Montana Water Resources Research Center
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Introduction

Research Program

Basic Information

Title:	Evaluation of the Fluorescent Amplified-Fragment Length Polymoprhism Method for Identifying Sources of Fecal Coliform in Grazed Watersheds
Project Number:	2000MT2B
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End Date:	4/30/2001
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Focus Category:	Non Point Pollution, Agriculture, Water Quality
Descriptors:	fecal coliform, livestock grazing, water quality, watershed monitoring
Principal Investigators:	Clayton Marlow , Donald Burgess

Publication

1. Higgins, M., 2001, Evaluation of the Fluorescent Amplified-Fragment Length Polymoprhism Method for Identifying Sources of Fecal Coliform in Grazed Watersheds, "in" Proceedings, General Meeting of the American Society for Microbiology, Orlando FL.
2. Finck, R. J., C.B. Marlow and J. Borkowski. 2000. Stubble height as a criteria for water quality. Riparian Ecology and Managment in Multi-land Use Watersheds. American Water Resources Association Specialty Conf. Aug. 29-31, 2000. Portland, OR, American Water Resources Association, Herndon, VA.
3. Marlow, C.B., R. Finck and H. Sherwood. 2000. Best Management Practices for Protection of Water Quality on Grazing Lands. IN: Watershed Management 2000 Conference. 9-12 July Vancouver, BC. Water Environment Federation, Alexandria, VA.
4. Higgins, M.R., C.B. Marlow and D.E. Burgess. 2001. Determination of the origin of Escherichia coli by FAFLP Analysis. Abstr. Amer. Soc. of Microbiol. Orlando, FL, May 2001.

Evaluation of the Fluorescent Amplified-Fragment Length Polymorphism Method for Identifying Sources of Fecal coliforms in Grazed Watersheds.

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Review of the detailed water quality database generated by Montana's efforts to meet provisions of the Clean Water Act indicates widespread presence of bacteria, specifically fecal coliforms, in the state's waters. While most of the samples fall well below state and Federal standards, some streams and rivers have fecal coliform levels that are cause for concern. From the point of view of Federal and state regulatory agencies and the general public sewage outfalls and livestock production facilities are the primary source of elevated fecal coliforms. However, monitoring results from a series of grazing trials conducted during the mid 1990s indicated that fecal coliform levels were often elevated in waters where cattle were absent. In one of the grazing trials little change in fecal coliform levels were detected after implementation of several grazing best management practices (Marlow et al. 2000). Supplementary water sampling indicated that elevated fecal coliform levels were related to periods of concentrated wildlife use and the presence of recreational homes. These results suggest that effective, long term reduction of unacceptable fecal coliform levels will require identification of the primary bacteria source. Identification of bacterial sources is complicated because microbiological techniques developed for identifying specific organisms within the fecal coliform complex, are not sensitive enough to determine whether the bacteria came from beaver, elk, cattle or human hosts.

With recent advancements in rapid DNA/RNA sequencing techniques there appeared to be an opportunity to incorporate one of these procedures into the water sampling protocol so that fecal coliforms host(s) could be identified. In April 2000 a cooperative effort to evaluate one promising RNA sequencing technique was undertaken by faculty from the Animal and Range Sciences and Veterinary and Molecular Biology Departments at Montana State University. The primary objective was to learn if a procedure referred to as *Fluorescent Amplified-Fragment Length Polymorphism* or FAFLP could differentiate between several species and populations of mammals. The FAFLP method was selected for evaluation based on previously published results (Arnold et al., 1999) suggesting the ability of this approach to clearly discriminate among strains of bacteria including *E. coli*, a major component in the fecal coliform group.

Methodology:

Because of the wide variety of organisms that are generally grouped under the category fecal coliform it was necessary to focus on a single genera or species to achieve a thorough evaluation of the methodology. *Escherichia coli* was chosen as the target

species because of the high level of concern over the potential for illness caused by a strain identified as *E. coli* 0157:H7.

Isolates of *E. coli* from multiple Montana stream sites, directly from cattle and cats, and laboratory strains were grown in a nutrient broth. DNA was extracted from each set of isolates and digested with MseI and EcoR1 restriction enzymes. The fragments were then litigated to selective oligonucleotides. This process had to be repeated several times until the appropriate volumes of MseI and EcoR1 for producing the highest number of oligonucleotides could be ascertained. Additional samples of *E. coli* were collected from the original sites/individuals and the process repeated with the new results. PCR primers matched to the selective oligonucleotides were used to obtain fluorescent PCR products that were then analyzed on a sequencing apparatus, ABI 377. Gel images obtained from the sequencer were used to produce genotype profiles of each isolate. Comparisons between the various isolate gel images were made with computer programs, Genescan, ABI and Genographer.

Principal Findings and Significance:

FAFLP patterns were identified from 27 gel bands for over 20 *E. coli* isolates (Fig. 1) and it was possible to discriminate between the various sources or isolates. There were gel bands uniquely associated with *E. coli* isolated from feline, bovine, laboratory and "wild" samples. This strongly suggests that FAFLP could be used to identify the source of fecal bacteria found in surface water samples. However, several limitations to the approach became apparent during the investigation.

First, it takes more time to analyze waterborne bacteria for their source than expected. Fecal coliforms have to be extracted from water samples through the filtration process. The filtrate then has to be grown on *E. coli* specific agars to produce enough material for DNA analysis. Then several runs of the enzyme digestion must be conducted to determine the most effective ratios for ultimate detection of bacteria source. Thus, FAFLP may not provide "quick turn" results for stream biologists, sanitation engineers or land managers.

Second, to take advantage of the results from FAFLP it is necessary to identify the bacterial DNA sequence for the mammal and avian species thought to occupy the watershed the affected stream drains. Studies conducted in the Mid-Atlantic Region of the US found that more than 200 DNA patterns had to be developed to successfully identify the source of waterborne pathogens in two separate watersheds (Rossen 2000). This suggests the need for a reference library of DNA sequences for various avian and mammalian species to enable broad use of methodologies like FAFLP. While researchers at the University of Minnesota (Dombek et al. 2000) have developed a "fingerprint" database for cows, pigs, horses, cats, dogs, chickens, turkeys, sheep, wild geese and ducks and deer, the "fingerprints" are only for *E. coli*. Similar efforts would have to be made for other bacterial genera. At the very least this suggests very high start up costs for state or private laboratories offering to "fingerprint" bacterial sources.

Lastly, in reviews of this project by faculty from the Veterinary and Molecular Biology Department there was some question as to why different host species should prompt genetic differences among populations of the same species of bacteria. In other words, why should *E. coli* from the gut of an Angus cow be genetically discernible from the *E. coli* inhabiting the intestines of an elk? While the answer to this specific question is not clear, it is clear from our results that strains of *E. coli* from different sources do have detectable differences at the DNA level and these results are in agreement with those of other investigators (Zhao et al., 2000).

Even with these cautionary notes this project produced results comparable to studies in Minnesota, Pennsylvania and Virginia , indicating that genetic fingerprinting can be used to detect the source of a fecal coliform contamination in surface waters. Development of a national DNA sequence database appears to be the next step in the development of a new tool for water quality monitoring.

Publications/Citations:

Finck, R.J., C.B. Marlow and J. Borkowski. 2000. Stubble height as a criteria for water quality. Riparian Ecology and Management in Mult-land Use Watersheds. American Water Resources Association Specialty Conf. Aug. 29-31, 2000. Portland, OR, American Water Resources Association, Herndon, VA.

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Higgins, M.R., C.B. Marlow and D.E. Burgess. 2001. Determination of the origin of *Escherichia coli* by FAFLP Analysis. Abstr. Amer. Soc. of Microbiol. Orlando, FL May 2001.

Student Support:

Melanie Higgins, Biotechnology Intern, Biotechnology-Animal Option
Robert Finck, Masters in Range Science; *Stubble Height Criteria for Water Quality Protection on Grazing Lands*. Masters Thesis, Animal and Range Sciences Department, Montana State University, Bozeman, MT

Notable Achievements and Awards:

Melanie Higgins – Recipient of ASM Student Travel Grant for 2001 General Meeting, Orlando FL. [Division C, Clinical Microbiology]

The results from this project were used in an application to the USDA's Initiative for Future Agriculture and Food Sustainability. Notification of award status will be made in August 2001.

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Arnold, C., L. Metherell, G. Willshaw, A. Maggs and J. Stanley. 1999. Predictive fluorescent
    amplified-fragment length polymorphism analysis of *Escherichia coli*: High-resolution
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    AFLP analysis. Microbes and Infection 2:107-113.

Basic Information

Title:	Ground-Water Age Dating as a Tool to Assess Land-Use Effects on Recharge and Water Quality in Intermontane Basins
Project Number:	2000MT6B
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Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Hydrology, Water Quality
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Principal Investigators:	John LaFave

Publication

1. LaFave, John I., 2000, Mantle flatulence and septic effluent: sampling the Missoula aquifer for environmental tracers. Presented at 2000 annual meeting of the American Water Resources Association (Montana Section), West Yellowstone, Montana, October 2000.
2. Pracht, K., LaFave, J., and Woessner, W., 2000, An attempt to age-date groundwater in the highly conductive Missoula aquifer, western Montana: accepted for presentation at the Geological Society of America Annual Meeting Reno, Nevada, November 2000.
3. Pracht Karl, 2001, Flow And Aquifer Parameter Evaluation Using Ground-Water Age-Dating, Geochemical Tools And Numerical Modeling; Missoula Aquifer, Western Montana : unpubl. MS thesis, Dept. of Geology, University of Montana, Missoula, MT.
4. LaFave, John I., 2002, Tracing Ground-Water Flow in the Missoula Valley Aquifer, Southwest Montana. Montana Bureau of Mines and Geology Ground-Water Assessment Open-File Report 17.
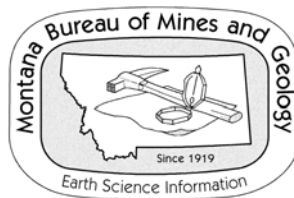
Tracing Ground-Water Flow in the Missoula Valley Aquifer, Southwest Montana

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Tracing Ground-Water Flow in the Missoula Valley Aquifer, Southwest Montana

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Abstract

Major-ion, chlorofluorocarbon (CFC), oxygen-18, deuterium, tritium and noble-gas data were used to evaluate water quality, and as environmental tracers to assess apparent ground-water ages and flow in the Missoula Valley aquifer between the Clark Fork and Bitterroot Rivers. Ground water was sampled at 10 sites, two with nested shallow-deep well pairs, along two transects of ground-water flow; water was also sampled from the Clark Fork River. Calcium and bicarbonate were the dominant ions in all the ground-water samples; total dissolved solids were less than 300 mg/L. Although the ground water is of excellent quality, constituents associated with human activities (sodium, chloride, and nitrate) generally increased along flow path. Seasonal variations of oxygen-18 were detected in surface and ground-water samples. Most of the sampled ground water had CFC concentrations in excess of air-water solubility, rendering the samples unsuitable for age dating; concentrations are markedly greater in unsewered than in sewered areas suggesting that septic effluent is a possible source of the excess CFC's. Tritium was detected in all samples, with concentrations ranging from 8.7 to 13.1 tritium units; tritium/helium-3 age dating shows that ground water in the Missoula Valley aquifer is young, with most of the samples (7 of 12) being less than 2 years old, the oldest age was 4.6 years. In general, the water age increased downgradient along flow path. The noble gas helium-4 is present in surprisingly large concentrations given the young age of the water, and distributed in a pattern opposite of expected flow path trends. Bulk hydraulic conductivity values determined from the age dating are in agreement with values obtained from conventional aquifer tests.

Introduction

Intermontane basins of the Northern Rocky Mountains contain alluvial aquifers that store and yield large quantities of water. In many basins alluvial aquifers represent the most productive aquifers and are important sources of municipal and domestic water (Kendy and Tresch, 1996). The basins also contain perennial streams and associated riparian habitats that are sustained by ground-water discharges. Population growth in the basins is occurring at an unprecedented rate resulting in increased demand for water (municipal/domestic) and a shift in land use from agricultural to residential/urban. The increased demand for water and the land-use shift have created a serious need for information and techniques to evaluate vulnerable hydrologic systems to assure water supplies, and to avid degradation of the ground-water resource.

This report presents the results of a study funded in part by the Montana Water Center and done in conjunction with the Montana Ground-Water Assessment Program at the Montana Bureau of Mines and Geology. The goals were to evaluate the use of environmental tracers, specifically tritium-helium isotopes, chlorofluorocarbons, and oxygen-18 and deuterium to trace ground water flow in the Missoula Valley aquifer. Some study results formed the basis of a University of Montana M.S. thesis to use tracers to refine hydraulic parameters used in ground-water management models for the Missoula Valley aquifer (Pracht, 2001). Previous studies have characterized the physical hydrogeology, modeled ground-water flow, and evaluated the water quality (McMurtrey and others, 1965; Geldon, 1979; Clark, 1986; Woessner, 1988; Miller, 1991).

Surficial glacial outwash and alluvium forms the Missoula Valley aquifer which is the main source of water for the city of Missoula. The primary objective of the study was to develop a better understanding the dynamics of ground-water flow through a part of the aquifer that is heavily

utilized, and in a part of the valley that is most susceptible to surface sources of contamination. The report presents a general description of the study area, the geology, the hydrogeology, and the results of the environmental tracer and water-quality analyses.

Acknowledgments

Description of the Study Area

The city of Missoula, home to about 57,000 people, has the second largest population in the state and grew by almost 33 percent between 1990 and 2000 (Montana Department of Commerce, 2001). Missoula is situated in the Missoula Valley, a wedge shaped intermontane basin, that is bounded on the northeast by the Rattlesnake Hills, on the southeast by the Sapphire Mountains and on the southwest by the Bitterroot Mountains (figure 1). The valley is drained by the west-flowing Clark Fork River and the north-flowing Bitterroot River. The part of the valley evaluated for this study lies between the Clark Fork and Bitterroot Rivers; the land use is mostly urban and/or residential. At the time of study, roughly half of the area was serviced by municipal sewer, with residences in the other half relying on septic tank systems (figure 2).

The mountains that surround and underlie the Missoula Valley are composed primarily of metasedimentary rocks of the Belt Supergroup. The basin is filled with up to 2,500 feet of Cenozoic fill, most of it Tertiary in age (McMurtrey and others, 1965). In the study area the Tertiary sediments are mantled by Quaternary alluvium and locally by glacial lake silts.

The climate of the Missoula Valley is characterized by warm summers and cool winters with the wettest months in the winter and spring. At the Missoula airport (altitude 3,200 ft) the average annual temperature is 44.3º F and average annual precipitation is 13.55 in. (Western Climatic Data Center data available online at: http://wrcc.sage.dri.edu/). Average monthly temperatures and monthly rainfall totals for the period of this study are presented on figure 3.

Missoula Valley Aquifer

The city of Missoula is underlain by unconsolidated Pleistocene deposits of the Missoula Valley aquifer, a designated sole-source aquifer by the U.S. Environmental Protection Agency (USEPA). Materials in the aquifer were deposited by glacial melt waters and range in size from fine sand and silt to gravel and cobbles. The aquifer is 100 to 150 feet thick and is bounded below by relatively impermeable, fine-grained Tertiary sediments (figure 4). Three lithologic units have been identified throughout most of the aquifer (Woessner, 1988): the top unit (unit one) is 10 to 30 feet thick, composed of very permeable coarse sand to boulders; the middle unit (unit two) is as much as 40 feet thick and composed of silt and fine sand and is a low permeability horizon within the aquifer; the basal unit (unit three) is composed of 50 to 100 feet of highly permeable, coarse-grained

sand and gravel (figure 5). Unit three is the most prolific zone in the aquifer, wells reportedly yield as much as 4,100 gallons per minute (gpm). Few wells penetrate the base of unit three, so the basal configuration of the aquifer is poorly known.

Ground-water in the Missoula Valley aquifer is unconfined, the water table ranges from 10 to 60 feet below the surface. Ground-water flow paths through the aquifer are important because they also describe paths that would likely be taken by contaminants. The potentiometric surface in June 1999 mimics the slope of the land surface; ground water flows from the Clark Fork River southwest toward the Bitterroot River and its confluence with the Clark Fork River; the gradient across the study area was 0.002 (figure 6a). In March 2000, water levels were 5 to 12 feet lower than they were in June 1999 (figure 6b); wells closer to the Clark Fork River show slightly larger declines. The general configuration of the potentiometric was similar to that of June 1999 with the same direction of ground-water flow, although the hydraulic gradient across the study area was slightly smaller.

