, the mulch treatment also reduced the total runoff and the peak runoff from the plots indicating that erosion rates may have been further reduced by a decrease in the rate of overland flow. Presumably the mulch layer acts much like the duff layer in an undisturbed forest soil profile, providing a temporary storage reservoir for rainfall which then infiltrates the ground over a longer time period.

We noted a tendency for mulch to be blown off the site by high winds, and this could be a problem when using mulch as an erosion control treatment. Many areas that were mulched in late May were completely bare by early August. Loss of the mulch would likely be less of a problem where a larger area was treated because mulch blown from one area would be replaced by mulch blown from elsewhere. However, periodic maintenance is needed to ensure that the mulch remains effective during the first summer after a fire, when vegetation cover is at a minimum. We reduced loss rates from the mulched plots by spreading nylon netting across the plots, and a similar approach could be employed in areas being treated on a larger scale.

Accumulation of wind blown silt in the plots prevented us from measuring erosion rates in the second year of the study. We were therefore unable to determine whether the treatments had a longer term effect on erosion rates. Seeding may have a positive effect in that it eventually provides more ground cover than on untreated sites. However, seeding can have a detrimental effect on long term recovery because it can inhibit the regrowth of native vegetation. None of our sites had any straw mulch visible in the second year due to a combination of decomposition and being blown away by wind. In-situ decomposition of mulch may enhance regrowth by increasing the soil's organic and nutrient content, but we did not quantify such an effect. More research is needed on the longer term effectiveness of erosion control treatments.

In addition to measuring the effectiveness of the treatments, the study provided insight to the infiltration and runoff process in burned areas. Increases in erosion from burned areas are due in part to an increased rate and frequency of overland flow, resulting in more sheet and rill erosion. The increased overland flow has been largely attributed to the presence of water repellent (hydrophobic) soils. In our study, most of the 2003 runoff hydrographs exhibited a declining runoff rate after the initial peak, indicating gradual wetting of a hydrophobic soil layer and resultant increase in the infiltration rate. This increase in infiltration with time is the opposite of what is typically observed in unsaturated hydrophytic soils, and has been observed in previous studies of post-fire infiltration (Benavides-Solorio and Mac Donald, 2001).

The 2004 runoff rates were substantially lower than those observed in 2003, indicating that the hydrophobic layer was at least partially broken down. In most cases, hydrophobic soils tend to disappear within one year after the fire, although the rate of breakdown varies with the initial fire intensity and the amount of precipitation. The increase in infiltration that accompanies breakup of the hydrophobic layer along with the increased vegetation cover will eventually lead to a decline in runoff and erosion rates from burned areas. However, studies conducted in Colorado indicate that higher erosion rates can persist for at least four years in burned areas, far beyond the period in which hydrophobicity is expected to persist (Dr. Lee MacDonald, *pers. comm.*). The implication is that reductions in infiltration after a fire may be due to factors other than the presence of water repellent soils, such as sealing of the surface by fine organic and mineral material. More research is needed to investigate the factors controlling post-fire infiltration and runoff.

CONCLUSIONS

Various methods are available to reduce post fire erosion, but there is relatively little quantitative information on the effectiveness of these techniques. A rainfall simulator was used to compare erosion and runoff rates from 0.5 m^2 plots treated with aerial grass seeding or straw mulch to that from untreated control plots in an area burned by the 2002 Fox Creek Fire in western Montana. The objective was to determine the effectiveness of these treatments for controlling post-fire runoff and erosion. The results indicate that seeding and mulch both reduce total runoff, peak runoff and erosion from burned areas. However, mulching is more than three times more effective in reducing erosion than seeding. Mulching may therefore be a more desirable treatment than seeding in situations where both treatments are being considered. Additional research is warranted to determine the longer term effectiveness of these treatments, and their effect on natural revegetation rates.

REFERENCES

Amaranthus M.P., 1989. Effect of grass seeding and fertilizing on surface erosion in two intensely burned sites in southwest Oregon. In: Berg N. (tech. coord.) Proceedings of the symposium on fire and watershed management. October 26-28, 1988. Sacramento, California. General Technical Report PSW-109. USDA Forest Service Pacific Southwest Forest and Range Experiment Station.

Benavides – Solorio J. and L.H. MacDonald, 2001. Post fire runoff and erosion from simulated rainfall on small plots, Colorado Front Range. Hydrological Processes 15:2931-2952.

DeBano L.F., 2000. Water repellency in soils: a historical overview. *Journal of Hydrology* 231-232:4-32.

Edwards L, J. Burney and R. DeHaan, 1995. Researching the effects of mulching on cool period soil erosion in Prince Edward Island, Canada. Journal of Soil and Water Conservation. 50:184-187.

Gee G.W. and J.W. Bauder, 1986. Particle size analysis. In: Klute A. (ed.), Methods of Soil Analysis: Part 1. American Society of Agronomy, Madison, Wisc.

Helvey J.D., 1980. Effects of a north central Washington wildfire on runoff and sediment production. *Water Resources Bulletin* 16(4):627-634.

Miles S.R., D.M. Haskins and W. Darrel, 1989. Emergency rehabilitation: cost, risk and effectiveness. In: Berg N. (tech. coord.) Proceedings of the symposium on fire and watershed management. October 26-28, 1988. Sacramento, California. General Technical Report PSW-109. USDA Forest Service Pacific Southwest Forest and Range Experiment Station.

Morris S.E. and T.A. Moses, 1987. Forest fire and natural soil erosion regime in the Colorado Front Range. *Annals of the American Association of Geographers* 77:245-254.

Orr H.K., 1970. Runoff and erosion control by seeded and native vegetation on a forest burn, Black Hills, South Dakota. USDA Forest Service Rocky Mountain Research Station, Res. Paper RM-60. Fort Collins, Colorado.

Ott, L., 1993. An introduction to statistical methods and data analysis, Fourth Edition. PWS Publishing Company.

Robichaud P.R., 2000. Fire effects on infiltration rates after prescribed fire in Northern Rocky Mountain forests, USA. *Journal of Hydrology* 231-232:220-229.

Robichaud P.R., J.L. Beyers and D.G. Neary, 2000. Evaluating the effectiveness of postfire rehabilitation treatments. *General Technical Report* RMRS-GTR-63, US Dept. of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, Colorado.

USDA, 1980. Soil Survey of Glacier County and part of Ponderosa County, Montana. USDA Soil Conservation Service.

USDA 1994. Soil Survey Manual. U.S. Department of Agriculture Handbook No. 18.

USDA, 1995. Burned area emergency rehabilitation handbook - Chapter 20. FSH 2509.13-95-6. USDA Forest Service, Washington DC.

Table 1. Mean plus or minus (±) one standard deviation of slope, moisture content, and vegetation cover in the control and treatment plots in 2003.

Treatment	**Slope (%)**	**Moisture content (%)**	**Vegetation cover (%)**
Control	27.2 ± 4.3	7.3 ± 1.5	14.8 ± 9.0
Mulch	25.3 ± 5.4	6.0 ± 1.9	16.6 ± 8.2
Seed	25.7 ± 4.1	6.3 ± 1.4	15.1 ± 9.9

Table 2. Total runoff, peak runoff and total erosion in treatment and control plots in 2003. Treatments: AG = aerial grass seeding, SM = straw mulching, UC = untreated control.

	Treatment	Range	Mean	Coefficient of Variability (%)
Time to equilibrium (minutes)	Control	7 – 28	15.5	42
	Mulch	8 – 26	16.0	33

	Seed	8 – 36	20.6	48
Total runoff (mm)	Control	12 – 64	44	40
	Mulch	1.4 – 56	28	69
	Seed	7.1 –52	30	46
Peak runoff (mm/hr)	Control	18 – 79	59	32
	Mulch	4 – 75	40	63
	Seed	12 – 62	41	39
Total erosion (kg/m^2)	Control	0.04 – 1.2	0.79	54
	Mulch	0.01 – 0.25	0.10	89
	Seed	0.01 – 1.75	0.59	83

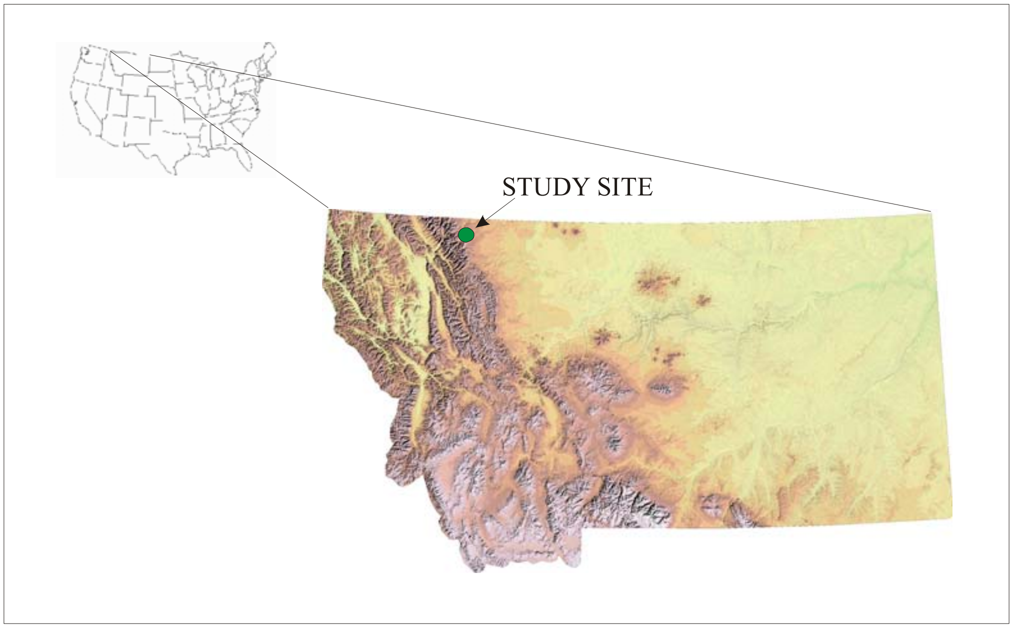


Figure 1. Location map of study area in northern Montana.

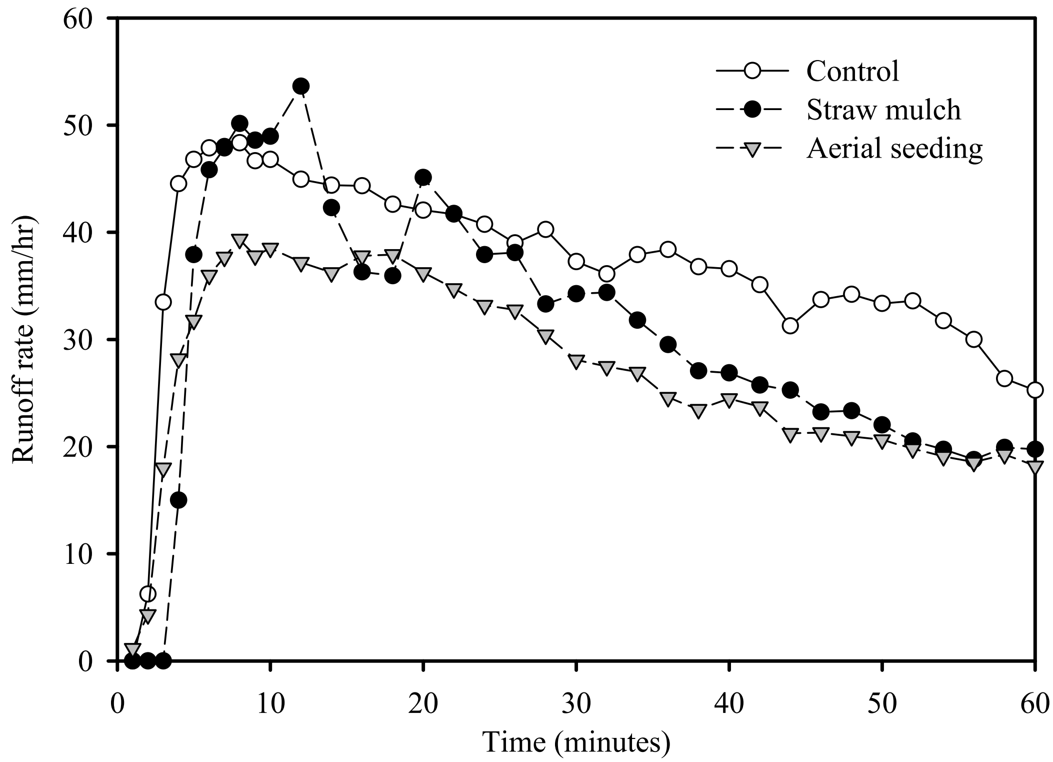


Figure 2. Mean runoff hydrographs for control, straw mulch and aerial seeding treatments in 2003.

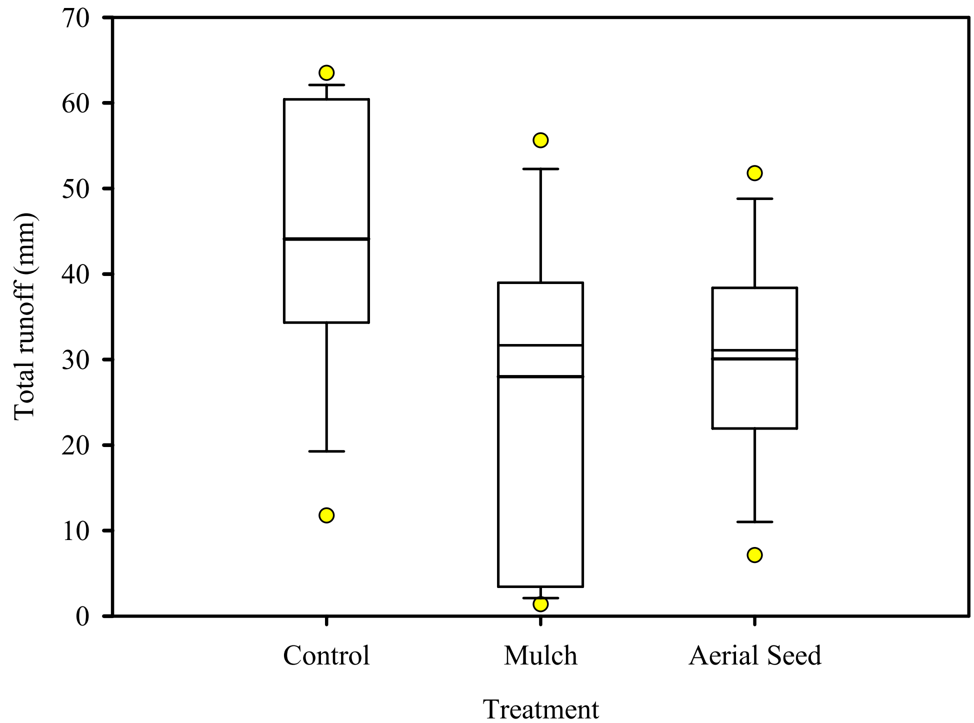


Figure 3. Total runoff from control, mulch and aerial seeded plots in 2003. Thin line inside box indicates mean, thick line indicates median. Box ends indicate 25th and 75th percentiles. Whiskers indicate 10th and 90th percentiles. Circles denote outliers.

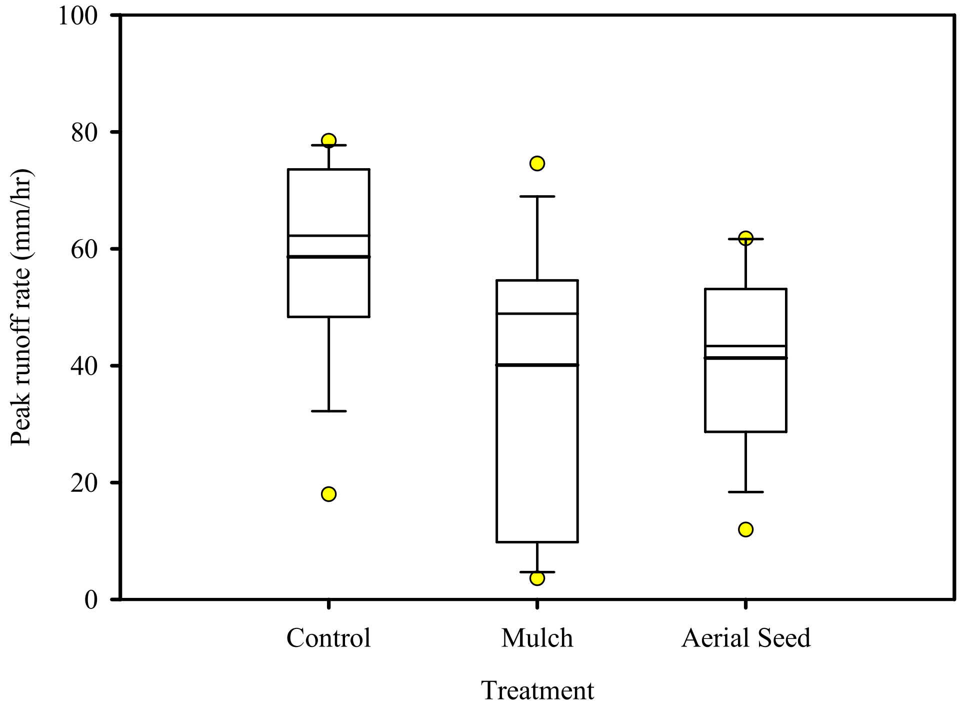


Figure 4. Peak runoff from control, mulch and aerial seeded plots in 2003. Thin line inside box indicates mean, thick line indicates median. Box ends indicate 25th and 75th percentiles. Whiskers indicate 10th and 90th percentiles. Circles denote outliers.

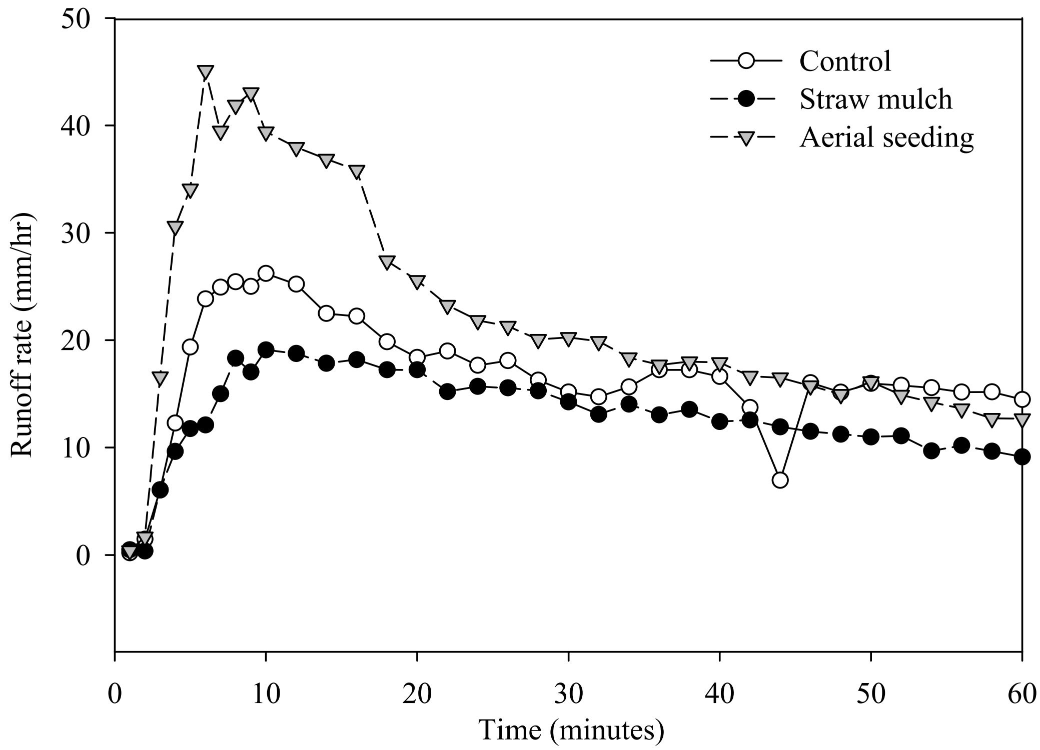


Figure 5. **Mean runoff hydrographs for control, straw mulch and aerial seeding treatments in 2004**

Investigation of microbial ecology, structure, and function in coalbed aquifers: Powder River Basin, Montana

Basic Information

Title:	Investigation of microbial ecology, structure, and function in coalbed aquifers: Powder River Basin, Montana
Project Number:	2004MT34B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	At large
Research Category:	Ground-water Flow and Transport
Focus Category:	Hydrogeochemistry, Ecology, Groundwater
Descriptors:	aquifer, preservation, methanogenisis, coalbed aquifers, sustainability, methane; Powder River Basin, Montana, microbes
Principal Investigators:	John R Wheaton, Patrick Ball

Publication

Investigation of microbial ecology, structure, and function in coalbed aquifers: Powder River Basin, Montana

Principal investigators: Pat Ball, PhD, University of Montana and John Wheaton, Montana Bureau of Mines and Geology

Start Date: March 1, 2004

End Date: February 28, 2005

Current Status: Preparing final report

Coalbeds supply three critical resources in southeastern Montana: 1) coal for energy; 2) water for domestic and agricultural uses; and 3) coalbed methane. Currently, these aquifers are being impacted by conventional coalbed methane (CBM) development. As concerns of global warming increase, speculation that these aquifers may serve as repositories for industrial CO_2 suggests that additional impacts are likely in the future. A comprehensive understanding of the relationships between the hydrogeologic terrains and the total microbial community at depth will help establish best management practices for methane production and potential CO_2 sequestration. Avoiding waste of the methane resource and the associated produced water is key to comprehensive resource sustainability that will preserve the aquifer yet allow for long-term recovery and utilization of methane and may help remediate the atmospheric CO_2.

Methane is held on cleat faces and micropore surfaces in coal by a combination of physical sorption and hydrostatic pressure from ground water in the coal (Law and Rice,1993; Rightmire, and others, 1984), and is released when the water pressure is reduced. To reduce hydrostatic pressure and capture released gas, water is pumped from wells drilled and completed in coalbeds .

The origin of CBM in the Powder River Basin (PBR) is the result of microbial processes (biogenic methanogenesis). The success of CO2 sequestration strategies will likely be a function of microbial activities as well. The purpose of this research is to begin the process of identifying the structure, diversity and presumptive function of the total microbial community and ecology within a specific methane-bearing coalbed aquifer in the PRB and conduct culture-based investigations that will help delineate the kinetic rates and pathways for methanogenesis. We foresee the value of data collected during this project as a means of moving us toward a philosophy of harvesting CBM rather than simply mining this resource at the expense of ground-water resources.

There are two distinct types of ground-water flow systems in the Powder River Basin, a deep regional system and a series of local flow systems. Ground water flows generally from the south to the north, with flow in the local systems reflecting topographic control. Recharge occurs at outcrop areas around the edges of the Basin in Wyoming and in high clinker-capped ridges such as the Wolf Mountains in Montana (Wheaton and Donato, 2004). Coal seams are the most continuous water-bearing geologic units and have hydraulic conductivity values equal to or slightly greater than those in sandstone aquifers.

Due to the geologic structure of the Powder River Basin, and the topographic relationship between generally higher elevations in Wyoming and lower elevations in Montana, coal seams outcrop along valley walls in Montana and ground-water discharges as springs at these outcrop areas. Additional ground-water discharge occurs as baseflow to streams and rivers in Montana.

The quality of ground water in the Powder River Basin reflects chemical and biological reactions that occur along flow paths. In deep coal beds, such as those that contain coalbed methane, chemical reactions have greatly reduced the amounts of sulfate, calcium, and magnesium, so that the water quality is dominated by moderate concentrations of sodium and bicarbonate. Coalbed methane can only exist in the sulfate-depleted, anaerobic conditions which occur in deeper coals. Therefore, all CBM production water is rich in sodium and much of it has a high SAR value.

It is understood that biogenic methane is produced as an end-product of a complex set of metabolic pathways represented by a consortium of microorganisms, including members of the domains *Eubacteria* and *Archaea*. This intricate and closely associated assemblage resides in anoxic zones depleted of typical electron acceptors found in many subsurface environments. Four groups of functionally diverse prokaryotes have been identified, as being necessary for the formation of methane under these conditions: 1) hydrolytic bacteria, 2) fermentative bacteria, 3) acetogenic bacteria and 4) methanogens (Whiticar, 1999). Each of these guilds of microorganisms is responsible for an important function in the methanogenic pathway. The hydrolysis of higher molecular weight substrates, such as, cellulose, high molecular weight proteins and mixed composition polysaccharides by hydrolytic and cellulolytic competent bacteria is a necessary first step in the decomposition of organic materials. It has been postulated that this represents the rate limiting step in the formation of methane in anoxic environments. Following their breakdown into monomeric subunits such as short-chain fatty acids, sugars, amino acids and additional substrates (e.g. ammonia and hydrogen sulfide), fermentation proceeds, mediated by assorted fermentative bacteria. The fermentation process produces a number of byproducts, including additional short-chain alcohols and acids (propionate and butyrate are common), as well as, acetate, formate and carbon dioxide

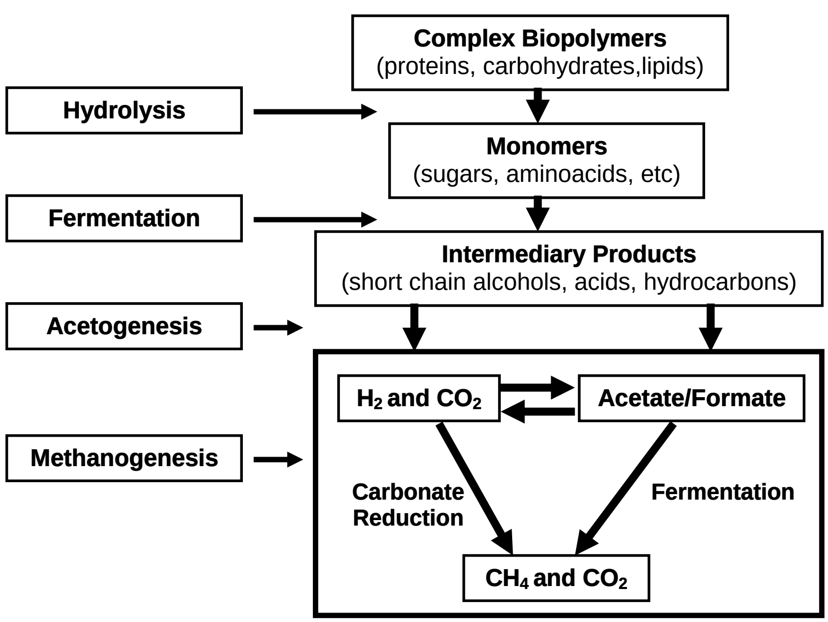


Figure 1. Anaerobic Degradation of organic compounds

(CO_2). Because methanogens metabolize a narrow range of compounds and are restricted to anoxic environments with redox potentials of Eh < -200 mV (Budwill, 2003), some further degradation is assumed to be required. Syntrophic acetogenic bacteria play an important role in consuming many of the short-chain acids that accumulate in the pathway, and the end products, predominately acetate and CO_2, become viable substrates for methanogenesis. The final step prior to conversion to methane is to convert any remaining alcohols and acids into acetate, carbon dioxide (methanol and methylamines may also be substrates for methanogenesis), hydrogen (H_2) and in some cases formate. This general scheme of anaerobic degradation of organic compounds to methane is diagrammed in figure 1.

Although the reduction of carbon dioxide by hydrogen is thought to be the most commonly used method for the production of methane in anoxic environments (Scott, 1999), the reduction of acetate (or a very limited methyl group containing hydrocarbon) provides a greater change in free energy and therefore is more favorable for energy conservation. This situation remains unclear, as the opposite is thought to be true in certain environments such as marine or open freshwater settings (Whiticarr, 1986). The two pathways may operate simultaneously under some circumstances and at differing stages of sedimentation of organic materials (Kotelnikova, 2002). While each of the two reductive processes produces methane, the two separate pathways may be active. The former by the (hydrogen mediated reduction of carbon dioxide) carbonate reduction pathway and the latter by the fermentation pathway. The general chemical equations for the respective pathways are illustrated below.

Carbonate reduction pathway: $4 H_2 + HCO_3^- + H^+ = CH_4 + 3H_2O$
($\Delta G°$ -3.2 KJ)*

Fermentation (methyl-group) pathway: $CH_3COO^- + H_2O = CH_4 + HCO_3^-$
($\Delta G°$ -24.7 KJ)*

*Reported free energy values vary from source to source.

Evidence supporting the notion that the two pathways operate at various times was reported by Chin et al, (2003). They described temporal changes in methanogen production in flooded rice paddies. Their findings indicate that structural changes in the methanogenic community lead to functional changes in methane production with time. Similarly Scheid et al. (2003) using rice roots as a community model for methanogenesis were able to show methanogenic community shifts when nitrate and sulfate were introduced. Apparently, the addition of alternative electron acceptors leads to changes in community substrate usage and may have broad effects on community structure and activity. Likely, competition between methanogens and sulfate and nitrate reducing bacteria led to these changes.

The use of culture independent molecular techniques for our investigation is crucial. It is generally accepted that classic culturing techniques may under represent microbial diversity in typical environments by two to three orders of magnitude (Torsvik, 1994; Torsvik et al., 1990). It is apparent that microbial communities and their associated populations play important roles in biogeo-chemical and physicochemical processes including methanogenesis and carbon cycling. Functional guilds of bacteria that have been associated with biogenic methane production include hydrolytic and cellulolytic bacteria, fermentative and acetogenic bacteria, as well as methanogens (Whiticar, 1999). However; Polman et al. (1993) reported that there were no viable microorganisms in three different ranked coals. Their observations were based on results of experiments attempting to grow bacteria in cultures. Vorres, (1990) reported that anaerobically preserved Argonne Premium Coals produced methane in sealed ampoules. Also these samples contained cultivable Clostridium species. In 1994, work by both Johnson et al. and Volkwein et al. noted that higher-rank coals produced low molecular weight organic acids when they were inoculated with presumptive anaerobic consortiums from various sources. Based on additional work completed by these groups, they concluded that the microorganisms collected from those environments (that were likely to contain methanogens and other consortium members) were responsible for the production of the methane. In Volkwein et al. (1994), although their cultures remained viable and continued to produce methane through five successive transfers, they were unsuccessful at identifying any of the microorganisms.

The purpose of the proposed research is to elucidate the diversity, composition, activity and function of the methane producing microbial community in coalbeds. These findings will have broader impacts than simply exploring the microbial ecology of a novel subsurface environment. Understanding the nature of the microbial ecology of coalbed seams will contribute knowledge toward management of enhanced microbial methane production and recovery and possibly contribute to CO_2 sequestration efforts, thereby impacting greenhouse gas mitigation strategies.

Sample collection

Two microbial samples were collected in Wyoming from the Tongue River Member, Big George coal seam. A water quality sample was collected at a nearby CBM discharge point from wells completed in the same coal seam, but different from the well where the coal samples were collected. The sites are in the Powder River watershed in east central Johnson county. The coal samples were collected during under reaming, using forward air rotary, of an already cased CBM well. The samples were gathered from the diverter pipe on the drill rig with a sample screen in less than one minute the samples were inserted into an anaerobic chamber with an oxygen consuming package then sealed. The coal samples were held in cold storage until they arrived at the laboratory at the University of Montana. The water sample was submitted to the Montana Bureau of Mines and Geology analytical laboratory for analysis.

The upper coal sample was collected while reaming from 1525 feet to 1530 feet. The second coal sample was collected just after reaching the base of coal (1596 feet) while cleaning the borehole. Since the well was cased, neither coal sample contained material from farther up the bore hole, and appeared clean and in good condition.

Analytical results of the water quality sample indicated total dissolved solids concentration of 2,056 mg/L, SAR of 25.5 and the sulfate concentration of less than 2.5 mg/L. The water quality is typical of all CBM production water in the PRB.

Molecular Analysis:

Nucleic Acid extraction

Aqueous Phase: To increase the biomass for molecular analysis, cells were collected by filtration onto three separate 142-mm Supor (Pall Corporation, Ann Arbor, MI) 0.2 μm membrane filters. Each filter received an approximate equal volume of groundwater (approximately 13 liters). Filters were placed in sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI) and frozen at -80° C. Prior to genomic DNA extraction, the frozen filter was crushed thoroughly within the collection bag. Processing of total community DNA from the filter was carried out by the direct lysis method of Holben (1997) with minor modifications.

Briefly, 20 ml of autoclaved extraction buffer (200 mM sodium phosphate buffer (NaPO4), 100 mM ethylenediamine tetra-acetate (EDTA) and 1.5% sodium dodecyl sulfate (SDS), pH 8.0) was added to sterile Oak Ridge tubes containing sterile glass beads (5 g of 0.2 mm and 5 g of 1 mm diameter) (Sigma Chemical Co., St. Louis, MO.). To this tube, one macerated filter was added, placed in a 70° C water bath for 30 min with frequent vortexing (5 min intervals). Tubes were then placed on a reciprocal platform shaker and shaken on high (approximately 100 oscillations/minute) for 30 min at room temperature. Filter, particulate and cell debris were removed by centrifugation (Sovall RC 5B Plus with SS34 rotor) at 10,000 RPM (7,796 x g) for 10 min at 10° C. Supernatant was transferred to clean Oak Ridge tubes and incubated on ice for 30 min to precipitate the SDS, then centrifuged as above to pellet SDS. Liquid was transferred to new 50 ml tubes with addition of 10% volume 3 M sodium acetate (pH 5.2) and 2.5 volumes 100% cold ethanol. After overnight incubation at -20° C, nucleic acids were collected by centrifugation, as described above. Nucleic acid pellets were resuspended in approximately 1 ml of sterile deionized water and precipitated by addition of 2.5 volumes 100% cold ethanol and placed in at -20° overnight. After centrifugation (as above) the resulting nucleic acid pellet was air dried and suspended in approximately 500 μl TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

Solid Phase: Anaerobic coal samples were subjected to direct nucleic acid extraction, as well as, used as inocula in both groundwater and growth media. Direct nucleic acid extractions were performed using Power Soil DNA Extraction Kits (Mo Bio, Solano Beach, CA) as per manufacture's suggestion. In additional to standard extractions, coal samples were subjected to further DNA purification which included the addition of chaotropic salts and silicon binding matrices. This method has proven to be beneficial when attempting to amplify various environmental samples.