Leakage from the Clark Fork River is estimated to provide 80 to more than 90 percent of the recharge to the aquifer (Woessner, 1988; Miller, 1991); other sources include underflow through Hellgate Canyon, and precipitation. Water leaves the aquifer as discharge to the Bitterroot River, evapotranspiration, and as pumpage from wells. Water levels fluctuate seasonally and are closely tied to discharge in the Clark Fork River (figure 7). Annual water-level fluctuations in wells are on the order of 5 to 10 feet; however, the fluctuations are more pronounced in wells near the Clark Fork River and become muted downgradient along the flow path.

Sample Collection

Sites for environmental tracer sampling were selected on the basis of location, depth, relative position along flow path, and accessibility. Thirteen monitor wells owned and maintained by the Missoula Valley Water Quality District were sampled for environmental tracers. The wells are completed along two transects of ground-water flow between the Clark Fork and Bitterroot Rivers and include two nested shallow-deep pairs (figure 8 and table 1).   The first round of samples were collected in June 1999 for common ions and trace elements, oxygen-18 (^{18}O), deuterium (D), chlorofluorocarbons (CFC's), tritium, and helium (and other noble gases). Samples for common ions and trace elements were collected from seven of the wells, after field measurements of specific conductance, pH and temperature had stabilized and at least three well-casing volumes were removed. Water samples for CFC's and noble gases were initially collected by lowering 0.25-in diameter copper tubes in the well, which were allowed to fill with water and then retrieved; a check valve on the bottom end of the tube prevented water from draining. Upon recovery to the surface the ends of the copper tube were sealed by metal pinch clamps. Water samples for tritium were collected in 1,000 ml glass bottles, samples for ^{18}O and D were collected in 250 ml plastic bottles. Monthly samples for ^{18}O were collected from the Clark Fork River and a nearby well (well 69055[1]) between June 1999 and December 2000; all the other wells (except well 151200) were sampled in June 1999 for ^{18}O and D, and again in March 2000 for ^{18}O by the Missoula Valley Water Quality District.

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[1]Unique Ground-Water Information Center (GWIC) well identification number. The GWIC database is on line at http://mbmggwic.mtech.edu/.

Subsequent samples for noble gases (obtained in December 1999 and from selected wells in August 2001) were collected using in-well diffusion samplers consisting of 1-in long lengths of copper tubing (0.25-in diameter). Each tube had one end sealed and the other end covered by a semi-permeable membrane; the membrane was permeable to gases but not to water. The diffusion samplers were lowered into the wells opposite the well screens and allowed to equilibrate with the dissolved gases in the ground water–generally for a period of about one week. Upon retrieval to the surface the open end of the copper tube was immediately sealed with a specially designed clamp to create a cold weld. Noble gas analyses results from the water samples and diffusion samplers were found to be comparable.

The tritium, CFC, and noble gas samples were analyzed by the University of Utah Noble Gas Laboratory. Tritium was determined using the helium ingrowth method (Clarke and others, 1976). Noble gasses (^{3}He,^{4}He, Ar, Kr, Ne) and reactive gases (O_2, N_2) were determined by mass spectrometer. The University of Waterloo Environmental Isotope Laboratory analyzed the ^{18}O and D samples by mass spectrometry. Analyses of common ions and trace metals were performed by the Montana Bureau of Mines and Geology's (MBMG) Analytical Laboratory. Water-quality data from the wells not sampled by MBMG were obtained from the Missoula Valley Water Quality District (MVWQD).

Discussion of the Results
Major Ions and Trace Metals
Water may be characterized by the type and concentrations of its dissolved constituents. Ground water in the Missoula Valley aquifer has a very consistent chemical make-up and is of very high quality (table 2). All of the sampled ground-water is a calcium-bicarbonate type; there is little variability in water samples (figure 9). The water is safe to drink and suitable for other uses based on USEPA drinking water standards for natural constituents; all total dissolved solids concentrations were less than 300 milligrams per liter (mg/L).

Although the overall composition of the water is consistent and of high quality, total dissolved solids and the concentrations of several constituents commonly associated with human activities increase downgradient along flow path. Figure 10 shows the distribution of specific conductance, used as a proxy for total dissolved solids; concentrations in wells near the Bitterroot River are more than 100 microsiemens per centimeter at 25°C (uS/cm) greater than those in upgradient wells near the Clark Fork River. Similarly, the concentrations of sodium, chloride, and nitrate generally increase downgradient in wells located further from the Clark Fork River (figures 11 - 13).

Water-quality data from the nested well pairs show that there are subtle yet regular differences in water quality with depth. Concentrations of sodium, chloride, and nitrate are all greater in the shallow wells. The increases are consistent with these constituents originating from the land surface. The most likely source of the elevated sodium and chloride is runoff from de-icing chemicals applied to streets, sidewalks and parking lots (MVWQD, 1997), and effluent from septic tanks (Woessner and others, 1995; MVWQD, 1996); likely sources of elevated nitrate include fertilizers applied to lawns and effluent from septic tanks.

The distribution of arsenic shows a different pattern than that of sodium, chloride and nitrate. Arsenic concentrations ranged from below the detection limit to 2.4 micrograms per liter (ug/L), with samples from the upgradient part of the aquifer near the Clark Fork River having larger

concentrations than samples from downgradient part of the aquifer (figure 14). Additionally, in samples from the nested well pair closest to the river (Well Pair A, figure 8), arsenic was not detected in the sample from the deep well. A sample collected in June 1999 from the Clark Fork River above Missoula (USGS gaging station 12340500) had an arsenic concentration of 2.2 ug/L (USGS online hydrologic data for Montana: http://mt.water.usgs.gov/), similar to that of the highest concentration measured in the ground water. Arsenic in the Clark Fork River is known to be elevated due to historic mining activity upstream of Missoula. The results suggest that arsenic in the aquifer is derived from the Clark Fork River water that recharges the aquifer.

Oxygen-18 and Deuterium

Oxygen-18 (^{18}O) and deuterium (^{2}H, or D) are the main isotopes that comprise the water molecule. Isotopic analyses are useful in hydrologic studies because waters of different ages, recharge areas, or hydrologic history are often isotopically distinctive which allows them to be used to show hydrologic connections. Variables such as temperature, altitude, distance from the ocean, and latitude have an influence on the isotopic composition of precipitation. Because the isotopic composition of ground water generally reflects the average isotopic composition of precipitation in a recharge area, spatial and temporal variations in the isotopic content of precipitation can be useful in evaluating ground-water recharge sources.

The ^{18}O and D concentrations are reported as δ values, which represent the difference in parts per thousand (per mill, ‰) between the ratios of ^{18}O/^{16}O (or D/H) of the water samples and that of standard mean ocean water (SMOW); δ values are calculated by:

$$(\text{in } \text{‰}) = (R_{sample}/R_{SMOW} - 1)1000$$

where "R" is the ratio of the heavy to light isotope. Therefore, the results are interpreted relative to SMOW. A positive δ value means that the sample contains more of the heavy isotope than standard ocean water; a negative δ value means that the sample contains less.

Isotopes of oxygen and hydrogen have been used to determine the sources and flow patterns of ground water (Muir and Coplen, 1981; Taylor and others, 1992), and the seasonal variability of the isotopes in surface water has been used to determine relative quantities and rates of ground-water recharge (McCarthy and others, 1992; Kennedy and others, 1986).

The δ^{18}O and δ D concentrations were measured in selected surface and ground-water samples (table 3). Monthly samples to assess the seasonal variation in ^{18}O were collected from the Clark Fork River at McCormick Park and a nearby monitor well (well 69055) between June 1999 and December 2000. The river was expected to show a seasonal difference between spring runoff when the river water is derived from snow melt (isotopic signature should be more depleted) and at other times when base flow conditions are predominant. Ground-water samples were collected from wells in June 1999 (peak flow) and March 2000 (low flow) to see if seasonal isotopic variations could be detected in the aquifer. Water from the June 1999 round of sampling, was analyzed for both isotopes (^{18}O and D). Subsequent surface and ground water samples were analyzed for ^{18}O only (table 3).

Seasonal variation of ^{18}O in ground water is typically muted due to relatively slow infiltration and mixing in the unsaturated zone (Clark and Fritz, 1997 and Coplen and others, 2000); however, given the hydrogeologic setting of the Missoula Valley aquifer (most of the recharge is infiltrated

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river water and the aquifer is highly transmissive) it was hypothesized that a seasonal signal might be detectable in the aquifer and provide an independent means to trace ground-water flow.

The results from the monthly sampling of the Clark Fork River and well 69055 are shown on figure 15. The $\delta^{18}O$ values from the river samples range from -17.86 to -16.46 per mill, the ground-water samples range from -17.86 to -16.29 per mill. In general, the surface and ground water samples show similar seasonal variations, with more depleted values in the cold winter and spring months and more enriched values in the warm summer months. All the surface water samples with $\delta^{18}O$ values greater than -17 per mill occur between June and December.

The $\delta^{18}O$ and δD results from the June 1999 sampling are shown in figure 16 along with the global and North American meteoric water lines (Coplen and others, 2000). The results plot along and between the two lines demonstrating the regular relationship between $\delta^{18}O$ and δD and demonstrating the meteoric origin of the water. The sample from the Clark Fork River plots slightly above the global line and has the most depleted $\delta^{18}O$ value; the ground water samples are all relatively enriched.

The spatial distribution of the June 1999 $\delta^{18}O$ values (figure 17) shows a gradient through the aquifer that reflects the hydraulic gradient of the flow system; values become more enriched (less negative) along flow path; the ground-water values ranged from -18 to -16.94 per mill, with a median of -17.26 per mill.

Figure 18 shows the results from the March 2000 samples. The March 2000 samples from all but one well are enriched relative to the June 1999 samples, the concentrations ranging from -17.52 to -15.82 per mill, with a median of -16.72 per mill. The amount of enrichment ranged from 0.20 to 1.12 per mill, with an average enrichment of 0.67 per mill (median 0.54 per mill). The magnitude of the change is on the same order as the seasonal change observed in the Clark Fork River samples. The $\delta^{18}O$ concentration gradient is greater than in June 1999, especially in the western part of the aquifer (figures 17 and 18), even though the hydraulic gradient is slightly less (figures 6a and 6b).

The overall enrichment and spatial distribution of $\delta^{18}O$ in the March 2000 samples suggests that water recharged from the Clark Fork River during warm months had invaded most of the aquifer. Another explanation of the observed changes is that seasonal pulses of isotopically enriched water move though the aquifer and the sampling frequency and spacing were not sufficient to identify multiple seasonal peaks. Clearly, the results indicate that a seasonal isotopic variability can be recognized throughout the aquifer, not just in the recharge area. Systematic sampling of ground and surface water over one or more years could help trace ground-water flow and assess ground-water residence times in the Missoula Valley aquifer.

Chlorofluorocarbons
Chlorofluorocarbons (CFC-11 and CFC-12) are synthetic organic compounds first produced in the 1930's, they have very low toxicity and have been used primarily as coolants in air conditioners and refrigerators, blowing agents in foams and insulation, propellents in aerosol cans, and as solvents (Plummer and Busenberg, 2000). Atmospheric concentrations of CFC's are uniform across large areas and have been steadily increasing since the 1940's. Atmospheric concentrations of CFC's have been monitored since 1978, and pre-1978 concentrations have been reconstructed from CFC production and rates of release (Cook and Solomon, 1997). Therefore, atmospheric input of CFC's to ground water can be determined for most localities, and CFC's provide excellent tracers

and dating tools for young ground water.

In ground water, CFC compounds are soluble and stable. Ground-water ages, or recharge dates, are determined by converting CFC concentrations in ground water to equivalent air concentrations using known solubility relationships and recharge temperature (Cook and Solomon, 1997). The equivalent air concentration is compared to known atmospheric concentrations to determine the recharge year. Limitations to the method include reducing conditions that can degrade CFC's in ground water, and non-atmospheric sources of CFC's (Oster and others, 1996). Under optimal conditions CFC's can be used to estimate ground-water age to within 1- to 2-years; however, accuracy generally decreases as age increases (Szabo and others, 1996; Stoner and others, 1997).

Several studies have used CFC's to age-date ground water and to trace ground-water flow (Busenberg and Plummer, 1992; Busenberg and others, 1993; Dunkle and others, 1993; Reilly and others, 1994; Cook and others, 1995). CFC ages have also been used to assess land-use effects on water quality (Bohlke and Denver 1996; Stoner and others, 1997), evaluate the timing of nitrate impacts to ground water in the Flaxville gravel and underlying aquifers in the northern plains of Montana (Nimick and Thamke, 1998), and to assess ground-water residence times and flow rates in shallow aquifers in west-central Montana (Nimick and others, 1996). Studies that have compared CFC to tritium-helium derived ages have shown generally good agreement between the methods (Ekwurzel and others, 1994; Szabo and others, 1996).

For the Missoula valley, concentrations of CFC-11 and CFC-12 were determined in ground-water samples collected from 12 wells and a sample from the Clark Fork River. The results were variable, ranging from less than 3 to more than 77 picomoles per kilogram (pmoles/kg) for CFC-12, and from less than 4 to 35 pmoles/kg for CFC-11 (table 4). All of the ground-water samples, except for the two closest to the Clark Fork River, were contaminated having CFC concentrations in excess of what would be expected from air-water solubility relationships. The elevated concentrations show that CFC's from non-atmospheric sources have been introduced into the aquifer, rendering the samples unusable for age-dating. The two ground-water samples that did not show elevated CFC concentrations were from wells at McCormick Park (well 69055) and near the Madison St. Bridge (well 151191), the recharge dates were 1989 (10 year old water) and 1999 (recent < 1 year old water), respectively. The sample from the Clark Fork River, collected at McCormick Park, returned a date of 1999 (< 1 year old water).

Although most of the CFC samples were not usable for age dating the ground water, the spatial distribution shows a pattern of increasing concentrations down flow path similar to the other parameters associated with human activities. The land use over a large part of the aquifer is unsewered residential; sewage effluent is a recognized source of CFC contamination to shallow ground water (Schultz and others 1976; Busenberg and Plummer, 1992; Plummer and Busenburg, 2000). Plotting the distribution of CFC-12 in relation to the location of known septic systems shows that concentrations increase markedly downgradient of the high density septic areas (figure 19). CFC concentrations in samples from the unsewered and upgradient parts of the sewered area are less than 10 pmoles/kg; downgradient of the high density septic areas concentrations range up to more than 75 pmoles/kg.

Samples from the shallow-deep well pair (Well Pair A, figure 8) in the upgradient, sewered area showed that there is no significant difference in CFC concentration between the shallow and deep well, the CFC-12 concentrations were 7.5 and 7.94 pmoles/kg, respectively. However, samples

from the well pair in the unsewered part of the area (Well Pair B, figure 8) near the end of the flow system showed that concentrations in the shallow well were almost 5 times greater than the deep well, 77.68 and 15.93 pmoles/kg, respectively. These observations suggest that septic effluent is a primary source of the excess CFC's.

Tritium

Tritium (^{3}H), the radioactive isotope of hydrogen with a half-life of 12.43 yr, is produced naturally in the upper atmosphere. Atmospheric testing of nuclear weapons between 1952 and 1963 injected large amounts of tritium into the atmosphere, overwhelming the natural production. Tritium concentrations in north American rainfall are estimated to have been in the range of 5 to 20 tritium units (TU) prior to above ground nuclear testing; during the early 1960s tritium concentrations in precipitation of more than 5,000 TU were recorded at several North American stations (Solomon and Cook, 2000). Most of the bomb-derived tritium has since been washed from the atmosphere and tritium levels in precipitation are now close to natural levels (Clark and Fritz, 1997). Tritium in precipitation fluctuates seasonally. In Ottawa, Canada where it is monitored monthly, tritium levels in precipitation since 1992 have ranged from about 10 to 30 TU (IAEA/WMO, 2001). Because of its short half life, tritium is an ideal marker of recent (post-1952) ground-water recharge.

Tritium concentrations in ground water and the Clark Fork River ranged from 8.7 to 13.1 TU (table 5 and figure 20). The results show that all the sampled water is modern (i.e. has been recharged since the advent of above ground nuclear testing) and are very consistent, less then 5 TU separate the high and low values. The tight range of values suggests that recharge water flushes through the aquifer relatively rapidly. There are no apparent flow path trends in the tritium data and there is no difference between the ground-water samples and the Clark Fork River sample (figure 20).

Tritium-Helium Ground-Water Ages

Tritium decays to the stable noble gas helium-3 (^{3}He). After water containing tritium enters the ground-water system and becomes isolated from the atmosphere, ^{3}He concentrations increase as the ground water becomes older. By determining the amount of tritium and tritiogenic ^{3}He in a ground-water sample, an age can be calculated according to the relationship (Plummer and others, 1993):

$$t = t_{1/2}/\ln 2 * \ln(1 + {}^3He_{trit}/{}^3H)$$

where t is the tritium-helium age, ^{3}He$_{trit}$ is the helium-3 in the sample derived from tritiogenic decay, ^{3}H is the tritium concentration, and $t_{1/2}$ is the tritium half life. Ratios of tritium to helium-3 have been used to accurately date shallow ground water with ages ranging from a few months to 50 years (Poreda and others, 1988; Solomon and Sudicky, 1991).

Tritium-helium dating has been used to understand flow constraints in an aquifer recharged by bank infiltration (Stute and others, 1997), and in other studies to determine ground-water recharge, to estimate variations in ground-water recharge, and to trace ground-water age and flow (Poreda and others, 1988; Solomon and Sudicky, 1991; Solomon and others, 1992; Solomon and others, 1993; Cook and others, 1996, Szabo and others, 1996). Tritium-helium dating has also been used to determine aquifer characteristics and to trace solute transport at contaminated sites (Cook

and others, 1996; Solomon and others, 1995). More recently tritium-helium ages have been used to improve ground-water flow models, and to estimate and constrain hydraulic parameters used in flow models (Sheets and others, 1998; Shapiro and others, 1998; Portniaguine and Solomon, 1998).

Tritium-helium-3 (^{3}H-^{3}He) apparent ages for the 12 ground-water samples from the Missoula Valley aquifer ranged from less than zero to 4.6 years, with estimated uncertainties of 1 to 1.5 years (table 5 and figure 21).

The results underscore one of the limitations of the ^{3}H-^{3}He method, namely for very young water accurate determinations of the amount of ^{3}He from atmospheric solubility and excess air are very important (Solomon, 2000). Obviously an age less than zero does not make sense. However, there are three main sources of ^{3}He in ground water, the atmosphere, excess air, and tritiogenic decay (^{3}He in ground water can also be derived from mantle and nuclear reactions, but for this study these sources were considered negligible). The total amount of ^{3}He can be expressed as:

$$^3He_{tot} = {}^3He_{atm} + {}^3He_e + {}^3He_{trit}$$

Where $^3He_{atm}$ is the helium-3 derived from dissolution of air in recharging ground water, ^3He_e is the component of helium-3 derived from the supersaturation of air in ground water, and $^3He_{trit}$ is the component derived from tritiogenic decay. What is measured in the laboratory is $^3He_{tot}$, the total amount of helium-3 in the sample. To apply this method the amount of $^3He_{trit}$ must be isolated by subtracting $^3He_{atm}$ and ^3He_e from $^3He_{tot}$. $^3He_{atm}$ is calculated from the recharge temperature, and equilibrium solubility relationships with He in the atmosphere. The excess air component is determined by the degree of neon supersaturation (the only source of neon is the atmosphere), the recharge temperature, and the atmospheric concentration. Once the amounts of $^3He_{atm}$ and ^3He_e have been accounted for the remaining ^3He is attributed to tritiogenic decay. The samples that returned "negative ages" are very young water in which the $^3He_{atm}$ and ^3He_e components overwhelm the $^3He_{trit}$ component, not enough time has elapsed to generate a significant amount of $^3He_{trit}$.

In general, the results show expected flow-path trends with ages increasing along flow path. Figure 22 shows the distribution of apparent ages from the samples collected along the eastern transect, the values range from less than 1 year near the river to more than 3 years at the end of the transect. There were no strong correlations with depth below the water table, although most of the samples were obtained near the water table (figure 23). In the nested well pair near the end of the flow system (Well Pair B) the sample from the deep well had the younger age; however, given the uncertainties associated with these determinations the ages can not be considered significantly different.

Using the data from Well Pair B, a horizontal ground-water flow velocity was determined using the equation:

velocity = distance/time

The well pair is approximately 15,000 feet downgradient from the Clark Fork River (figure 8); the apparent ground-water ages were 4.6 years (well 151201) and 3.3 years (well 157210). Assuming most of the ground water is recharged from the river, the velocity through this part of the aquifer ranges from about 7 to 18 feet per day. These velocity estimates can be used in conjunction with the hydraulic gradient and porosity to estimate values of bulk hydraulic conductivity for the

aquifer using a form of Darcy's Law:

$$K = (vel*n)/I$$

Where K = hydraulic conductivity, vel = ground-water velocity, n = effective porosity, I = hydraulic gradient. Using the measured hydraulic gradient of 0.002, and an assumed effective porosity value of 0.25, the estimated hydraulic conductivity of the aquifer ranges from about 900 to 2,300 feet per day (ft/d). This range agrees favorably with, although it is slightly lower than, ranges published by McMurtrey and others (1965), 830 - 1,608 ft/d; Woessner (1988), 1,400 - 3,400 ft/d; and Miller (1991), 1,100 - 18,000 ft/d.

Helium-4

Recharging ground water contains atmospheric ^{4}He in an amount that depends on recharge temperature and air-water solubility relationships. As water moves through the subsurface, ^{4}He concentration will rise due to additions of terrigenic ^{4}He produced within the aquifer solids (Solomon, 2000). Terrigenic ^{4}He (^{4}He$_{ter}$) is derived mostly from the alpha (α) decay of uranium and thorium series elements in rocks and sediments, and has been used to trace ground-water flow. The general theory behind the method is that the longer the ground water is in contact with uranium and thorium bearing minerals the greater the ^{4}He concentration. Therefore, as ground water moves down flow path, ^{4}He concentrations increase; if the release rate is known then ^{4}He concentrations should be proportional to ground-water travel times and can be used to trace ground-water flow (Solomon, 2000). The method has been used to trace ground water in regional bedrock aquifers in the range of 10^3 - 10^8 years (Andrews and Lee, 1979; Torgersen and Clarke, 1985; Stute and others, 1992). However, Solomon and others (1996) observed ^{4}He concentrations increased with travel time in a shallow, unconsolidated aquifer where the ground-water age was less than 50 years, and the concentrations were 300 times greater than what can be supported by in situ decay of uranium and thorium. They postulated that the large concentrations were due to the release of residual helium that had accumulated in the protolith prior to its erosion and deposition as aquifer materials. Furthermore, they showed that by quantifying the release rate, ^{4}He could be used to trace ground water over a time scale of 10 to 10^3 years in some aquifers.

The ^{4}He$_{ter}$ concentrations determined for the ground-water samples and the sample from the Clark Fork River ranged from 0.2 x10^{-8} to 5.45x10^{-8} cubic centimeters at standard temperature and pressure per gram (ccSTP/g) (table 5 and figure 24). The sample from the Clark Fork River had a relatively small amount (0.22 x10^{-8} ccSTP/g), while samples from wells near the river, in the upgradient part of the flow system, and below the central part of town had the largest concentrations. A plot of the ^{4}He$_{ter}$ concentrations with distance form the Clark Fork River (i.e. down flow path) shows that concentrations decrease down flow path (figure 25), a trend opposite of what has been reported in the literature (Solomon and others, 1996; Solomon, 2000). There were no apparent correlations of ^{4}He$_{ter}$ with depth (figure 26), one of the samples from a deep well near the Clark Fork River had a large concentration (well 157208, 5.45x10^{-8} ccSTP/g), but the other deep well located near the end of the flow system did not (well 157210, 2.10x10^{-8} ccSTP/g). To verify the results, four of the wells were resampled, the results (table 5) confirmed the elevated concentrations and the concentration distribution (figure 27).

The results of the helium analyses indicate a significant source of terrigenic helium in the

Missoula Valley aquifer and a concentration distribution that defies conventional interpretation. Large concentrations of terrigenic ^{4}He have been detected in other aquifers in the region. Plummer and others (2000) observed excess terrigenic helium, believed to be derived from a mantle source, in the eastern Snake River Plain aquifer in south-central Idaho. However, the largest excesses were detected in water with tritium concentrations generally < 1 TU, ground water that contained large fractions of irrigation water (derived from the Snake River) had low excess ^{4}He. Pope and others (1999) reported significant terrigenic helium in a basin-fill aquifer near Dillon, in southwest Montana. The terrigenic ^{4}He was shown to increase in relation to depth and ground-water age, however there was insufficient data to determine if the source was diffusion from the mantle or from aquifer solids.

All of the samples from the Missoula Valley aquifer have terrigenic ^{4}He concentrations well above what could be possible from the in-situ decay of uranium and thorium. If the source is the release of residual helium then it would appear that the release rate is not uniform through the aquifer. Whatever the source, the presence of such large concentrations in ground water less than 5 years old, and a concentration distribution that does not account for known flow paths presents an unsolved problem.

Summary and Conclusions

A study to assess the use of environmental tracers and water quality in the Missoula Valley aquifer was undertaken as part of the Montana Ground-Water Assessment Program and funded in part by the Montana Water Center.

The ground water in the aquifer is a calcium-bicarbonate type with low dissolved solids concentrations; none of concentrations of inorganic constituents exceeded public drinking water standards. However, concentrations of total dissolved solids, sodium, chloride and nitrate were higher in samples from the downgradient wells, indicating that concentrations of these constituents increase as ground water flows from the Clark Fork to the Bitterroot River. Samples from two shallow-deep well pairs show that concentrations of these constituents are slightly greater in the shallow wells (near the water table). Much of the area overlying the aquifer in the study area is urban, or high- and medium density residential (sewered and unsewered), and is covered by streets, driveways, parking lots and lawns. Likely sources of sodium and chloride in this environmental setting include de-icing chemicals and septic effluent; likely sources of the nitrate include fertilizers applied to lawns and gardens, and septic effluent.

Monthly sampling of the Clark Fork River shows that concentrations of δ^{18}O and δD vary seasonally, generally the water is more isotopically enriched during the warmer months. Ground-water samples from June 1999 show δ^{18}O increasing fairly uniformly down flow path. The results from a repeat sampling in March 2000, show all of the samples, except one, were enriched relative to the June 1999 samples and the concentration gradient was steeper. The amount of enrichment was on the order of the seasonal variability observed in the Clark Fork River samples. The seasonal variability in the Clark Fork River most likely explains the spatial variability observed within the aquifer and the shift in δ^{18}O values in the ground water between June 1999 and March 2000 because the river is the primary source of recharge. The observed temporal and spatial variability could be useful for tracing ground-water flow and determining ground-water residence times in the Missoula Valley aquifer.

Evaluation of CFC concentrations in context of the ground-water flow system and land use,

specifically the distribution of septic systems, indicate that septic effluent is a probable significant source of CFC's to the Missoula Valley aquifer. The results indicate that CFC's can not be used in this environmental setting to date ground water because of CFC contamination. Although unsuitable for age dating, CFC's may be useful for monitoring the effectiveness of sewering on the ground-water quality. Parts of the study area are scheduled to be sewered, resampling the wells for CFC's after the septic systems are no longer in use may provide a way to document the effect of sewering on ground-water quality.

Ground-water ages determined by the ^{3}H/^{3}He method were for the most part hydrologically consistent; ages generally increase with distance down flow path. The oldest apparent age was 4.6 years with an uncertainty of +\- 1 yr. Dating of the ground water was problematic because most of the water is so young. Young water is more sensitive to atmospheric concentrations of ^{3}He which results in increased uncertainty without accurate determinations of atmospheric components of ^{3}He. However, the results are significant because they demonstrate the presence of young water throughout the flow system between the Clark Fork and Bitterroot Rivers, and they are consistent with the high transmissivity of the aquifer as measured by aquifer tests. Comparison of bulk hydraulic conductivity values estimated from ^{3}H/^{3}He ages agree favorably with values determined from aquifer tests.

Large concentrations of terrigenic ^{4}He were present in the ground-water samples from the Missoula Valley aquifer. The sample from the Clark Fork River did not contain significant ^{4}He, suggesting that the excess ^{4}He is derived from the aquifer solids or possibly a deep regional flux. However, the spatial distribution of ^{4}He does not show expected flow path trends that would support either of these hypotheses. Ground-water samples very close to the Clark Fork River were highly enriched in ^{4}He and concentrations generally decreased along flow path, samples from the deep wells were not consistently more enriched in ^{4}He than shallow samples.

The water quality trends and the young age of the ground water highlight the overall vulnerability of the Missoula Valley aquifer to contamination. It is recommended that the ongoing monitoring efforts of the Missoula Valley Water Quality District continue in order to preserve the current high-quality ground water in the aquifer.