Resulting DNA from both solid and aqueous phases was subsequently subjected to DNA amplification by the Polymerase Chain Reaction (PCR) using both generally conserved primers and methanogen specific primer sets 16S rDNA primers (536fc and 907r) as well as the methanogen-specific primer pair 23fc and 440r). More recently, the genomic DNA was amplified with primer set ME1 and ME2, which are specific for the *mcr* (methyl coenzyme M reductase) gene (alpha subunit). The expected product is approximately 750 kb. The result of this gave correct size product, which were gel purified and are to be used to align with groups of other amplicons derived from Powder River Basin coal samples and associated aquifer. From these alignments “coal specific” methanogen primers will be constructed, which will be used to amplify any samples that are coal related. For the first two sets of primers, the amplicons generated from the PCR was subjected to analysis by Denaturing Gradient Gel Electrophoresis (DGGE). This method of analysis separates double stranded DNA amplicons run in an acrylamide matrix based on sequence differences. Therefore in figure 2, (below) each individual band theoretically represents an individual organism.

Culturing Methods:

Coal samples were also used as inocula in both onsite reduced groundwater and growth media were allowed to incubate in the dark at room temperature. The growth medium for culturing core samples and for growing anaerobic consortia consisted of a modified mineral salts solution (Fedorak and Hrudey, 1984). Cultures were incubated in a headspace gas of 20% CO_2 and 80% N_2. Bottles were sealed with butyl rubber stoppers and crimped down with aluminum seals and received 0.35 g $NaHCO_3$/100 ml. All anaerobic work was completed in an oxygen free atmosphere to ensure anaerobic conditions prevailed.

Results of Analysis:

The results indicate relatively low diversity of the total microbial community (see lane 1) compared to that of a typical subsurface or soil environment. Sequencing of several prevalent bands (indicated by arrows) indicated that all presumptive identities, based on known sequences in the Ribosomal Database (RDP II), had relevant metabolic capabilities consistent for their presumed role in coal formations that generate methane. The diversity of the methanogen community (as indicated by the number of bands in lane 2) appeared quite high, but the five bands sequenced all had similar phylogenetic affiliations. Each sequence was closely associated with the genus *Methanolobus*

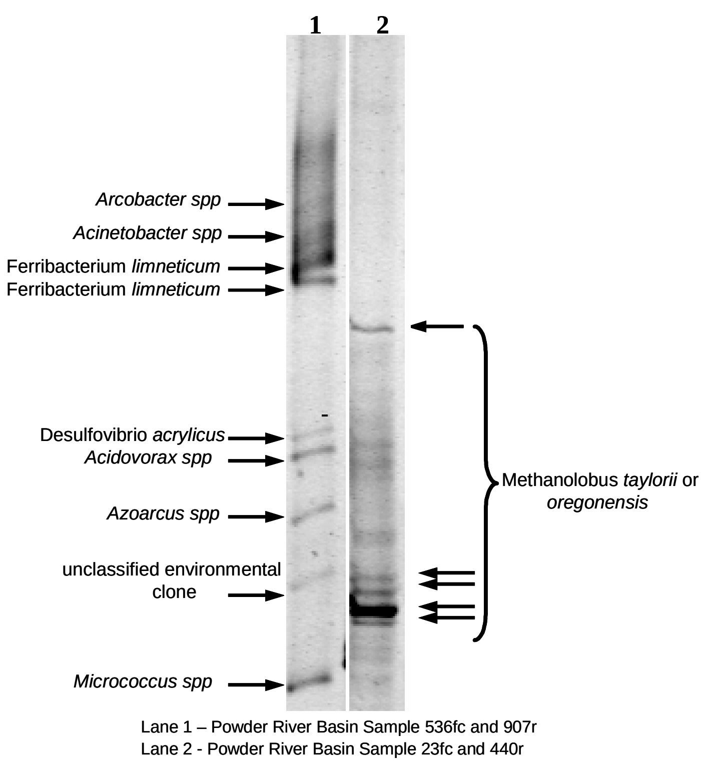


Figure 2. DGGE analysis of the microbial community of PRB coal associated aquifer water.

within the order *Methanosarcinales*. In addition, all five were most closely related to the species *M. taylorii* or *M. oregonensis* (averaging 93% homology). Interestingly and unexpectedly, this group is typically linked with marine environments. However, the geological formation and shallow depth where this sample was taken have not been associated with ancient marine origins. This evidence supports the concept that this environment may sustain novel members of the methanogen group. More recently, an additional survey of coal methanogens (using ME1 and ME2) produced seven sequences all related to members of the *Methanosarcinales* or *Methanobacteriales* orders. Six appear to be unique from each other and their closest matches are to environmental clones from various origins. Additionally, many of the important members of the consortia may be underrepresented in terms of numbers, but may be dominant in terms of activity. If this were the case, it suggests that there are a number of minority microbial populations are present in coalbeds, and that to more fully understand the community ecology an extensive and intensive investigation must be undertaken.

In addition to the molecular data we are currently in the midst of incubating coal samples in our laboratory. As of yet there is little evidence of growth, based on turbidity and direct observations. However this is not surprising as methanogen growth is typically very slow and may indeed take a period of time well beyond the time frame or this study. However these culture attempts will continue and a molecular analysis of these samples will be undertaken.

Conclusions and recommendations:

This initial investigation proved to be an excellent starting point for continuing efforts toward unraveling the microbial community complexity responsible for biogenic methane production. To fully underpin the microbial community in this environment a more comprehensive study must be undertaken which would include the following:

- Continue with sequencing efforts on the microbial community within coal samples
- Develop conceptual model of the microbial community present based on molecular analysis
- Design primers appropriate for real-time PCR
- Conduct culturing experiments and isolate pure cultures to confirm the presence of novel organisms
- Design amendment/perturbation experiments for laboratory (later for *in situ*?)
- Develop activity studies aimed at determining active microbial populations responsible for methane production (future stable isotope experiment)

Mountain front groundwater recharge: groundwater-surface water exchange across an alpine-valley bottom transition

Basic Information

Title:	Mountain front groundwater recharge: groundwater-surface water exchange across an alpine-valley bottom transition
Project Number:	2003MT9B
Start Date:	5/1/2003
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	At-large
Research Category:	Ground-water Flow and Transport
Focus Category:	Hydrology, Wetlands, Groundwater
Descriptors:	groundwater recharge
Principal Investigators:	Brian Leonard McGlynn, Richard Sojda

Publication

1. Covino, T., B.L. McGlynn, R. Sojda, and B. Edwards. 2004. Mountain front groundwater recharge: groundwater-surface water exchange across an alpinevalley transition. American Geophysical Union Fall Meeting.
2. Covino, T, B.L. McGlynn, R. Sojda and B. Edwards. 2004. Groundwater-surface water exchange across an alpine-valley transition. Montana Chapter of the American Water Resources Association Annual Meeting, Helena, Montana.

Mountain Front Groundwater Recharge: Groundwater-Surface Water Exchange Across an Alpine-Valley Bottom Transition

Timothy P. Covino, Brian L. McGlynn, and Richard S. Sojda

Abstract

Mountain front recharge (MFR) contributes significantly to valley aquifer recharge in mountainous regions, yet adequate understanding of this process is lacking. GW recharge at the mountain front and subsequent GW discharge in the valley bottom are important hydrological processes in mountain watersheds. Interactions between GW and SW are gaining increasing recognition as an outstanding research need. In many valleys, streams change in both space and time from *losing* water to GW to *gaining* water from GW as they flow toward the valley-bottom. Alpine to valley bottom transition zones play a key role in regulating the amount, timing, and chemistry of stream water exiting the mountains and reaching the valley floor. We hypothesize that alpine-valley transitions function as hydrologic and biogeochemical buffers and both GW recharge and discharge zones. More specifically, we hypothesize that streams often recharge GW near the mountain front and receive older stored GW further downstream. To investigate these hypotheses we applied physical hydrology techniques, tracer injections, and geochemical hydrograph separations in the Humphrey Creek watershed in southwestern Montana. A network of four stream gauging stations, 19 wells, and 18 piezometers were installed for monitoring physical hydrology. Our intensive instrumentation network allowed us to assess the spatial and temporal variability of mountain front GW recharge and GW-SW interactions across an alpine-valley transition. Geochemical signatures were used to partition stream flow into alpine and GW sources, and tracer injections were used to quantify GW recharge/discharge over various reaches. When investigating complex GW-SW interactions it is necessary to use multiple lines of evidence to understand these processes. Our results demonstrate that much of the alpine streamwater recharges GW at the mountain front and that older GW of a different chemical composition sustains down-valley stream discharge. Down-valley stream discharge was dominated by GW inputs and responded to GW stage. A critical GW stage height was necessary to sustain down-valley channel flow, as this is the only major input to channel flow during early and late season base flow. Conversely, GW contributed little to stream flow in the upper reaches (MFR zone) of the study area. Much of the water exiting the mountains recharged GW in MFR zone throughout the summer. Water exiting the mountains as channel flow and water reaching the lake as channel flow were not the same water and had different sources and geochemistry (alpine water versus older stored groundwater). This was due to GW-SW exchange occurring in the MFR zone and across the valley floor, which controlled stream water geochemistry and buffered hydrograph response in the valley bottom. This exchange resulted in significant changes in SW chemistry moving from alpine, to MFR zone, to the valley bottom, and muted fluctuations in channel flow, both at high and low flow. Implications are that mountain front GW recharge magnitudes over long timescales control valley aquifer storage state which combined with alpine runoff magnitude control stream water quantity and geochemical composition downstream.

Research Objectives

Many valley bottoms receive significant inputs of water from mountain front groundwater (GW) recharge, yet this process is not well understood. The precise definition of the mountain front has been ambiguous in the literature (Wilson and Guan, 2004). For the purposes of this project the mountain front recharge (MFR) zone will be defined as the zone between the mountain block-piedmont break in slope and the piedmont-valley bottom break in slope. GW-surface water (SW) exchange that occurs in the MFR zone, and across the valley bottom, is important in controlling SW chemistry, and stream discharge magnitude. Although GW-SW exchange has received increasing attention as a research need, adequate understanding of these processes has not been attained. Stream discharge and valley aquifer storage in mountainous regions are largely dependent on MFR and GW-SW exchange, and understanding these hydrological processes is important. The goals of this project were to use physical hydrology, injected stream tracers, and geochemical hydrograph separations to understand mountain front GW recharge and GW-SW exchange in the Humphrey Creek watershed in south-western Montana (Figure 1). Specifically, we sought to:

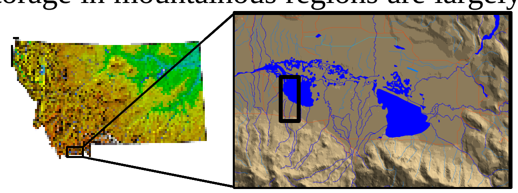


Figure 1. Location of the Humphrey Creek watershed in south-western Montana.

(1) elucidate areas of GW recharge and discharge,
(2) quantify GW recharge/discharge over specific reaches,
(3) determine source water contributions to stream flow and how they impact SW chemistry and discharge magnitude,
(4) determine how physical relationships between GW and SW impact all of these processes.

Furthermore, we sought to know how all of the above listed objectives varied both spatially and temporally.

Methodology

Physical hydrology, injected stream tracers, and geochemical hydrograph separations were used to investigate the questions and objectives proposed above. A network of 19 wells, 18 piezometers, and four gauging stations were installed in the Humphrey Creek watershed to investigate the physical hydrology (Figure 2). Injected stream tracers were used to quantify GW recharge/discharge over specific reaches of Humphrey Creek. Geochemical hydrograph separations were used to determine source water contributions to stream flow. These methods were combined and used together to help answer the questions posed in this project. Studying complex GW-SW exchange processes requires a combined approach in order to correctly identify dominant mechanisms controlling these processes.

Study Site

The Humphrey Creek watershed is located in the Red Rock Lakes National Wildlife Refuge in southwestern Montana (Figure 1). Humphrey Creek flows from south to north, originating in the Centennial Mountains and flows into Lower Red Rock Lake draining a 351 ha watershed. The headwaters of the creek begin above tree line in the alpine region of the watershed. Humphrey Creek then flows through sub-alpine mixed coniferous forest, exits the forest and flows through upland grasses, willows, and shrubs and enters the valley bottom where the vegetation consists of sedges, rushes, grasses and willows. The area of instrumentation begins where Humphrey Creek exits the coniferous forest and continues to the lake edge (Figure 2). The soils in the study site range from cobbly silts in the upland, and peat, to clay, to sand, to gravel in the lowland. The geology of the Humphrey Creek watershed consists of tertiary volcanics, underlain by upper cretaceous, Mesozoic, Paleozoic, and pre-cambrian rocks in the alpine zone and landslide debris, lake sediments, and alluvial deposits in the valley bottom.

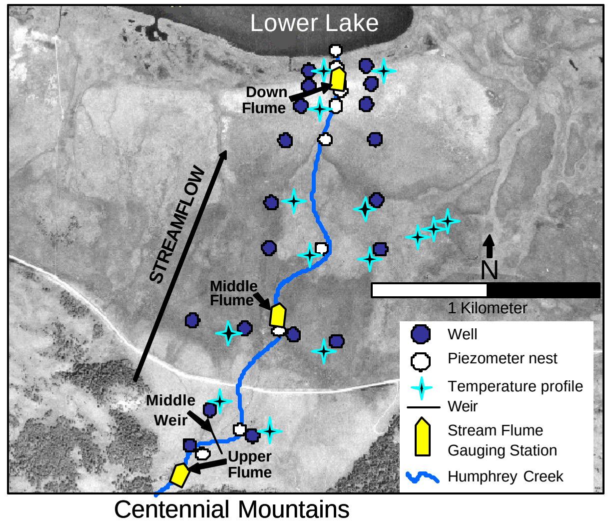


Figure 2. Study site instrument layout for the Humphrey Creek watershed. Humphrey Creek flows to the north, draining the Centennial Mountains into Lower Red Rock Lake.

<u>Stream Gauging</u>

Three inch Parshall flumes were installed in Humphrey Creek during the spring of 2004 (Figure 3) for stream gauging purposes. Flumes were anchored in the bed and banks of the stream to force stream water through the flume. This was done by excavating an appropriate area necessary to insert the flume wingwalls and subsequently backfilling the area. In total three flumes were installed; one in the upper reach of the study area (referred to as upper flume), a second in the middle reach of the study area (referred to as middle flume), and a third in the lower reach of the study area (referred to as down flume). Each flume was instrumented with data loggers (either Druck pressure transducers connected to Campbell CR10 data loggers, or Tru-Track capacitance rods) installed in the stilling well to collect stage measurements on ten minute intervals. Discharge was calculated based on stage-discharge rating curves we

Figure 3. Example of three inch Parshall flume installed in Humphrey Creek.

developed. Measurements began in the end of April or early May and continued until the end of August or September, 2004 (dependent on cessation of channel flow).

A rectangular weir was installed in Humphrey Creek prior to the project, and was also utilized for stream gauging. This weir is located between the upper and middle flumes and is referred to as middle weir. A section of stream behind the weir was widened and deepened to create a stilling pool. A stilling well was built on the upstream side of the weir and was instrumented with a Tru-Track capacitance rod. Stage measurements were recorded on a ten minute interval, and were taken from the end of April to the end of September, 2004. Again, a stage-discharge rating curve was developed.

Velocity-area gauging was used at each flume and the weir to help calibrate the rating curves for the flumes and the weir. This method was also used to gauge the stream in locations where a flume or a weir did not exist, in order to get an estimate of discharge at that location. A Marsh-McBirney Flo-Mate 2000 portable flow meter was used in the velocity-area gauging of the stream. Velocity-area gauging occurred on a regular basis (nearly daily) from the beginning of May to the end of August, 2004.

Dilution gauging with sodium chloride (NaCl) was used to help calibrate flume and weir rating curves. Breakthrough curves were obtained with Campbell CS547A conductivity and temperature probes connected to Campbell CR10 data loggers. Measurements were taken every 5 seconds during dilution gauging experiments. Integration of the area under the breakthrough curve yields discharge.

Wells and Piezometers

Wells were installed in transects perpendicular to the stream from the mountain front to the valley bottom lake edge (see Figure 2) to capture the shape of the local GW table surrounding Humphrey Creek. Most wells were instrumented with Tru-Track data loggers which recorded GW height measurements every ten minutes. Hand measurements of the wells occurred regularly. Hand measurements included GW height, conductivity (specific conductance in mS cm^{-1}), and temperature. Nested piezometers were installed in the channel bed of Humphrey Creek to determine the vertical gradients in the GW table at each transect (see Figure 2). Again, most piezometers were equipped with Tru-Track capacitance rods recording measurements on ten minute intervals. Hand measurements were the same as for wells. These measurements began in March, 2004 and continued through September, 2004.

Water Sampling

GW samples were taken from wells, piezometers and springs for later chemical analysis. GW samples were obtained with a hand held peristaltic pump, and lines were always pumped and purged before sample was taken. Clean plastic bottles were rinsed with sample three times before filling. Samples were filtered through 0.45 µm polypropylene filters and stored in the dark at 4°C until analysis. SW samples were taken from stream gauging locations either by hand or by ISCO auto samplers. ISCO auto samplers pump sample via a peristaltic pump and pump and purge lines before filling clean plastic sample bottles. Hand SW samples were taken in clean plastic bottles, and bottles were rinsed with sample three times before filling. All SW samples were filtered through 0.45 μm polypropylene filters and stored in the dark at 4°C until analysis.

Data Loggers

We installed Campbell CR10X data loggers at upper, middle, and down flumes. Data collected was stream stage, SW conductivity (SC in mS cm^{-1}), SW temperature, soil moisture, and air temperature. Stream stage was measured with a Druck PDCR 1230 pressure transducer, stream water conductivity and temperature was measured with a Campbell CS547A conductivity and temperature probe, soil moisture was measured with a Campbell CS616 water content reflectometer, and air temperature was recorded with Thermocron I-buttons. A Campbell TE525 tipping bucket rain gauge was installed at middle flume to collect rain data. Campbell data loggers collected measurements from each sensor every ten minutes, except for rain data which was registered each time the bucket tipped. Tru-Track capacitance rod data loggers were also installed in flume and weir stilling wells, GW wells, and piezometers. Tru-Track data loggers measured water height, water temperature, and logger temperature on ten minute intervals.

Figure 4. Example of Campbell data logger set-up with solar panel. Logging stream stage, conductivity, temperature, and soil moisture.

Chemical Analysis

Water samples were analyzed for major ions with a Metrohm-Peak compact ion chromatograph. Sodium (Na), ammonium (NH_4), potassium (K), calcium (Ca), and magnesium (Mg) were measured on a Metrosep C-2-250 cation column. Nitrate (NO_3), nitrite (NO_2), chloride (Cl), bromide (Br), phosphate (PO_4), and sulphate (SO_4) were measured on a Metrosep C-2-250 anion column. And silica (Si) was measured as silicate (SiO_4) on a Hamilton PRP-X100 anion column.

Hydrograph Separation and Uncertainty

Hydrograph separations were used to determine contributions to stream flow from the alpine zone (AL) and the valley bottom GW. Real time separations were made using conductivity. GW conductivity was known and AL and SW conductivity were measured real time on ten minute intervals. Chemical analysis of samples and regression of ion concentration versus conductivity was used to validate this separation. A two-component separation can be solved by simultaneously solving equations one and two.

$$Q_{AL} = \left[\frac{C_{ST} - C_{GW}}{C_{AL} - C_{GW}}\right] Q_{ST} \ \textbf{(1);} \quad Q_{GW} = \left[\frac{C_{ST} - C_{AL}}{C_{GW} - C_{AL}}\right] Q_{ST} \ \textbf{(2);} \quad Q_{ST} = Q_{GW} + Q_{AL} \ \textbf{(3)}$$

Where Q_{AL} is the contributions to discharge from alpine waters, Q_{GW} is the contributions to discharge from valley bottom GW, Q_{ST} is the stream discharge, and C_{AL}, C_{GW}, and C_{ST} are alpine conductivity, GW conductivity, and stream conductivity, respectively. Uncertainty analysis was applied to the hydrograph separations following the methods of Genereux (1998) using equations four and five.

$$W_{f_{AL}} = \left\{ \left[\frac{C_{GW} - C_{ST}}{(C_{GW} - C_{AL})^2} W_{C_{AL}} \right]^2 + \left[\frac{C_{ST} - C_{AL}}{(C_{GW} - C_{AL})^2} W_{C_{GW}} \right]^2 + \left[\frac{-1}{C_{GW} - C_{AL}} W_{C_{ST}} \right]^2 \right\}^{1/2} \quad \textbf{(4)}$$

$$W_{f_{GW}} = \left\{ \left[\frac{C_{AL} - C_{ST}}{(C_{AL} - C_{GW})^2} W_{C_{GW}} \right]^2 + \left[\frac{C_{ST} - C_{AL}}{(C_{AL} - C_{GW})^2} W_{C_{AL}} \right]^2 + \left[\frac{-1}{C_{AL} - C_{GW}} W_{C_{ST}} \right]^2 \right\}^{1/2} \quad \textbf{(5)}$$

Where $W_{f_{AL}}$ is the uncertainty in the AL component, $W_{f_{GW}}$ is the uncertainty in the GW component, W_{AL}, W_{GW}, and W_{ST} are the analytical errors in AL, GW, and stream conductivity measurements, and C_{AL}, C_{GW}, and C_{ST} are AL, GW, and stream conductivities.

Salt Tracer Experiments

Salt tracer experiments occurred in May, June, July, and August, 2004. Sodium chloride (NaCl) was used for salt tracer experiments. Salt was injected as a slug above upper flume at the edge of the coniferous forest. Breakthrough curves were gathered at five downstream locations: upper flume, middle weir, a transect between the road and middle weir (referred to as south transect 1), a transect between the road and the middle flume (referred to as north transect 0), and middle flume. Data at the three most upstream locations (upper flume, middle weir, and south transect 1) were collected with Campbell data loggers and Campbell CS547A conductivity and temperature probes on five second intervals. At the two most downstream locations (north transect 0, and middle flume) measurements were made with a YSI conductivity, temperature, and pH probe on ten second intervals. Salt tracer injections allow us to accurately assess the stream discharge at each measurement location by knowing the mass injected and the accumulated change in concentration. Accurate discharge measurements at five measurement locations allows us to apply a water balance technique to estimate the amount of water being lost from the stream as GW recharge.

Principal Findings

Results indicate that significant GW recharge occurs at the mountain front, GW-SW exchange is dynamic (spatially and temporally), and GW recharge and GW-SW exchange control valley bottom aquifer storage, SW chemistry, and stream discharge magnitude.

Stream Discharge and Conductivity

Stream discharge was highest in the upper reaches of the study site, in the mountain front GW recharge zone (Figure, 5). Much of the water exiting the mountains as channel flow in this zone was lost from the stream as GW recharge. Stream discharge subsequently decreased at downstream locations (Figure, 5). Stream discharge responded to rain events with pulsed increases in channel flow. However the bulk of the discharge seems to be driven by snowmelt. Peak discharge occurred on June, 9th at upper and middle flumes, and on June, 10th at down flume. Down flume, located near the lake edge in the valley bottom, had a flashy hydrograph and was very responsive to rain events. This is presumably due to the limited storage available in this part of the landscape. GW

GW was close to the ground surface in the area around down flume and soils were moist, which may account for the responsiveness of stream discharge to rain events at down flume. Conversely, at middle and upper flumes the stream hydrograph was not as responsive to rain events. This is presumably due to greater available storage in these zones. The water table was far deeper in these reaches and soils were dry. As such, moisture inputs from rain events contributed to recharging GW and soil moisture, and less input was directed to the stream. Stream water conductivity at upper and middle flumes were similar. The conductivity at these locations was near 0.2 mS cm^{-1} during the rising limb and peak of the hydrograph. Conductivity rose slightly during late season base flow. Rain events caused spiked decreases in conductivity, as low conductivity water contributions to stream flow were increased. At down flume the early season conductivity was much higher, compared to middle and upper flumes. Conductivity was near 0.6 ms cm^{-1} when channel flow began in May at down flume and had a convex shape. It was high during the early season, fell during peak flow, and began climbing again during late season flow. This shows high GW contributions during early season flow, increased AL contributions during peak flow, and high GW contributions to

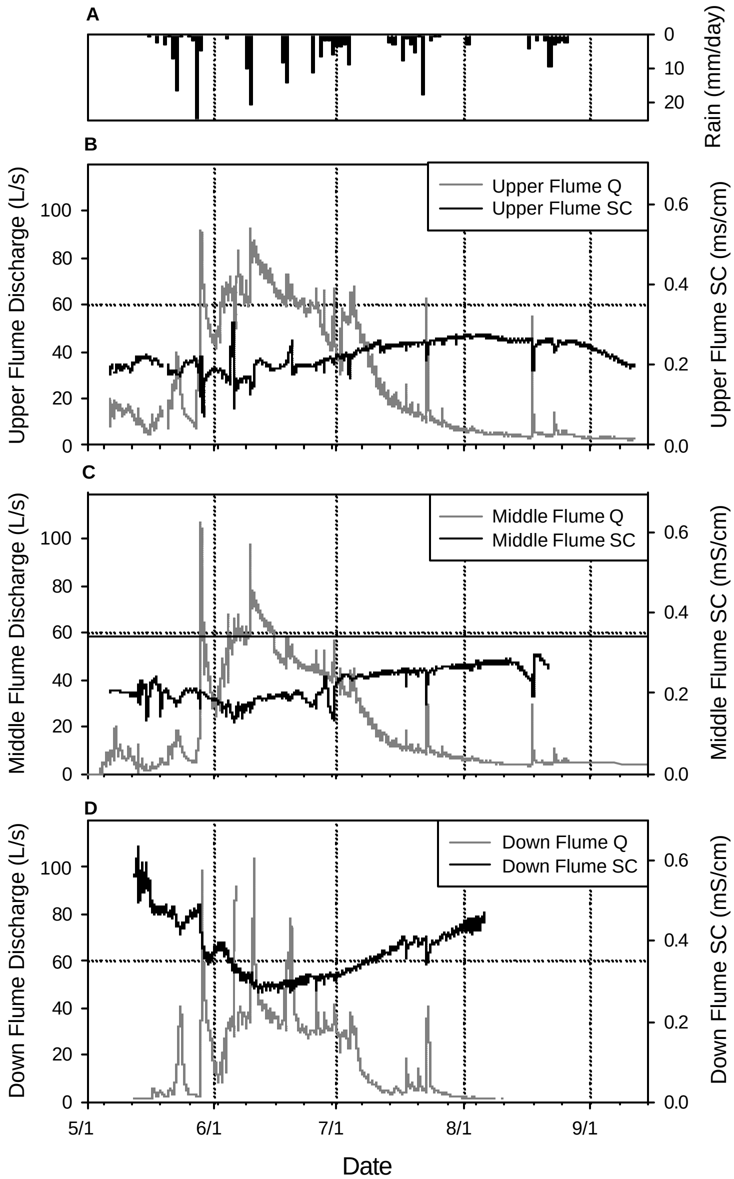


Figure 5. (A) Daily rain totals (mm/day). Stream hydrograph and conductivity (SC) for upper flume (B), middle flume (C), and down flume (D) from 5/1-9/15/2004.

stream flow during late season flow. Interestingly, peak daily flow due to diurnal fluctuations occurred between 10:00 and 12:00, and low flow occurred near 20:00. This suggests alpine controls on water exiting the mountains and a lag time in response moving downstream. If local evapotranspiration (ET) were controlling diurnal fluctuations, expectations would be that peak flow would occurr late at night or early in the morning. This data suggests that alpine ET, occurring late at night or early in the morning, is controlling transition zone and valley bottom discharge, and there is a lag time associated with water moving out of the mountains to the transition zone. Fluctuations in conductivity lag behind discharge fluctuations which suggests a particle transport mechanism (conductivity transport) versus a pressure wave propagation transport (discharge transport). Hydrologic lag times associated with ET have been observed by other studies (Bond et al., 2002), as have differences between water (pressure wave propagation) and tracer (particle transport) movement through watersheds (USGS, 1997). Stream discharge peaked before lake stage peaked (Figure 6). This shows that stream discharge is controlling lake response. Stream and GW gradients were into the lake and drove lake response.

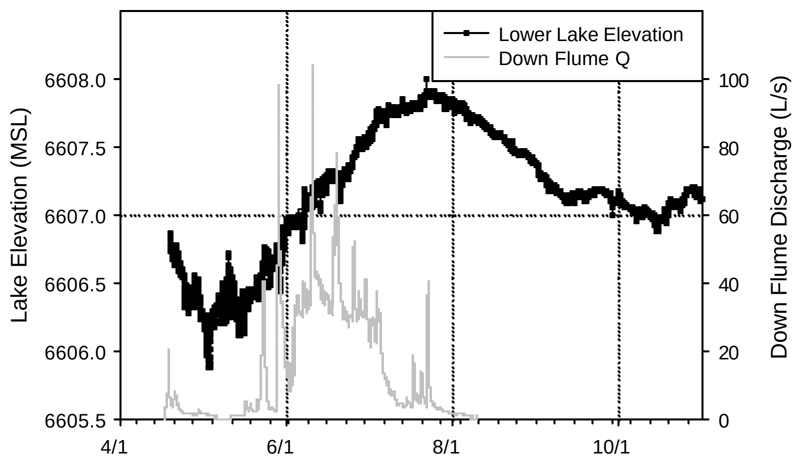


Figure 6. Time series of Lower Red Rock Lake elevation and stream discharge at down flume. Stream peak occurred much earlier in the summer than did lake peak.

<u>Wells and Piezometers</u>

GW levels were deep relative to ground surface in the MFR zone (first two lateral well transects) of the study area (Figure 7). Wells in this zone were completed to depths between 1.7 and 2.8 meters, and were dry for most of the season (April to September, 2004). Well completion depths were determined by the depth to which hand augering was possible. GW levels in this area rose during peak discharge and GW was able to be seen in wells. GW hydrographs in this zone largely followed the shape of the stream hydrograph. GW was often disconnected from stream water in this zone, showing that depths to GW were greater than the depth of the stream bed. This makes GW inputs to channel flow not possible and gradients were consequently out of the stream. Thus, the stream was losing in the MFR zone and contributed to GW and soil moisture recharge. Depths to GW relative to ground surface in the valley bottom were shallow, and GW was often at or near the ground surface in this zone (Figure 8). In north well 71 (NW 71) and north well 72 (NW 72) a sharp rise in GW levels can be seen around March, 20. Prior to this sharp increase in GW levels there was significant water ponding on top of frozen soils in the valley bottom. It seems that thawing soils allowed significant amounts of water to infiltrate to the GW table at this time. Water inputs were due to spring snowmelt in the valley bottom which occurs prior to melt of snow higher in the watershed. This event contributed significantly to local GW recharge, and also initiated channel flow in the valley bottom. A similar increase in GW levels could have occurred in the upper reaches

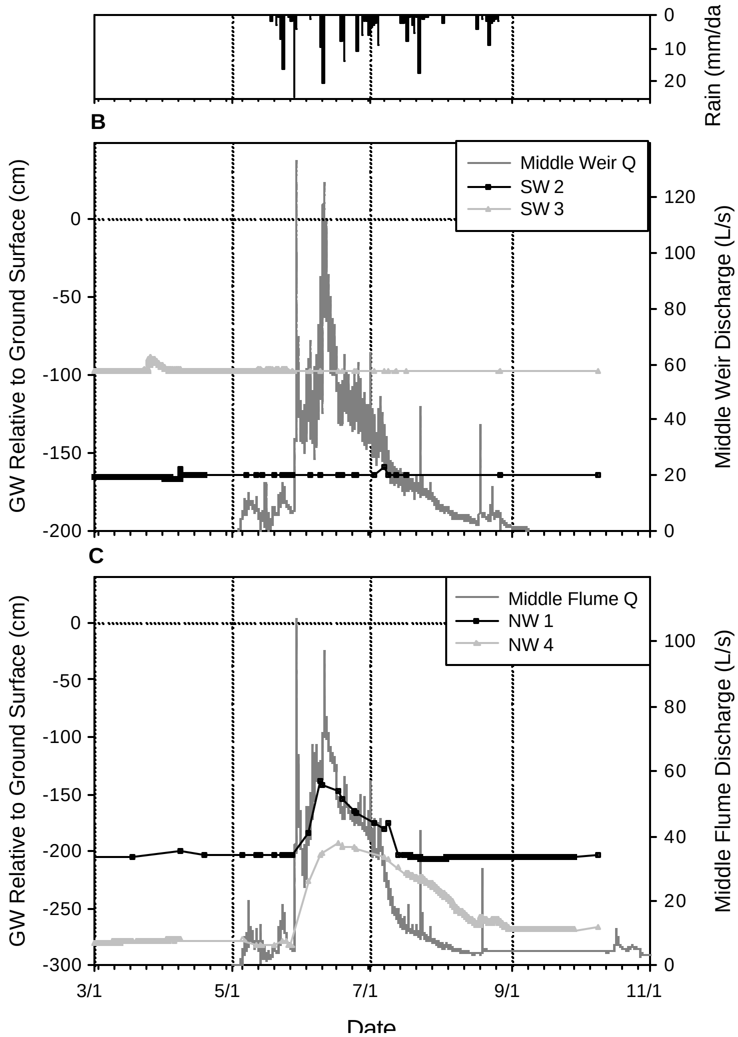


Figure 7. (A) Daily rain totals (mm/day). (B) GW levels for SW 2 & 3, with stream discharge from middle weir; and, (C) GW levels for NW 1 & 4, with discharge from middle flume for 3/1-11/1/2004. Flat lines in wells signify well completion depths. These wells are from the two upper-most transects of wells in the study site.

of the study area (MFR zone), however, due to great depths to GW, wells in this zone were dry during this period and there is no data to assess this possibility.