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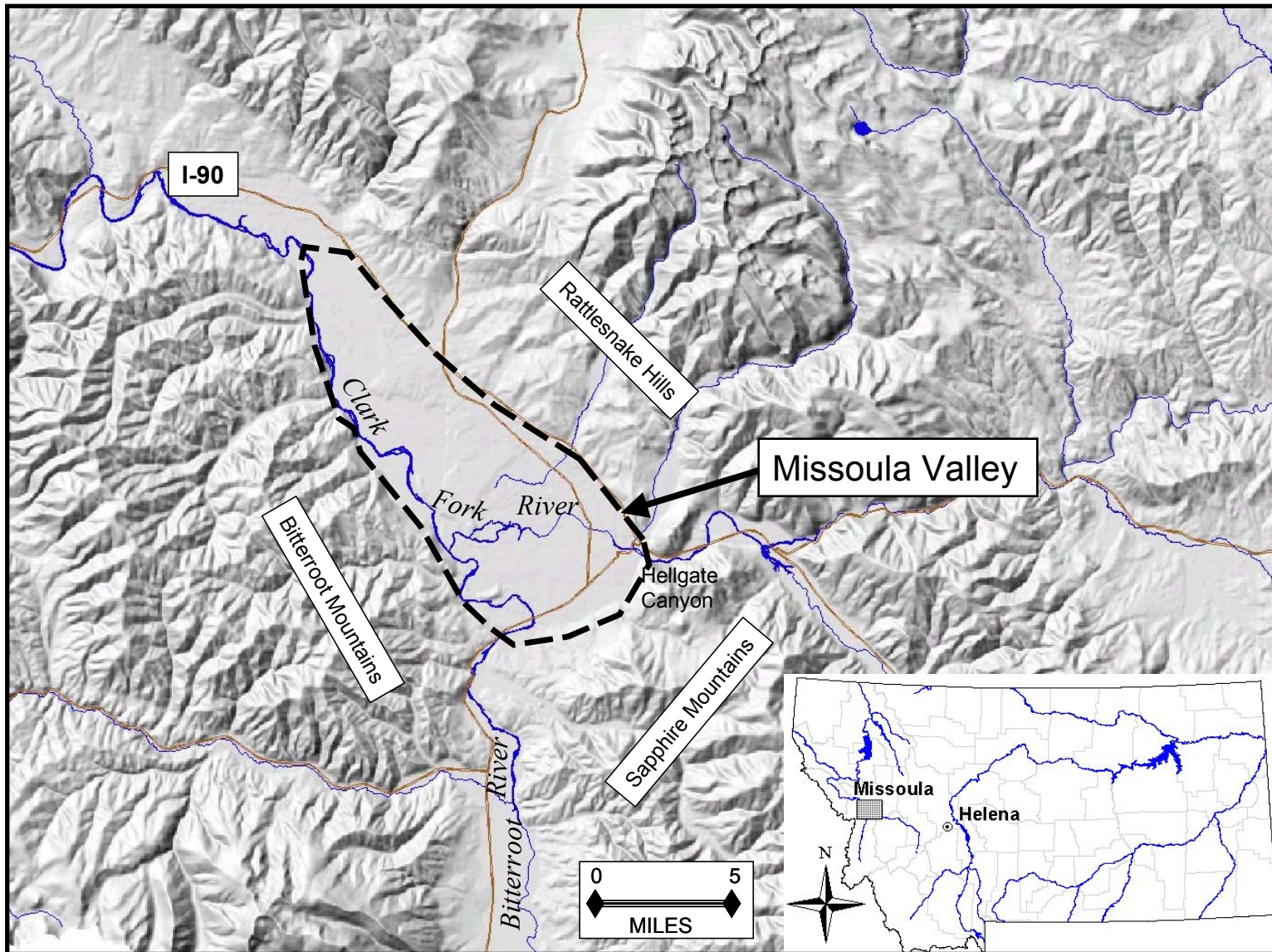
Figure 1. The Missoula Valley is located in southwest Montana near the confluence of the Clark Fork and Bitterroot rivers.
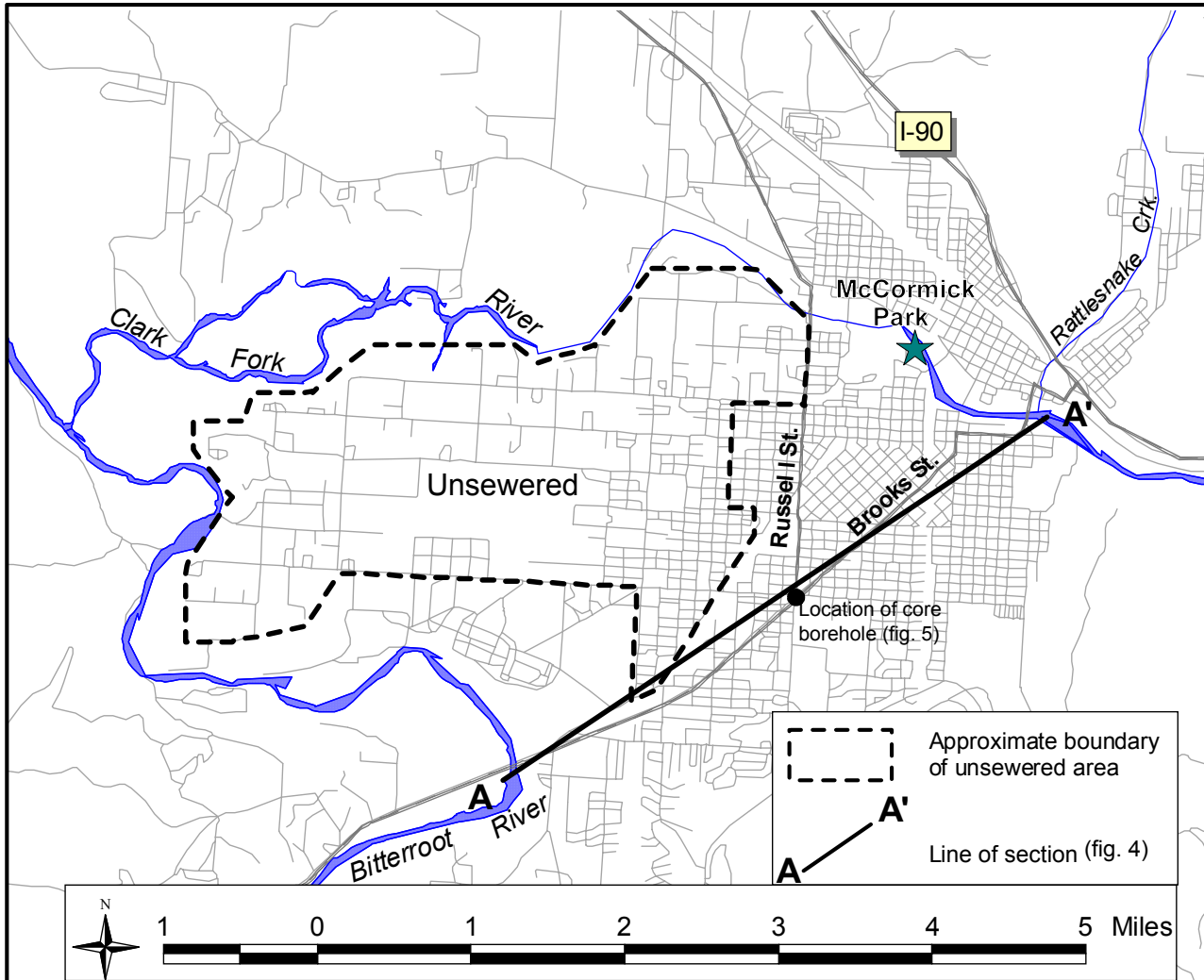
Figure 2. The study area includes the part of the basin between the Clark Fork and Bitterroot Rivers; land use is predominately urban and residential. A large part of the study area is not serviced by municipal sewer.
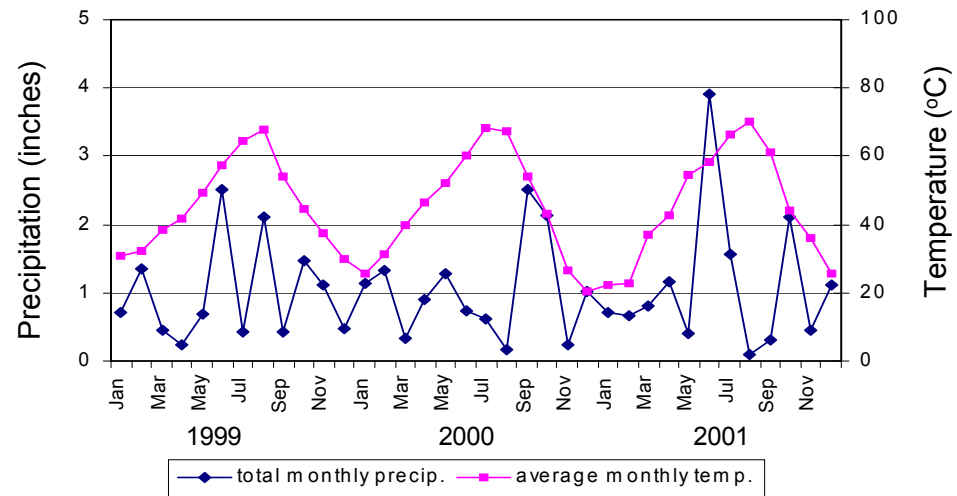
Figure 3. Monthly temperature averages and rainfall totals recorded at the Missoula airport during the study period.
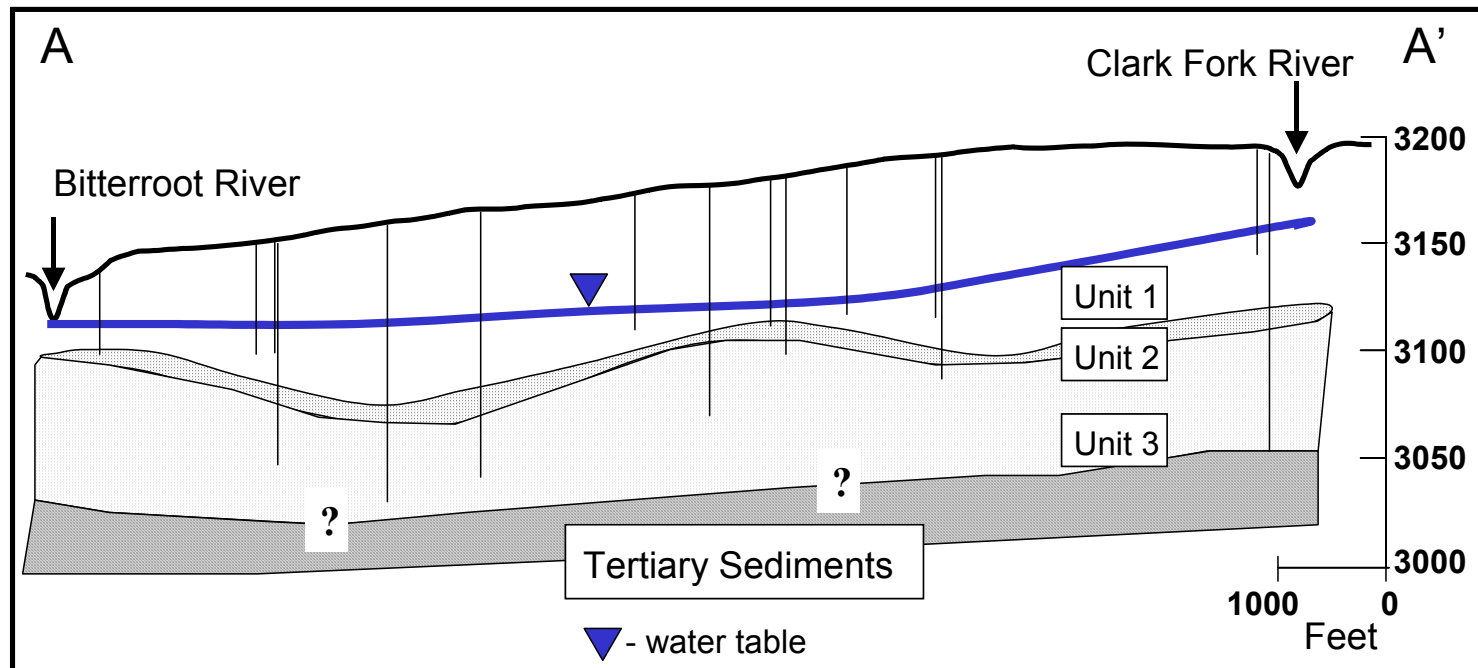
Figure 4. Generalized cross section along the direction of ground-water flow between the Clark Fork and Bitterroot Rivers, showing the three units of the Missoula Valley aquifer. The basal Unit 3 is the most productive part of the aquifer. Borehole data used to construct the cross section are shown by vertical lines. (Line of section A-A' is shown on figure 2.)

Figure 5. Photograph of core samples showing the fine sand of Unit 2, and the coarse gravels and cobbles of Unit 3. Core came from borehole drilled near the intersection of Russell and Brooks Streets (figure 2). Unit 2 sample is from 75 to 77 feet below land surface. Unit 3 sample is from 107.5 to 110 feet below land surface.
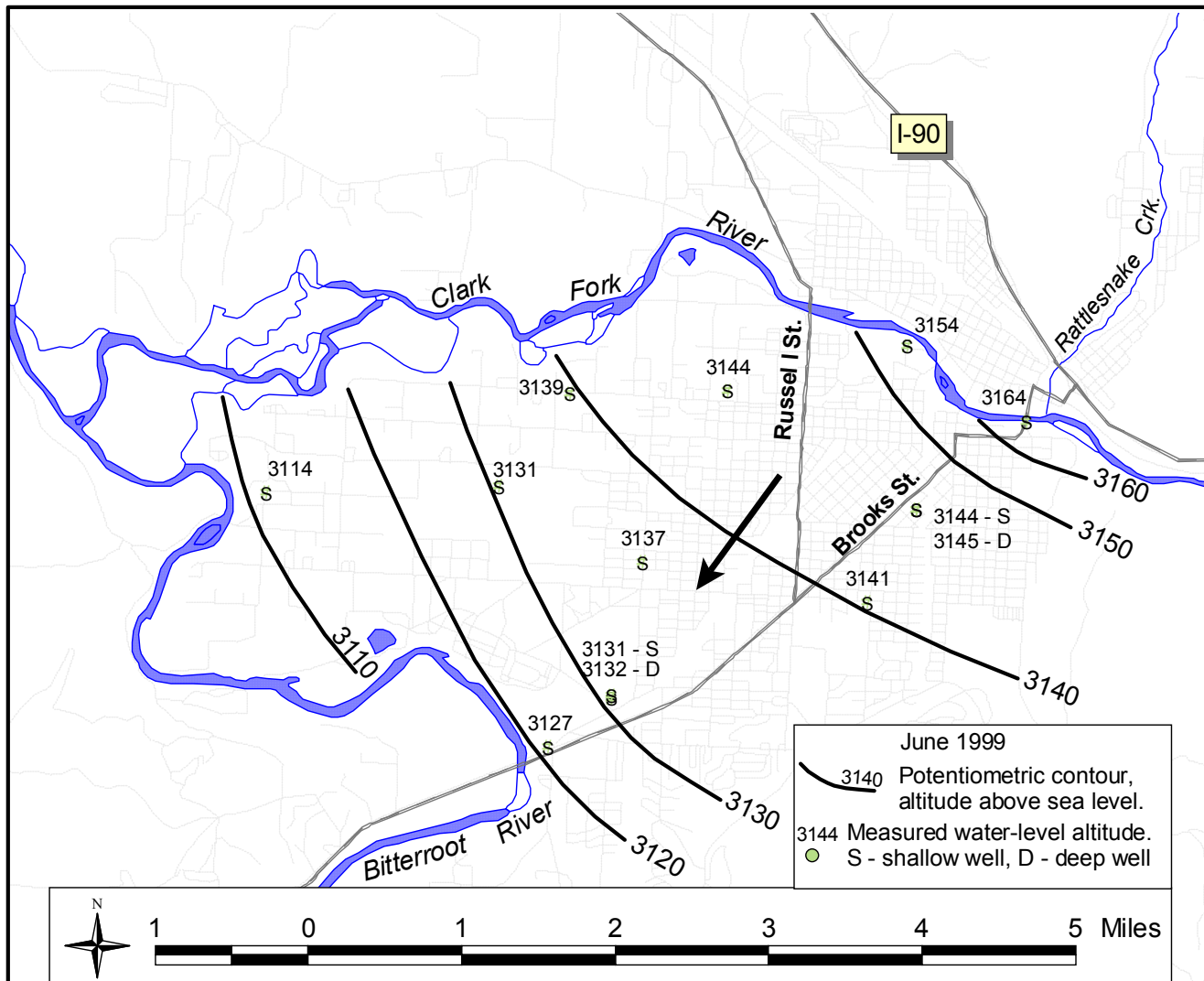
Figure 6a. Potentiometric surface of the Missoula Valley aquifer, June 1999. Ground-water flows to the southwest (arrow), roughly parallel to Brooks St., between the Clark Fork and Bitterroot Rivers.
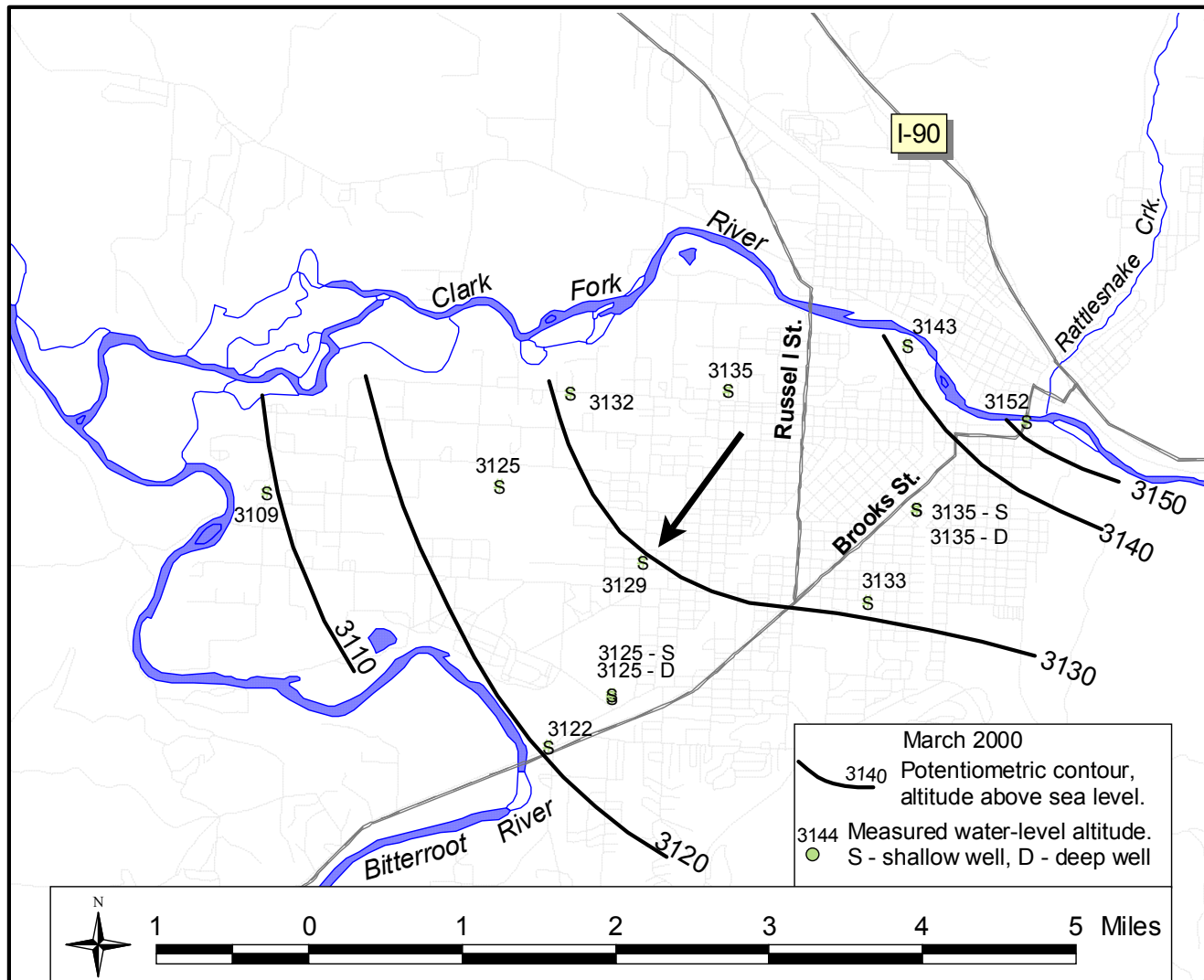
Figure 6b. Potentiometric surface of the Missoula Valley aquifer, March 2000. Ground-water flow direction (arrow) was similar to June 1999, however water levels were lower and the hydraulic gradient across the area was slightly less.
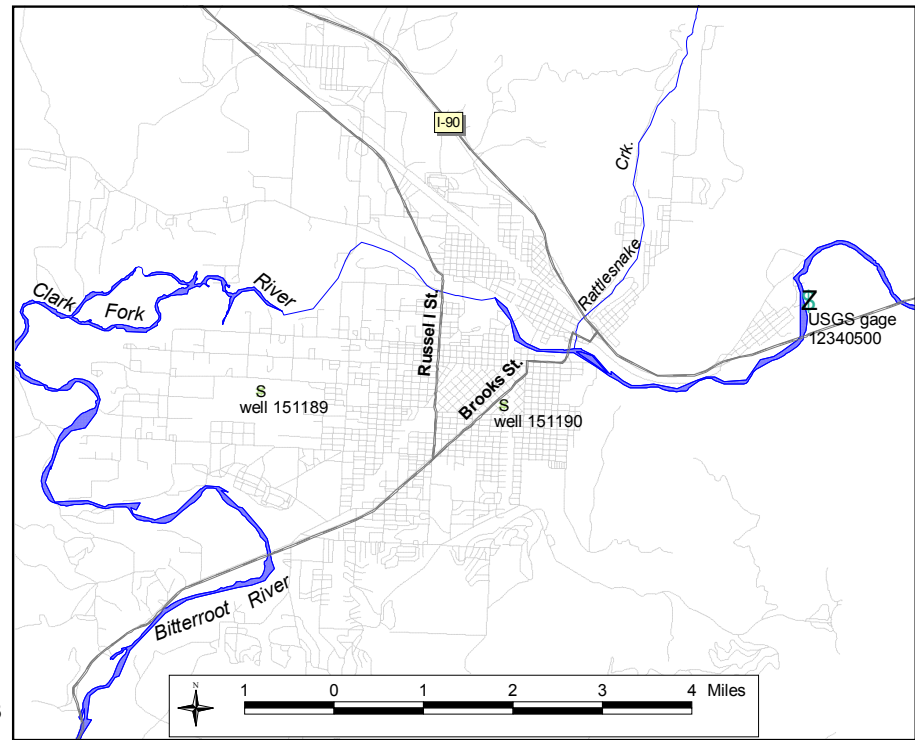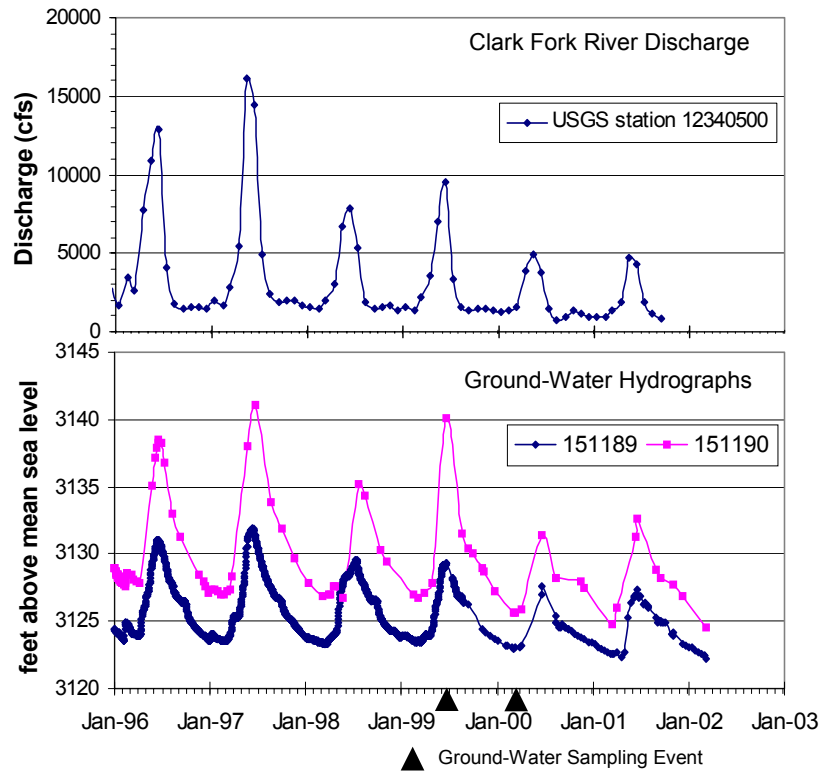
Figure 7. Ground-water levels in the Missoula Valley aquifer closely follow variations in the Clark Fork River discharge highlighting the interconnection of the surface and ground water. Major ground-water sampling events occurred during peak and low flow periods.
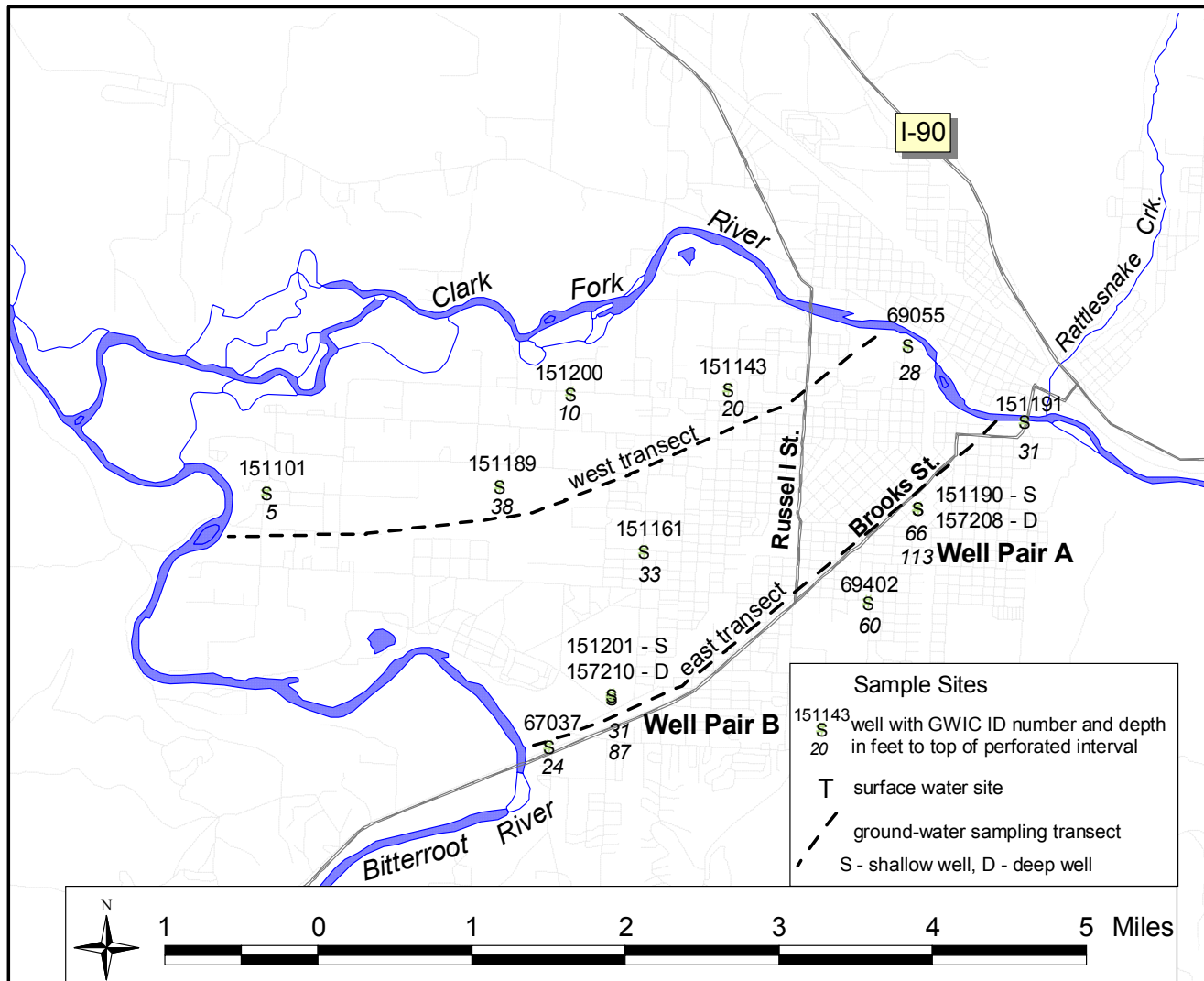