Nested piezometers were installed in the stream channel. Vertical GW gradients were upward at north piezometers 60 (NP 60) and 61 (NP 61), which are approximately half way between the MFR zone and Lower Red Rock Lake (Figure 9). Further downstream towards the lake, GW gradients were mostly lateral (Figure 9). This results in substantial GW contributions to stream flow near NP60 and 61. Lateral GW gradients make subtle gradients in and out of the stream possible and some GW-SW mixing may occur in this situation. GW inputs to channel flow near NP 60 and 61 and subsequent exchange between GW and SW substantially altered the chemistry of valley bottom SW compared to SW in the MFR zone. South piezometers 1 (SP 1) and 2 (SP2) and north piezometers 1 (NP 1) and 2 (NP 2) which are located in the MFR zone generally did not have water in them (Figure 10). This shows that GW depths in the MFR zone were deeper than the channel bed and gradients were out of the stream. Changes in GW gradients and GW-SW exchange across the study area impacted SW chemistry and attenuated hydrograph response. In some reaches (MFR zone) GW was being recharged by stream water, while in other reaches GW was discharging to the stream. These interactions seem to control much of the SW chemistry and stream hydrograph response in the watershed.

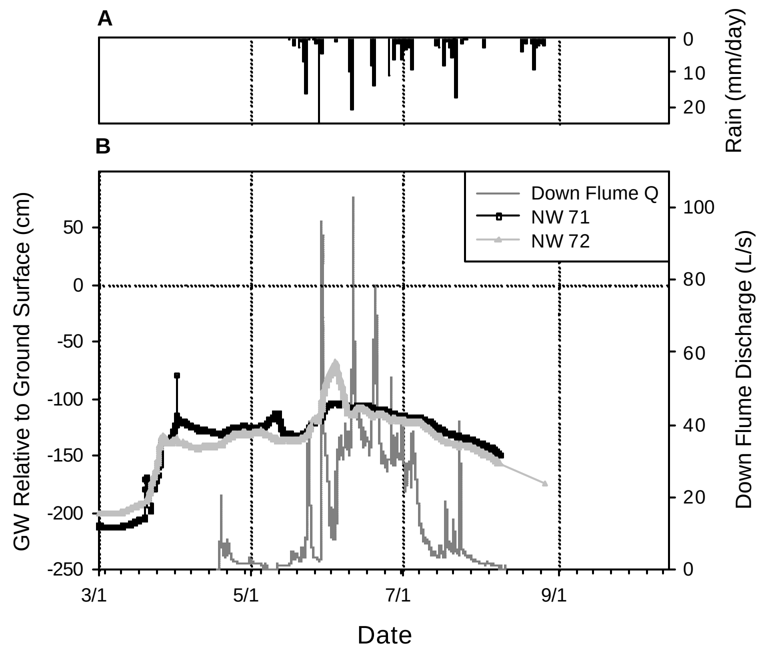


Figure 8. (A) Daily rain totals (mm/day). (B) GW levels for NW 71 & 72 with stream discharge for down flume from 3/1-10/15/2004. NW 71 & 72 are located in the valley bottom near down flume.

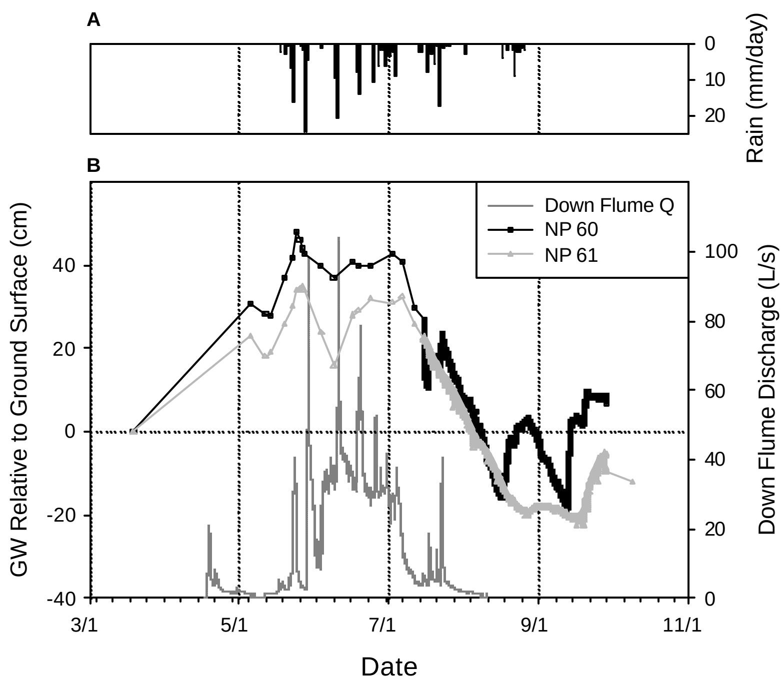


Figure 9. (A) Daily rain totals (mm/day). (B) GW levels for piezometers NP 60 & 61 and stream discharge for down flume from 3/1-11/1/2004. NP 60 is the deeper of the two nested piezometers indicating upward gradients.

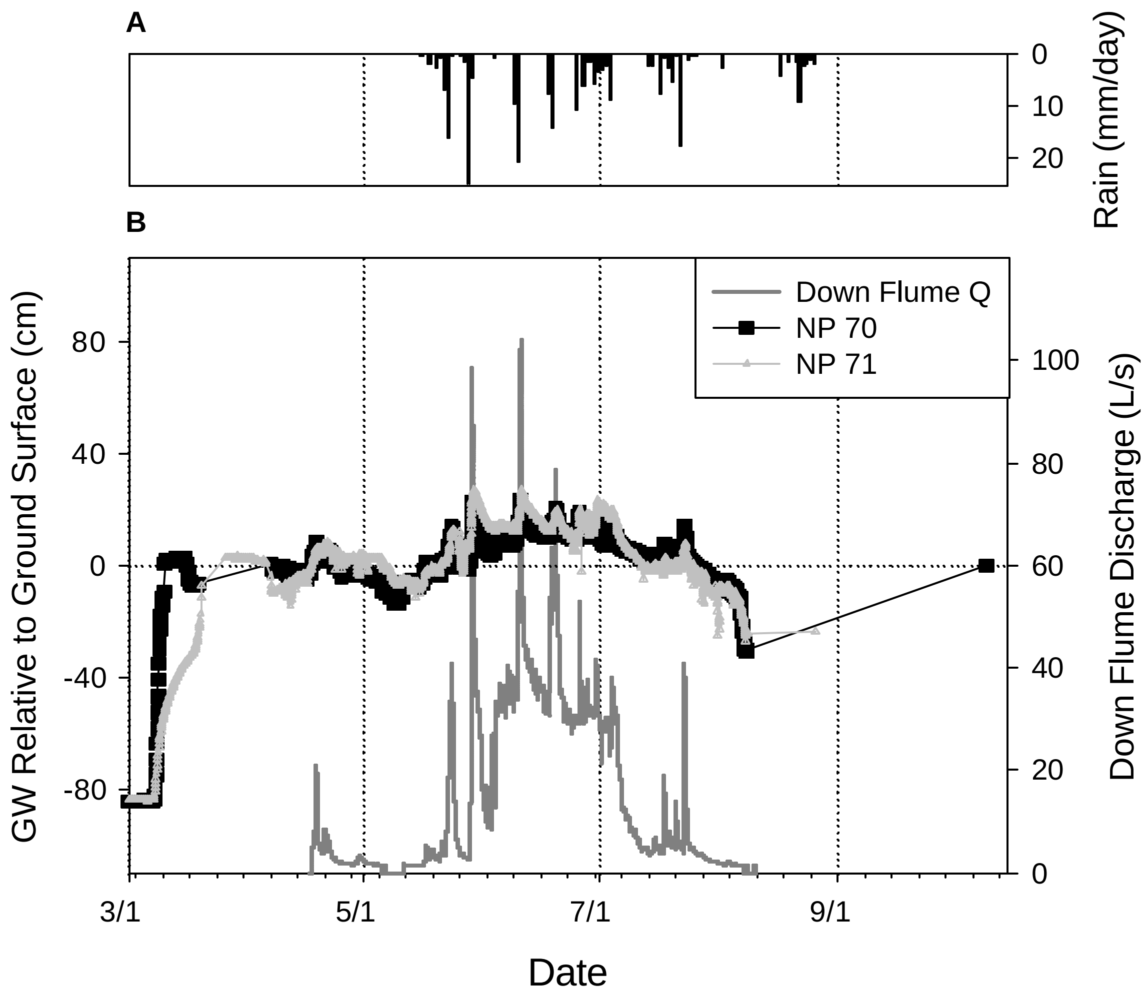


Figure 10. (A) Daily rain totals (mm/day). (B) GW levels for piezometers NP 70 & 71 with down flume stream discharge from 3/1-10/15/2004. NP 70 & 71 are located in the valley bottom near down flume. NP 70 is the deeper piezometer, gradients are lateral.

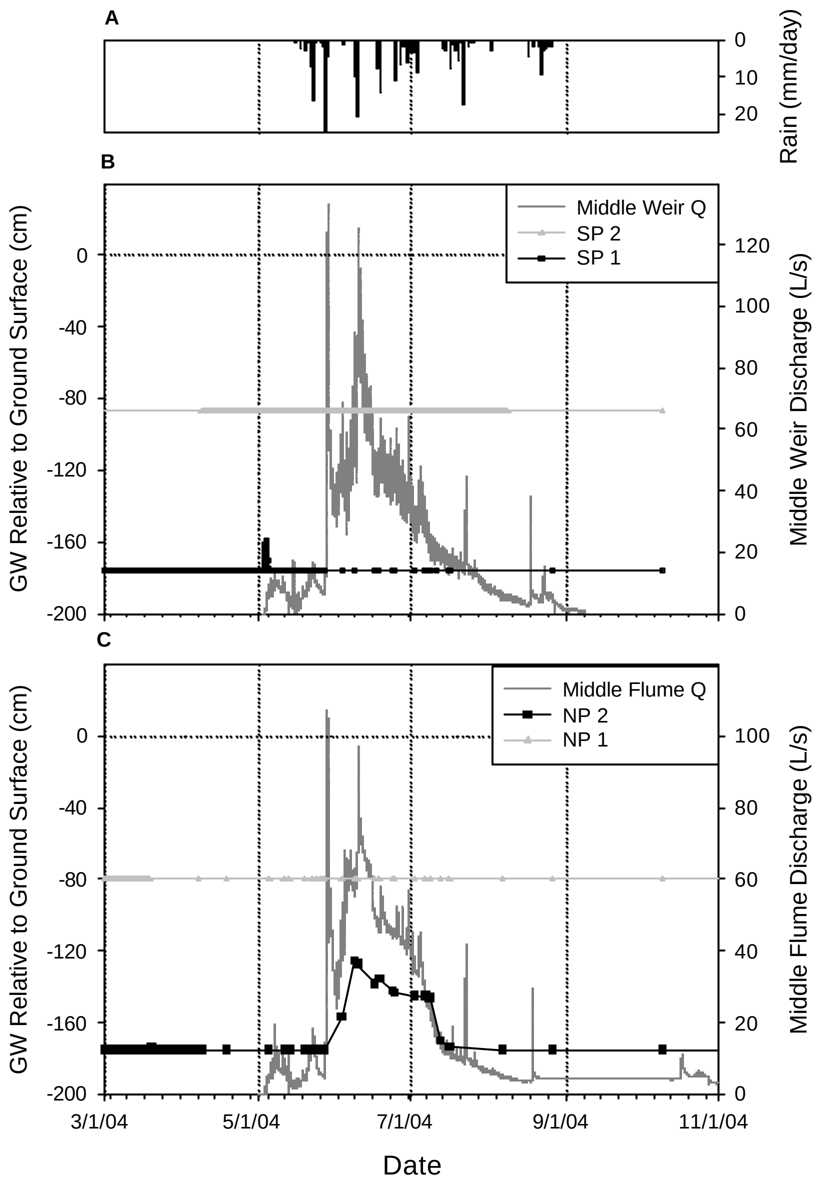


Figure 11. (A) Daily rain totals. (B) GW levels for piezometers SP 1 & 2, and stream discharge for middle weir, and (C) GW levels for piezometers NP 1 & 2 and stream discharge for middle flume. Black symbols are the deeper piezometers (SP 1 and NP 2). SP 1, SP 2, and NP 1 were dry for nearly all of the year. These piezometers are located in the upper reaches of the study area in the MFR sone.

Hydrograph Separations

Hydrograph separations showed marked changes in contributions from AL and GW to stream flow between middle and down flumes (Figure 11). Water in the channel at middle flume was composed mostly of AL water, conversely, water in the channel at down flume had substantial GW contributions to total discharge. From hydrograph separations we can determine how much water would be expected in the channel if there were not GW contributions to channel flow at down flume. There would be significantly less discharge at down flume and the period of time that channel flow occurred would be shorter. GW sustains early and late season base flow in this reach. As such GW-SW exchange serves to change the chemistry of water found in the channel between middle and down flumes, increases the amount of stream discharge at down flume, and lengthens the amount of time that channel flow occurs at down flume. Since stream discharge was less at down versus middle flume we might had assumed that Humphrey Creek was

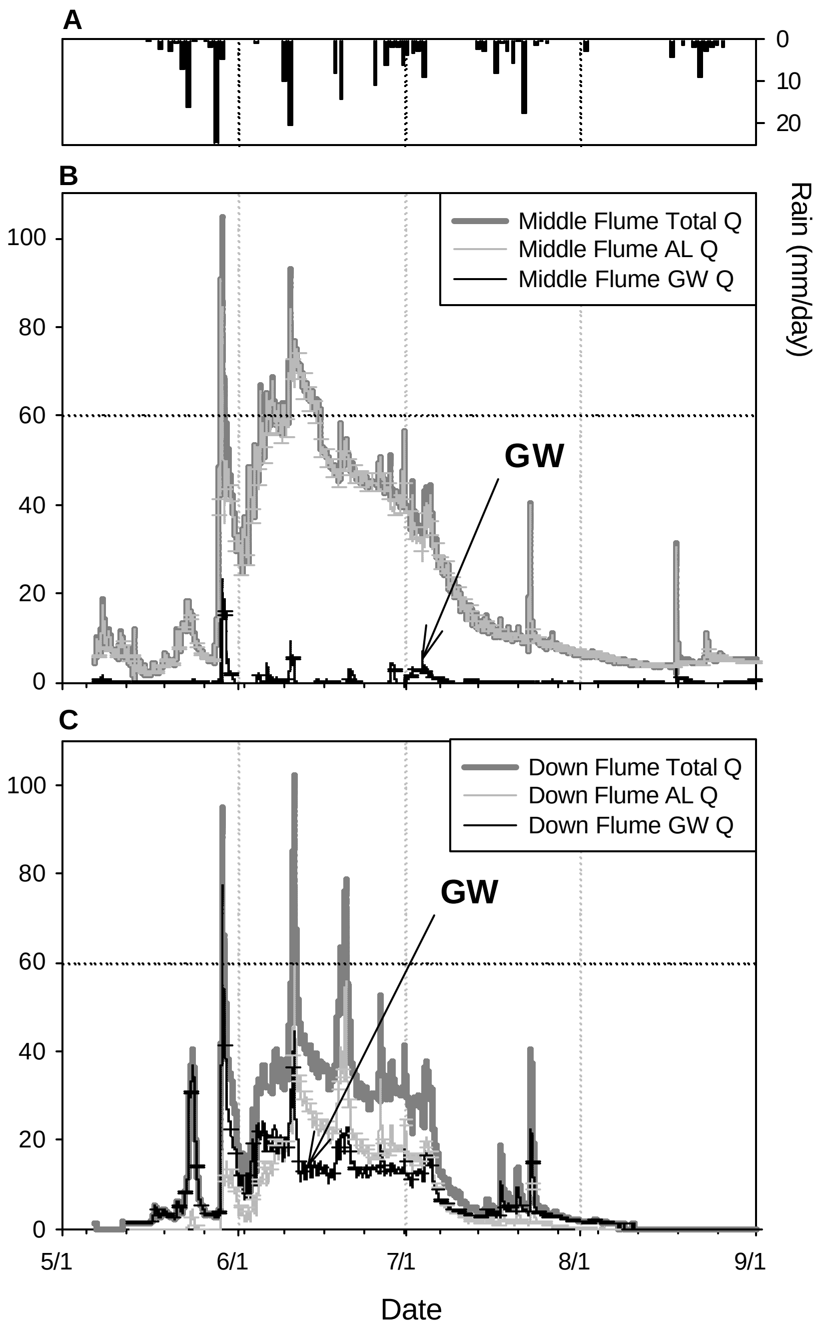


Figure 12. (A) Daily rain totals (mm/day). (B) Hydrograph separation for middle flume (GW and AL components of total discharge), and (C) hydrograph separation for down flume. Error bars on separation indicate the uncertainty in the separation.

simply a losing stream between these two reaches if we had not applied hydrograph separations. Hydrograph separations show us that although there is less water in the channel at down flume versus middle flume, Humphrey Creek actually gains significant GW in the down flume reach. The dynamic interaction between GW and SW, where GW recharge is occurring in ceratin reaches and GW discharge in others, seems to have significant impact on hydrological processes in the Humphrey Creek watershed. It is possible that similar interactions are important in other mountain watersheds, and mountain front GW recharge and GW-SW exchange might be a significant driver of hydrological processes in these settings. Combining physical hydrology with geochemical hydrograph separations allows researches to more thoroughly investigate the hydrology of these watersheds.

Salt Tracer Injections

Analysis of salt tracer experiment data is still occurring and will be completed shortly. This data will allow us to assess the amount of GW recharge and GW discharge occurring over specific reaches, and to estimate the volume of water exiting and entering the channel over these reaches. This data will be used in concert with physical hydrology data, and hydrograph separations to further analyze and investigate mountain GW recharge and GW-SW exchange in the Humphrey Creek watershed and the impact these mechanisms have on hydrological processes.

Publications & Citations

Covino, T., McGlynn, B.L., Sojda, R., and B. Edwards. *Mountain Front Groundwater Recharge: Groundwater-Surface Water Exchange Across an Alpine–Valley Transition*. American Geophysical Union Fall Meeting. Fall, 2004.

Covino, T., McGlynn, B.L., Sojda, R., and B. Edwards. *Groundwater-Surface Water Exchange Across an Alpine–Valley Transition*. Montana Chapter of the American Water Resources Association Annual Meeting. Fall, 2004. **FIRST PLACE IN STUDENT PAPER COMPETITION.**

This work is currently be finalized in a Masters thesis titled *Mountain Front Groundwater Recharge: Groundwater-Surface Water Exchange Across an Alpine-Valley Bottom Transition*, and will be submitted for publication in peer reviewed journals such as *Water Resources Research* or *Journal of Hydrology*. This work has also led to ideas that will be pursued in future proposals to further this research.

Notable Achievements & Awards

TIM COVINO, FIRST PLACE IN STUDENT PAPER COMPETITION: Montana Chapter of the American Water Resources Association Annual Meeting. Fall, 2004.

Covino, T., McGlynn, B.L., Sojda, R., and B. Edwards. *Groundwater-Surface Water Exchange Across an Alpine–Valley Transition*. Montana Chapter of the American Water Resources Association Annual Meeting. Fall, 2004. **FIRST PLACE IN STUDENT PAPER COMPETITION**

TIM COVINO, 2005-2006 Montana USGS 104b Research Fellow

This work has supported one Undergraduate Scholars Project (USP) student, MSU undergraduate Brian Edwards.

LITERATURE CITED

Bond, B.J., J. A. Jones, G. Moore, N. Phillips, D. Post, and J.J. McDonnell, The xone of vegetation influence on baseflow revealed by diel patterns of streamflow and vegetation water use in a headwater basin, *Hydrol. Process.*, 16, 1671-1677, 2002.

Genereux, D., Quantifying uncertainty in tracer-based hydrograph separations, *Water Resour. Res.*, 34(4), 915-919, 1998.

U.S. Geological Survey, The Colorado River in Grand Canyon: how fast does it flow?, FS-168-97, www.gcmrc.gov/files/pdf/fs-168-97.pdf, December, 1997.

Wilson, J.L. and H. Guan, "Mountain-Block Hydrology and Mountain-Front Recharge," in Groundwater Recharge in a Desert Environment: The Southwestern United States, edited by F.M. Phillips, J.F. Hogan, and B. R. Scanlon, 2004, American Geophysical Union, Washington, DC, October 2004.

Understanding and predicting changes in the microbial ecology of mine tailings in response to the addition of dissolved organic carbon

Basic Information

Title:	Understanding and predicting changes in the microbial ecology of mine tailings in response to the addition of dissolved organic carbon
Project Number:	2003MT10B
Start Date:	5/1/2003
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	At-large
Research Category:	Engineering
Focus Category:	Water Quality, Toxic Substances, Treatment
Descriptors:	acid mine drainage, microbial ecology
Principal Investigators:	Paul Sturman

Publication

1. Two manuscripts are nearing submission to professional journals.

Abstract

Recent field- and laboratory-scale experimentation at MSU and elsewhere has indicated that microbial populations within acid-producing mine tailings can be influenced by the addition of dissolved organic carbon. Heterotrophic bacteria can be stimulated to consume dissolved oxygen from infiltrating water, thus decreasing the oxidation-reduction (redox) potential throughout the tailings pile and promoting the activity of anaerobic sulfate reducing bacteria (SRB). However, an unintended consequence of the addition of organic carbon may be the stimulation of heterotrophic populations within the mine tailings that are also capable of iron reduction. The stimulation of these populations via organic carbon addition is detrimental to remediation efforts. The research reported herein quantifies the specific response of SRB and iron-oxidizing bacteria (IOB) to commonly used organic carbon sources. Results indicate that both SRB and IOB populations may be stimulated by the addition of whey, molasses, or methanol. While IOB populations were much higher than SRB initially in untreated tailings from both highly weathered and fresh mine tailings, SRB were stimulated 3-5 orders of magnitude in some cases, whereas IOB were stimulated less than 100-fold in all cases. Bacterial population increases were not found to be well correlated with organic carbon concentration, but whey and molasses were clearly better substrates for SRB growth than methanol. Bacterial DNA examined using denaturing gradient gel electrophoresis (DGGE) indicated both that a high level of microbial diversity was present in tailings prior to treatment, and that this increased following treatment. DGGE also shows more microbial diversity in highly weathered tailings than in freshly placed tailings.

Background

Acid rock drainage (ARD) arises from waste rock and mine tailings containing sulfide minerals and lacking adequate acid-consuming carbonate minerals. Sulfide minerals, such as pyrite (FeS_2) are oxidized to form ferrous iron (Fe^{2+}), sulfate, and acidity when oxygenated water infiltrates tailings. The role of iron- and sulfur-oxidizing bacteria (IOB/SOB), such as *Acidithiobacillus (At.) ferrooxidans*, in accelerating the production of acidic drainage from sulfide-containing mine tailings has been known for decades (Silverman and Lundgren, 1959). IOB accelerate acid production by cycling ferrous iron to ferric iron (Fe^{3+}), which then reacts with pyrite to form more iron, sulfate, and acidity. The activity of IOB/SOB is estimated to result in increased acid production of up to six orders of magnitude over abiotic tailings oxidation (Brierley, 1978).

ARD from abandoned hard rock mine lands is a major environmental problem that impacts both ground- and surface water throughout the Western United States and is a major contributor to loss of habitat for fisheries. In Montana alone, there are estimated to be over 20,000 abandoned mines, many of which generate ARD which impacts over 1000 miles of streams. Many abandoned mines are located on public land (State, US Forest Service, or US Bureau of Land Management) or on patented parcels enclosed by public lands. It is therefore in the public interest to foster innovative, cost-effective solutions to ARD.

A remedial strategy that has received significant attention over the past 5 years is the addition of organic carbon directly to mine tailings either in solid form or dissolved in water applied to the tailings surface. Common solid additives include compost, manure, agricultural wastes and sewage sludge. Dissolved materials which have been studied include food processing wastes such as molasses and whey, as well relatively inexpensive non-waste products such as methanol and ethanol. These strategies seek to manipulate indigenous microbial populations in the mine tailings; specifically, stimulating sulfate-reducing bacteria.

Recent field- and laboratory-scale experimentation at MSU and elsewhere has indicated that microbial populations within acid producing mine tailings can be influenced by the addition of easily assimilable dissolved organic carbon. Results from this work have shown that heterotrophic bacteria can be stimulated to consume dissolved oxygen from infiltrating water, thus decreasing the oxidation-reduction (redox) potential throughout the tailings pile and promoting the activity of anaerobic sulfate reducing bacteria (SRB). This work has also shown that mine tailings mineralogy and pH can be favorably altered as a result of the activity of populations of general heterotrophic bacteria (GHB) and SRB. The control of microbial growth within tailings piles can potentially result in significant reductions in the rate and extent of mineral oxidation and subsequent acid production. However, an unintended consequence of the addition of organic carbon may be the stimulation of heterotrophic populations within the mine tailings that are also capable of iron and/or sulfur reduction. Other researchers (Johnson et al., 2001) have recently identified several phylogenetically distinct groups of Gram-positive bacteria which are capable of both heterotrophic growth and simultaneous iron oxidation. The stimulation of these populations via organic carbon addition may be detrimental to remediation efforts. In past work at the Center for Biofilm Engineering (partially funded by the Montana Water Center) stimulation of these populations following the addition of dissolved molasses and whey was periodically observed. Successful implementation of this technology at the field scale requires a more thorough understanding of the presence, activity, and stimulation of these potentially detrimental populations, as well as beneficial populations (e.g. SRB). In particular, it is necessary to understand and predict the response of iron-oxidizing and sulfate-reducing populations to various organic carbon addition strategies. The research reported herein seeks to determine the specific response of these microbial populations to commonly used organic carbon sources. Mine tailings from the long-abandoned Mammoth Mine (Boulder River, MT) and recently deposited tailings from the Golden Sunlight Mine (Cardwell, MT) were fed varying concentrations of whey, molasses, and methanol in 6 week microcosm experiments. SRB and IOB populations were assessed before and after organic carbon treatment to determine the responses of these populations.

Methods and Materials

Mine tailings from both the Golden Sunlight Mine and the Mammoth Mine were collected from tailings piles in 5-gallon plastic containers and transported to the Center for Biofilm Engineering (CBE) at Montana State University. The Mammoth mine was operational during the early 20th century, and has been abandoned for approximately 70 years, while the Golden Sunlight Mine is in current operation. The tailings from these mines (which are approximately 15 miles apart) therefore represent highly weathered (Mammoth) and unweathered (GSM) conditions.

Microcosm Construction

Tailings were air dried for 1 day by spreading on clean plastic trays. 25 g aliquots of either Mammoth or GSM tailings were weighed and placed into 120 ml serum bottles. 100 ml growth media was then added. Microcosms that were to be incubated aerobically received sterile phosphate buffer solution (PSB), and microcosms that were to be incubated anaerobically received sterile modified Postgate C medium containing no added organic carbon. Organic carbon in the form of cheese whey, molasses, or methanol was then added to each of the microcosms to final concentrations of 100 mg/l, 1 g/l, or 5 g/l. Control microcosms received no added organic carbon. A full description of each microcosm, including tailings source, carbon source, and incubation condition is shown in Table 1. After the addition of organic carbon, aerobic microcosms were sealed with a gas-permeable foam stopper and anaerobic microcosms were nitrogen purged and crimp-sealed to maintain anaerobic conditions. All microcosms were then incubated in the dark at room temperature on a shaker table.

Microcosm Sampling and Analysis

Liquid phase samples were removed from the microcosms at days 1, 21, and 42 of incubation. The day 1 sample represents the pre-treatment condition of the tailings while the day 21 and 42 samples measure the progression of bacterial growth over the course of the experiment. During each sampling event, 1 ml of liquid solution was removed from each microcosm using a sterile syringe. From this sample, IOB and SRB were enumerated, and denaturing gradient gel electrophoresis (DGGE) was performed. IOB were enumerated using the most-probable number (MPN) technique (SM 9240 D.1F) and SRB were enumerated using API recommended practice 38/SRB MPN.

Table 1. Microcosm tailings source, carbon source, and incubation conditions.

#	Code	Tailings Source	Carbon Source	Carbon Conc.	Incubation conditions
1	MCO+	Mammoth	None	--	Aerobic
2	MCO-	Mammoth	None	--	Anaerobic
3	MW10+	Mammoth	Whey	100 gm/l	Aerobic
4	MW10-	Mammoth	Whey	100 gm/l	Anaerobic
5	MW20+	Mammoth	Whey	1 g/l	Aerobic
6	MW20-	Mammoth	Whey	1 g/l	Anaerobic
7	MW30+	Mammoth	Whey	5g/l	Aerobic
8	MW30-	Mammoth	Whey	5g/l	Anaerobic
9	MM10+	Mammoth	Molasses	100 gm/l	Aerobic
10	MM10-	Mammoth	Molasses	100 gm/l	Anaerobic
11	MM20+	Mammoth	Molasses	1 g/l	Aerobic
12	MM20-	Mammoth	Molasses	1 g/l	Anaerobic
13	MM30+	Mammoth	Molasses	5g/l	Aerobic
14	MM30-	Mammoth	Molasses	5g/l	Anaerobic
15	MA10+	Mammoth	Methanol	100 gm/l	Aerobic
16	MA10-	Mammoth	Methanol	100 gm/l	Anaerobic
17	MA20+	Mammoth	Methanol	1 g/l	Aerobic
18	MA20-	Mammoth	Methanol	1 g/l	Anaerobic
19	MA30+	Mammoth	Methanol	5g/l	Aerobic
20	MA30-	Mammoth	Methanol	5g/l	Anaerobic
21	GCO+	GSM	None	--	Aerobic
22	GCO-	GSM	None	--	Anaerobic
23	GW10+	GSM	Whey	100 gm/l	Aerobic
24	GW10-	GSM	Whey	100 gm/l	Anaerobic
25	GW20+	GSM	Whey	1 g/l	Aerobic
26	GW20-	GSM	Whey	1 g/l	Anaerobic
27	GW30+	GSM	Whey	5g/l	Aerobic
28	GW30-	GSM	Whey	5g/l	Anaerobic
29	GM10+	GSM	Molasses	100 gm/l	Aerobic
30	GM10-	GSM	Molasses	100 gm/l	Anaerobic
31	GM20+	GSM	Molasses	1 g/l	Aerobic
32	GM20-	GSM	Molasses	1 g/l	Anaerobic
33	GM30+	GSM	Molasses	5g/l	Aerobic
34	GM30-	GSM	Molasses	5g/l	Anaerobic
35	GA10+	GSM	Methanol	100 gm/l	Aerobic
36	GA10-	GSM	Methanol	100 gm/l	Anaerobic
37	GA20+	GSM	Methanol	1 g/l	Aerobic
38	GA20-	GSM	Methanol	1 g/l	Anaerobic
39	GA30+	GSM	Methanol	5g/l	Aerobic
40	GA30-	GSM	Methanol	5g/l	Anaerobic

DGGE Method

Microbial diversity was assessed using DGGE. Genetic material was extracted from liquid samples and amplified by polymerase chain reaction (PCR) using conserved, 16S rDNA-targeted primers. Nucleic acids were extracted and purified using a FastDNA Spin Kit and a FastPrep FP120 beadbeater (BIO101 Systems; Qbiogene, Inc, Carlsbad, CA). DNA was subsequently amplified using polymerase chain reaction (PCR). Complimentary regions of the 16S rDNA were amplified using 1µL each of 5' and 3' primer sets mixed with 25 µL Accuprime SuperMix II, 23 µL nuclease-free water (Sigma Chemical, St. Louis, MO) and 1 µL sample. Reagents were mixed in a 0.2 mL Thermowell tube and transferred to a programmable Thermal Blok II thermocycler (Lab-Line, Melrose Park, IL) for replication. PCR products were separated using denaturing gradient gel electrophoresis (DGGE), which was performed using a Bio-Rad DCode universal mutation detection system and PowerPac 300, 100V power supply (Bio-Rad Laboratories, Hercules, CA). Acrylamide gradient gels (40%-70%) were poured using a Bio-Rad Model 485 Gradient Former and allowed to set up for 1 hour. Wells were loaded with PCR product (approximately 10 µL) and run for 17 hr at 100V. The gel was then stained with SYBR Green for 30 minutes and gel images were recorded using a FluorChem 8800 imaging system (Alpha Innotech, San Leandro, CA).