Figure 8. Ground-water samples were collected from monitor wells located along two transects of ground-water flow, surface water samples were collected near the boat ramp at McCormick Park. Ground Water Information Center (GWIC) identification numbers and depths to the top of perforated intervals are shown.
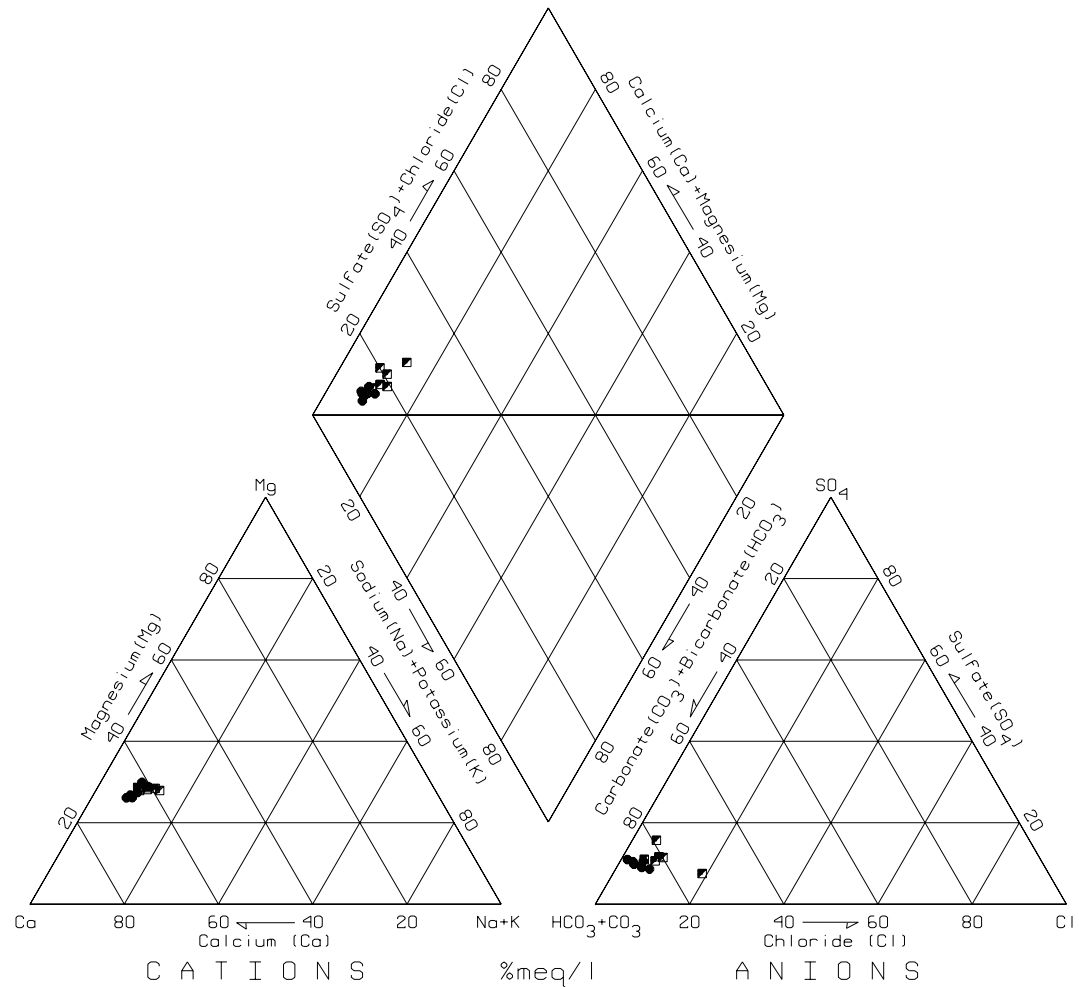
Figure 9. The Missoula Valley aquifer contains a consistent calcium-bicarbonate type water. Samples collected in June, 1999; squares represent data from the Missoula Valley Water Quality District.
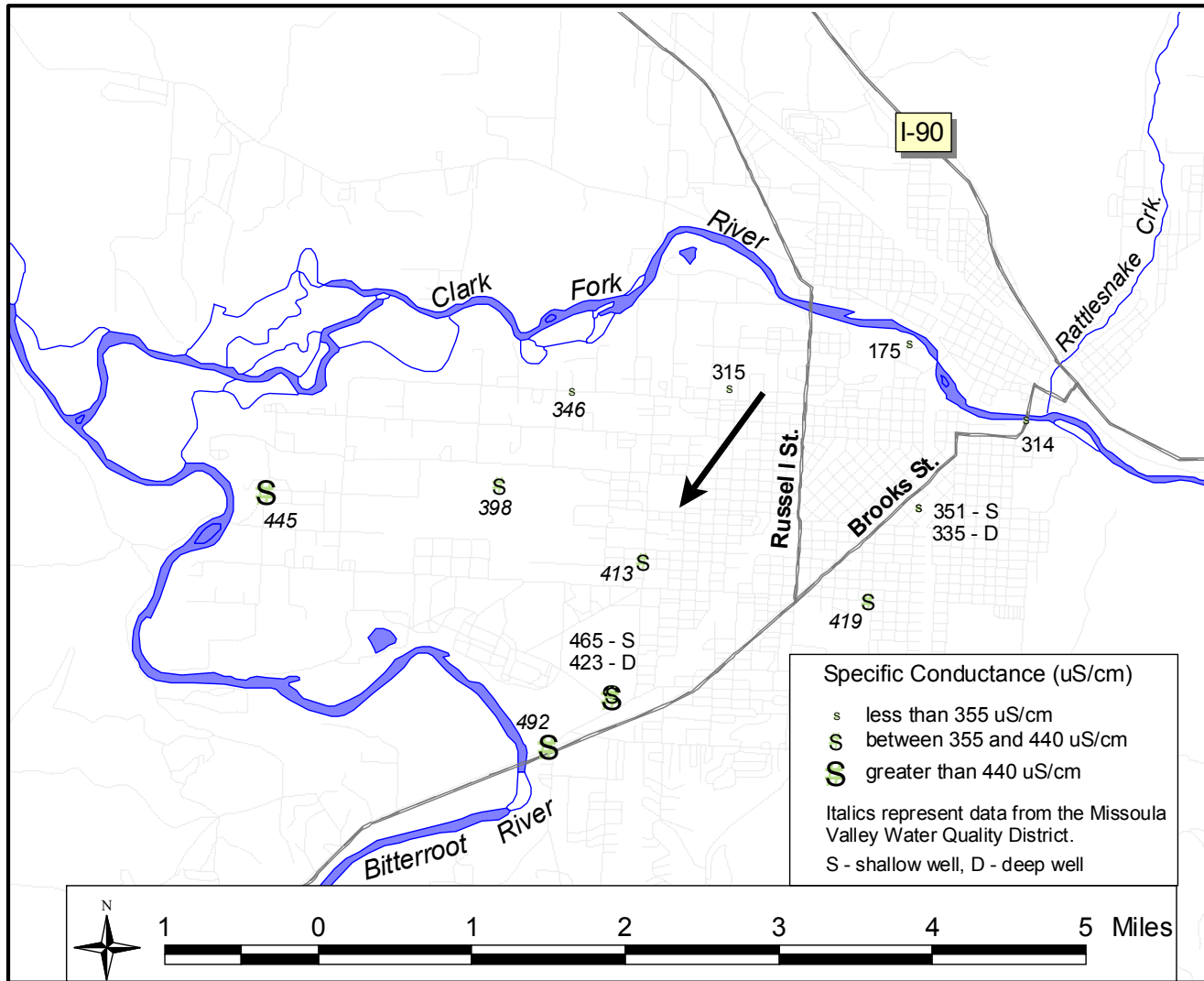
Figure 10. The distribution of specific conductance shows that the concentration of dissolved solids in the aquifer increases along ground-water flow path (arrow) between the Clark Fork and Bitterroot Rivers.
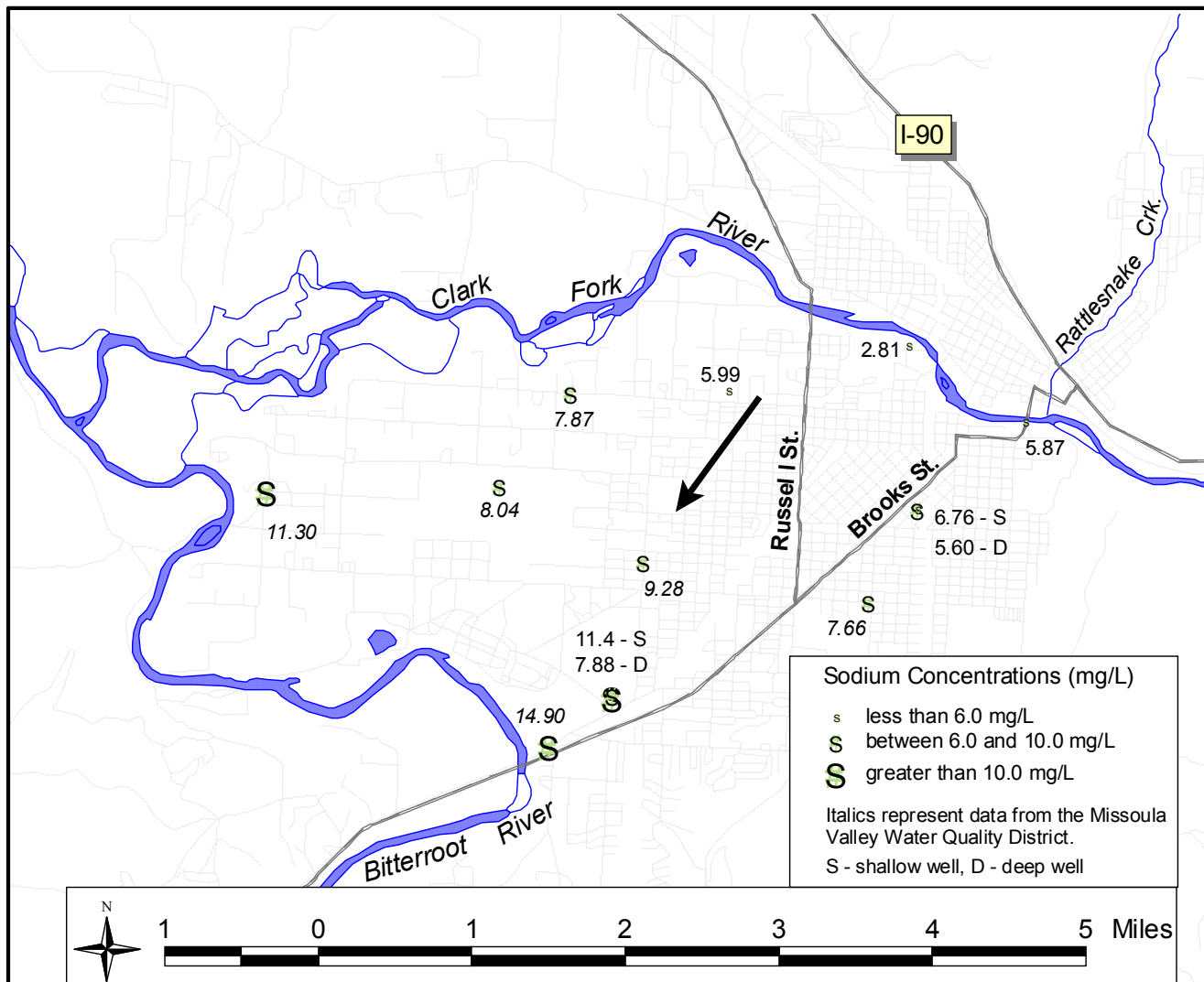
Figure 11. The concentration of sodium in the aquifer increases along ground-water flow path (arrow) between the Clark Fork and Bitterroot Rivers.
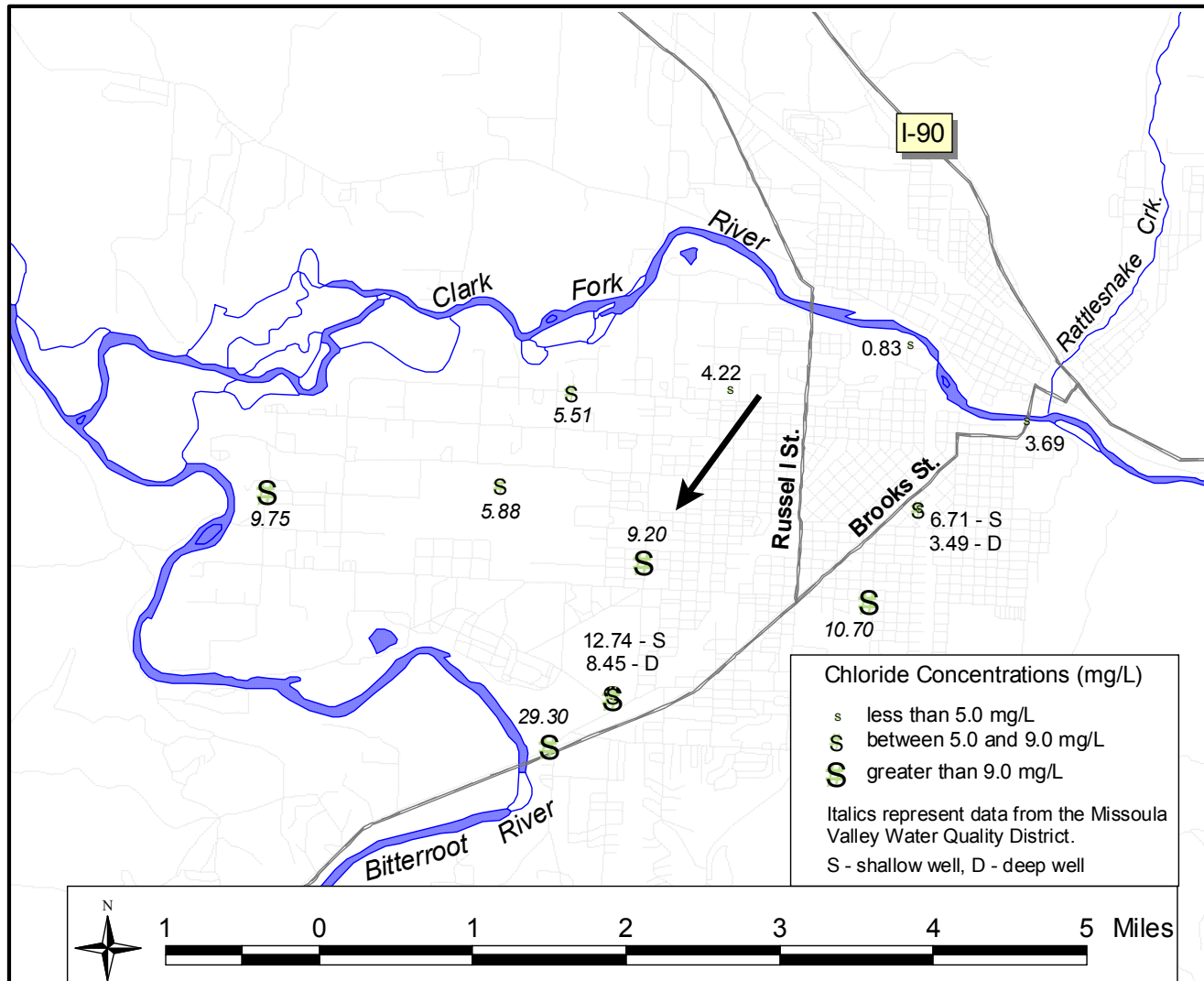
Figure 12. The concentration of chloride in the aquifer increases along the ground-water flow path (arrow) between the Clark Fork and Bitterroot Rivers.
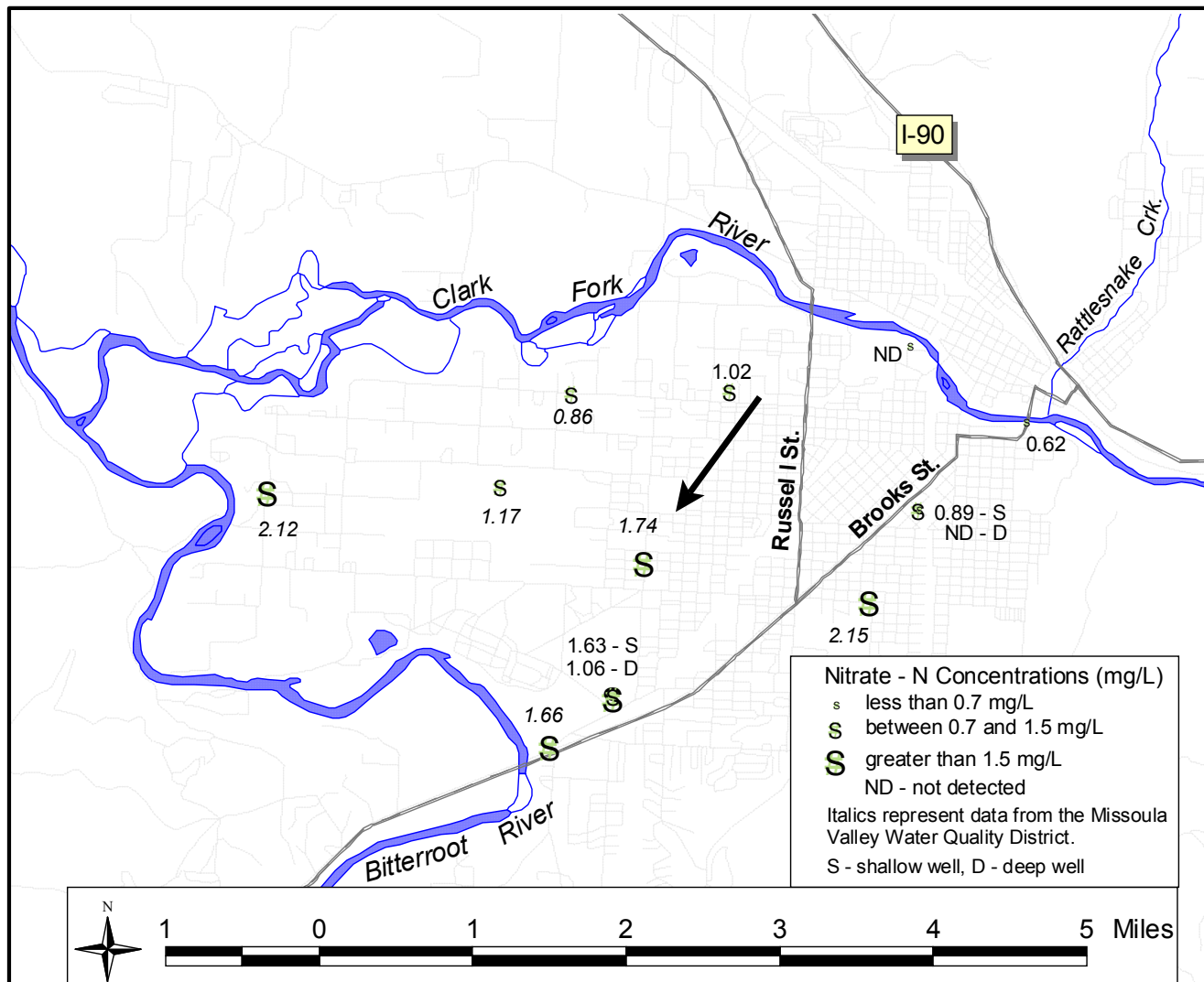
Figure 13. The concentration of nitrate in the aquifer increases along ground-water flow path (arrow) between the Clark Fork and Bitterroot Rivers.