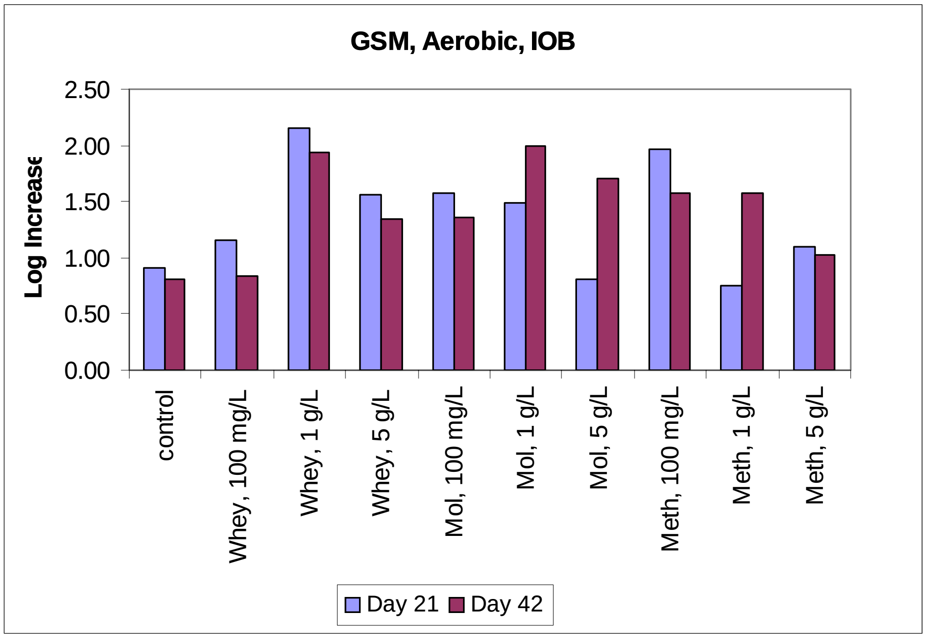
Results and Discussion

Iron Oxidizing Bacteria

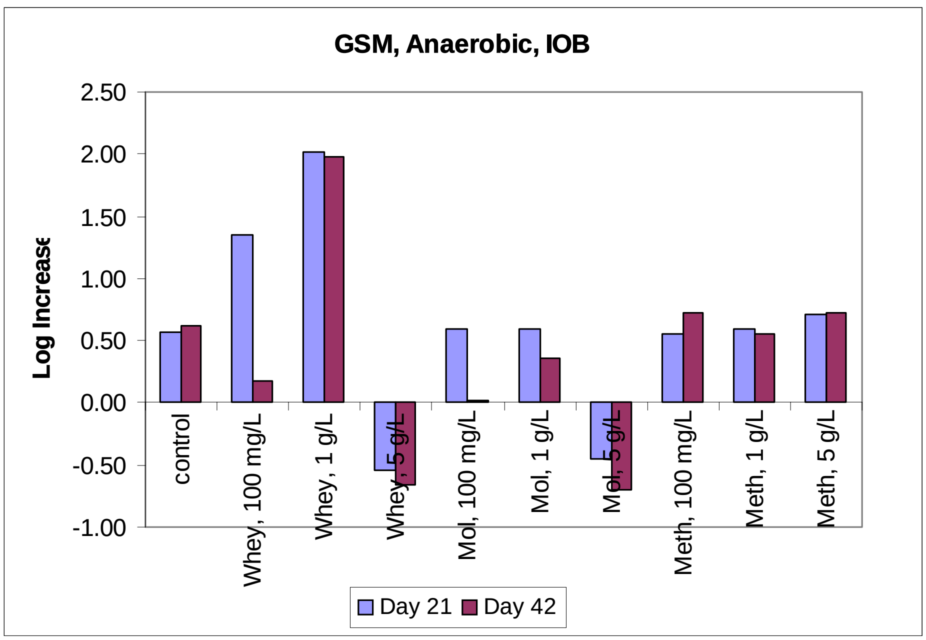
In Golden Sunlight Mine (GSM) tailings, IOB increased as a result of all organic carbon treatments when the microcosms were incubated aerobically. In contrast, anaerobic incubation conditions were more favorable than the control for IOB only in those GSM microcosms treated with 1 g/l whey (Figure 1). The day 42 IOB populations were typically very similar to the day 21 populations, with several notable exceptions where the populations fell between days 21 and 42 (e.g., 100 mg/l whey and 100 mg/l molasses). IOB population growth was not well correlated with organic carbon concentration for any of the three carbon sources tested.

In the Mammoth Mine tailings, aerobically incubated microcosms showed generally lower levels of IOB population increase compared to those observed with GSM tailings. In most cases, IOB population increases in carbon-amended microcosms were not significantly greater than the non-fed controls (Figure 2). In the Mammoth microcosms incubated anaerobically, several whey and molasses treatments resulted in 1.5-2 log increases in IOB populations. As with the GSM tailings, there was not apparent correlation between organic carbon loading and IOB population change.

Collectively, these data suggest that IOB populations can be expected to increase under aerobic conditions following organic carbon treatment, but that it is difficult to predict the response of IOB populations under anaerobic conditions. Although IOB have traditionally been considered chemolithotrophic (gaining energy from the transfer of electrons from ferrous iron to oxygen and utilizing CO_2 as their carbon source), recent research has shown that IOB are actually a metabolically diverse group that includes autotrophs, mixotrophs, and heterotrophs (Johnson, 1998). Because mine tailings are typically oligotrophic environments, with ferrous iron and

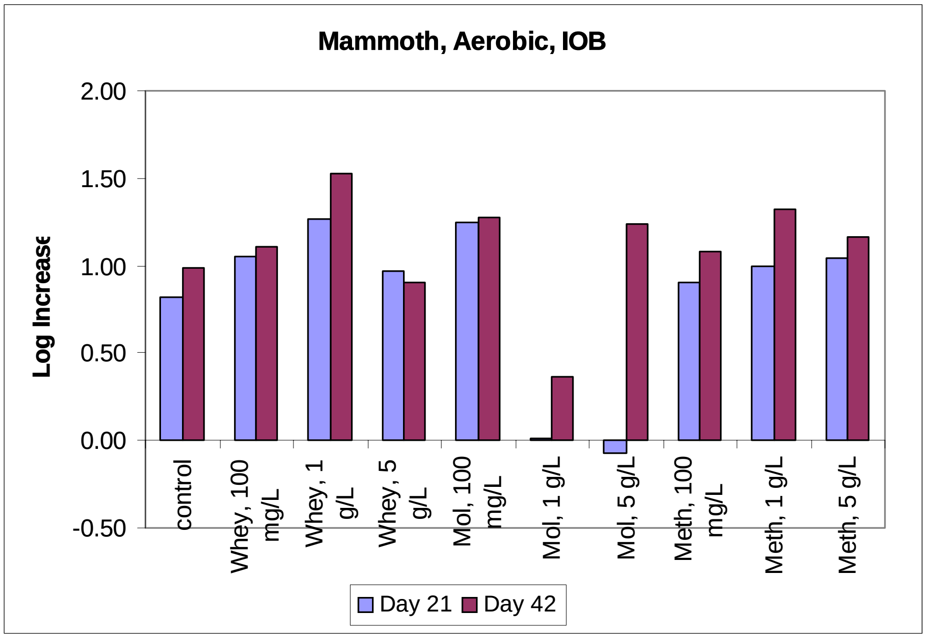


a)

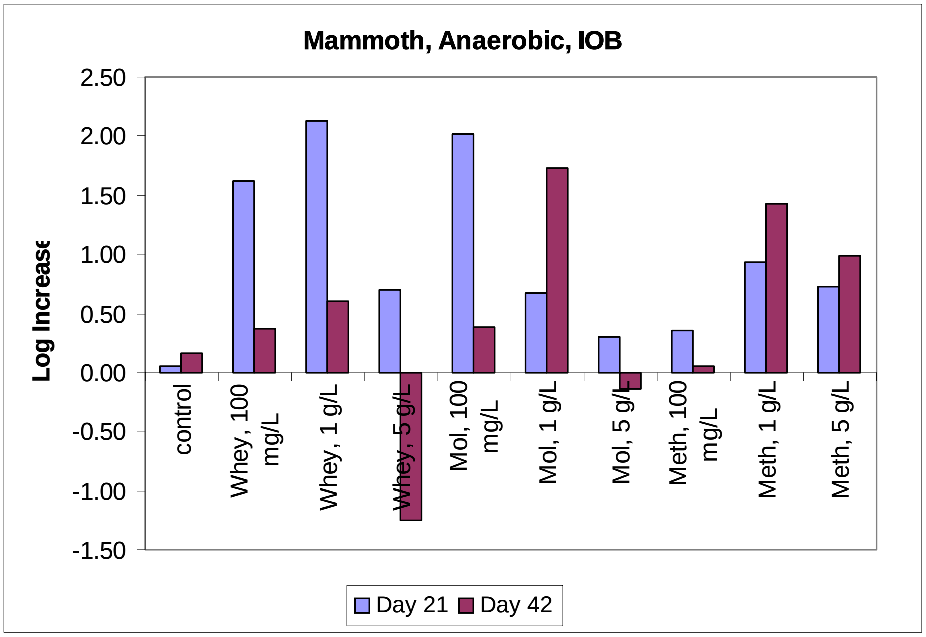


b)

Figure 1. Iron oxidizing bacteria (IOB) from Golden Sunlight Mine tailings 21 and 42 days following treatment with various organic carbon sources. Microcosms were incubated under a) aerobic and b) anaerobic conditions.



a)



b)

Figure 2. Iron oxidizing bacteria (IOB) from Mammoth Mine tailings 21 and 42 days following treatment with various organic carbon sources. Microcosms were incubated under a) aerobic and b) anaerobic conditions.

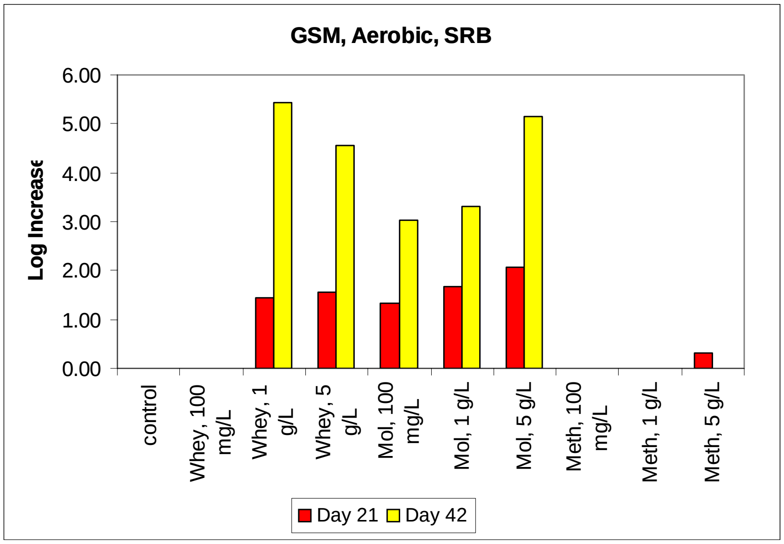
oxygen readily available, chemolithotrophy has distinct competitive advantages over heterotrophy under carbon-limited conditions. However, where organic carbon is available, some IOB have been observed to assimilate it for cell mass, while still oxidizing ferrous iron for energy (mixotrophy) (Pronk et al., 1991). These organisms do not require organic carbon for growth, but will utilize it if available. Members of this group include mesophiles such as *At. ferrooxidans* and moderate thermophiles such as the Gram-positive *Sulfobacillus acidophilus* and *S. thermosulfidooxidans* as well as members of *Acidimicrobium* (Johnson, 1998**;** Johnson, 2001). Some acidophilic iron oxidizers are obligate heterotrophs, being unable to fix CO_2. *Ferrimicrobium acidiphilum*, a Gram-positive IOB and *Sphaerotilus sp.* are representative of this group (Johnson et al., 1992**;** Johnson, 2001). Some obligately heterotrophic iron-oxidizers are relatively sensitive to high concentrations of organic carbon, and may be inhibited under carbon-rich conditions (Bacelar-Nicolau and Johnson, 1999).

Clearly, the individual microbial ecology of a tailings environment is an important determining factor in the response of this community to organic carbon addition. In light of past research, it is not surprising that IOB populations were, under some conditions, stimulated by treatment. This situation is potentially problematic for application of organic carbon as a treatment method. IOB are principally responsible for acid and metals generation from tailings. Treatments that increase their population density are likely to increase ARD from tailings unless some alternative mechanism of control is present, as described below.

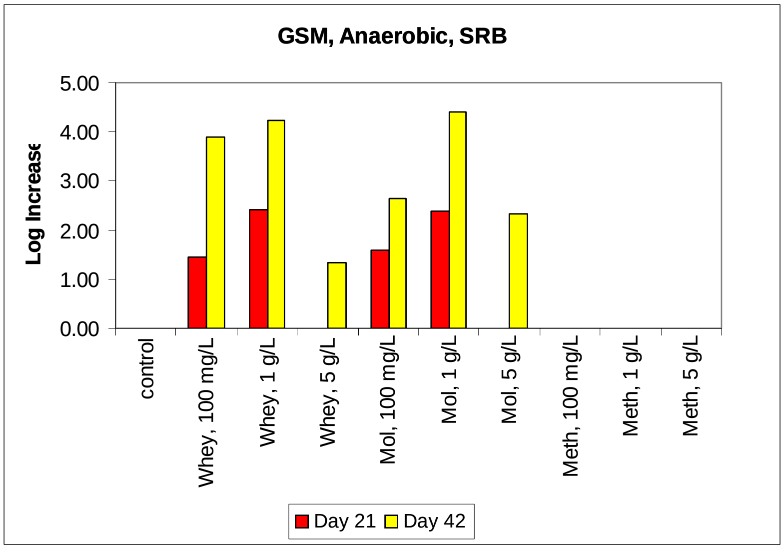
Sulfate Reducing Bacteria

Through the generation of hydrogen sulfide, and subsequent precipitation of metals as metal sulfides, ARD is mitigated by the activity of SRB. SRB have been widely used for the treatment of ARD in engineered bioreactors, where influent organic carbon, pH, and oxidation-reduction (redox) potential can ostensibly be controlled to the benefit of SRB populations. The oxidized and acidic conditions present in most sulfidic mineral tailings would seem prohibitive to SRB, which are generally considered acid intolerant (Postgate, 1984). Nonetheless, SRB have been isolated from tailings piles (Fortin et al., 1995; Fortin et al., 1996; Fortin and Beveridge, 1997). In addition, SRB have been recovered from sediments deposited from mine waste effluent streams (Wielinga et al., 1999**;** Gyure et al., 1990**;** Herlihy and Mills, 1985) and their presence has been inferred from genetic sequencing of some of the most acidic drainage streams on Earth (Bond et al., 2000).

Results from GSM microcosms incubated both aerobically and anaerobically indicate the growth of SRB under all whey and molasses treatment conditions (Figure 3). Methanol, conversely, did not stimulate SRB in GSM microcosms under the conditions tested. In contrast to IOB, which in most cases did not continue to increase in number between days 21 and 42, SRB in most cases did, particularly when incubated aerobically. SRB have generally been considered obligately anaerobic bacteria, able to tolerate oxygen, but unable to grow in its presence. Recent evidence suggests that some SRB are capable of utilizing oxygen as an electron acceptor. Under aerobic conditions, no sulfate reduction would occur, yet the population would maintain the ability to reduce sulfate when conditions again become favorable. These data (Figure 3) indicate that SRB present in the GSM tailings increased in population when fed whey and molasses and incubated both aerobically and anaerobically (subsequent SRB enumeration was completely under anaerobic conditions). Results were similar with the Mammoth tailings, with the exception that methanol also stimulated SRB populations, albeit generally not as well as whey and molasses (Figure 4). As with IOB, SRB population increases were not well correlated with organic carbon concentration. This suggests that SRB (and IOB growing heterotrophically) were not carbon limited during this experiment. It is likely that the levels of carbon used provided adequate carbon for the duration of the experiment. The lack of SRB growth under methanol addition in GSM tailings, and the lower SRB increases in Mammoth under methanol addition could result from the antimicrobial properties of methanol. At high concentrations, methanol (like ethanol) is toxic to bacteria. The threshold of this toxicity varies among populations, but could certainly account for the attenuated response to methanol observed here.

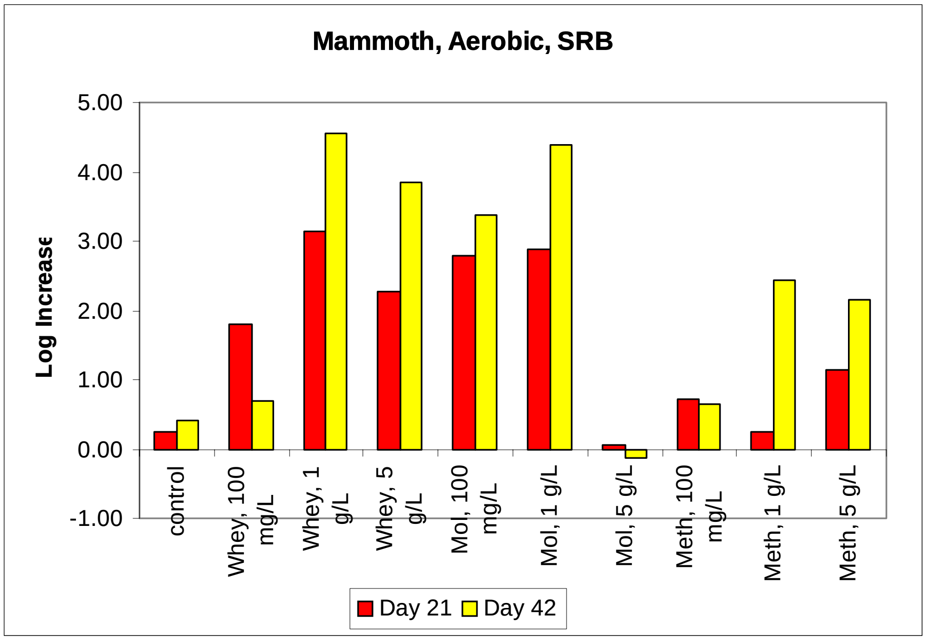


a)

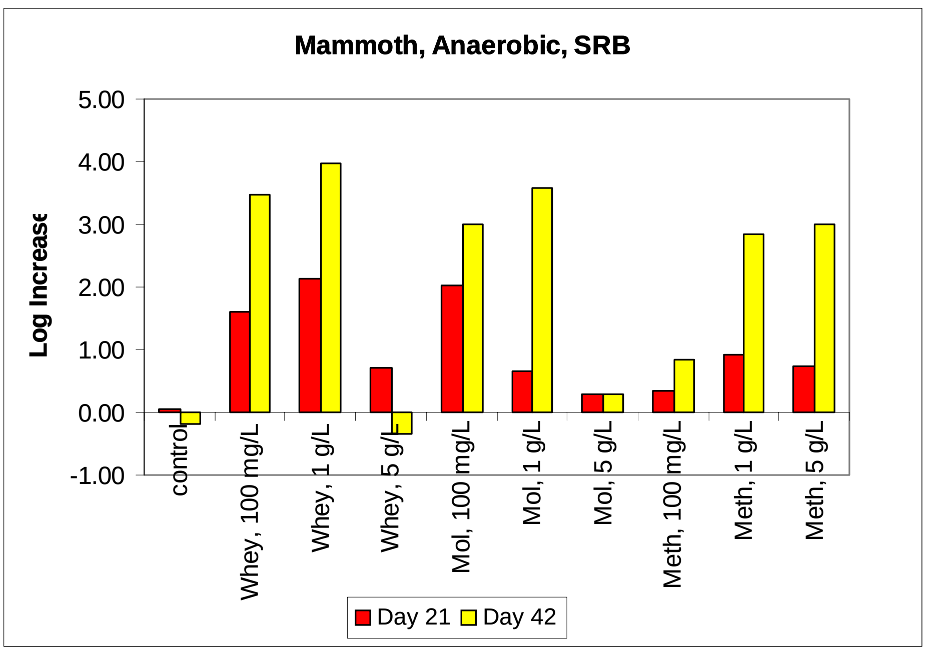


b)

Figure 3. Sulfate reducing bacteria (SRB) from Golden Sunlight Mine tailings 21 and 42 days following treatment with various organic carbon sources. Microcosms were incubated under a) aerobic and b) anaerobic conditions.



a)



b)

Figure 4. Sulfate reducing bacteria (SRB) from Mammoth Mine tailings 21 and 42 days following treatment with various organic carbon sources. Microcosms were incubated under a) aerobic and b) anaerobic conditions.

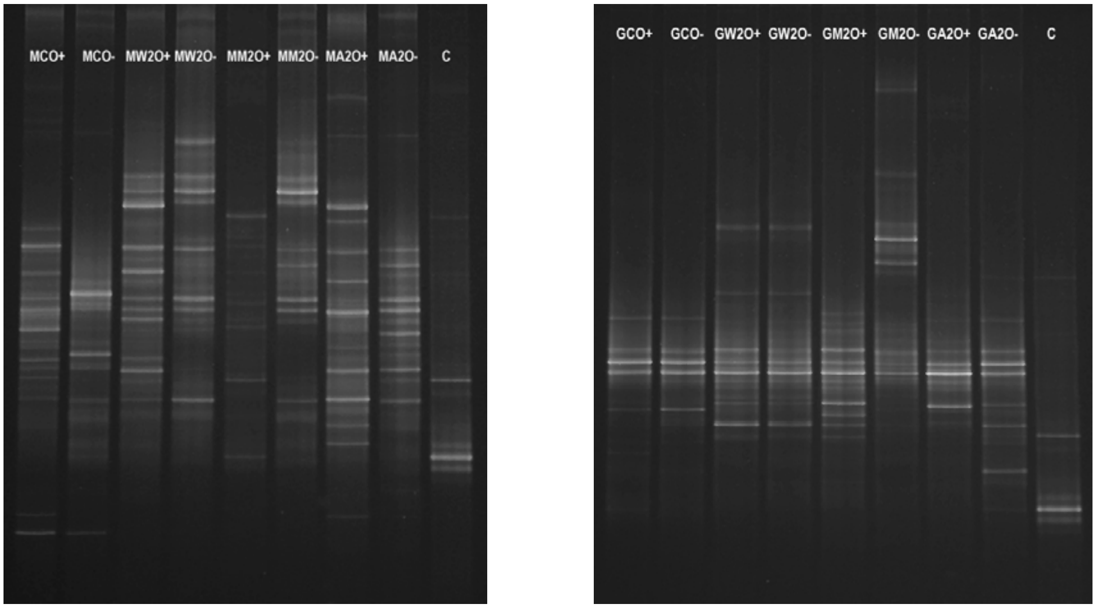
Denaturing Gradient Gel Electrophoresis Analyses

DGGE can be a powerful tool to determine the composition of microbial communities. In this study, it was used primarily as a means to qualitatively assess community differences between the various samples from both Mammoth and GSM. DGGE gels run from selected samples taken at day 42 illustrate distinct differences both between tailings types (GSM vs. Mammoth) and between organic carbon treatments. Each band in the various samples shown in Figure 5 represents an individual bacterial species. Time and resource constraints prevented the identification of each band; however, it is apparent that significant microbial diversity is present, particularly in the Mammoth samples. Comparison of the control samples from each tailings type (listed as "MCO+" and "GCO+" in Figure 5) suggests more diversity in unamended Mammoth samples. This is expected based on the length of time (> 70 yrs) the Mammoth tailings have been exposed to the open environment. GSM tailings, conversely, are relatively young (several months old). Mine tailings undergo a succession of bacterial colonization after placement. This progression may start in the meso-acidic range with filamentous IOB, such as *Metallogenium* (Walsh and Mitchell, 1972) and progress to the more acid-tolerant Gram-negative IOB, such as the *Thiobacilli and Acidithiobacilli* (Johnson, 2001), and progress to the archaea under highly acidic conditions. The rate of this progression is dependent on the mineral character and texture of the tailings, temperature, water infiltration, and site biota. Organic carbon addition clearly further promotes microbial diversity in samples, as indicated by the increasing number of bands in treated samples compared to the non-treated controls (Figure 5).

Close scrutiny of the banding patterns in Figure 5 reveals that, not surprisingly, different populations are stimulated when incubation occurs under aerobic vs. anaerobic conditions, particularly in Mammoth samples. Among GSM samples tested via DGGE, only those treated with molasses at 1 g/l ("GM2O+" and "GM2O-") showed significantly different banding patterns between the aerobic and anaerobic samples. These data further suggest that the bacterial consortium present in both tailings samples is capable of either aerobic or anaerobic growth. The Mammoth DGGE gel suggests more population diversity, and a broader initial population from which individual populations (e.g., IOB or SRB) can grow. SRB populations were higher in Mammoth tailings than GSM at the beginning of the experiment, but not dramatically so, and IOB populations were of similar magnitude in both samples (Table 2). Given this parity of IOB populations, it is surprising that the GSM DGGE gel doesn't show more diversity. This may be attributable to problems in the DGGE analysis, but this seems unlikely since the DGGE bands for GSM samples were very similar among samples.

Table 2. Initial Colonization of GSM and Mammoth Tailings at Day 0.

	SRB	IOB
GSM	2.89E+00	7.09E+05
Mammoth	1.14E+01	3.66E+05



a) b)

Figure 5. DGGE microbial community profile of treated a) Mammoth and b) GSM microcosms at day 42. Code names for treatments correspond to Table 1.

Conclusions

Treatment of mine tailings from two distinct sources, highly weathered tailings and freshly placed tailings, showed both similarities and differences in IOB and SRB response to organic carbon treatment. IOB, the activity of which is detrimental to ARD control efforts, were stimulated, albeit inconsistently, by organic carbon treatment. Under most organic carbon conditions, IOB populations in aerobic microcosms grew approximately 10-fold in Mammoth tailings and 10- to 100-fold in GSM tailings. Under anaerobic conditions, IOB population growth was much less consistent, with some treatments causing a drop in IOB numbers. Fortunately, SRB populations in both Mammoth and GSM tailings responded positively to both whey and molasses treatments. Methanol was not universally effective in SRB stimulation, however.

These experiments illustrate both the promise of using organic carbon to abate ARD through SRB stimulation and the potential to inhibit remedial efforts through the inadvertent stimulation of IOB. Although increases in bacterial populations were not well correlated with the concentration of organic carbon, it is apparent that even relatively low levels of whey and molasses addition can stimulate SRB populations over 1000-fold.

References

Bacelar-Nicolau, P. and D.B. Johnson. 1999. Leaching of pyrite by acidophilic heterotrophic iron-oxidizing bacteria in pure and mixed cultures. Appl. Environ. Microbiol. 65:585-590.

Bond, P.L., S.P. Smriga and J.F. Banfield. 2000. Phylogeny of microorganisms populating a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid mine drainage site. Appl. Environ. Microbiol. 66:3842-3849.

Brierley, C.L. 1978. Bacterial leaching. Critical Reviews in Microbiology. 6.207-262.

Fortin, D., B. Davis, G. Southam and T.J. Beveridge. 1995. Biogeochemical phenomena induced by bacteria within sulfidic mine tailings. J. Indust. Microbiol. 14:178-185.

Fortin, D., B. Davis and T.J. Beveridge.1996. Role of *Thiobacillus* and sulfate-reducing bacteria in biocycling in oxic and acidic mine tailings. FEMS Microbiology Ecology. 21:11-24.

Fortin, D. and T.J. Beveridge. 1997. Microbial sulfate reduction within sulfidic mine tailings: formation of diagenetic Fe sulfides. Geomicrobiology Journal. 14:1-21.

Gyure, R.A., A. Konopka, A. Brooks and W. Doemel. 1990. Microbial sulfate reduction in acidic (pH 3) strip-mine lakes. FEMS Microbiol. Ecol. 73:193-202.

Herlihy, A.T. and A.L. Mills. 1985. Sulfate reduction in freshwater sediments receiving acid mine drainage. Appl. Environ. Microbiol. 49:179-186.

Johnson, D.B., M.A. Ghauri and M.F. Said. 1992. Isolation and characterization of an acidophilic, heterotrophic bacterium capable of oxidizing ferrous iron. Appl. Environ. Microbiol. 58:1423-1428.

Johnson, D.B. 1998. Biodiversity and ecology of acidophilic microorganisms. FEMS Microbiol. Ecol. 27:307-317

Johnson, D.B., M.A. Dziurla and A. Kolmert. 2000. Novel approaches for bioremediation of acidic, metal-rich effluents using indigenous bacteria. In: Proceedings from the Fifth International Conference on Acid Rock Drainage, pp. 1209-1217. Society for Mining, Metallurgy, and Exploration, Inc. Littleton, CO.

Johnson, D.B. 2001. Importance of microbial ecology in the development of new mineral technologies. Hydrometallurgy. 59:147-157.

Johnson, D.B. 2001. Role of pure and mixed cultures of Gram-positive eubacteria in mineral leaching. In: Proceedings of the International Biohydrometallurgy Symposium, pp. 401-410. Ouro Preto, Minas Gerais, Brazil. Elsevier Science.

Postgate, J.R., 1984, The Sulphate-reducing Bacteria, 2nd Edition, 208 pp. Cambridge University Press, Cambridge.

Pronk, J.T., W.M. Meijer, W. Hazeu, J.P. van Dijken, P. Bos and J.G. Kuenen. 1991a. Growth of *Thiobacillus ferrooxidans* on formic acid. Appl. Environ. Microbiol. 57:2057-2062.

Silverman, M.P. and D.G. Lundgren. 1959. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. J. Bact. 77:642-647.

Wielinga, B., J.K. Lucy, J.N. Moore, O.F. Seastone and J.E. Gannon. 1999. Microbial and geochemical characterization of fluvially deposited sulfidic mine tailings. Appl. Environ. Microbiol. 65:1548-1555.

Walsh, F. and R. Mitchell. 1972. A pH-dependent succession of iron bacteria. Environ. Sci. Technol. 6:809-812.

Potamopyrgus antipodarum and baetid mayflies: temporal variation and community-level consequences

Basic Information

Title:	Potamopyrgus antipodarum and baetid mayflies: temporal variation and community-level consequences
Project Number:	2003MT11B
Start Date:	5/1/2003
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	At-large
Research Category:	Biological Sciences
Focus Category:	Ecology, Surface Water, Conservation
Descriptors:	New Zealand mud snails, Potamopygrus antipodarum, baetid mayflies, temperature variences
Principal Investigators:	Billie Kerans

Publication

1. Cada, C. A. and B. L. Kerans. Submitted. Community response to Potamopyrgus antipodarum invasion. Biological Invasions.
2. Cada, C. A. and B. L. Kerans. In preparation. Competitive interactions between Potamopyrgus antipodarum and baetid mayflies.
3. Cada, C. A., B.L. Kerans, and J. Smith. In preparation. Trophic effects of the New Zealand mud snail, Potamopyrgus antipodarum, on trout and a sculpin.
4. Cada, C. A. and B. L. Kerans. 2004. Competitive interactions between the invasive gastropod Potamopyrgus antipodarum and baetid mayflies. Annual Meeting of the North American Benthological Society. Vancouver, B.C.
5. Cada, C., J. Smith, and B. L. Kerans. 2003. What about the fish? 3rd Annual Potamopyrgus antipodarum Conference. Montana State University - Bozeman.

Abstract

We investigated the consequences of the introduction of *Potamopyrgus antipodarum* to Darlinton Spring Creek (Gallatin County, Montana), a popular trout spring-creek fishery where *Potamopyrgus* was recently introduced and their range has expanded. Our overall goal was to examine if and how *Potamopyrgus* changes macroinvertebrate and periphyton assemblages and whether growth of *Salmo trutta*, *Cottus bairdi,* and *Oncorrhynchus mykiss* differs between stream reaches with varying *Potamopyrgus* abundances. We examined *P. antipodarum* and baetid mayfly densities and biomasses, as well as periphyton biomass and fish diet and growth in reaches containing high and low densities of *P. antipodarum.* We also determined the strength of competitive interactions between *P. antipodarum* and baetid mayflies using two *in situ* competition experiments. Densities of baetid mayflies did not respond as strongly to high-densities of *Potamopyrgus* as we expected, and we observed no statistically significant differences in baetid density between high and low snail density reaches. *Potamopyrgus* exerted a negative effect on periphyton biomass, the hypothesized resource for which competition between *Potamopyrgus* and baetids occurs, but we did not observe a clear difference between *Potamopyrgus* and *Diphetor* or *Baetis* in their abilities to depress periphyton biomass. In competition experiments, baetid mayflies negatively affected *Potamopyrgus* survivorship but not growth. Similarly, *Potamopyrgus* negatively affected the survivorship but not the growth of the mayflies *Diphetor hageni* and *Baetis tricaudatis.* In the fish growth experiments, *C. bairdi* lost less weight in low densities of *P. antipodarum* compared to high densities of *P. antipodarum.* On the other hand, there was no difference in mean growth for *S. trutta* or *O. mykiss* between low and high densities of *P. antipodarum.* We found only 1 *Potamopyrgus antipodarum* in 1 stomach of *S. trutta in 2003* and 2 out of out of 15 contained *P. antipodarum* in 2004. However, *P. antipodarum* was eaten frequently by *O. mykiss* and sometimes in large quantities (up to 27 *P. antipodarum* per individual).

Statement of water problem:

Nonindigenous species pose one of the largest threats to biodiversity and are a major cause of endangerment or extinction of native species (Coblentz 1990, Jenkins 1996). Invasive species seriously threaten the integrity of ecosystems by altering interactions among species (Crooks 2002). For example, invasive predators can change the dynamics among resident predators and their prey, and invasive competitors can displace resident species. Such changes in interactions among species may propagate to other levels of biological scale altering population, community and ecosystem dynamics (e.g., the zebra mussel; Rappaport and Whitford 1999).