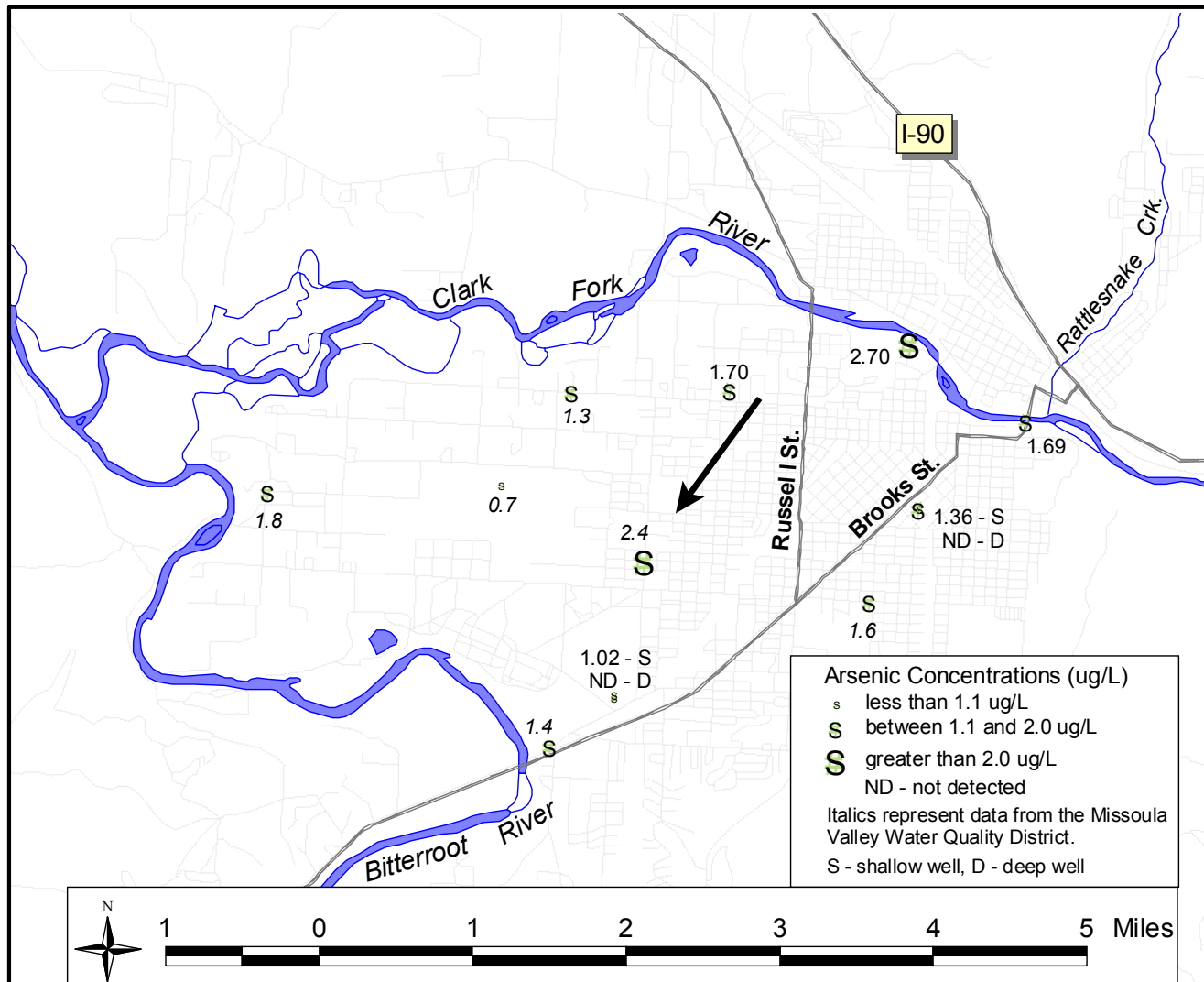
Figure 14. Arsenic concentrations (shown in micrograms per liter) in the upgradient part of the aquifer are slightly greater than concentrations near the end of the flow system. Arrow shows direction of ground-water flow.
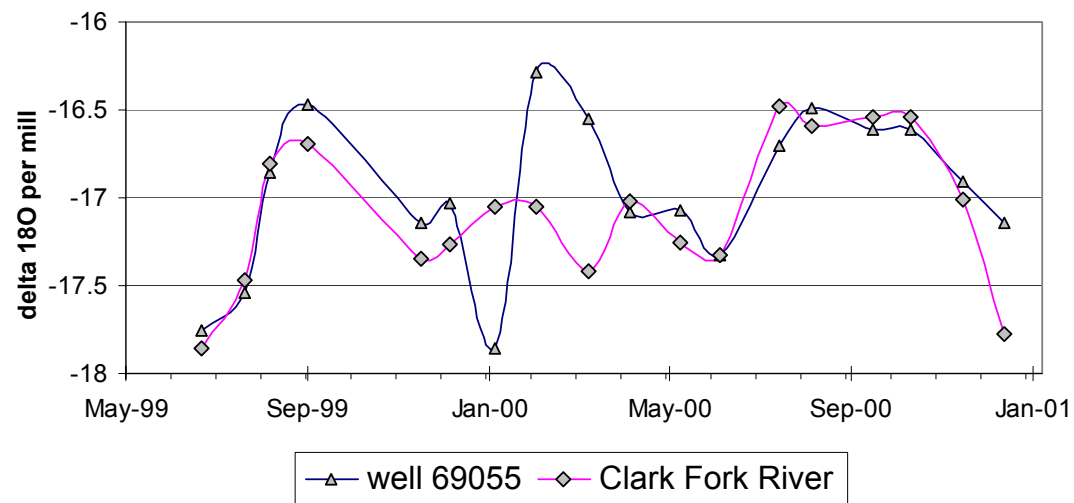
Figure 15. The $\delta^{18}O$ in the ground water (well 69055) tracks the surface water fairly closely. The values are generally more enriched during the warmer months. The variation in the well signal for Jan.- March 2000 is unexplained.
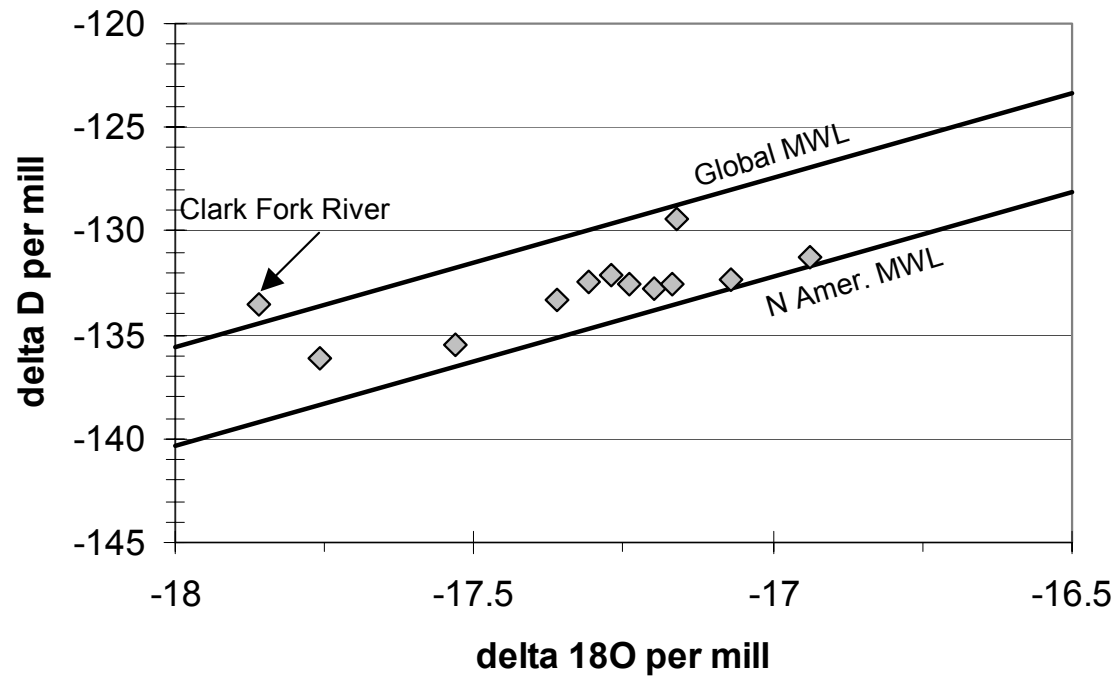
Figure 16. The $\delta^{18}O$ and δD of the June 1999 surface and ground-water samples plot along the global and North American meteoric water lines (MWL's).
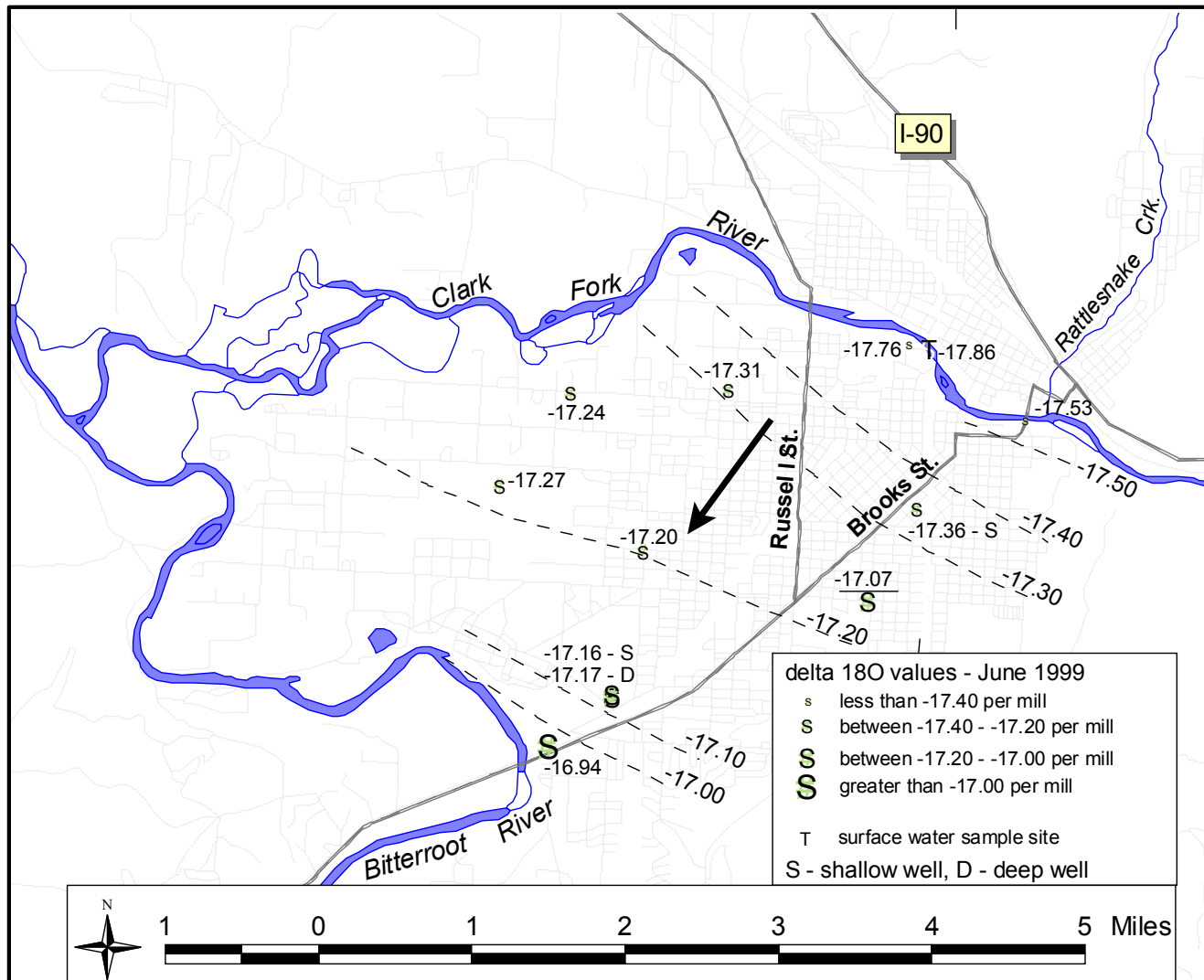
Figure 17. The δ¹⁸O values from the June 1999 samples become enriched along the ground-water flow path (arrow).
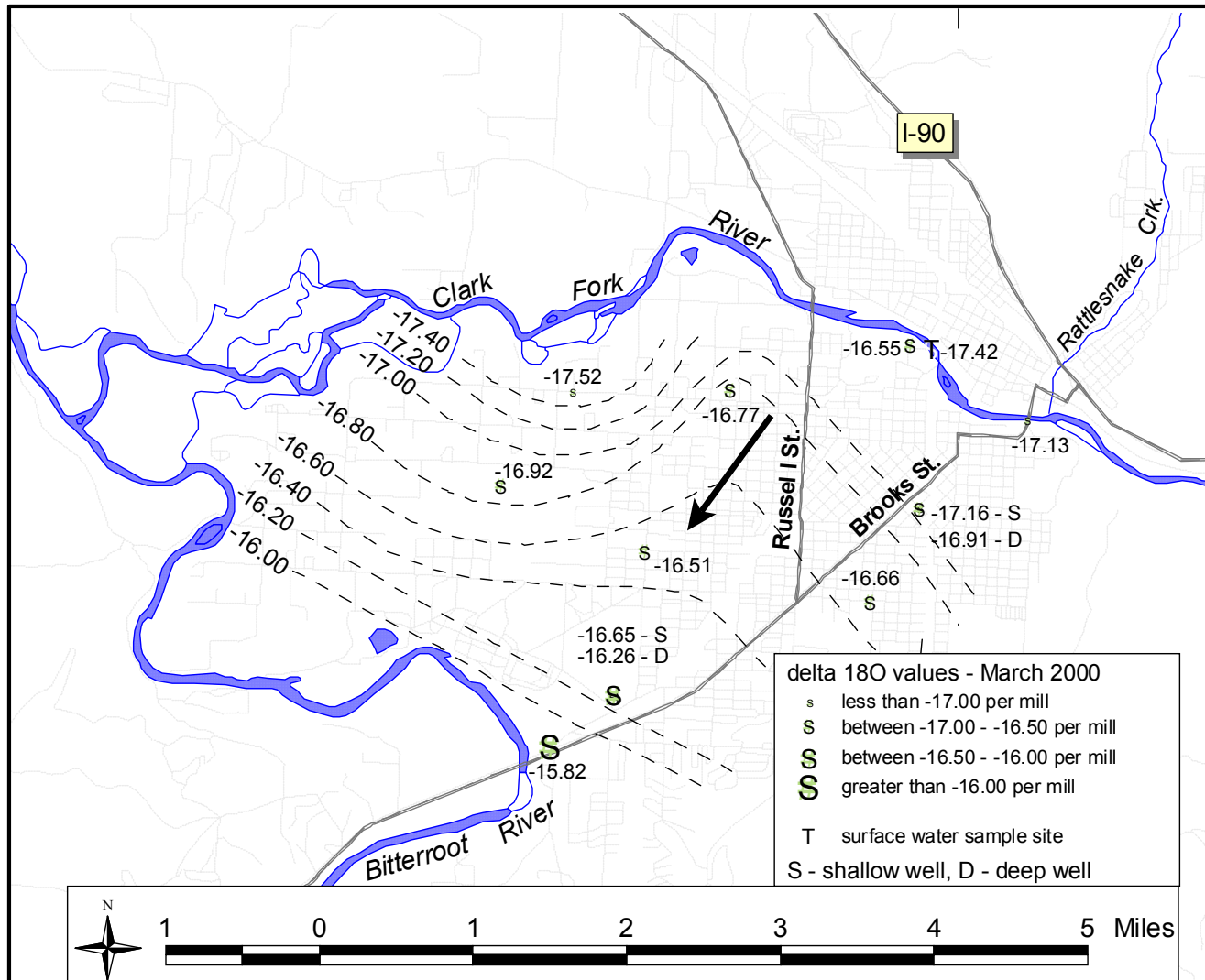
Figure 18. The δ18O values from March 2000 are enriched relative to the June 1999 samples and the concentration gradient is greater (Figure 17). Arrow shows the direction of ground-water flow.
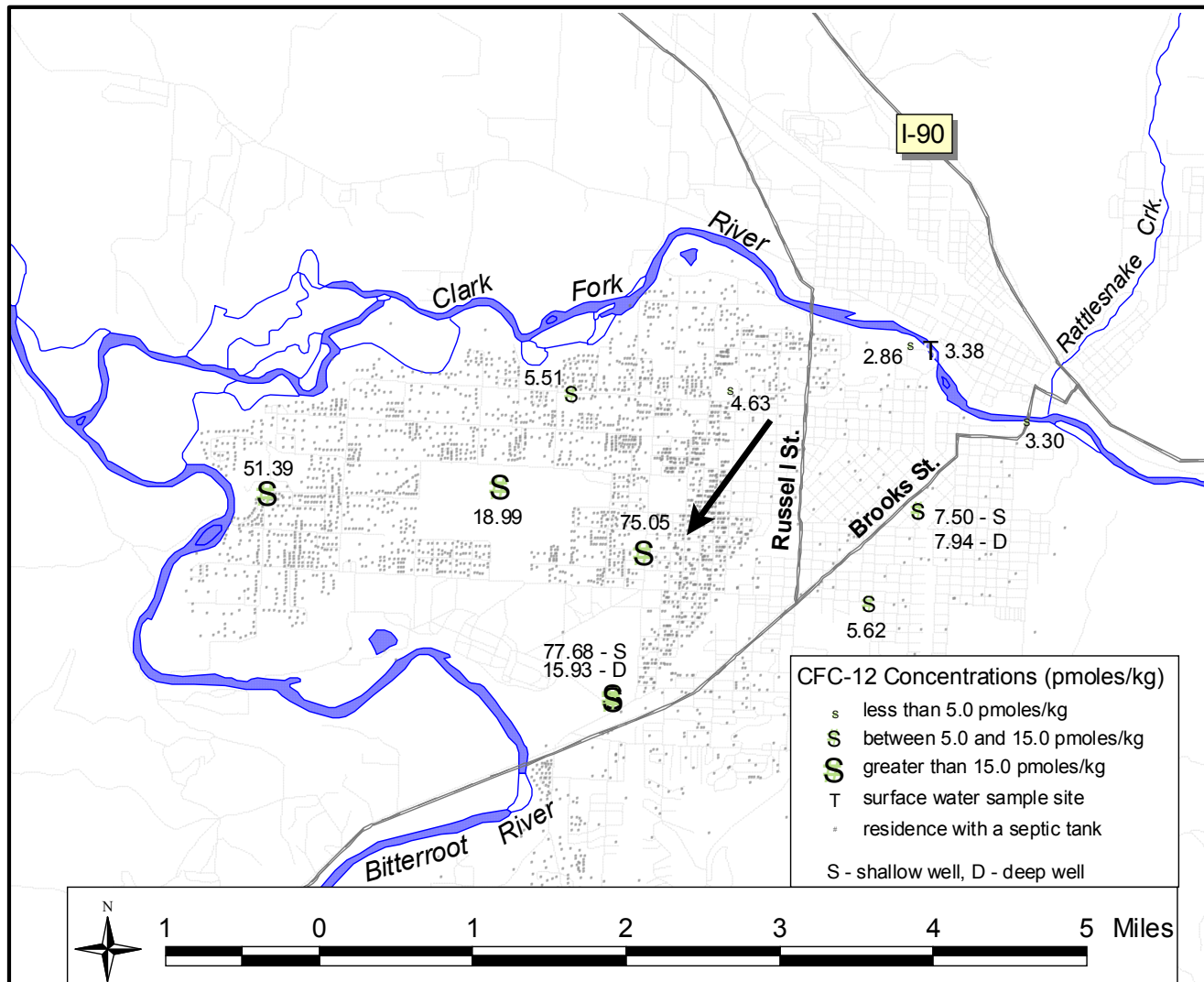
Figure 19. The ground-water concentrations of CFC-12 increase markedly in areas downgradient (arrow) of high density septic areas. (Septic tank location data from the Missoula Valley Water Quality District)