The New Zealand Mud Snail, *Potamopyrgus antipodarum*, has recently invaded freshwater ecosystems in the United States including southwestern Montana (Zaranko et al. 1997; Hall et al. 2003; Kerans et al. 2005). The high densities, feeding ecology, and reproductive biology of *Potamopyrgus* suggest that it will compete with other grazing macroinvertebrates potentially causing detrimental effects to other trophic levels including fish populations (e.g., Haynes and Taylor 1984, Dorgelo 1987, Fox et al. 1996). In addition, invasive species cost the American economy about $137 billion per year (Pimentel et al. 2000). *Potamopyrgus antipodarum* might be detrimental to local economies such as the fly-fishing industry in the Bozeman area which generates about $3.5 million annually (The River's Edge, Bozeman).

We investigated the consequences of the introduction of *Potamopyrgus* to Darlinton Spring Creek (Gallatin County, Montana), a popular trout spring-creek fishery. *Potamopyrgus* was recently introduced into the creek where their population has increased and their range has expanded. Darlinton is an ideal location to study the effects of this invader because it supports a simple aquatic community amenable to experimental manipulation and because reaches with similar habitat properties contain varying abundances of *Potamopyrgus*. Thus, we were able to compare aquatic assemblages under varying stages of invasion but where the habitat was similar. Furthermore, our earlier, less extensive studies showed that macroinvertebrates and grazer food resources declined as *Potamopyrgus* abundances increased (Cada and Kerans, submitted).

Research Objectives:

Our overall goal was to examine if and how *Potamopyrgus* changes macroinvertebrate and periphyton assemblages and whether fish growth differs among areas with varying *Potamopyrgus* abundances. Our specific objectives were: 1) quantify the differences in the abundances of grazing mayflies as abundances of *Potamopyrgus* varies, 2) quantify the magnitude of inter- and intraspecific competition between grazing mayflies and *Potamopyrgus* varies, 3) determine how periphyton biomass changes as abundance of *Potamopyrgus* varies, and 4) explore whether insectivorous fishes fed on *Potamopyrgus* and whether growth of those fishes was lower in areas where the abundances of *Potamopyrgus* was high.

Methods:

We conducted this study in Darlinton Spring Creek at the Montana Fish, Wildlife and Parks Cobblestone fishing access site in south-central Montana, USA (45.8638°N, 111.4947°W). We have conducted research in the past in this area (Cada and Kerans submitted) and found that baetid mayflies, which were dominant members of the scraper/collector-gather functional feeding group of the macroinvertebrate community, declined in abundance in the presence of *Potamopyrgus* (Cada and Kerans submitted). Thus, in this more extensive study, we examined how *Potamopyrgus* influenced mayflies in the family Baetidae.

Objective 1: Macroinvertebrate field sampling—We examined *P. antipodarum* and baetid densities and biomasses in reaches with high and low densities of *P. antipodarum.* We expected baetid density and biomass and periphyton biomass to be greater in low-snail than in high-snail reaches. Both macroinvertebrate and periphyton samples were collected monthly (April 2002 to May 2003, plus July, August and October 2003) from two downstream high-snail reaches and two upstream low-snail reaches. We sampled macroinvertebrates using cobble samples to target the collecting/grazing community (Kerans et al. 1995), which we expected to be most influenced by *P. antipodarum*. Thirty-two cobbles, 8 per reach with 2 reaches per snail density, were taken each sampling date. To reduce loss of organisms due to drift when disturbed, we placed a Surber sampler (132-µm-mesh) downstream of the rock and then gently lifted both in unison from the water (Kerans et al. 1995). Cobbles were brushed and rinsed to remove organisms, which were then preserved in Kahle's solution (Pennak 1978). Dimensions of cobbles were measured according to Graham et al. (1988) for subsequent calculation of surface area and macroinvertebrate density.

We identified and enumerated invertebrates to species using a dissecting scope at 6.3X to 40X magnifications (Merritt and Cummins 1996). We calculated densities for each sample by dividing the taxa abundance by surface area of the corresponding cobble.

For baetids, we measured head capsule width to 0.01mm using an ocular micrometer at 40X magnification of randomly chosen individuals (n=20 per species per reach and sampling date), categorized individuals into developmental stages based on wing-pad size (I, II, III, or IV) as defined by Deluchi and Peckarsky (1989), and recorded sex of stage III-IV individuals based on the presence of the enlarged second pair of compound eyes of males (Peckarsky et al. 1993). We measured shell length similarly to baetid head widths and determined both reproductive status and fecundity by dissecting randomly chosen *P. antipodarum* (n=40 per reach and sampling date). Reproductive status was defined as the presence or absence of embryos in a brood pouch, whereas fecundity defined as a count of embryos present in the brood pouch.

To satisfy assumptions of normality and equality of variance, density data for all species were transformed using the natural log of x + 1, where x represents any datum point. All statistical analyses were performed using SAS 9.0 for Windows (SAS Institute Inc., Cary, North Carolina, USA). To evaluate densities and biomasses of *P. antipodarum* and baetid species and the biomass of periphyton, we used repeated measures, nested 2-way MANOVA (Von Ende 2001) with the response variable repeated over time. The 2 main factors (levels listed in parentheses) included snail-density (low or high) and reach (A or B) nested within snail-density. We were particularly interested in the time*snail interaction to determine whether the snail effect differed across time for any of the response variables.

<u>Objective 2: Periphyton food resources</u> —We compared periphyton biomass between high and low-snail reaches using chlorophyll *a* from small cobbles. We collected 8 additional cobbles per reach per sampling date, which were frozen and stored in the dark

until chlorophyll extraction. We extracted chlorophyll *a* in 90% ethanol by submerging each cobble and using spectrophotometric analysis to measure concentration (Cada and Kerans, submitted). Direct extraction of chlorophyll *a* was chosen over other periphyton sampling methods such as scraping or brushing of the cobbles primarily because these methods can underestimate biomass through loss of tightly adhered diatoms (Aloi 1990, Cattaneo and Roberge 1991). Biomass was calculated as the product of the extract's concentration and volume divided by the estimated surface area. We estimated surface area of the cobbles as noted for macroinvertebrates. Chlorophyll *a* and pheophytin biomasses were analyzed in the same manner as macroinvertebrate densities using repeated measures nested 2-way ANOVA.

Objective 3: Competition experiments— To determine the strength of competitive interactions between *P. antipodarum* and baetid mayflies, we conducted two *in situ* experiments in artificial chambers stocked with various density combinations of baetid mayflies and *P. antipodarum* in late summer (28 July – 13 August 2003, Experiment 1) and early winter (23 October – 11 November 2003, Experiment 2) to compare the magnitude of competition between seasons. Experiments occurred in different seasons (summer and winter) to compare the magnitude of competition between seasons. Experiment 1 was 4 days shorter than Experiment 2 because invertebrate growth is temperature dependent and body growth of individuals should have accumulated more quickly in Experiment 1. Additionally, because emergence increased over time in Experiment 1, we wanted to limit the loss of mayflies before sample size became too small.

The circular chambers were 11 cm in diameter x 14 cm in depth with two 4 x 7 cm holes covered by 500-µm nytex mesh on opposing sides of the chamber to allow water exchange. Chambers were mounted in polystyrene floats (1.2 m x 0.6 m x 0.05 m, 4 chambers per float) that were secured in the stream channel with rebar and protected from debris by 0.64 cm wire-mesh attached to the rebar upstream of the floats. Each chamber received 3 similarly sized pebbles (total surface area of about 125 cm^2) prior to invertebrate stocking. We collected the pebbles from the stream channel and carefully removed visible invertebrates to minimize disturbance of periphyton. Extra pebbles were collected and frozen for analysis to determine periphyton biomass at the beginning of the experiment (n=4 for Experiment 1 and n=18 for Experiment 2). We measured water velocity at the upstream and downstream edges of each float and at two depths (0.6X channel depth and 5 cm below the water's surface, which corresponded with the depth of the water-exchange holes) with a Swoffer 3000 flow meter. Onset® temperature probes, secured at the upstream-most and downstream-most floats, recorded water temperature at 1-hr intervals throughout the experiments.

Stocking abundances reflected the range of densities observed at Darlinton Spring Creek (10,000-20,000 m^{-2}). In Experiment 1, we compared *Diphetor* and *Potamopyrgus*, whereas in Experiment 2, we compared *Baetis* and *Potamopyrgus*. We used *Diphetor* rather than *Baetis* in Experiment 1 because most *Baetis* I collected were too small and might have escaped the chambers, but *Diphetor* was within the appropriate size range. Experiment 1 examined competitive interactions where intra- and interspecific competition cannot be separated (Goldberg and Scheiner 2001) and consisted of 3 treatments in an substitutive design where the total number of individuals in a replicate was constant at 250: *Diphetor* alone (D), *Potamopyrgus* alone (P), and *Diphetor* plus *Potamopyrgus* together (D+P). In contrast, Experiment 2 estimated both intraspecific and interspecific interactions and was comprised of 7 treatments in a response-surface design (Table 1). Assignment of treatments to chambers was completely randomized across floats.

Invertebrate stocking of the experimental chambers occurred over 2 d. We collected invertebrates using kick nets and pipetted a known number of individuals into temporary containers. We chose *P. antipodarum* ~2 mm length and young baetid nymphs (wing-pads present but not darkened or thickened) for stocking. These sizes precluded prior embryo development by *P. antipodarum* (Richards et al. 2001) in addition to allowing growth by both species and field identification.

Maintenance of chambers and floats occurred every ~3 days. We cleaned the nytex and wire meshes of debris to aid water exchange and removed dead invertebrates by pipetting to prevent deterioration of water quality. For Experiment 2, maintenance included removal of snow and ice from the surfaces of chambers and floats. At the end of each experiment, we enumerated and preserved live individuals in Kahle's solution. Additionally, pebbles from the experimental chambers (n=3 per chamber) were frozen for chlorophyll and pheophytin analysis and calculation of periphyton biomass (see methods in field surveys).

We quantified the effect of competition using two characters related to fitness—daily survivorship and daily per capita body growth. Because *Potamopyrgus* reproduced in some replicates in Experiment 1, the response variable in that case is per capita population growth rather than survivorship. We calculated survivorship or per capita population growth according to equation 1.

Daily survivorship or per capita population growth = ln[(final number of species y alive at experiment end)/(initial number of species y added at the beginning of the experiment)] / (number of days in experiment) Eq.1

In Experiment 1, survivorship of *Diphetor* was corrected for loss of individuals due to emergence (i.e., mean daily emergence was added to each final abundance).

We calculated the second fitness characteristic, daily per capita growth, for both species according to equation 2.

Daily per capita body growth = ln[(biomass of species y alive at experiment end)/(biomass of species y added at the beginning of the experiment)] / (number of days in experiment) Eq. 2

To estimate initial and final biomasses, we measured shell length or head-capsule width and converted these measurements to dry-mass according to equations 3 from Cada and Kerans (submitted) and 4 from Benke et. al (1999).

Potamopyrgus dry weight [mg] = length $[mm]^{2.3697} * 0.117$ Eq. 3

Diphetor or *Baetis* dry weight [mg] = width $[mm]^{3.326} * 1.2688$ Eq. 4

For initial biomass, we measured 40 individuals per species, which we subsampled from the individuals available for stocking. For final biomass, we measured up to 40 individuals per species per replicate, depending on survivorship of the invertebrates.

In Experiment 1, we used 1-way ANOVA to test for a treatment effect for each response variable (survivorship and growth) for each competitor. Factor levels were *Diphetor* alone (D) or *Potamopyrgus* alone (P) and *Diphetor* plus *Potamopyrgus* (D+P). Because the response variables were not independent of each other, we used Bonferroni corrections. Additionally, we compared overall survivorship and growth between competitors using two-sample t-tests. To determine whether treatment-levels affected chlorophyll *a* or pheophytin biomass through differential grazing pressure, we used 1-way ANOVA with 5 factor levels. This analysis included an "initial" factor level that represented periphyton from the stream channel at the start of the experiment and a "control" factor level that represented periphyton biomass from experimental chambers with no invertebrates, in addition to the invertebrate treatments D, P or D+P. Chlorophyll *a* and pheophytin data were ln transformed

In Experiment 2, we used 2-way ANOVA for each competitor for each response variable with treatment ("solitary" or "B+P") and density ("low" or "high") as the factors. Because the response variables were not independent of each other, we used Bonferroni corrections. We compared overall survivorship and growth between competitors using two-sample t-tests.

<u>Objective 4: Fish feeding and growth</u>— We examined whether fish fed on *Potamopyrgus* and estimated the effects *P. antipodarum* density (referred to as "low snail" or "high snail") on the growth rates and body condition of *Salmo trutta, Cottus bairdi,* and *Oncorhynchus mykiss* using *in situ* enclosure experiments in 2003 and 2004. Enclosures were constructed from 2.5 x 2.5 cm pine frames to dimensions of 61 x 61 x 30.5 cm for *C. bairdi* and 61 x 91.5 x 91.5 cm for *S. trutta* and *O. mykiss.* They were wrapped with

0.85 cm nylon-netting or 0.64 cm hard-wire cloth, respectively. Bottoms and tops of enclosures were covered with nylon window-screening, except the enclosures in 2004 were covered with hard-wire cloth. All mesh was secured with staples. In 2003, a total of 6 brown trout enclosures and 6 sculpin enclosures were placed in high-snail and low-snail reaches. In 2004, we placed 6 brown trout and 6 rainbow trout enclosures in high-snail and low-snail reaches. We placed trout enclosures so that water would flow through the chambers, but we also added several large cobbles to provide a flow-refuge (Wilzbach et al. 1986). In 2004, we added pebbles and cobbles to cover the bottoms of the enclosures, and we secured bundles of live willow (*Salix* sp.) branches in the front of the enclosures to add additional refugia for fishes. Sculpin enclosures were placed in riffles and the bottom was covered with pebbles to simulate their habitat preference. Both enclosure types were secured to rebar posts driven into the streambed. The rebar posts, about 30 cm upstream of each enclosure, also supported chicken-wire that served to reduce clogging of the enclosures' mesh and improve water flow within enclosures. All mesh, including that on the enclosures, was cleaned of debris every 2-3 days throughout the duration of the experiment. We measured water flow at the front and rear of each enclosure using a Swoffer 3000 and measured physicochemical water conditions at each enclosure using a Yellow Springs Instrument (YSI).

We collected one-year old *S. trutta* (~7 cm length) and *C. bairdi* (7-12 cm length) by electrofishing 1 July 2003. We electrofished for *S. trutta* on 29 June 2004. We obtained four month old *O. mykiss* (Eagle Lake strain) from the National Fish Hatchery in Ennis, MT on 22 June 2004. Fishes were anesthetized using MS-222 for handling. For each individual, we measured fork length (nearest mm) and wet mass (nearest 0.1g) at the beginning and the end of the experiment (Wilzbach et al. 1986). Three sculpin per enclosure were stocked 1 July 2003, and 5 *S. trutta* per enclosure were stocked on 2 July 2003, after being held overnight within Darlinton Spring Creek. We stocked 5 *S. trutta* and 5 *O. mykiss* per enclosure in 2004. Because high flow events between 9 July and 14 July 2003 washed-out two trout enclosures (one high-snail and one low-snail), individuals were redistributed within their snail-treatment and enclosures thereafter contained only 3 trout. We terminated the sculpin experiment on 31 July 2003 and the first trout experiment on 6 August 2003. The experiments in 2004 were terminated 9 August (*O. mykiss*) and 17 August (*S. trutta*).

To determine how diet of *C. bairdi*, *S. trutta*, and *O. mykiss* differed between high-density and low-density snail reaches and to determine the extent to which *P. antipodarum* was fed on, we used gastric lavage to remove the stomach contents of all individuals (Bowen 1983) after collection or when the experiment was terminated. Invertebrates with at least a head capsule present were identified to family. Diet composition was calculated as both the relative abundance of invertebrates in the diet and the frequency of fish containing each invertebrate family.

We estimated daily growth of *S. trutta, O. mykiss* and *C. bairdi* as the difference in weight from the start and end of the experiment divided by the number of days in the experiment. Growth was transformed by ln (x + 1). We used 2-way ANOVA to compare the difference in growth between species (levels: sculpin and brown or rainbow and brown) and between snail-treatments (low and high density).

To determine the density of *Potamopyrgus* during the experiments, we sampled macroinvertebrates from low-snail and high-snail reaches on 9 July and 6 August 2003 as well as 30 June and 19 August 2004. Additionally, macroinvertebrate densities within sculpin enclosures were sampled using cobble samples (n=3 per enclosure) as noted earlier.

Principal Findings:

Objective 1: Macroinvertebrate field sampling —*Potamopyrgus* densities peaked during summer months of 2002 (24,750 m^{-2}) but reached their lowest levels in spring 2002 and 2003 (< 1000 m^{-2}) (Figure 1, Table 2). In general, densities were lower in 2003 than in 2002, perhaps because of the biology and life history of *Potamopyrgus* or as a consequence of invasion dynamics. *Potamopyrgus* may be sensitive to cold temperatures (Hylleberg and Siegismund 1987) and an early, particularly low-temperature event may have decreased survival of individuals in late winter and early spring 2003. In support of this hypothesis, minimum and maximum temperatures in October were nearly three degrees cooler in 2002 than in 2003 (2.76-14.11 ºC and 5.42-17.59 ºC, respectively). Alternatively, many invasive species exhibit dynamic population behavior with large cycles or experience a "boom and bust" where populations decline markedly after initial high abundances (Williamson and Fitter 1996). However, large intra-annual changes in densities have been observed for this species (Dorgelo 1987, Schreiber et al. 1998), suggesting population density variation may have been within the normal range for *P. antipodarum*. For example, density in Darlinton Spring Creek dropped from nearly 28,000 m^{-2} in November 2000 to almost 9,000 m^{-2} in June 2001 (Cada and Kerans, submitted). *Potamopyrgus* reproduced year-round and did not exhibit clear cohorts, which is consistent with other findings on *P. antipodarum* reproduction

(Winterbourn 1970). Thus, it seems more likely that this population fluctuates temporally as some function of the winter environment (e.g., low temperature, low productivity).

All three mayfly species exhibited patterns of abundance and size-class distributions consistent with univoltine life cycles (Figure 2, Table 3). Young *Baetis* individuals (stage I) formed a large proportion of the population as early as July and were the dominant life stage in fall and early winter. *Baetis* individuals close to emergence and maturity (stage IV) were present over a wide range of months from late-winter through mid-summer suggesting that emergence occurred throughout these months and was not tightly synchronized. In contrast with *Baetis*, young *Diphetor* and *Acerpenna* individuals did not comprise a large proportion of the population until September and consisted of more than 90% of the population through February. This indicates eggs began hatching in late summer and may have continued throughout winter. In addition, little if any individual growth occurred during winter months as mean head width did not change during that time period. Stage IV individuals of *Diphetor* and *Acerpenna* occurred from late spring throughout the summer, indicating emergence occurred primarily in summer months and *Diphetor* may have emerged slightly before *Acerpenna*. Differential timing of emergence between *Diphetor* and *Acerpenna* may be caused by different developmental requirements such as degree days or could be a result of past competitive interactions and temporal habitat partitioning (Connell 1980).

Densities of baetid mayflies did not respond as strongly to high-densities of *Potamopyrgus* as we expected (Figures 1 and 2, Tables 2 and 3); i.e., we expected mayfly densities to be higher in low-snail reaches than in high-snail reaches at least during fall months as we observed in November 2000 for a similar magnitude of snail densities (Cada and Kerans, submitted). High variability undoubtedly decreased our ability to detect statistical differences between mean mayfly densities in high-snail and low-snail reaches.

While there were no statistical differences in mayfly densities between high and low snail reaches, we think it worthwhile to explore the trends observed because they may be biologically significant. *Baetis* densities appeared greater in low-snail reaches than in high-snail reaches during late winter and relatively late within larval development (Figure 3). *Diphetor* densities tended to be greater in low-snail reaches than in high-snail reaches in late fall and early winter, before larvae began to develop wing pads. In contrast to *Baetis* and *Diphetor*, *Acerpenna* seemed to be positively affected (densities greater in high-snail reaches than in low-snail reaches) beginning in late fall and continuing through early spring. These trends suggest that the interaction between *Potamopyrgus* and baetids may be biologically significant at certain time periods. Additionally, these trends agree with previous field research that showed a strong effect of *Potamopyrgus* on the density and biomass of baetid mayflies in November 2000 (Cada and Kerans, submitted).

It is important to point out that “high” *Potamopyrgus* densities within our field study do not represent the range of densities that *Potamopyrgus* reaches in other locations (Kerans et al. 2005, Hall et al. 2003). In a broader perspective, the densities observed in Darlinton Spring Creek would more correctly be considered “moderate”. As a result, the effect of *Potamopyrgus* on baetid mayflies in locations of “high” (i.e., > 50,000) and extremely high (i.e., > 150,000) densities could be much stronger and more apparent than we observed in this study.

Objective 2: Periphyton food resources —In the field survey, both chlorophyll a and pheophytin a biomasses varied over time and seemed to reach the greatest biomass in fall months (Figure 3; Table 4). Chlorophyll *a* was marginally higher in low snail density reaches than in high-snail density reaches (Figure 3; Table 4, snail effect, $P < 0.06$). Since we did not observe a clear effect of *Potamopyrgus* on baetid mayflies in the field study, it seems likely that *Potamopyrgus* did not depress resources sufficiently to limit resources and strongly influence baetid densities. Periphyton is probably not the only resource for which *Potamopyrgus* may compete with baetid mayflies. Space is likely to be an important factor because high densities of *Potamopyrgus* should limit habitat availability.

In Experiment 1, Chlorophyll *a* and pheophytin biomasses were greater in the initial and control treatments than in D, P or D+P treatments (chlorophyll *a*: $F_{5,19} = 8.58$, $P = 0.0004$; pheophytin: $F_{4,19} = 11.58$, $P <0.0001$; Tukey's HSD $P < 0.05$) (Figure 4). *Potamopyrgus* and *Diphetor* did not differ in their effect on periphyton biomass (Figure 4).

In Experiment 2, mean chlorophyll *a* biomass was somewhat lower in *Baetis*-only treatments (Figure 5) (species: $F_{2,92} = 2.73$, $P = 0.0703$) in comparison to the initial and control levels as well as to *Potamopyrgus*-only and B+P treatments, indicating that chlorophyll *a* biomass was depressed in the experiment only when *Baetis* grazed by itself. Density did not affect chlorophyll *a* biomass (density: $F_{1,92} = 0.01$, $P = 0.9396$; species*density: $F = 1.98$, $P = 0.1432$). Similarly, mean pheophytin *a* biomass was lower in *Baetis*-only treatments (species: $F_{2,92} = 4.25$, P = 0.0172) in comparison to all other treatment levels, indicating that only grazing by *Baetis* was able to depress pheophytin biomass lower than the initial and control levels of biomass. An interaction effect indicates that pheophytin *a* biomass was lower in the high B+P treatment in comparison with the low B+P treatment (density: $F_{1,92} = 0.10$, $P = 0.752$; treatment*density: $F_{2,92} = 4.43$, P = 0.0145). Additionally, both chlorophyll a and pheophytin a biomass results suggest that *Baetis* may be able to graze algae to lower levels than *Potamopyrgus.*

Although *Baetis* may be better able to graze periphyton to lower levels than *Potamopyrgus*, *Baetis*' behavioral decisions may change the interaction in the natural environment. That is, *Baetis* is thought to actively enter the drift when food levels reach a certain threshold (Kohler and McPeek 1989), and rather than remaining in an area of decreased periphyton biomass that results from the presence of *Potamopyrgus*, *Baetis* may choose to drift and seek areas of higher food availability. By choosing to drift, *Baetis* increases its probability of death by predation, decreases the relative amount of time spent foraging, and runs the risk of drifting to an unsuitable habitat, all of which may ultimately decrease fitness.

Objective 3: Competition experiments— In Experiment 1, *Potamopyrgus* survivorship was greater than survivorship of *Diphetor* (t_{14}=6.51, $P < 0.0001$; Figure 6a). Survivorship was greater for both species in the intraspecific treatment than in the interspecific treatment (*Potamopyrgus*: $F_{1,6}$ =50.14, $P = 0.0004$; *Diphetor:* $F_{1,6} = 9.61$, $P = 0.0211$), indicating the interspecific competition was greater than intraspecific competition. Additionally, mean individual growth per day (Figure 6b) did not differ between treatments for either species (*Potamopyrgus*: $F_{1,316} = 0.51$, $P = 0.4757$;

Diphetor: $F_{1,132}$ =1.26, *P* = 0.2640), but *Diphetor* growth was greater than that of *Potamopyrgus* (t_{199}=5.78, *P* < 0.0001).

In Experiment 2, the overall survivorship of *Potamopyrgus* was greater than that of *Baetis* (t_{37}=8.2, *P* < 0.0001, Figure 7). The mean survivorship for *Potamopyrgus* was greater when maintained only with conspecifics than when combined with *Baetis* (i.e., B+P; Figure 7) (treatment: $F_{1,18}$ = 287.31, *P* < 0.0001). Density negatively affected survivorship only when *Potamopyrgus* was combined with *Baetis* (B+P) (density: $F_{1,18}$ = 8.44, *P* = 0.0095; treatment*density: $F_{1,18}$ = 6.30, P = 0.007). Similarly, the mean survivorship of *Baetis* was greater when with conspecifics than in combined treatments with *Potamopyrgus* (Fig 7) (treatment: $F_{1,20}$ = 24.01, P < 0.0001). However, *Baetis* survivorship did not differ between low and high densities (density: $F_{1,20}$ = 0.0, P = 0.9520; treatment*density: $F_{1,20}$ = 0.0, P = 0.9609).

The overall mean daily growth of *Potamopyrgus* did not differ from that of *Baetis* (t_{1341}=1.14, *P* = 0.2533, Figure 7). *Potamopyrgus* growth did not differ between solitary and mixed treatments (Figure 7, $F_{1,873}$ = 0.36, *P* = 0.5487). However, increased density negatively affected *P. antipodarum* growth (density: $F_{1,873}$ = 91.55, *P* < 0.0001; treatment*density: $F_{1,873}$ =0.79, *P* = 0.376). In contrast, *Baetis* growth did not differ between solitary and mixed treatments nor between low and high densities (Figure 7, treatment: $F_{1,430}$ = 0.15, *P* = 0.7019; density: $F_{1,430}$ = 1.68, *P* = 0.1961; treatment*density: $F_{1,430}$ = 0.40, *P* = 0.5260).

One reason we did not detect any effects of *Potamopyrgus* on the growth of *Diphetor* or *Baetis* was the low survivorship of both mayflies in the experiments. Low survivorship resulted in fewer individuals from which to estimate growth in each replicate; i.e., a small sample size. Additionally, if we assume that only the most healthy individuals survived, these many be less affected by competition than by unhealthy individuals and result in a biased sample.

Objective 4: Fish feeding and growth — Diet analysis of 29 *S. trutta* and 17 *C. bairdi* removed from a reach containing high snail densities (>50,000 m^{-2}) in 2003 yielded only 1 *Potamopyrgus antipodarum* in the stomach of a *S. trutta* (greater than 23 cm length). This *P. antipodarum* individual appeared to be a newly hatched juvenile less than 1mm in length. Additionally, diet composition of trout and *C. bairdi* held in experimental cages seemed to change between low- and high density reaches with *Potamopyrgus.* That is, *S. trutta* tended to eat more amphipods in low-snail than in high-snail density reaches (Figure 8a). *Cottus bairdi* tended to eat a more varied diet in high-snail than in low-snail reaches, where only two taxa (Isopoda and Chironomidae) were eaten (Figure 8b).

In 2004, the diets of 34 *S. trutta* that were caught in a reach with high densities of *P. antipodarum* prior to the experiment did not contain *P. antipodarum*. However, 2 out of 15 individuals recovered from the high-snail enclosures after the experiment was terminated had 1 *P. antipodarum* each in their stomachs. Additionally, 11 out of 15 *O. mykiss* that were recovered from high-snail enclosures contained at least one *P. antipodarum* individual. The mean number of *P. antipodarum* found in *O. mykiss* was 4.8 ± 1.82 (mean ± SE).

In the 2003 fish growth experiment, *S. trutta* gained weight whereas *C. bairdi* lost weight (Figure 9; species effect $F_{1,6} = 14.16$, $P = 0.0094$). It seems probable that *C. bairdi* lost weight in this experiment due to density-dependent effects because three *C. bairdi* were held in each cage. Although growth for both species appeared higher in the low-snail reaches, there were no differences between snail reaches for either species (snail effect $F_{1,6} = 1.58$, $P = 0.2553$; species*snail interaction $F_{1,6} = 0.0$, $P = 0.9646$). High variability of trout growth in the low-density *P. antipodarum* reaches reduced our ability to detect any effect of *P. antipodarum* on *S. trutta*.

In 2004, the growth rate of both fish species did not differ between low-snail density (0.070 g d^{-1}) and high-snail density (0.069 g d^{-1}) reaches (snail effect, $F_{1,8} = 0.00$, $P = 0.95$). Additionally, the growth rate did not differ between *S. trutta* (0.061 g d^{-1}) and *O. mykiss* (0.078 g d^{-1}) (species effect, $F_{1,8} = 0.76$, $P = 0.41$; snail X species effect, $F_{1,8} = 0.00$, $P = 0.98$), even though *O. mykiss* individuals were larger than *S. trutta* at the start and the end of the experiment.

Significance of findings:

Contrary to previous research (Cada and Kerans, submitted), this study does not demonstrate a strong effect of *Potamopyrgus* on the density or biomass of baetid mayflies in the field. Factors such as high levels of patchiness (Simon and Townsend 2003) and environmental variation, in combination with the invasion process, may decrease the ability to detect the effects of species interactions at a larger spatial scale (Kerans et al. 2005). In addition, field observations did not separate different types of interactions between *Potamopyrgus* and baetid larvae, which could be a combination of both negative and positive interactions of differing magnitudes that sum to a smaller net negative interaction (Berlow 1999). Similarly, competitive interactions between closely related species, such as between baetid species, may obscure the responses of baetids to *P. antipodarum.* Furthermore, periphyton is not the only resource for which *Potamopyrgus* may compete with baetid mayflies. Space is likely to be an important factor because high densities of *Potamopyrgus* will limit habitat availability (Zaranko et al. 1997, Kerans et al. in 2005).

In contrast to the field observations, the competition experiments demonstrated a negative effect of *Potamopyrgus* on baetid mayfly survivorship. Decreased survivorship may affect population dynamics of baetid species and may ultimately have negative implications for the persistence of some mayfly populations in the presence of *Potamopyrgus*. However, these experimental results do not agree with field observations, which indicated no effect of *Potamopyrgus* on baetids. Experimental results do not always agree with observational studies because factors operating at a large spatial scale may overwhelm the importance of small-scale factors (Peckarsky et al. 1997, Thrush et al. 1997). Additionally, extrapolation of results from an experiment to the population or community level may also be affected by species interactions (Billick and Case 1994) that are not included within the experiment.

Competition experiments also demonstrated a negative effect of baetid mayflies on *Potamopyrgus* survivorship, which may adversely affect the degree of success of *Potamopyrgus* populations. That is, *Potamopyrgus* densities in Darlinton Spring Creek may be limited, at least in part, by competition with baetids. This relationship is known as the “biotic resistance hypothesis” and has been proposed as one way to understand why invasion success varies (Baltz 1993).

Experiments and observations in this report also showed that *Potamopyrgus* can depress periphyton food resources, but whether to a level that limits other species will depend upon biological attributes and competitive abilities of each species.

Because this study does not demonstrate an effect of *Potamopyrgus* on baetid mayflies in the field, but does indicate that *Potamopyrgus* can negatively affect baetid survivorship, it forces the question—“under what circumstances might *Potamopyrgus* affect baetids, as well as other macroinvertebrates?” One working hypothesis is that *Potamopyrgus* does not negatively affect baetids until densities reach a certain level—perhaps >50,000 or >100,000 m^{-2}. An additional hypothesis may be that the effect of *Potamopyrgus* on baetids may change over time, having a greater effect during times of lower productivity (winter) or during different developmental ages of baetid larvae. For *Potamopyrgus*, as well as other invasive species, this question is important to ask and answer so that accurate predictions about the consequences of the invasive species can be made. Furthermore, we caution against interpreting the results of this study to mean that *Potamopyrgus* will not have an effect in other invaded locations.

Finally, this study does not demonstrate a strong effect of *Potamopyrgus* on the growth of either *S. trutta, O. mykiss,* or *C. bairdi*, but it does suggest that insectivorous fishes may adjust their diet according to changes in macroinvertebrate abundances caused by *Potamopyrgus*. Furthermore, *O. mykiss* frequently fed on *P. antipodarum.* However, the amount of sustenance that *P. antipodarum* contributes to the growth of *O. mykiss* individuals is still unknown. At least 50% of *P. antipodarum* were recovered from intestines rather than the stomachs and were relatively undigested (C. Cada, personal observation). This suggests that *O. mykiss* gut-retention time was not sufficient to digest *P. antipodarum* and that they gained little energy or nutrition from *P. antipodarum*.

Publications/Citations:
The research offered in this report has been presented publicly at two conferences and at an informal gathering between biology/ecology departments at Montana State University and University of Montana. With the exception of the 2004 fish experiment, these data were published in the Master's thesis of Chelsea A. Cada in 2004.

Cada, C. A. and B. L. Kerans. Submitted. Community response to *Potamopyrgus antipodarum* invasion. Biological Invasions.

Cada, C. A. and B. L. Kerans. In preparation. Competitive interactions between *Potamopyrgus antipodarum* and baetid mayflies.

Cada, C. A., B.L. Kerans, and J. Smith. In preparation. Trophic effects of the New Zealand mud snail, *Potamopyrgus antipodarum,* on trout and a sculpin.

Cada, C. A. 2004. Interactions between the invasive New Zealand Mud Snail, *Potamopyrgus antipodarum,* baetid mayflies, and fish predators. Master's thesis, Department of Ecology, Montana State University, Bozeman.

Cada, C. A. and B. L. Kerans. 2004. Competitive interactions between the invasive gastropod *Potamopyrgus antipodarum* and baetid mayflies. Annual Meeting of the North American Benthological Society. Vancouver, B.C.

Cada, C., J. Smith, and B. L. Kerans. 2003. "What about the fish?" 3rd Annual *Potamopyrgus antipodarum* Conference. Montana State University—Bozeman.

Student Support:
These funds provided a monthly stipend support for Chelsea A. Cada as a graduate student pursuing her Master's Degree in Biology. Additionally, it supported two part-time undergraduate technicians: Jeremy Smith and Karlee Thorpe.

References Cited

Aloi J (1990) A critical review of recent freshwater periphyton field methods. Canadian Journal of Fisheries and Aquatic Sciences 47: 656-670

Baltz DPM (1993) Invasion resistance to introduced species by a native assemblage of California stream fishes. Ecological Applications 3: 246-255

Benke A, Huryn A, Smock L and Wallace J (1999) Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. Journal of the North American Benthological Society 18: 308-343

Berlow E, Navarrete S, Briggs C, Power M and Menge B (1999) Quantifying variation in strengths of species interactions. Ecology 80: 2206-2224

Billick I and Case TJ (1994) Higher order interactions in ecological communities: what are they and how can they be detected? Ecology 75: 1529-1543

Bowen SH (1983) Quantitative description of the diet. In: Nielsen LA and Johnson DL (eds) Fisheries Techniques, pp 325-336. American Fisheries Society, Bethesda, Maryland

Cattaneo A and Roberge G (1991) Efficiency of a brush sampler to measure periphyton in streams and lakes. Canadian Journal of Fisheries and Aquatic Sciences 48: 1877-1881

Coblentz BE (1990) Exotic organisms: A dilemma for conservation biology. Conservation Biology 4: 261-265

Connell JH (1980) Diversity and the coevolution of competitors, or the ghost of competition past. Oikos 35: 131-138

Crooks J (2002) Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos 97: 153-166

Deluchi CM and Peckarsky B (1989) Life history patterns of insects in an intermittent and a permanent stream. Journal of the North American Benthological Society 8: 308-321

Dorgelo J (1987) Density fluctuations in populations (1982-1986) and biological observations of *Potamopyrgus jenkinsi* in two trophically differing lakes. Hydrobiological Bulletin 21: 95-110

Fox J, Dybdahl M, Jokela J and Lively C (1996) Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (*Potamopyrgus antipodarum*). Evolution 50: 1541-1548

Goldberg DE and Scheiner SM (2001) ANOVA and ANCOVA, field competition experiments. In Scheiner SM and Gurevitch J (eds), Design and Analysis of Ecological Experiments, pp 77-98. Oxford University Press, Oxford

Graham A, McCaughan D and McKee F (1988) Measurement of surface area of stones. Hydrobiolgia 157: 85-87

Hall R, Tank JL and Dybdahl MF (2003) Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. Frontiers in Ecology and the Environment 1: 407-411

Haynes A and Taylor B (1984) Food finding and food preference in *Potamopyrgus jenkinsi* (EA Smith) (Gastropoda:Prosobranchia). Archiv fur Hydrobiologie 100: 479-491

Hylleberg J and Siegismund HR (1987) Niche overlap in mud snails (Hydrobiidae): freezing tolerance. Marine Biology 94: 403-407

Jenkins PT (1996) Free trade and exotic species introductions. Conservation Biology 10: 300-302

Kerans BL, Dybdahl MF, Gangloff MM and Jannot JE (2005) *Potamopyrgus antipodarum*: distribution, abundance, and effects on native macroinvertebrates in the Greater Yellowstone Ecosystem. Journal of the North American Benthological Society 24: 123-138

Kerans BL, Peckarsky B and Anderson C (1995) Estimates of mayfly mortality: is stonefly predation a significant source? Oikos 74: 315-323

Kohler SL and McPeek MA (1989) Predation risk and the foraging behavior of competing stream insects. Ecology 70: 1811-1825

Merritt R and Cummins K (1996) An Introduction to the Aquatic Insects of North America.

Peckarsky BL, Cooper S and McIntosh A (1997) Extrapolating form individual behavior to populations and communities in streams. Journal of the North American Benthological Society 16: 275-390

Peckarsky B, Cowan C, Penton M and Anderson C (1993) Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. Ecology 74: 1836-1846

Pimentel D, Lach L, Zuniga R and Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. BioScience 50: 53-65

Pennak RW (1978) Freshwater invertebrates of the United States. 2nd edition. Wiley, New York

Rappaport D and Whitford W (1999) How ecosystems respond to stress - Common properties of arid and aquatic systems. Bioscience 49: 193-203

Richards DL, Cazier LD and Lester GT (2001) Spatial distributuion of three snail species, including the invader *Potamopyrgus antipodarum* in a freshwater spring. Western North American Naturalist 61: 375-380.

Schreiber ESG, Glaister A, Quinn GP and Lake PS (1998) Life history and population dynamics of the exotic snail *Potamopyrgus antipodarum* (Prosobranchia : Hydrobiidae) in Lake Purrumbete, Victoria, Australia. Marine and Freshwater Research 49: 73-78

Simon KS and Townsend CR (2003) Impacts of freshwater invaders at different levels of ecological organisation, with emphasis on salmonids and ecosystem consequences. Freshwater biology 48: 982-994

Thrush SF, Cummings VJ. Dayton PK, Ford R, Grant J, Hewitt JE, Hines AH, Lawrie SM, Pridmore RD, Legendre P, McArdle BH, Schneider DC, Turner SJ, Whitlach RB and Wilkinson MR (1997) Matching the woutcome of small-scale density manipulation experiments with larger scale patterns: an example of bivalve adult/juvenile interactions. Journal of experimental marine biology and ecology 216: 153-169

Von Ende CN (2001) Repeated-measures analysis. In: Scheiner SM and Gurevich J)eds) Design and Analysis of ecological experimetns, pp 134-157. Oxford University Press, New York

Williamson MH and Fitter A (1996) The characters of successful invaders. Biological Conservation 78: 163-170

Wilzbach ME, Cummins KW and Hall JD (1986) Influence of habitat manipulations on interactions between cutthroat trout and invertebrate drift. Ecology 67: 898-911

Winterbourn M (1970) Population studies on the New Zealand freshwater gastropod, *Potamopyrgus antipodarum* (Gray). Proceedings of the Malacological Society of London 39: 139-149

Zaranko DT, Farara DG and Thompson FG (1997) Another exotic mollusc in the Laurentian Great Lakes: the New Zealand native *Potamopyrgus antipodarum* (Gray 1843) (Gastropoda, Hydrobiidae). Canadian Journal of Fisheries and Aquatic Sciences 54: 809-814

Table 1. Experimental design of field competition Experiment 2 indicating the species and density combinations for each treatment, the number of individuals stocked per chamber, and the number of replication for each treatment. B=*Baetis tricaudatis*. P=*Potamopyrgus antipodarum*.

Species	Density	# Individuals	n
Baetis	low	120B	6
Potamopyrgus	low	120P	5
Baetis + Potamopyrgus	low	60B + 60P	6
Baetis	high	240B	6
Potamopyrgus	high	240P	5
Baetis + Potamopyrgus	high	120B + 120P	6
Control	na	0	5

Table 2. Results of repeated measures 2-way MANOVA for *P. antipodarum* (a) and Baetidae mayflies (pooled by family) (b) where density and biomass were repeatedly measured over time. Note that "+" indicates that instead of λ, the value reported is the F-statistic from the between-subjects' effect repeated measures analysis

.

a. *Potamopyrgus antipodarum*

Source of variation	df	Wilk's lambda	*P*
	Density		
Time	13, 16	0.106	**<0.0001**
Snail	1, 28	1926.7^{+}	**<0.0001**
Time*Snail	13, 16	0.141	**0.0001**
Time*Reach(Snail)	26, 32	0.208	0.1511
	Biomass		
Time	13,16	0.203	**0.0019**
Snail	1, 28	3346.6^{+}	**<0.0001**
Time*Snail	13,16	0.198	**0.0016**
Time*Reach(Snail)	26, 32	0.091	**0.0027**

b. Baetidae

Source of variation	df	Wilk's lambda	*P*
	Density		
Time	13, 16	0.0354	**<0.0001**
Snail	1, 28	0.24^{+}	0.6248
Time*Snail	13, 16	0.542	0.4624
Time*Reach(Snail)	26, 32	0.0621	**0.0003**
	Biomass		
Time	13, 16	0.0357	**<0.0001**
Snail	1, 28	0.18^{+}	0.675
Time*Snail	13, 16	0.55	0.488
Time*Reach(Snail)	26, 32	0.0524	**<0.0001**

Table 3. Results of repeated measures 2-way MANOVA for *Baetis tricaudatis, Diphetor hageni* and *Acerpenna pygmaea,* where density and biomass were repeatedly measured over time. Note that "$^{+}$" indicates that instead of λ, the value reported is the F-statistic from the between-subjects' effect repeated measures analysis.

Density				Biomass			
Source of variation	df	Wilk's lambda	*P*	Source of variation	df	Wilk's lambda	P
Baetis							
Time	13, 16	0.059	**<0.0001**	Time	13,16	0.044	**< 0.0001**
Snail	1, 28	0.1+	0.7545	Snail	1, 28	0.09+	0.763
Time*Snail	13, 16	0.485	0.3023	Time*Snail	13,16	0.444	0.2043
Time*Reach (Snail)	26, 32	0.065	**0.0004**	Time*Reach (Snail)	26, 32	0.057	0.0002
Diphetor							
Time	13, 16	0.066	**<0.0001**	Time	13, 16	0.054	**< 0.0001**
Snail	1, 28	0.51+	0.4790	Snail	1, 28	0.66 +	0.4218
Time*Snail	13, 16	0.409	0.1368	Time*Snail	13, 16	0.408	0.1358
Time*Reach (Snail)	26, 32	0.137	**0.0239**	Time*Reach (Snail)	26, 32	0.129	**0.0181**
Acerpenna							
Time	13, 16	0.055	**<0.0001**	Time	13, 16	0.059	**< 0.0001**
Snail	1, 28	1.72+	0.1998	Snail	1, 28	1.74 +	0.1979
Time*Snail	13, 16	0.453	0.2239	Time*Snail	13, 16	0.482	0.2932
Time*Reach (Snail)	26, 32	0.059	**0.0002**	Time*Reach (Snail)	26, 32	0.06	**0.0002**

Table 4. Results of repeated measures 2-way MANOVA for chlorophyll *a* and pheophytin *a* biomass where periphyton biomass was repeatedly measured over time. Table 4. Note that "+" indicates that instead of λ, the value reported is the F-statistic from the between-subjects' effect repeated measures analysis.

Source of variation	df	Wilk's lambda	*P*
		Chlorophyll *a*	
Time	13, 11	0.1049	**0.0012**
Snail	1, 23	3.9600 +	0.0587
Time*snail	13, 11	0.2671	0.0850
Time*reach(snail)	26, 22	0.1164	0.1230
		Pheophytin *a*	
Time	13, 11	0.0491	**< 0.0001**
Snail	1, 23	1.0600 +	0.3136
Time*snail	13, 11	0.2740	0.0939
Time*reach(snail)	26, 22	0.0541	**0.0085**

Figure 1. - Temporal trends in the densities and biomasses (mean ± 1 SE) of *Potamopyrgus antipodarum* and Baetidae mayflies in high-snail (filled circles) and low-snail reaches (open circles). For each snail type and each time period, 16 cobble samples were taken for a total of n = 384 invertebrate samples.

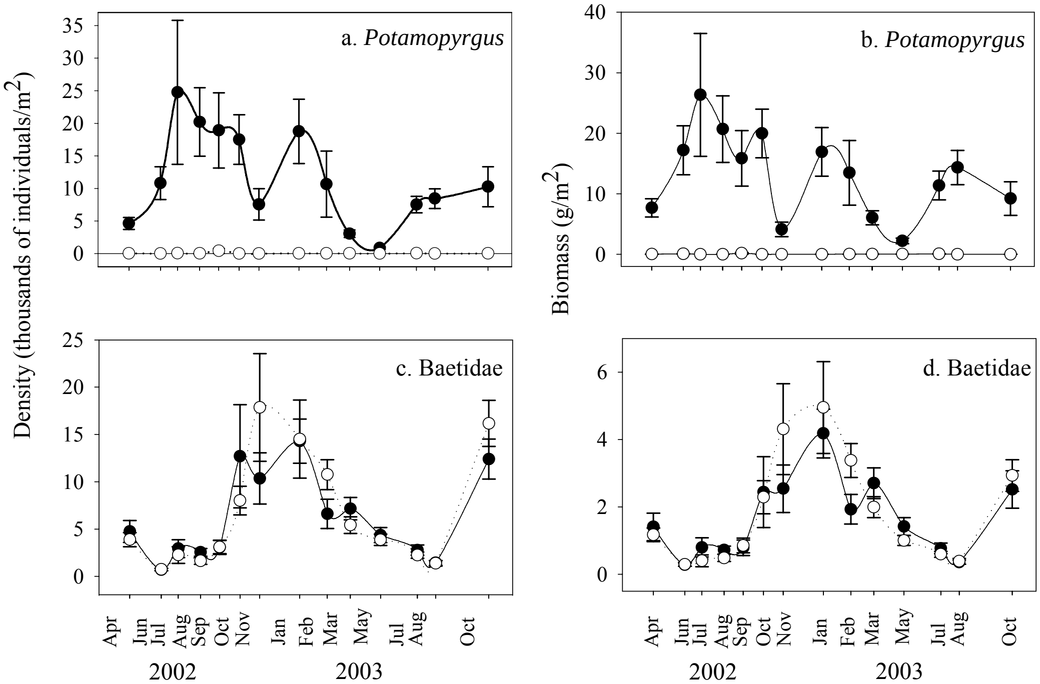


Figure 2 - Temporal trends in the densities (mean ± 1 SE) of *Baetis tricaudatis*, *Diphetor hageni*, and *Acerpenna pygmaeus* in high-snail (filled circles) and low-snail reaches (open circles). For each snail type and each time period, 16 cobble samples were taken for a total of n = 384 invertebrate samples.

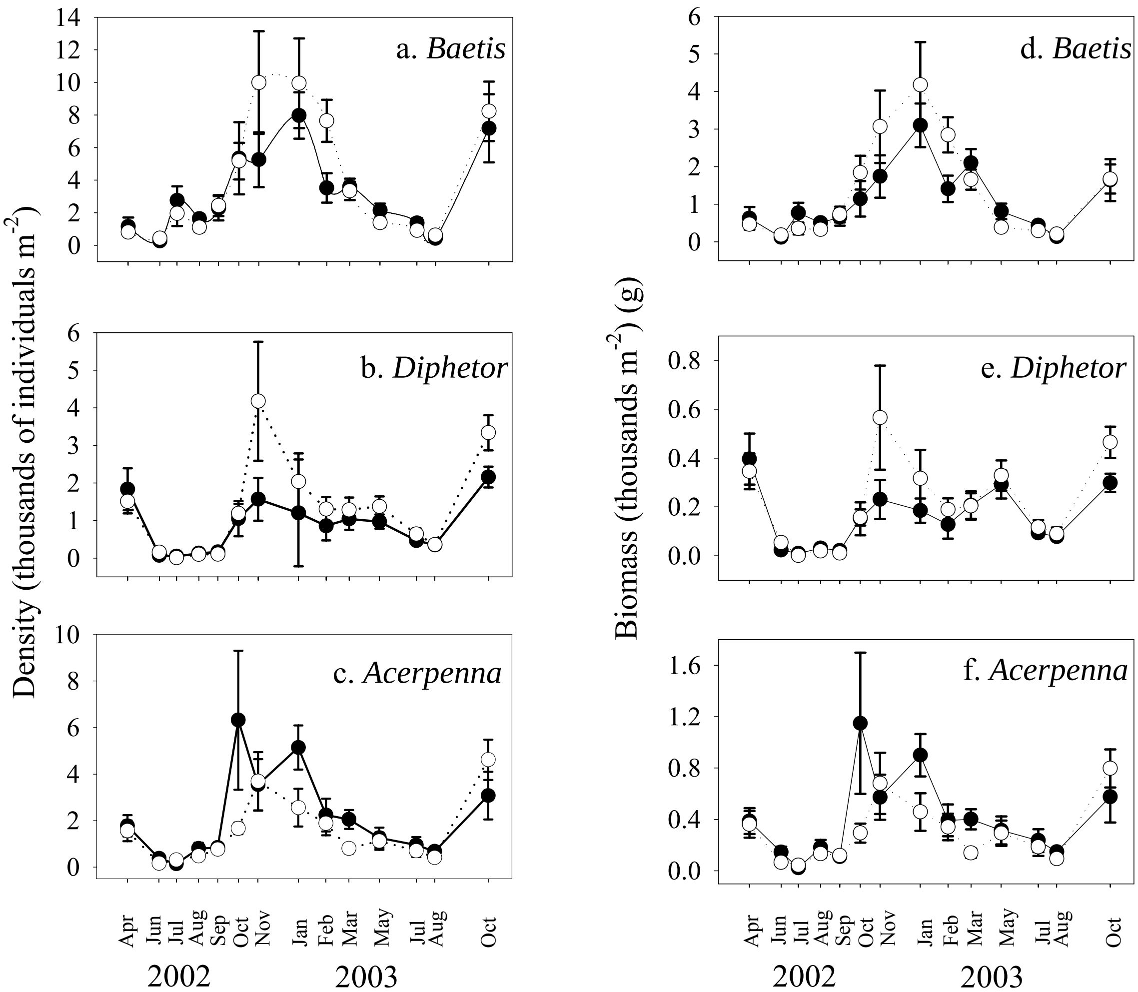


Figure 3 - Chlorophyll *a* biomass (mean ± 1 SE) (a) and pheophytin *a* biomass (b) compared between high-snail and low-snail reaches over time from the field surveys. The filled symbols represent high-snail reaches and open symbols represent low-snail reaches.

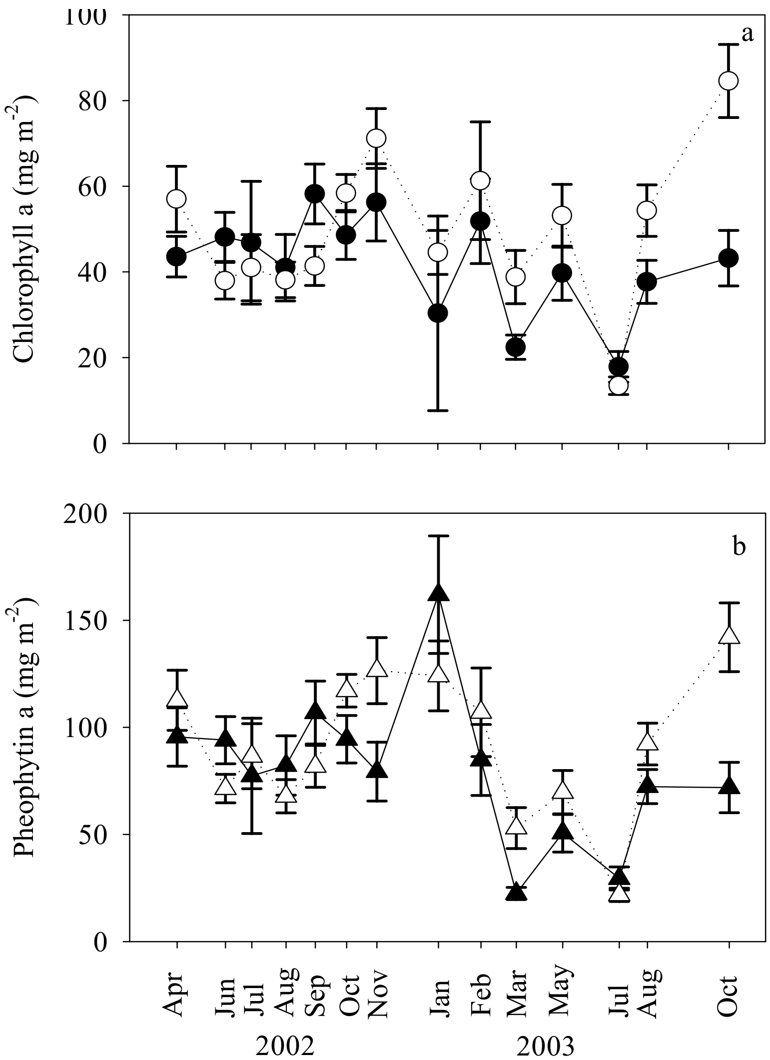


Figure 4 - Chlorophyll *a* and pheophytin *a* biomass (mean ± 1 SE) from competition Experiment 1. Treatments included biomass from the stream channel at the start of the experiment (Initial), a control with no invertebrates added (Control), *Diphetor* only (D), *Potamopyrgus* only (P), or both species (D+P). Horizontal lines indicate those treatment means that are statistically similar to each other from Tukey's HSD multiple comparisons ($p < 0.05$).

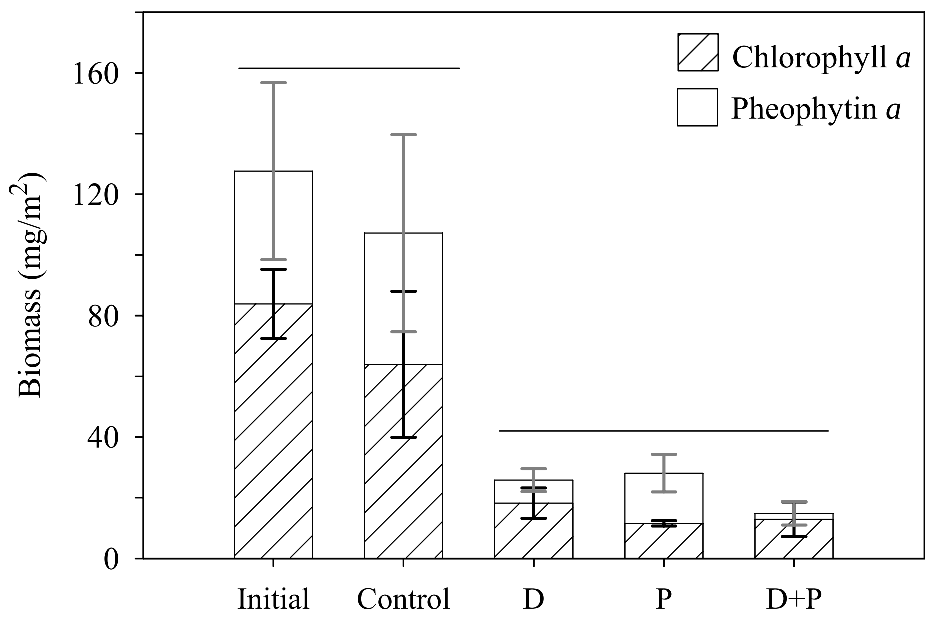


Figure 5 - Chlorophyll *a* (shaded) and pheophytin *a* (open) biomasses (mean ± 1 SE) from competition Experiment 2. Treatments included biomass from the stream channel at the start of the experiment (Initial), a control with no invertebrates added (Control), *Baetis* only at both densities (B), *Potamopyrgus* only at both densities (P), or both species at both densities (B+P).

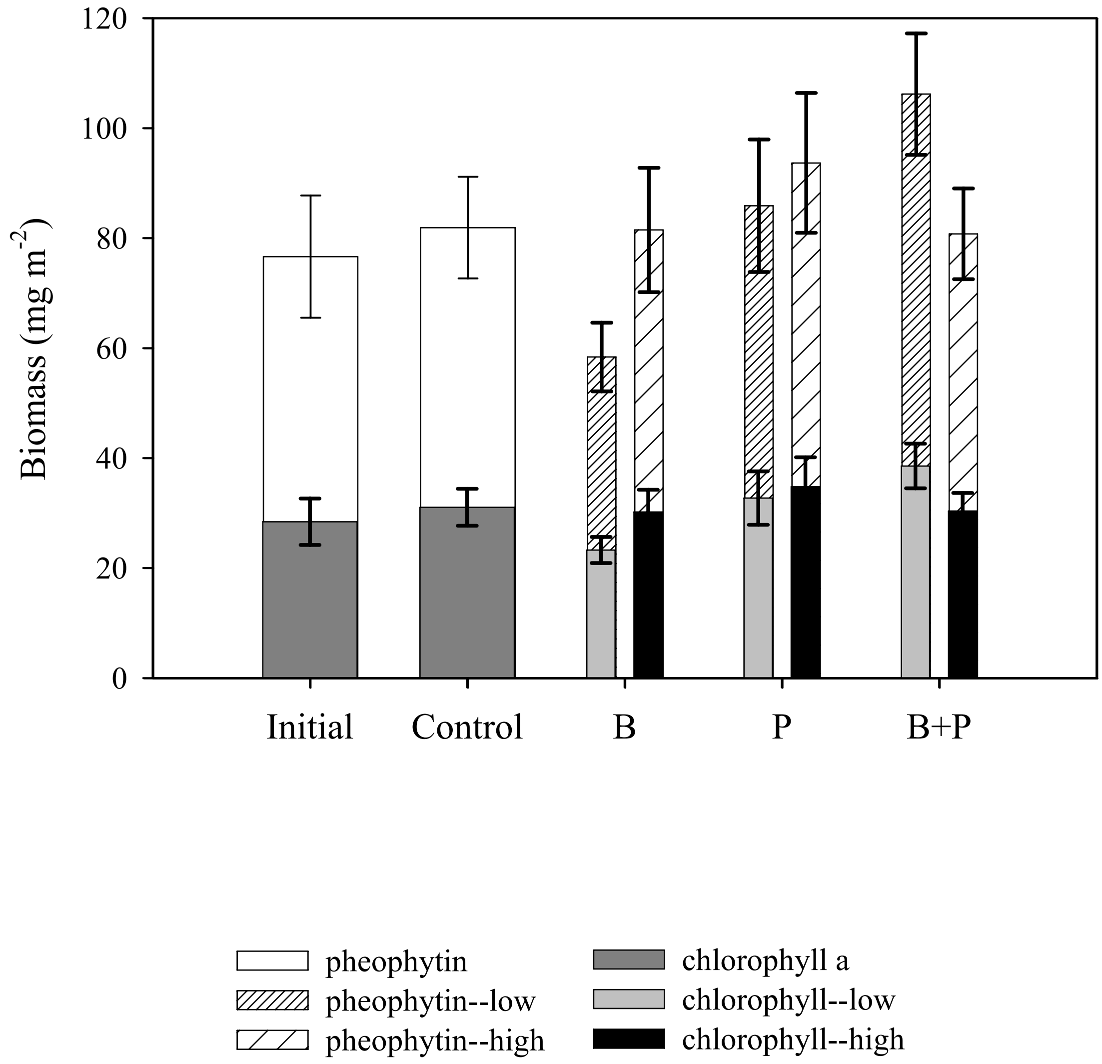


Figure 7 - Survivorship (a & b) or growth (c & d) (mean ± 1 SE) for *Potamopyrgus* and *Baetis* from competition Experiment 2. Filled circles represent *Baetis* whereas open circles represent *Potamopyrgus*. Treatments included *Baetis* only at low and high densities and *Potamopyrgus* only at low and high densities ("solitary"), or both species at low and high densities (B+P). Different upper-case letters above data points indicate differences among means using Tukey's HSD multiple comparisons ($p <$ 0.05).

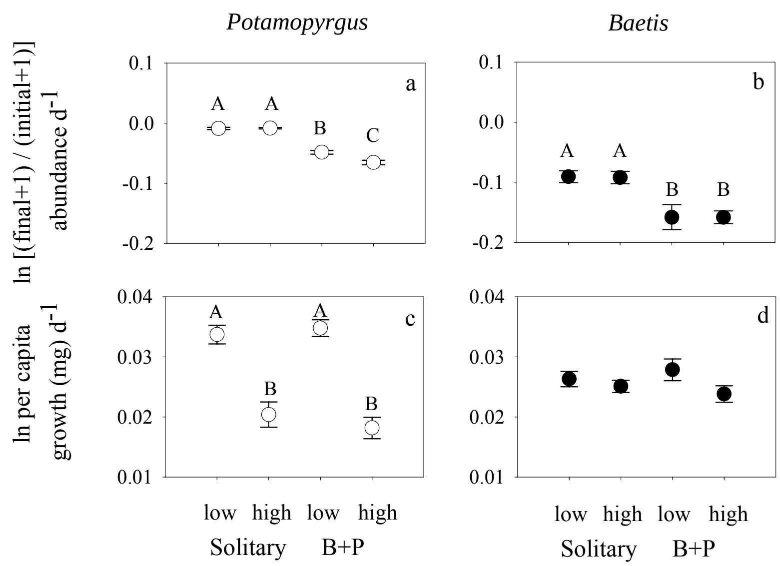


Figure 8 - The relative abundance of macroinvertebrates in stomach samples from the end of the enclosure experiment for *Salmo trutta* in high- (a) and low-snail (b) reaches and for *Cottus bairdi* in high- (c) and low-snail (d) reaches.

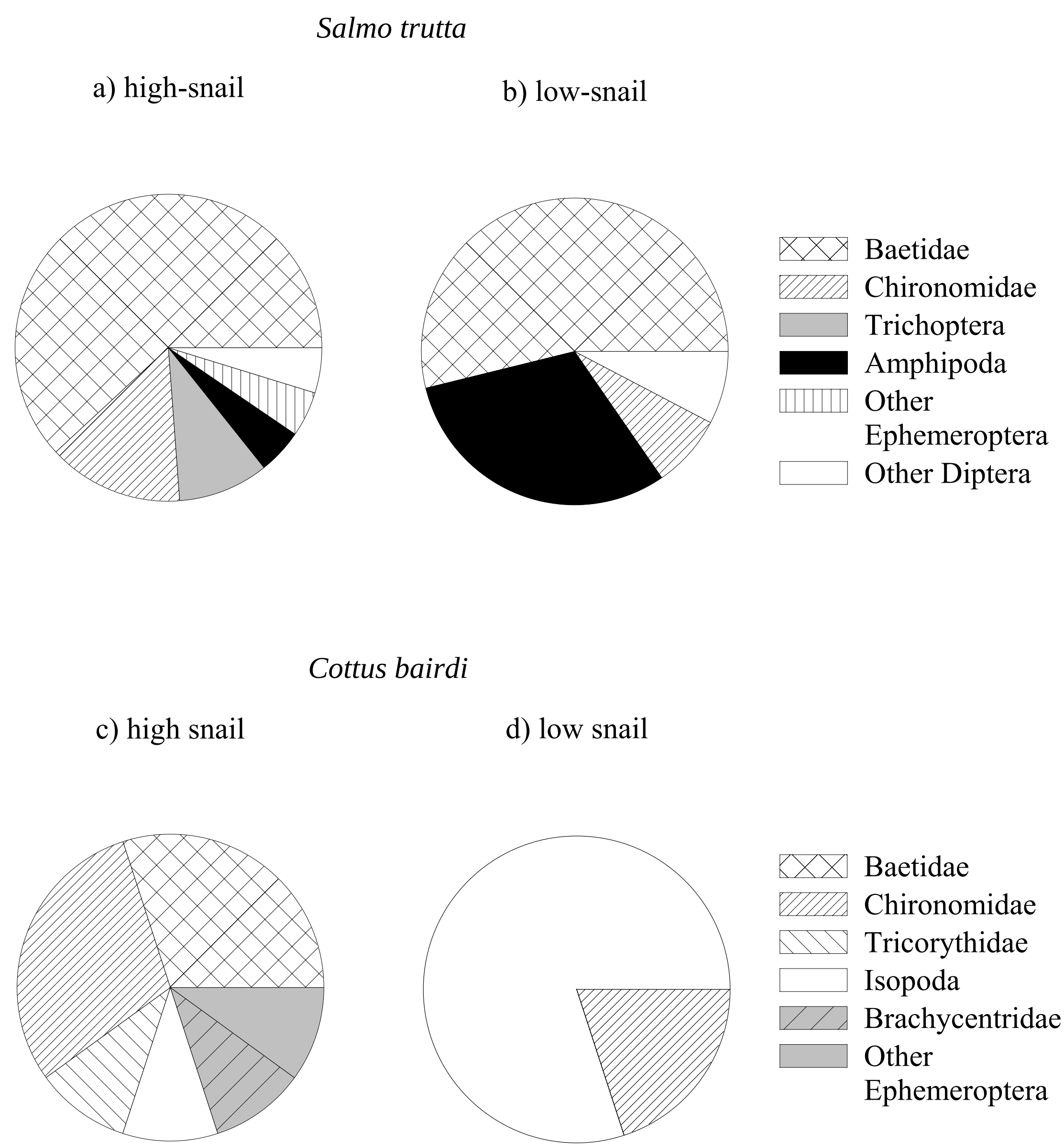
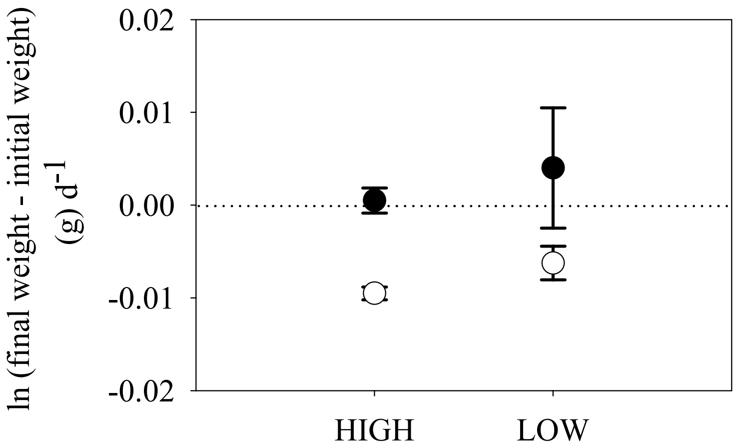


Figure 9 - Comparison of fish growth in high-snail and low-snail density reaches from the enclosure experiment for *Salmo trutta* (filled circles) and *Cottus bairdi* (open circles). The dotted line represents no growth; above it is weight gain and below it is weight loss.



Pharmaceuticals in Septic System Effluent and a Preliminary Evaluation of Environmental Fate

Basic Information

Title:	Pharmaceuticals in Septic System Effluent and a Preliminary Evaluation of Environmental Fate
Project Number:	2002MT3B
Start Date:	3/1/2002
End Date:	9/30/2005
Funding Source:	104B
Congressional District:	at-large
Research Category:	Water Quality
Focus Category:	Water Quality, Hydrology, Toxic Substances
Descriptors:	septic system, effluent, pharmaceuticals, Trimethoprim, Diltiazem, Cimetidine, Acetaminophen, Caffeine, Carbamazpine
Principal Investigators:	William W. Woessner, Garon C. Smith

Publication

1. Godfrey, Emily and William W. Woessner. 2004. Screening level study of pharmaceuticals in septic tank effluent and a wastewater treatment plant waste stream. Proceedings of the 4th International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, held October 13-15, 2004, in Minneapolis, Minnesota (copyright 2004 by the National Ground Water Association, ISBN 1-56034-114-9): 296-308.
2. Godfrey, Emily. 2004. Screening Level Study of Pharmaceuticals in Septic Tanks, Ground Water and Surface Water in Missoula, Montana. Unpublished Master of Science Thesis, Dept. of Geology, University of Montana.
3. As the 2005 Birdsall Dreiss Distinguished Lecturer for the Geological Society of America, PI William Woessner has incorporated study results in one of the two talks he is invited to present around the country (~15 universities as of May, 2005). Talk Title is: Occurrence, Transport and Fate of Viruses and Pharmaceuticals in Groundwater Impacted by Septic System Effluent: The Hydrogeologists and Human Health.

Screening Level Study of Pharmaceuticals in Septic Tank Effluent and a Wastewater Treatment Plant Waste Stream

Emily Godfrey and William W. Woessner

Department of Geology, University of Montana, Missoula, Montana.

Abstract

Evaluating how pharmaceuticals are entering the environment has been the focus of recent research. Two principal pathways requiring investigation are wastewater treatment plants and septic systems. This study attempts to examine the occurrence and estimate the concentrations of selected pharmaceuticals in these waste systems. Thirty-two single family and ten multiple family septic tanks, as well as the influent and effluent wastewater from the community wastewater treatment plant (WWTP) in Missoula, Montana, were sampled. Samples were analyzed by Time-of-Flight High Performance Liquid Chromatography/ Mass Spectrometry for 19 drug residues and three drug metabolites of both prescription and non-prescription drugs. Only 18 of the 22 pharmaceuticals were present in the septic tanks, 12 were detected in the WWTP influent, and nine were detected in the WWTP effluent. The most frequently detected (>50%) non-prescription drugs were, acetaminophen, caffeine, and nicotine, as well as metabolites of caffeine (paraxanthine) and nicotine (cotinine). Median concentrations of these compounds were 219-ug/L, 80-ug/L, 8.7-ug/L, 175-ug/L, and 4.7-ug/L, respectfully. Prescription drugs were detected less than 30% of the time, with the exception of warfarin, which was detected in approximately 77% of the samples. Prescription drugs found most frequently were codeine, trimethoprim and carbamazepine. This work suggests that concentrations of pharmaceuticals, originating from both septic effluent and wastewater treatment plant effluent could be leaving these treatment systems and entering the associated surface water or ground water resources in Missoula.

INTRODUCTION

During the last three decades, an increased focus on water pollution from organic chemicals such as toxic/carcinogenic pesticides and industrial byproducts has emerged (Christensen 1998). In recent years, pharmaceuticals and personal care products (PPCP's) and their metabolites have been detected in the environment (Raloff 1998; Buser et al. 1999; Hartig et al.1999; Seiler et al. 1999; Heberer 2002a and 2002b; Holm et al. 1995; Kolpin et al. 2002; Scheytt et al. 1998; Eckel et al. 1998; McQuillan et al. 2000, Buerge et al. 2003; Clara et al. 2004; Petrovic et al. 2003). To date, efforts have focused principally on the detection and fate of PPCP's in surface water. Only a few studies (Holm et al. 1995, Umari et al. 1995, Eckel et al. 1998, Seiler et al. 1999, Heberer 2002, Drewes et al. 2003, Verstraeten et al. Draft, Benotti et al. 2003, Cordy et al. 2004) have examined the concentration of pharmaceuticals in raw sewage. As of this writing, no published research has examined pharmaceutical concentrations from individual septic systems. In the US, approximately 25-30% of households use septic systems for wastewater disposal (Verstraeten et al. Draft). This raises concerns that trace pharmaceuticals could enter the ground water underlying these systems.

GOALS AND OBJECTIVES

This study characterizes the occurrence and estimates the concentration of a selected group of pharmaceuticals in septic system effluent and wastewater inflows and outflows from a municipal sewage treatment plant. The specific study objectives were to: (1) identify target compounds; (2) develop sampling and analyses procedures; (3) characterize individual and community septic tank effluent, and compare these results to the character of municipal sewage wastewater.

METHODS

Identify target compounds of concern

Pharmaceuticals selected for this study were based on the following criteria: 1) they are commonly used drugs; 2) compounds found in the environment reported by other studies; 3) they ionize well under positive electron spray mode (analytical consideration). Certain compounds, like ibuprofen, that fit criteria one and two, were not included as they cannot be easily detected using the chosen analytical technique. Target compounds including 19 pharmaceuticals, both prescription and non-prescription drugs, and three metabolites were selected for evaluation (Table 1).

Table 1. Pharmaceuticals selected for analyses. The last two columns report the maximum recommended dose for an adult and maximum urinary excretion percentage

Compound	Type	Use	Maximum Recommended Dose for adults	Maximum Urinary Excretion (%) (Goodman and Gilman, 1990)
Acetaminophen	Non-prescription drug	Antipyretic	1000mg, 4-6hrs***	3 +/- 1
Antipyrine (Phenazone)	Prescription	Analgesic	54mg, 3 times/day**	ND*
Caffeine	Non-prescription drug	Stimulant	210-440mg coffee (Buerge et al. 2003), 200mg, 3-4 hrs pill***	1.1 +/- 0.5
Carbamazepine	Prescription drug	Anticonvulsant, antineuralgic, antimanic, antidepressant, antipsychotic	75-300mg/day**	<1, 3**
Cimetidine	Non-Prescription drug	Antiasthmatic	200mg, 12hrs**	62 +/- 20
Codeine	Prescription drug	Analgesic (anti-cough)	10-60mg, 1-4 times/day**	Negligible
Cotinine	Metabolite	Nicotine metabolite	Metabolite	ND*
Diltiazem	Prescription drug	Antihypertensive	480 mg/day**	<4
Erythromycin-18	Metabolite of Prescription drug	Antibiotic	Metabolite	12 +/- 7
Fenofibrate	Prescription	Lipid Metabolism Regulator	201 daily**	ND
Fluoxetine	Prescription drug	Antidepressant, antiobsessional, and antibulimic	80mg/day**	<2.5
Hydrocodone	Prescription drug	Analgesic (anti-cough) and antitussive	7.5/day**	ND*
Ketoprofen	Non-Prescription	Anti-inflammatory	12.5mg, 4-6hrs***	<1
Metformin	Prescription drug	Antihyperglycemic	2550mg/day**	ND*
Nicotine	Non prescription drug	Stimulant	21mg/hr patch***	16.7 +/- 8.6
Nifedipine	Prescription drug	Antianginal (blood pressure control)	120mg/day**	~0
Paraxanthine (1,7-dimethylanthine)	Metabolite	Caffeine metabolite	Metabolite	ND*
Ranitidine	Non- Prescription drug	Histamine	75mg, 12hr***	69 +/- 6
Salbutamol	Prescription drug	Relax restricted airways	5mg/day**	ND*
Sulfamethoxazole	Prescription drug	Antibiotic	80mg, 3-4hrs**	14 +/-2
Trimethoprim	Prescription Drug	Antibiotic	20mg, 3-4 times/day**	69 +/- 17
Warfarin	Prescription drug	Anticoagulant	10mg/day**	<2

ND*= no data, **= Physicians desk reference 1999, ***=Physicians desk reference 2001

Field Sampling and Site Description

Two types of wastewater were sampled for pharmaceuticals: 1) individual and community septic systems and 2) the city wastewater treatment plant. Thirty-two single-family and ten community septic tanks were sampled in the City of Missoula (Figure 1).

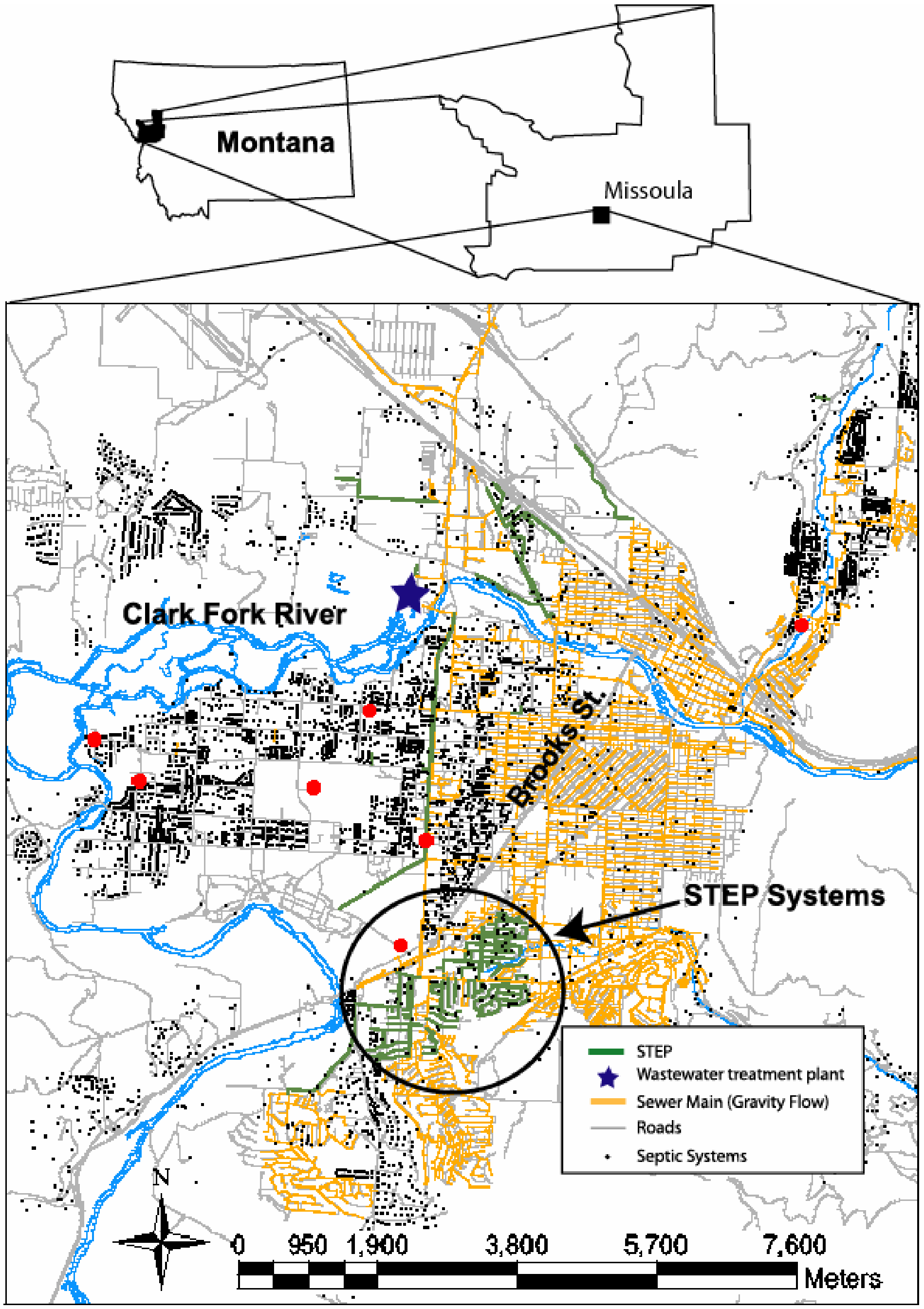


Figure 1. Location map of the City of Missoula. Shown are sewer systems (gravity flow and STEP), unsewered areas and the wastewater treatment plant in the city of Missoula, Montana (Map source: Department of Water Quality Missoula, Montana)

tank's liquid effluent reaches a volume of 2,600-L, it is pumped from the septic tank to the city sewer line. Solids that settle to the bottom of the tank are pumped out as needed (Figure 2). Community STEP tanks function the similar to single-family STEP systems except community tanks hold 11,300 to 30,300-liters of effluent.

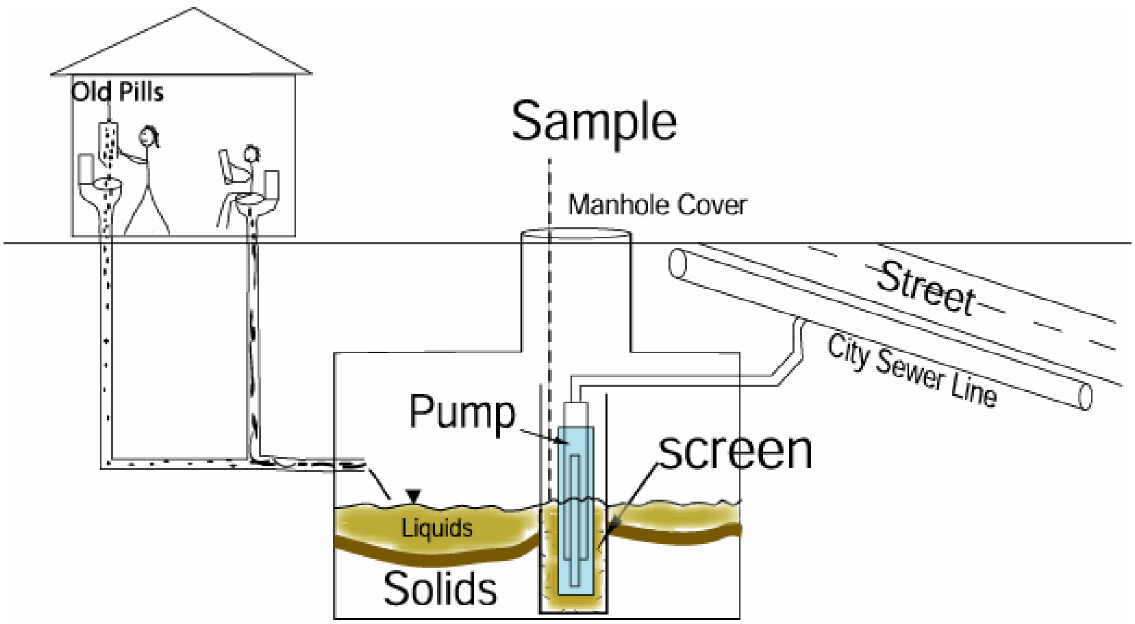


Figure 2. Schematic diagram of STEP (Septic Tank Effluent Pumping) system for a single-family residence

Each septic tank effluent sample was collected from STEP systems using a parastolic pump equipped with new 30-cm length of silicon tubing and 1.5 to 7.6-m new 0.6-cm diameter polyethylene tubing. Samples pumped from the tanks were collected in a 2.5-L glass bottle. All bottles were pre-washed with methanol and Milli-Q water and dried overnight. All sampling tubing was discarded after sample collection.

The municipal wastewater treatment plant (WWTP) in Missoula, Montana is connected to about 57,000-population equivalents. The WWTP utilizes commonly used treatment steps, preliminary sedimentation followed by activated sludge treatment and final clarification by chlorination. After primary sedimentation, three influent samples were obtained at the WWTP by submersing a 2.5-L glass bottle into the liquid flowing into the secondary treatment basin. As an advanced wastewater treatment, Missoula WWTP uses ultraviolet treatment during the summer months to further treat photoreactive compounds. Two effluent samples were taken before and after ultraviolet treatment. Effluent from the WWTP is then discharged into the Clark Fork River.

Sample Preparation

At this time no standardized procedure has been adapted for sample preparation and analysis. Samples were placed on ice in the field and refrigerated at 4ºC in the lab. They were prepared within 1-3 days of collection for analysis using adjusted methods described by Kolpin et al. (2002) (pharmaceutical extraction method 3). This method was designed to target human prescription and non-prescription drugs and their metabolites. In brief, first a pre-filtration step was initiated by passing the sample through a 0.45-um glass fiber filter (Whatman, 47-mm). Then one-liter of sample was processed on a solid phase extraction (SPE) cartridge that contained 6-cc, 500-mg of sorbant Hydrophilic-Lipophilic-Balance (Oasis, HLB) at a flow rate of 15 to 25-mL/min. Next, compounds were extracted from the SPE cartridge using two 3-mL aliquots of methanol (CH_3OH) and two 3-mL aliquots of methanol acidified with trifluoroacetic acid (0.1% trifluoroacetic acid, $C_2HF_3O_2$). Compounds were slowly reduced to near dryness under N_2 gas and then brought to a 1-mL solution volume with 10-mM ammonium formate/formic acid, (pH=3.7). All effluent samples were filtered with a 0.2-um PTFE (Polytetrafluoroethylene) syringe filter, and then diluted to a 10% solution, prior to analysis. As part of the method development and to maximize the resolution and sensitivity of the HPLC-TOF-MS, three samples were prepared at sample concentrations of 10%, 50% and 100% solution. The 10% diluted sample solution was chosen for its ability to produce chromatograms with the least amount of matrix interference and a discernable internal standard peak. Thus for all samples, prior to HPLC analysis, a 10% diluted sample solution was used.

Compounds were separated and measured by Time-of-Flight, High Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-TOF-MS, Waters HPLC system) in the Marine Sciences Research Center laboratory at Stony Brook University, the State University of New York, using a polar (neutral silanol) reverse-phase octylsilane (C8) HPLC column (Metasil Basic 3-um, 150*2.0-mm; Metachem Technologies). This preparation procedure was used for all samples (Benotti et al., 2003).

For quality control, one internal standard, $^{13}C_3$ labeled caffeine was used (Cambridge Isotope Laboratories in Cambridge, Ma). Pharmaceutical standards were obtained from Aldrich and prepared by the personnel of the Marine Sciences Research Center laboratory. Analyses were conducted in ESP+ mode with a selected mass range of 100 to 800 Da. A lock mass, leucine enkephalin (Sigma #P9003), was added post-column at a flow rate of 1-uL/min, with a concentration of 5-ng/mL. After analysis, all sample chromatograms were corrected by using a single point correction of the base calibration file with the lock mass (Benotti et al. 2003, Ferrer and Thurman 2003). Quantification of compounds was estimated from the internal standard ($^{13}C_3$ labeled caffeine) injected into the sample prior to analysis.

ANALYTICAL CHALLENGES

As this research effort was a screening level study and minimally funded, the analytical approach involved sample preparation in Missoula and preliminary runs of samples for selected compounds at the University of Montana Liquid Chromatography Lab in the Chemistry department. However, due to an absence of environmental QA/QC protocols and shared use of the instrument, analytical assistance was sought by Professor Bruce Brownawell and Ph.D. student Mark Benotti at the Marine Science Research Center, Stony Brook University in New York. A guest arrangement allowed us to travel to the lab with an HPLC column and operate the equipment under their guidance using a provided 20 compound standard. After analyzing all samples in New York, we returned to the University of Montana to process the results.

The standards examined during sample analysis (11/03) did not produce reliable results, so standards were remade and analyzed on a later date (01/04). The reason for unreliable standards, analyzed on 11/03, is a result of human error during standard preparation. Due to changing the detector voltage prior to analyzing the samples, it is impossible to compare the stability of the machine before and after samples were analyzed. In attempt to demonstrate the stability over a length of time, responses for standards from February 2004 are 109 +/- 12 (n=6) and May 2004 are 108 +/- 20 (n=6). The standard responses compare favorably over a four-month period. Moreover the response for standards, used for sample quantification on January 2004 was 107 +/- 13.

Analytical difficulty also occurred during sample preparation and SPE concentration. Using the stated preparation methodology, target compounds were captured from a one-liter filtered effluent sample using a 6-cc, 500-mg HLB sorbant. The ability for the HLB cartridges to capture all target compounds was evaluated by passing one sample through two HLB cartridges in series. Compounds such as acetaminophen, caffeine, cotinine and paraxathine were detected after the second processing of the one-liter samples, while ketoprofen, nicotine and warfarin were not detected (Table 2).

Table 2. Double runs through cartridges. Samples A and B are samples from two septic tanks. A1 and B1 are the results of effluent processed on one HLB cartridge and A 2 and B 2 are processed on a second HLB cartridge. **All values represent minimum concentrations.**

Samples	Acetaminophen	Caffeine	Cotinine	Ketoprofen	Nicotine	Paraxathine	Warfarin
	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L
A 1	1.09	8.26	Nd	Nd	Nd	67.67	1.81
A 2	0.64	1.39	0.12	Nd	Nd	40.88	Nd
B 1	140.01	60.84	5.36	147.64	0.87	71.84	5.84
B 2	427.73	13.621	2.44	Nd	nd	196.43	Nd

In an effort to examine the reproducibility of our analytical method, nine splits were prepared and analyzed (Table 3).

Table 3. Sample splits. These are reported by compound, total mean % comparisons, number of positive identified compounds ( ). **All values compared represented minimum concentrations**

Compound (n=)	Acetaminophen (9)	Caffeine (9)	Carbamazepine (3)	Cimetidine (2)	Cotinine (8)	Diltiazem (2)
Total mean (%)	88.4	83.2	78.1	91.7	83.9	83.2
Compound (n=)	**Erythromycin-18 (3)**	**Codeine (4)**	**Hydrocodone (1)**	**Ketoprofen (1)**	**Metaformin (3)**	**Nicotine (8)**
Total mean (%)	87.5	90.9	90.4	70.6	87.5	81.7
Compound (n=)	**Paraxathine (9)**	**Ranitidine (1)**	**Sulfamethoxazole (2)**	**Trimethoprim (3)**	**Warfarin (6)**	
Total mean (%)	83.8	69.4	46.0	80.6	70.7	

All compounds exhibited reproducibility above 50% with the exception of sulfamethoxazole, which was only detected in two samples.

During evaporation of the samples, a residue formed in some the test tubes. Visually, these samples were a dark brown color and collected on the bottom and sides of the glass vial. Adding the mobile phase (10-mM ammonium formate/formic acid, pH=3.7) to the near dry sample re-dissolved a portion of the solid phase, but in some samples the solid phase remained in the vial. It is possible that the residue remaining in the sample vial contained target compounds. These conditions may have created analytical results that are lower than actual values for these samples.

These analytical challenges limit the accuracy to report compound concentrations. This study attempted to characterize pharmaceutical concentrations in an environmental compartment for which little data exist (septic tanks). Generally speaking, pharmaceuticals in septic tanks exhibit a wide range of concentrations (from ng/L to high μg/L). While this offers interesting discussion, it must be noted that both the extraction procedure and HPLC-TOF-MS analysis applied in this study were designed to study trace levels of contaminants. Thus, reported concentrations, especially high values, represent a low-end concentration. The actual value cannot be quantitatively determined because phenomenon such as over-loading of SPE cartridges, ionization suppression/enhancement, and detector saturation impact analytical results, especially the determination of high concentrations (Benotti et al. 2003, Godfrey 2004). Although studies to qualify detector saturation and ionization suppression were outside the scope of this project, observation of such phenomenon indicate that concentrations from 10–500-ng/L are within the error of the analysis. Systematic error was assumed to linearly increase for concentrations that exceed 500-ng/L, probably underestimating the highest concentrations.

RESULTS

Single Family and Community Septic Tanks

This study analyzed for 22 pharmaceuticals in each sample. Of those, only 18 were found above their detection limit (Figure 3).

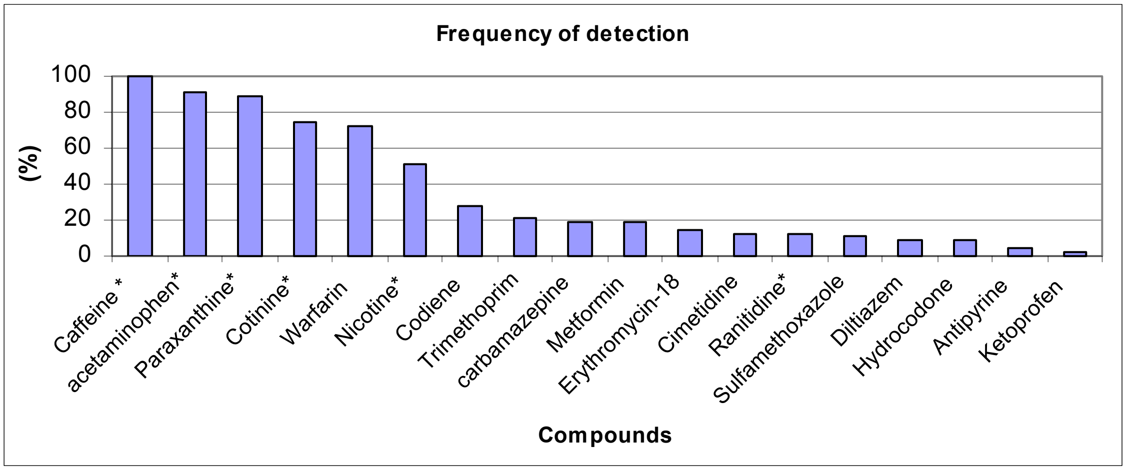


Figure 3. Most frequently detected compounds in raw sewage samples (community, single family and WWTP influent). Marked (*) compounds are nonprescription drugs and/or their metabolites.

Concentration ranges and frequency of occurrence data are provided for all compounds detected in community and single-family septic tank effluent (Figures 4 and 5). Compounds not detected were fenofibrate, fluoxetine, nifedipine and salbutamol. In all community tank effluent the most detected compounds (>60%) were acetaminophen, caffeine, cotinine, paraxanthine and warfarin (Figure 4). In single-family tanks the most detected compounds (>60%) were caffeine, acetaminophen, cotinine, paraxanthine and warfarin (Figure 5).

a. b.

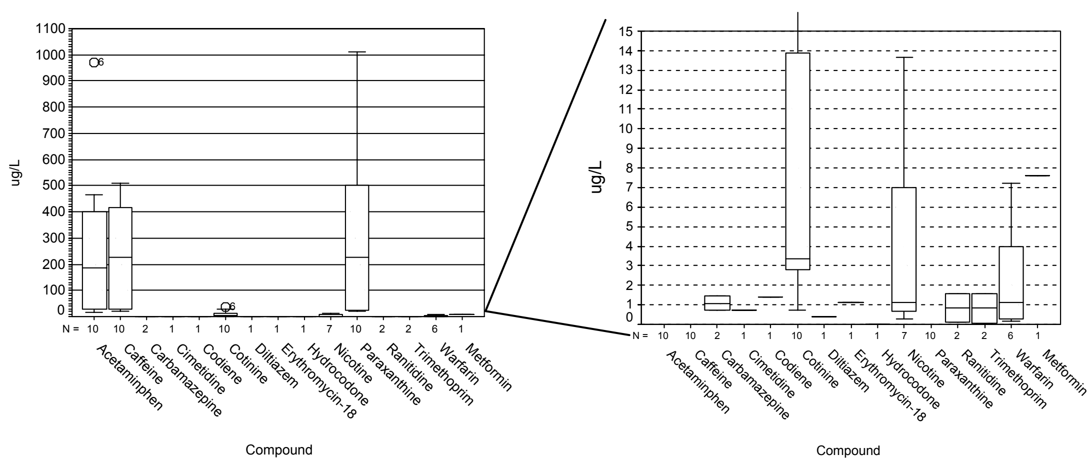


Figure 4. Pharmaceuticals detected in community septic tanks. Box plots report median, 75%, 25% quantities and maximum and minimum values and O_{xx} represent outliers. The numbers of detections in samples are reported above the compound name. Two box plots are used to show all concentration ranges of samples (a) higher concentrations and (b) lower concentrations. **All values represent minimum concentrations.**

a. b.

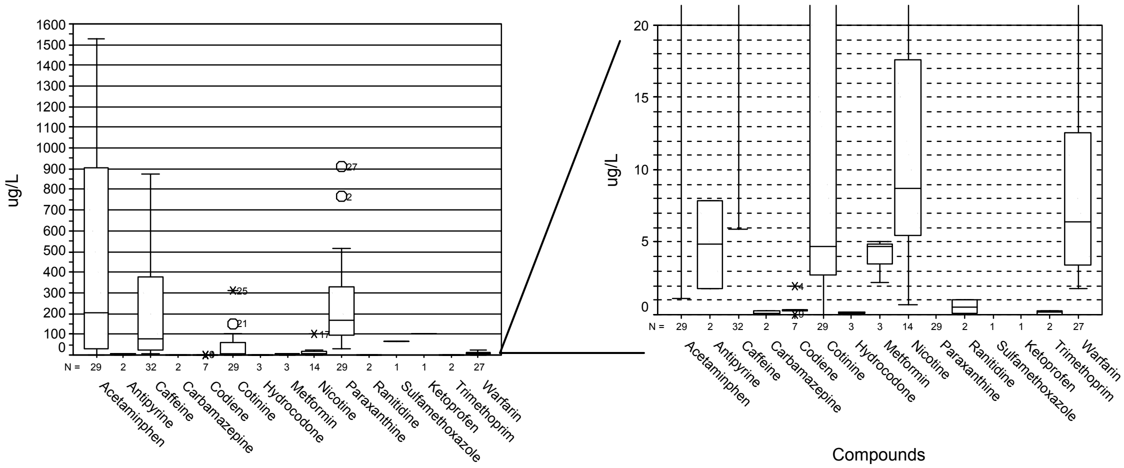


Figure 5. Pharmaceuticals detected in single-family septic tank. Box plots report median, 75%, 25% quantities and maximum and minimum values. O_{xx} and represent outliers and $*_{xx}$ represent extreme values. The numbers of detections in samples are reported above the compound name. Two box plots are used to show all concentration ranges of samples (a) higher concentrations and (b) lower concentrations. **All values represent minimum concentrations.**

Wastewater Treatment Plant

Comparisons of pharmaceutical concentrations from influent and effluent sewage of the city's WWTP are reported, including concentrations of before and after ultraviolet treatment (Figure 6). Acetaminophen, diltiazem, nicotine, paraxathine and warfarin were not detected in the outflow of the WWTP.

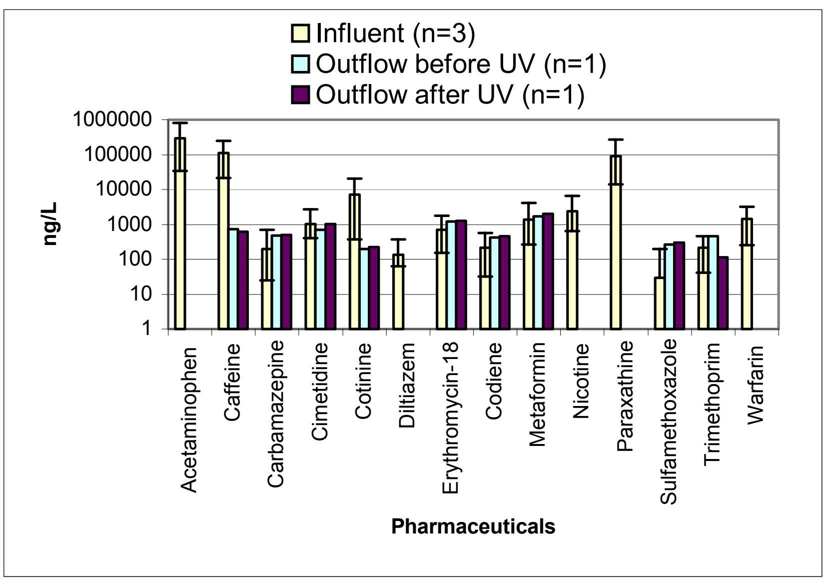


Figure 6. Concentrations of pharmaceuticals at the WWTP. Error Bars in the influent column represent a range of three separate sampling periods. Two concentrations are plotted of outflow samples before and after ultraviolet treatment. **All values represent minimum concentrations.**

DISCUSSION

Effluent samples
(Community, single family and WWTP samples)

Non-prescription drugs

Non-prescription drugs examined in this study include acetaminophen, caffeine, nicotine, ranitidine, paraxanthine (caffeine metabolite), and cotinine (nicotine metabolite). Five of these compounds were among the most frequently detected compounds in sewage (Figure 3). In community and single-family tanks acetaminophen, caffeine and paraxanthine were detected most frequently, with concentrations estimated at greater then 1530-ug/L, 877-ug/L, and 1010-ug/L, respectively (Figures 4 and 5). High concentrations detected in WWTP were lower than those found in septic effluent (acetaminophen at 525-ug/L, caffeine at 137-ug/L, and paraxanthine at 183-ug/L).

Concentrations of target compounds in septic systems appear to be more variable (have a larger range) than samples from the WWTP. Variations in concentrations are likely the result of the septic tank effluent's susceptibility to fluctuation and/or perturbations based on homeowner pharmaceutical use. It is likely that WWTP's have more stable concentrations and fluctuations are subtle as the waste integrates pharmaceutical use by a large diverse population.

The greater frequency of detection and higher concentrations of non-prescription drugs compared to prescription drugs in both septic waste and WWTP influent is related to their suspected greater annual use (Kolpin et al. 2002). Kolpin et al. (2002) observed similar findings when testing streams and rivers across the US. They report that non-prescription drugs were detected more frequently than other organic contaminants such as antibiotics, prescription drugs and reproductive hormones. They also frequently detected concentrations of drug metabolites and noted the importance of expanding analysis to include the possible degradates of parent compounds (Kolpin et al. 2002). For example, there are more than 20 metabolites of caffeine produced in the human liver (Buerge et al. 2003).

Prescription Drugs

Prescription drugs in effluent were detected less than 30% of the time, with the exception of warfarin, which was detected in approximately 77% of the samples (Figure 3). The highest concentrations of prescription drugs found in both single-family and community tank effluent were estimated to be greater than 6.4-ng/L for carbamazepine, 64-ug/L for sulfamethoxazole, 1.5-ug/L for trimethoprim, and 23-ug/L for warfarin (Figures 4 and 5). The apparent lower concentrations and frequency of detection for prescription drugs could be the result of their limited use and accessibility. Heberer (2002a) states that a reliable predictor of environmental concentrations of pharmaceuticals is the overall consumption and the fate of individual compounds in the human body. This study supports the fact that overall consumption of drugs plays a major role in the concentrations of pharmaceuticals found in the environment.

Wastewater treatment plant

The effluent samples at the WWTP were taken synoptically. However, pharmaceutical concentrations entering the plant were generally higher than levels leaving the plant (Figure 6). Ultraviolet treatment did not seem to significantly alter the apparent pharmaceutical concentrations (Figure 6). Acetaminophen, diltiazem, nicotine, paraxanthine and warfarin were below detection limits in WWTP outflow samples. This could be the result of degradation processes by microorganisms, elimination by the wastewater treatment process or the stated analytical recovery issues. Ternes (1998) noted the lack of acetaminophen in surface water due to high removal efficiencies by WWTP's. Buerge et al. (2003) and Heberer et al. (2002b) reported ~99.3% and 99.9% removal rates of WWTP for caffeine, respectively.

Analytical Difficulties

There are thousands of tons of pharmaceuticals produced and used in human and veterinary medicinal practices (Daughton and Ternes 1999). This can lead to potentially thousands of different molecules belonging to different chemical classes, structures and behaviors that could re-enter the environment. It would be unrealistic and costly to produce analytical methods for measuring all pharmaceuticals in the environment. To date no single analytical procedure has been set as an accepted method to measure quantities of pharmaceuticals in the environment (Castiglioni et al. 2004).

Due to the analytical difficulties mentioned earlier, this study reports a range of concentrations for septic tank effluent samples (Figures 4 and 5) with the exception of demonstrating analytical changelings with the HLB cartridges (Table 2). Reasons for error include: 1) over saturation of the 500-mg, 6-cc HLB sorbant by sewage effluent samples; 2) loss of target compounds during filtration 3) loss of target compounds to the glass vial; and 4) concentrations of target compound over saturating the detector, causing suppression of ions during analysis.

Recovery data for raw sewage effluent matrix are not reported in this paper, yet Ternes et al. (2001) reports a limited number of recoveries of pharmaceuticals from a raw sewage effluent matrix. Ternes et al. (2001) reported 70% recovery of caffeine in sewage treatment plant effluent with other pharmaceuticals ranging from 30-142% recovery. Clearly, additional effort is needed to standardize analytical techniques.

FURTHER RESEARCH

The presence of pharmaceuticals in our waterways and ground water is a growing concern. With increased sensitivity of analytical equipment, we are able to report concentrations in the low ng/L range (Benotti et al. 2003). This low level of detection requires a methodology to ensure clean glassware and proper sample preparation in a raw sewage matrix are in need. In addition, other compounds that may be important to evaluate in ground water and wastewater include: primidone, naproxen, gemfibrozol, and metoprolol (Scheytt 1998; Ternes 1998; Drewes et al. 2003; Heberer 2002a; Castiglioni et al. 2004). Certainly a follow up study of Missoula's ground water that more clearly quantifies the occurrence and concentration of pharmaceuticals and personal care products should be conducted. This screening level study should be used to design such an effort.

CONCLUSION

Based on the analysis of all sewage effluent samples, 18 of the 22 compounds studied were detected above the detection limit. These 18 compounds include both prescription and non-prescription drugs, with prescription drugs being most frequently detected. This is most likely the result of greater annual use by the general population.

Acknowledgements

This work was supported by a grant from the Montana Water Center and the University of Montana. We would like to recognize the assistance of Professor Bruce J. Brownawell and Ph.D. student Mark Benotti of the Marine Science Research Center, Stony Brook University, at the State University of New York. They provided us access to their environmental laboratory and equipment. We greatly appreciate their assistance without which this project could not have been completed. Thanks also to Dr. Traugott Scheytt for his frank discussions regarding our results.

REFERENCES

Benotti, M., Ferguson, P.L., Rieger, L.A. Iden, C.R. Heine, C.E and Brownawell, B.J., 2003, Liquid Chromatography Mass Spectrometry/Mass Spectrometry, MS/MS and Time-of-Flight MS: Analysis of Emerging contaminants: American Chemical Society Symposium Series 850, Oxford University Press, New York, USA, , chapter 7: pp 109-127.

Buerge, I., Poiger, T., Muller, M. and Buser, H., 2003 *Caffeine, an anthropogenic marker for wastewater contamination of surface waters*: Environmental Science and Technology 27: pp 691-700.

Buser H.R., Poiger T. and Muller M.D. 1999., *Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and in wastewater:* Environmental Science and Technology 33, pp 2529-2535.

Castiglioni, S., Fanelli, R., Calamari, D., Bagnati, R. and Zuccato, E., 2004, *Methodological approaches for studying pharmaceuticals in the environment by comparing predicted and measured concentrations in River Po, Italy:* Regulatory Toxicology and Pharmacology, IN PRESS.

Clara, M. Strenn, B. and Kreuzinger, N., 2004, *Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behavior of carbamazepine in wastewater treatment and during ground water infiltration*: Water Research 38, no. 4, pp 947-954.

Cordy, G.E., Duran, N.L, Bouwer, H., Rice, R.C., Furlong, E.T., Zuagg, S.T., Meyer, M.T., Barber, L.E. and Koplin, D.W., 2004, *Do pharmaceuticals, pathogens, and other organic wastewater compounds persist when wastewater is used for recharge?:* Ground Water Monitoring and Remediation 24, no.2, pp 58-69.

Daughton, C.G and Ternes, T.A. 1999, *Pharmaceuticals and personal care products in the environment: agents of subtle change:* Environmental Health Perspectives 107, no. 6, pp 906-938.

Drewes, J.E., Heberer, Rauch, T., and Reddersen, K., 2003, *Fate of pharmaceuticals during ground water recharge*, Ground Water Monitoring and Remediation 23, no 3, pp 64-72.

Eckel, W.P, Ross, B. and Isensee, R.K., 1998, *Pentobarbital found in ground water*: *Ground Water* 31, no.5, pp 801-804.

Ferrer, I. and Thurman, M., 2003, *Liquid chromatography/ time-of-flight/mass spectrometry (LC/TOF/MS) for the analysis of emerging contaminants*: Trends in Analytical Chemistry 22, no.10, pp 750-756.

Godfrey, E., 2004, *Screening level study of pharmaceuticals in septic tanks, surface water and ground water in Missoula, Montana*. Masters Thesis, unpublished, University of Montana: pp 10-15.

Goodman, L.S. and Gilman, A.G., The pharmacological basis of therapeutics. XVI 8th edition, New York, Pergamon Press, 1990, pp 1180.

Hartig C., Storm T., Jekel M., 1999, D*etection and identification of sulphonamide drugs in municipal wastewater by liquid chromatography coupled with electrospray ionization tandem mass spectrometry*: Journal of Chromatography A 854, pp 163-173.

Heberer, T., 2002a, *Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data:* Toxicology Letters, 131, pp 5-17.

Heberer, T., 2002b, *Tracking persistent pharmaceutical residues from municipal sewage to drinking water*: Journal of Hydrology 266, issues 3-4, pp 175-189.

Holm, J.V., Rugge, K., Bjerg, P.L., and Christensen, T.H. 1995. Occurrence and distribution of pharmaceutical organic compounds in the ground water down gradient of a landfill (Grindsted, Denmark). *Environmental Science and Technology* 29, no. 5: pp 1415-1420.

Knowles, G., 1998, SepticStats An Overview. West Virginia University Research Corporation. Available: http://www.nesc.wvu.edu/images/septicstat.pdf, obtained March 2004.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M, Zaugg, S.D, Barber, L.B., and Buxton, H.T., 2002, *Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance*: Environmental Science and Technology 36, pp 1202-1211.

McQuillan, D., Parker, J., Chapman, T.H., Sherrell, K. and Mills, D., 2000, *Drug Residues in Ambient Water: Initial Surveillance in New Mexico, USA*, 3rd International conference on pharmaceuticals and endocrine disrupting chemicals in water 2003, National Ground Water Association, OH.

Petrovic, M. Gonzalez, S. and Barcelo, D., 2003, *Analysis and removal of emerging contaminants in wastewater and drinking water: Trends in Analytical Chemistry* 22, no 10, pp 685-696.

PDR 2001, Physicians Desk Reference for non-prescription drugs and dietary supplements. Medical Economics Company, Inc., Montvale, NJ.

PDR 1999, Physicians Desk Reference. Medical Economics Company, Inc., Montvale, NJ.

Raloff, J., 1998, *Drugged Waters: Does it matter that pharmaceuticals are turning up in water supplies?:* Science News 153, pp 187-189.

Scheytt, T., Grams, S. and Fell, H., 1998, *Occurrence and behavior of drugs in ground water:* Gambling with Ground Water- Physical, Chemical and Biological Aspects of Aquifer-Stream Relations, pp 13-18.

Seiler, R.L., Zaugg, S.D., Thomas, J.M., and Howcroft, D.L., 1999, *Caffeine and pharmaceuticals as indicators of wastewater contamination in wells*: Ground Water 37, no. 3, pp 405-410.

Ternes, T., 1998, *Occurrence of drugs in German sewage treatment plants and Rivers*. Water Research 32, No 11, pp 3245-3260.

Ternes, T. Hirsh, R. and Mueller, J., 1998, *Methods for the determination of neutral drugs as well as betablockers and beta2-sympathomimetics in aqueous matrices using GC/MS and LC/MS/MS:* Fresenius Journal Analytical Chemistry, 362, pp 329-340.

Umari, A.M.J., P.Martin, R.A. Schroeder, L.F.W. Duell Jr., and Fay, R., 1995, *Potential for ground-water contamination from movement of wastewater through the unsaturated zone, Upper Mojave River Basin, California*: USGS Water-Resources Investigations Report 93-4137.

Verstraeten, I.M., Fetterman, G.S., Meyer, M.T., Bullen, T. and Sebree, S, 2004, Use of tracers and isotopes to evaluate vulnerability of water in domestic wells to septic waste, Draft.

Biographical Sketches

Emily Godfrey has a bachelor's degree in geology/chemistry from Union College, NY and recently completed her Master of Science in Geology at the University of Montana, Missoula. Her work has focused on analysis of pharmaceuticals in waste systems and ground water. Address: Department of Geology, University of Montana, Missoula, Mt 59812. (406) 728-0084

William W. Woessner received his B.A. from the College of Wooster, M.S. in geology from the University of Florida, M.S. in Water Resource Management and Ph.D in Geology from the University of Wisconsin. He is a Regents' Professor of Geology teaching courses in Hydrogeology, Advanced Hydrogeology, and Ground Water Modeling at the University of Montana. His research interests included ground water and health, surface water/ground water interactions, and linking ground water and biological systems. Address: Department of Geology University of Montana, Missoula, Mt 59812. (406) 243-5698

Recharge Assessment of the Anaconda Mine near Belt, Montana

Basic Information

Title:	Recharge Assessment of the Anaconda Mine near Belt, Montana
Project Number:	2002MT4B
Start Date:	3/1/2002
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	At-Large
Research Category:	Water Quality
Focus Category:	Groundwater, Geochemical Processes, Toxic Substances
Descriptors:	acid mine drainage
Principal Investigators:	Jon C. Reiten

Publication

1. Duaime, Ted, Sandau, Ken, Vuke, Susan, Hanson, Jay, Reddish, Shawn, and Reiten, Jon. 2004. Reevaluation of the Hydrogeological system in the vicinity of the Anaconda Coal Mine at Belt, Cascade County, Montana. MBMG Open File Report 504.
2. Reddish, Shawn. 2003. A continued study of the hydrogeologic characterization of AMD production along Belt Creek near Belt, Montana. Poster Presentation, American Water Resource Association annual Montana Conference October 2, 2003, Livingston, Montana.
3. Reddish, Shawn. 2004. Age dating of the aquifers in the Belt area. Poster Presentation, American Water Resource Association annual Montana Conference October 20, 2004.

ABSTRACT

Decades of underground coal mining have resulted in acid mine drainage (AMD) that has contaminated ground-water and surface-water resources in Belt, Montana. The AMD has lowered the pH of Belt Creek and increased trace metals concentrations in the stream. The overall goal of work in the Belt area was to define the hydrogeologic regime in the vicinity of Belt so that recharge to old mine workings, the source of acid mine drainage, can be delineated with a reasonable level of certainty. This Water Center project provided seed funding for additional work funded by the Montana Department of Environmental Quality MDEQ 319 Program and the MDEQ Remediation Division-Abandoned Mine Lands.

This project consisted of a phased approach to define and mitigate water quality problems in Belt Creek near the town of Belt, which is 23 miles southeast of Great Falls. Phase 1 is a hydrogeologic investigation to determine contaminant sources and their relative contributions, and to identify and evaluate mitigation measures. Phase 2 will be based on a later proposal to apply specific measures to reduce recharge to the Anaconda Coal Mine and monitor their success.

Work conducted by Shawn Reddish, under the supervision of Jon Reiten has documented hydrogeologic conditions surrounding the abandoned Anaconda Coal Mine (ACM) near Belt. Specific tasks included inventorying, sampling for water quality and collecting samples for age dating water from wells, springs, adits and seeps. These tasks were conducted to determine if the recharge to the mine workings is local or regional. The inventory process included collecting GPS coordinates of pertinent locations, measuring specific conductivity, pH, oxidation-reduction potential, dissolved oxygen; and determining the geologic source of water in the well. These field data were then evaluated to screen for the most useful sampling sites; all information was entered into a database accessible by the public.

Water levels at 31 wells and discharges at 3 springs were measured monthly for about 2 years to monitor the fluctuations of local aquifers. Several of these wells and springs have been sampled for tritium, helium-3/tritium and chlorofluorocarbons (CFC) to determine the age of the water. All sampled wells have tritium concentrations greater than background pre-nuclear testing levels. This suggests a modern (post nuclear testing) age for ground water in the alluvial, Kootenai, Morrison, Swift, and Madison aquifers. CFC samples also indicated that all of the recharge is relatively recent. Several samples from the Madison aquifer were supersaturated with CFC’s, but the cause of this

supersaturation is unknown. The results of helium-3/tritium dating of two water samples also confirms the relatively young age of water in aquifers near Belt.

Stream flows at 9 sites were also measured monthly in the study area. Differences in flows between measuring sites were used to evaluate gaining or losing reaches of the streams. Field parameters, including specific conductivity, pH, oxidation-reduction, and dissolved oxygen were measured at each site. The AMD discharge was monitored at 5 sites on a monthly basis for about 2-years, the measurements included flow and field parameters. In addition to monthly measurements, an H-flume set up with a pressure transducer recorded the AMD discharge from the mine adit. Based on this work and other ongoing MBMG research, the direct loading to Belt Creek from AMD was estimated to be 94,500 pounds per year of iron and 59,279 pounds per year of aluminum. Indirect loading to the Creek from other AMD sources that moves through alluvial sediments before reaching Belt Creek was estimated to be 48,880 pounds per year of iron and 25,254 pounds per year of aluminum. The main direct source of AMD is the discharge from the ACM that averages about 132 gpm or about 213 acre feet per year. The primary purpose of this work has been to identify the source of water recharging the mine workings and recommend methods to reduce the recharge which would decrease or possibly eliminate the AMD loading to Belt Creek.

Several possible sources of recharge were suggested when this project started and others developed as new information became available. The possible sources include 1) recharge from regional aquifers such as the Madison aquifer 2) upward seepage from deep aquifers along fault planes 3) localized recharge from precipitation directly overlying the mines, or up-gradient recharge areas 4) water loss from Box Elder Creek, and 5) focused recharge through shallow depressions overlying the mines. Water-level data from wells completed below the mine workings and in areas surrounding the mine, in the Madison aquifer, indicate the static water level in the Madison aquifer is about 400 feet below the mine voids. Therefore, the Madison aquifer is not hydrologically connected to the workings nor is it a likely source of recharge to the mines. Other regional aquifers do not appear to be likely sources although these have not been completely ruled out. Upward seepage along fault planes does not appear to be a likely source of recharge based on the downward hydraulic gradients. Box Elder Creek is at a higher elevation than the mine workings and therefore has a potential for losses to the mine. Flow data along Box Elder are currently inconclusive to document stream losses. The most likely source of recharge to the mines is infiltration of precipitation on the land surface overlying the mine workings, including up-gradient areas that recharge the localized Kootenai aquifer system.

Based on initial interpretations, a significant source of water to the Anaconda Coal Mine appears to be from the overlying Kootenai Formation which is about 260-feet thick in the Belt area. Figure 1 is a surficial geologic map of the area above and adjacent to the Anaconda mine.

A potentiometric-surface map of the Kootenai Formation was constructed based on well inventory and monitoring measurements. This map was contoured using measurements from 44 wells and springs near the mine. The Kootenai potentiometric surface map combines head data from aquifers in both the Sunburst and Cutbank Members of the Kootenai Formation. As a result, this map shows only general water-level conditions in the mapped area. Additional wells at critical locations will be needed to accurately depict ground-water flow. Figure 2 is a 3-dimensional view of the predominant recharge area to the mine. Ground water is interpreted to flow from a divide located about 3.5 miles south of the ACM. The ground-water divide south of the mine appears to be both topographically and structurally controlled. The topographically high area forming the ground-water divide is located just north of a paired anticline-syncline structure that trends north 45 degrees east. Only precipitation falling north of this divide has the potential to move towards the mine. Once recharge infiltrates vertically to the saturated zone, ground-water flow is generally to the north perpendicular to the potentiometric contours depicted in Figure 2. The upland area between Belt Creek and Box Elder Creek is highly dissected by tributaries of the two streams. These tributaries plus the main stems of the two streams are discharge areas for ground water moving out of the Kootenai Formation. The potential recharge area covers about 2,100 acres overlying and up gradient of the mine. The highly dissected nature of the upland appears to cause much of the precipitation falling on the upland to recharge a shallow ground-water flow system and discharge to the surface water drainages as seeps and springs in the valley walls. Several of the springs coincide with the contact of the Sunburst Sandstone Member (aquifer) and the underlying unnamed fine-grained unit (aquitard).

A final report is currently being prepared. Based on the data collected it appears that recharge to the ACM is likely locally derived. As a result, the volume of AMD discharging from the mine may be able to be reduced or possibly eliminated by changing land use in the recharge area. Growing alfalfa or other water consumptive crops would have the potential to reduce infiltration and possibly decrease the AMD.

Publications/Citations-

Duaime, Ted, Sandau, Ken, Vuke, Susan, Hanson, Jay, Reddish, Shawn, and Reiten, Jon, 2004, Reevaluation of the Hydrogeological system in the vicinity of the Anaconda Coal Mine at Belt, Cascade County, Montana, MBMG Open File Report 504.

Reddish, Shawn, 2003, A continued study of the hydrogeologic characterization of AMD production along Belt Creek near Belt, Montana, Poster Presentation, American Water Resource Association annual Montana Conference October 2, 2003, Livingston, Montana.

Reddish, Shawn, 2004, Age dating of the aquifers in the Belt area, Poster Presentation, American Water Resource Association annual Montana Conference October 20, 2004.

Student Support – Shawn Stevens, MSU-B Environmental Studies student.

Additional Funding Sources- The Montana Department of Environmental Quality provided most of the funding for work in the Belt area through the MDEQ 319 Program and several Task orders through the MDEQ Remediations Division-Abandoned Mine Lands Bureau.

Figure 1: Geologic Map of the Anaconda Mine Area, Belt Montana

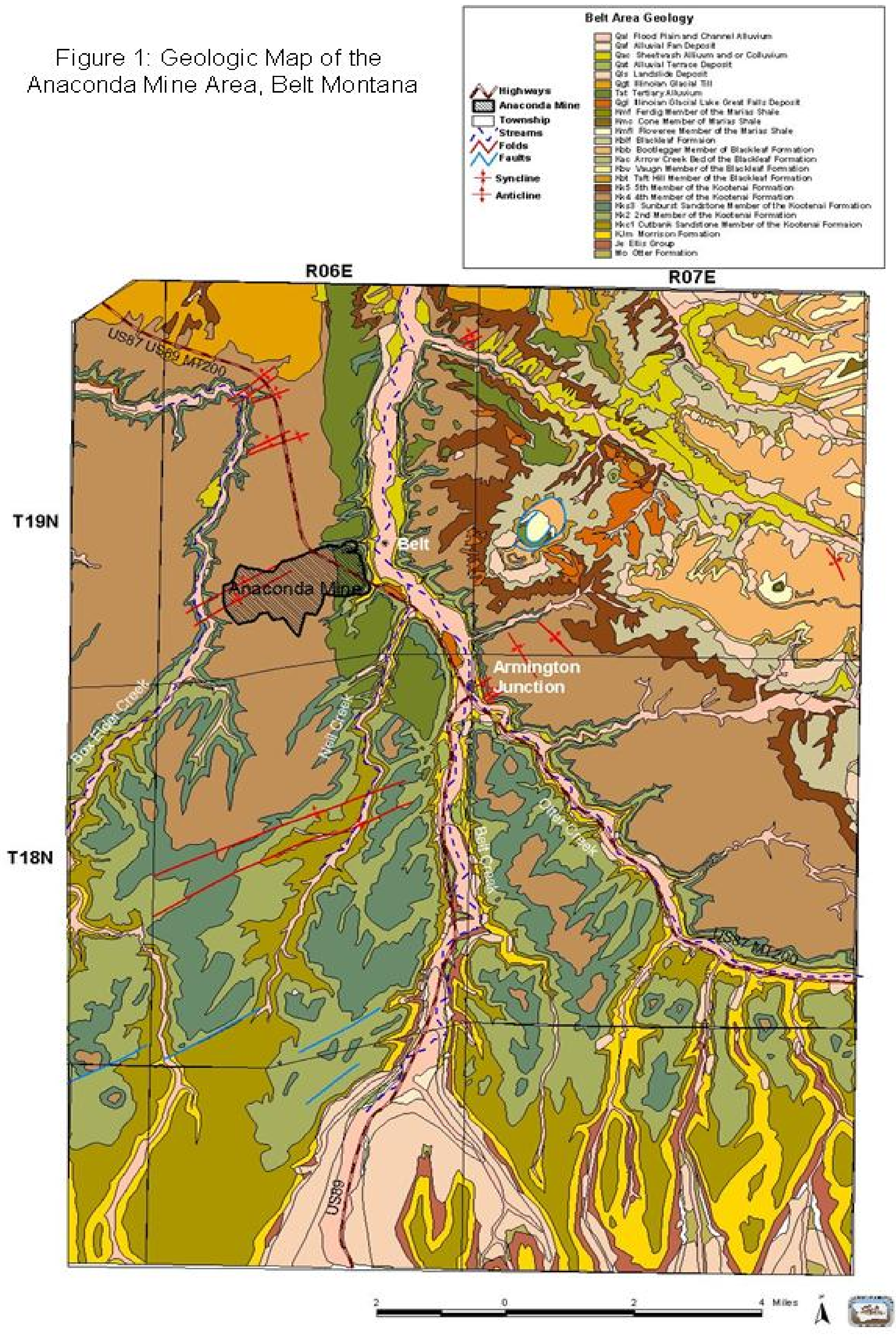
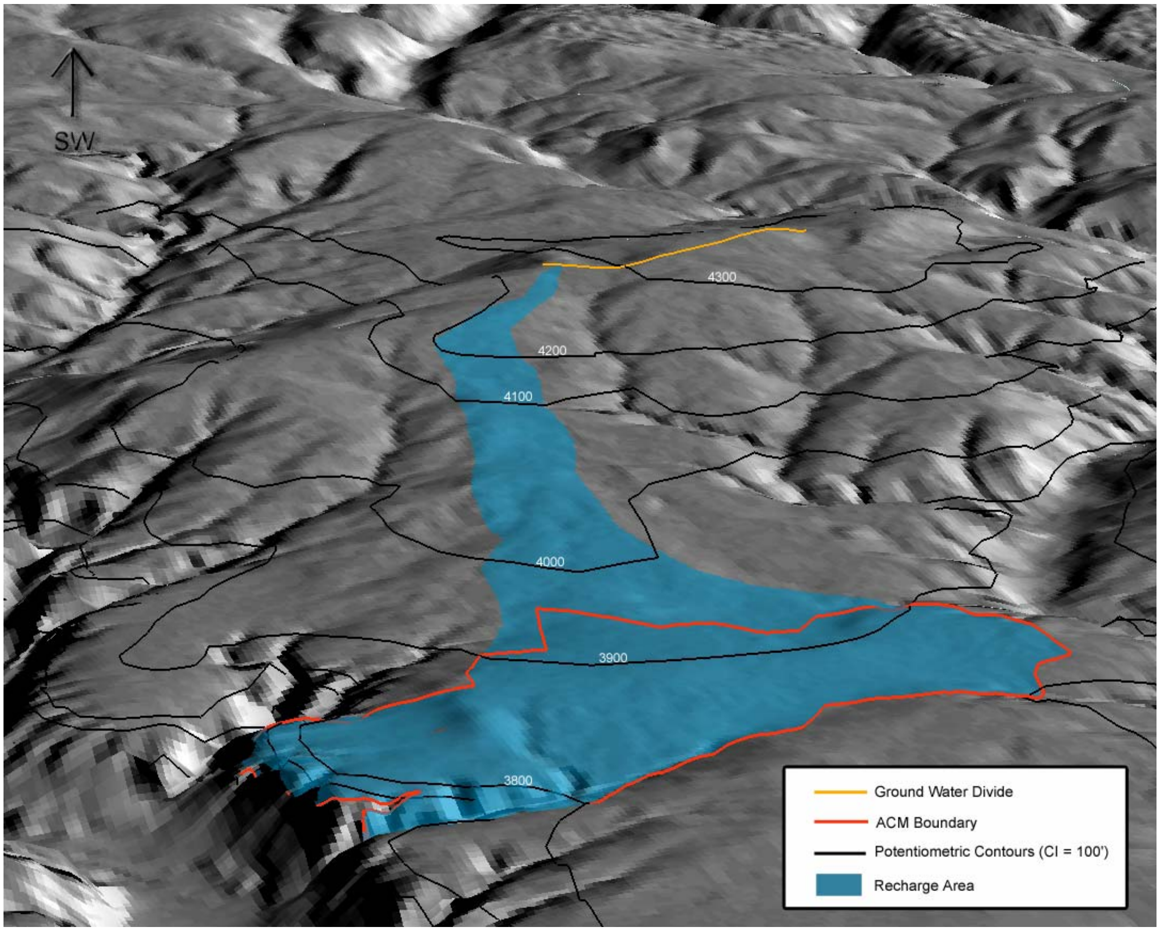


Figure 2. Recharge area for ACM near Belt shown on a 3-D image of the land surface. View is upslope towards the Southwest, with the ACM in the foreground.



Information Transfer Program

During FY 2004 (March 1, 2004 through February 28, 2005), the Montana Water Center developed or sponsored many programs and tools to carry out its mission to mobilize the resources of Montanas public university researchers to resolve the states water problems. Because of support from the USGS, the Center was actively involved in these water information transfer activities:

1. Planned and organized the annual meeting of the 54 directors of the National Institutes for Water Resources in Washington D.C. The meeting was conducted on March 5-8, 2005. Organizational activities included development of the meeting web site, development of program and registration materials, and communications will all directors. More information about the meeting is found at http://water.montana.edu/niwr.

2. Organized an International Drinking Water Colloquium of water experts in Bozeman, Montana, May 9-12, 2004. Though funded primarily through a grant from the Environmental Protection Agency, our USGS funding helped with outreach. The colloquium was designed as a forum to share observations on what works and what doesnt work in small systems in different developed nations using practices developed in individual nations as case studies. Forty-six invited people from 15 nations participated in the colloquium with a broad representation from the drinking-water microbiology, system operation, management, and regulation disciplines. Participants came from academia, regulatory agencies, public water utilities, and technical assistance organizations. Following plenary sessions, the participants split into three facilitated groups, where they deliberated for two days. At the end of the colloquium, all participants re-convened and shared their observations. The colloquium proceedings is located at http://water.montana.edu/colloquium.

3. Administered the 104b research grants, and promoted interest in the 104g research grant program, with assistance from the Centers Water Research Advisory Committee.

4. Developed and circulated an RFP for a new competitive student fellowship program, and ultimately offered a $5,000 research award to a University of Montana graduate student. This effort served as a model for our current and expanded student fellowship program.

5. Encouraged and enabled student involvement through internships, research opportunities, trainings, and other efforts that provide practical experience for future water professionals.

6. Distributed monthly Montana Water e-newsletters a database of over 1,000 people.

7. Continued to maintain and expand MONTANA WATER, the Montana Water Centers web information network at http://water.montana.edu. This website includes an events page, news updates, an online library, water-resource forums, a Montana watersheds projects database, an expertise directory, water facts and more.

8. Produced the Montana Water Centers 2003-2004 Annual Report, covering all of the programs accomplished through the Centers $2.3M budget;

9. Coordinated two live teleconferences sponsored by the American Water Works Association. About 40 water-system professionals attended at downlink sites in Missoula, Havre, Great Falls, Billings, Helena, Butte and Bozeman. The March 2004 teleconference focused on Emerging Issues in Water Utility

Operations, and the November 2004 teleconference was titled Water Resource Alternatives: The Future of Sustainable Utility Practices.

10. Conducted the state-wide water research meeting in Helena, Montana on October 4 and 5, 2004 for exchange of research information among water professionals. Montanas Water Outlook: Current and Future Challenges offered more than 30 papers and 20 poster presentations. The web-based archive of this meeting is found at http://www.awra.org/state/montana; full proceedings can be downloaded at http://awra.org/state/montana/pdfs/Proceedings2004.pdf.

11. Served as a liaison among the university community and water professionals and decisionmakers in local, state, and tribal and federal governments, including attendance at all Montana Legislative Environmental Quality Council meetings.

12. Participated in the 71st Annual Water School training for water and wastewater managers and operators has been offered for seven decades. Each year, operators from throughout Montana can receive four days of water and wastewater training for managing their local systems. The program features workshops and presenters from private consulting, industry, academia and government. At the close of the training, operators may sit for the water/wastewater certification exam administered by the Montana Department of Environmental Quality (DEQ). Along with DEQ, this program is conducted by the Montana Environmental Training Center, the Montana Water Center, and the MSU Department of Civil Engineering.

13. Created the first annual black-and-white water facts and photos calendar for general circulation entitled Montana Water 2005. Each month was dedicated to a different Montana water topic.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	5	0	0	0	5
Masters	10	0	0	1	11
Ph.D.	2	0	0	0	2
Post-Doc.	0	0	0	0	0
Total	17	0	0	1	18

Notable Awards and Achievements

This year the Center initiated a Student Water Research Fellowship Program. Offered for the first time by the Water Center, the award is made to an undergraduate or graduate student at a Montana institution conducting research on a critical water resource issue in the region. Megan McBride, University of Montana graduate student, was chosen from a pool of 14 competitive applications to receive $5,000 to conduct her research entitled "Recreation on the Upper Yellowstone River: Use and Place Attachment." This program was so successful, that the Montana Water Center will now annually offer this opportunity (twelve Fellowships, averaging $3,000 each, were awarded in FY 2005).

Tim Covino, a student supported through 104b research funding (with Montana State University principal investigator, Dr. Brian McGlynn) received two student presentation awards at the Annual Montana meeting of the American Water Resources Association meeting Helena, September 2004.

Already addressed in the information transfer section, we are pleased with outcomes of two meetings organized by the Montana Water Center, First, on May 9-12, 2004 in Bozeman, Montana, the Montana Water Center organized and facilitated an international colloquium entitled Protecting Public Health in Small Water Systems. The discussions drew on the expertise of 45 invited experts from the United States, Europe, Canada, and Oceania. Proceedings can be downloaded at http://water.montana.edu/colloquium. Later, on February 5-8, 2005, in Washington D.C., the Montana Water Center hosted the Annual Meeting of the National Institutes for Water Resources Directors from the fifty states and four U.S. territories. The Directors deliberated on a strategic plan for the organization and on future collaborative efforts.

Publications from Prior Projects

1. 2001MT241B ("Determination of the maximum weight radio transmitter that can be implanted in westslope cutthroat trout without affecting swimming performance: a challenge to the 2% rule") - Articles in Refereed Scientific Journals - Zale, Alexander, Carrie Brooke and William Fraser. 2005. Effects of surgically implanted transmitter weights on growth and swimming stamina of small adult westslope cutthroat trout. Tranactions og the American Fisheries Scoiety 134:653-660.

2. 1999MT101B ("Publications from 104 b and 104g research conducted between 1997 and 2002 in Montana.") - Articles in Refereed Scientific Journals - As listed in Montana’s Five Year Report submitted in 2004, a new listing of publications will appear here shortly.