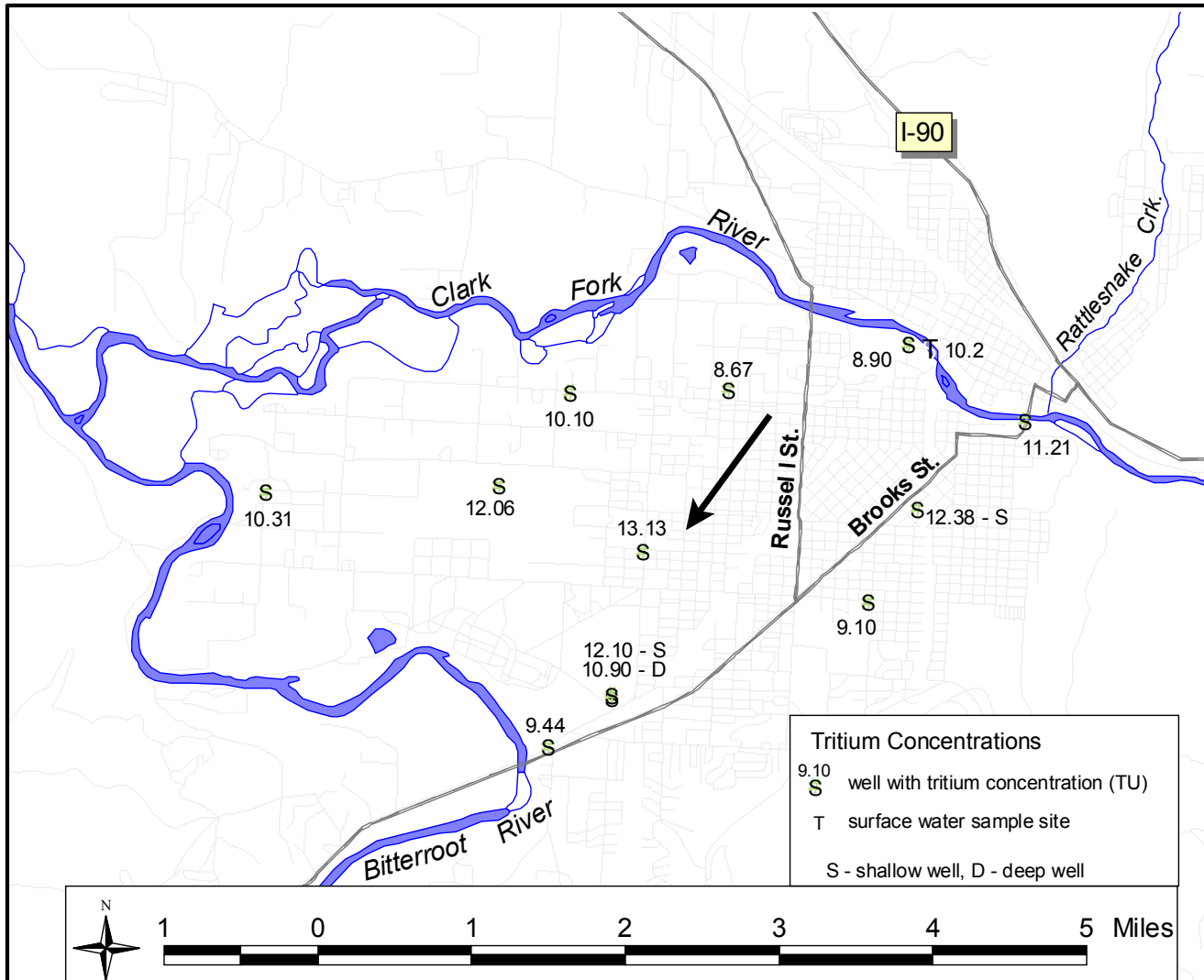
Figure 20. Tritium concentrations are fairly uniform through the aquifer. Arrow shows the direction of ground-water flow.
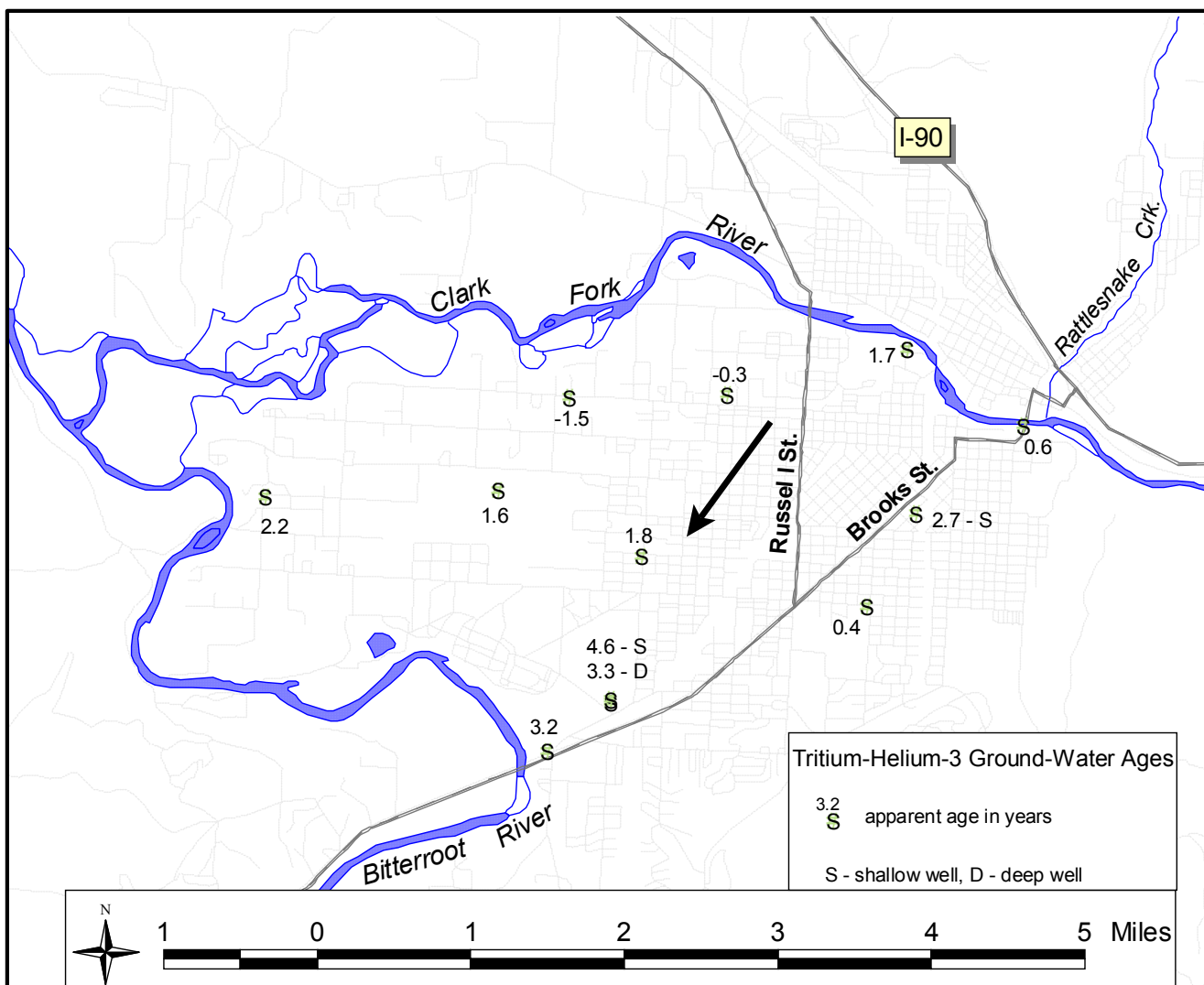
Figure 21. In general, the ^{3}H/^{3}He apparent ages increase along the ground-water flow path (arrow). For an explanation of negative ages see text.
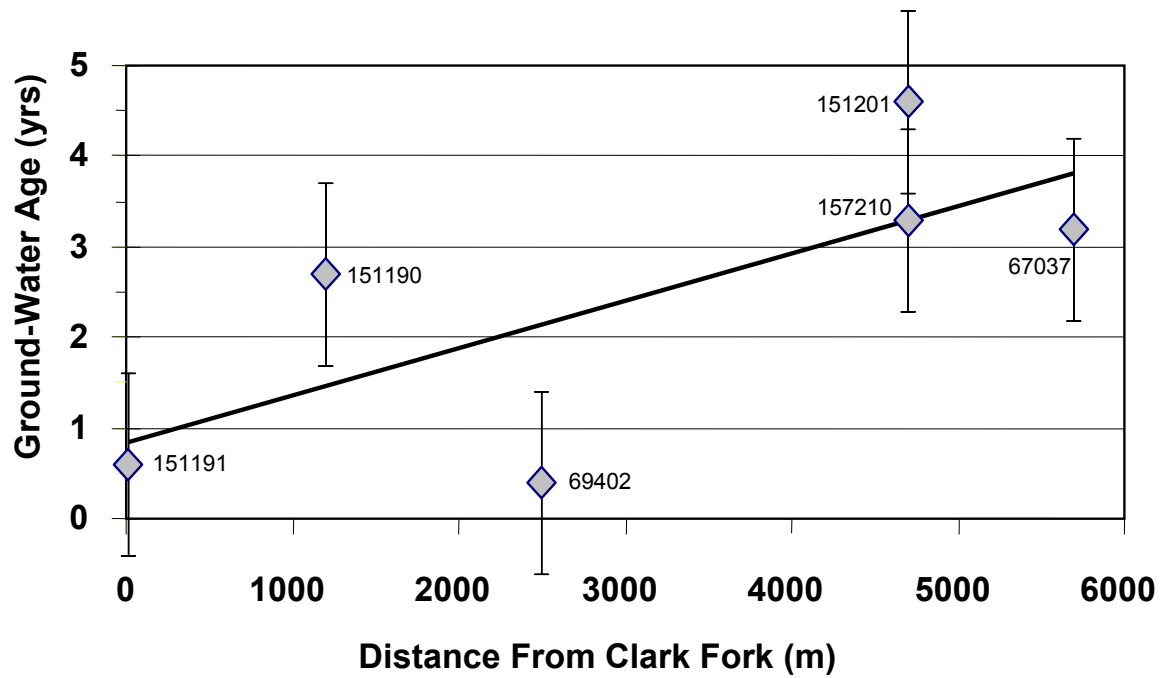
Figure 22. In general, apparent $^3H/^3He$ ages increase with distance from Clark Fork River along the east transect. GWIC identification numbers and error bars are shown.
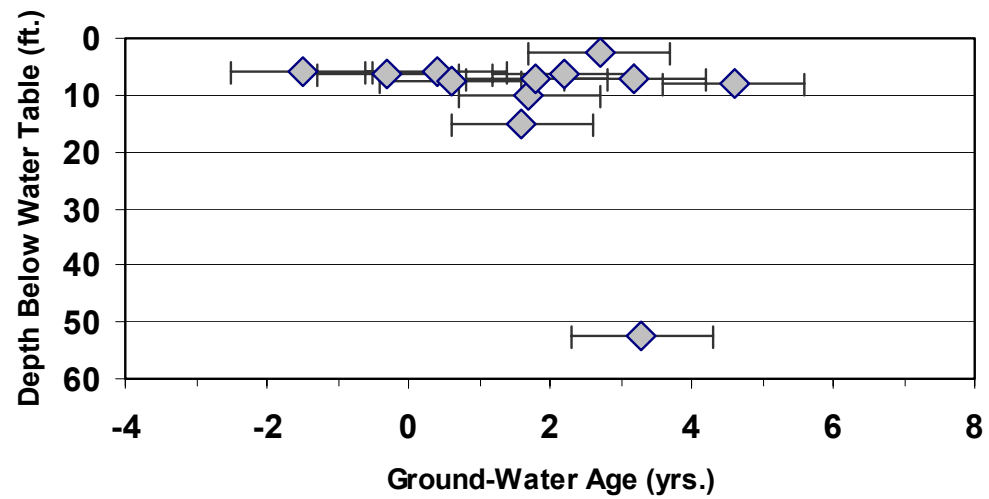
Figure 23. Most of the samples for ^{3}H/^{3}He age dating were collected within 10 feet of the water table, there were no apparent trends in ground-water age with depth. Error bars are shown.
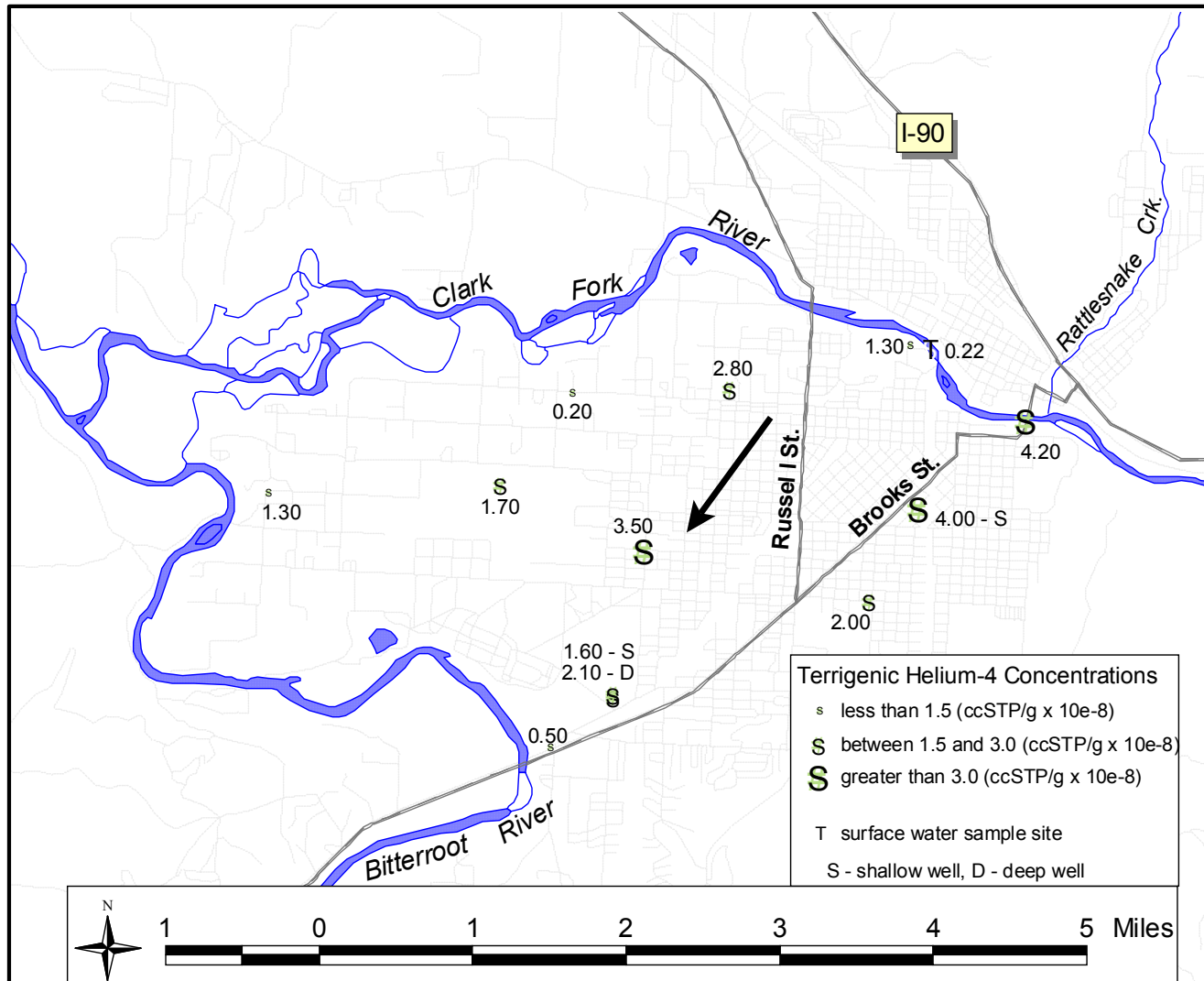
Figure 24. Elevated concentrations of terrigenic ^{4}He occur in the upgradient part of the flow system. Arrow shows the direction of ground-water flow.
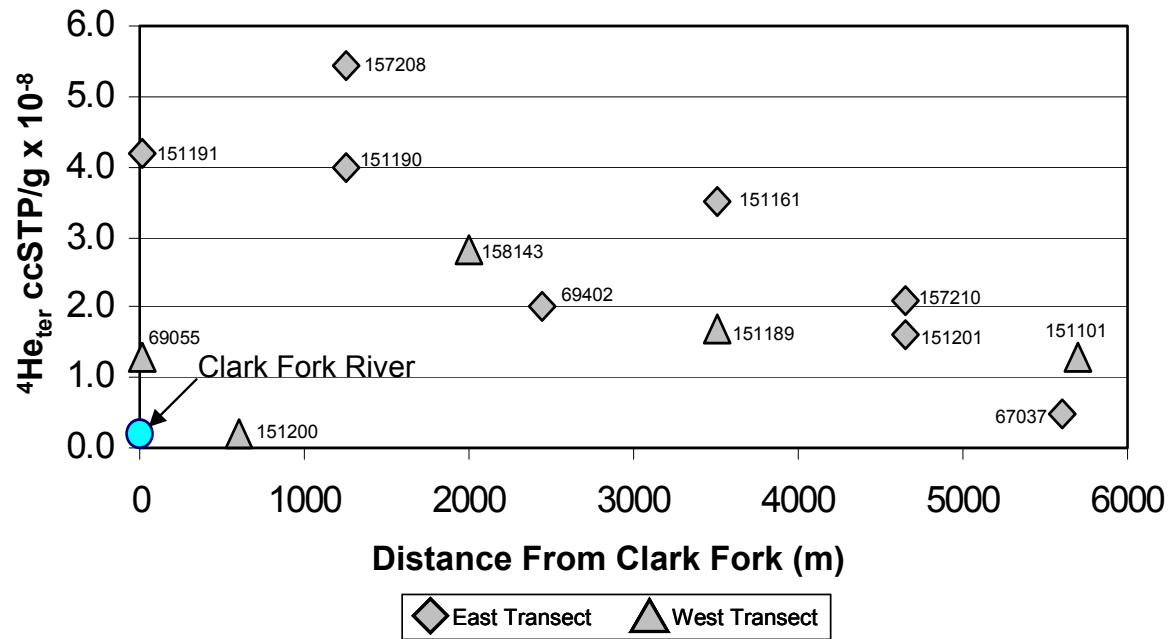
Figure 25. Plot of terrigenic ^{4}He with distance from the Clark Fork River. In general, terrigenic ^{4}He decreases with distance from the Clark Fork, down flow path. GWIC identification numbers are shown.
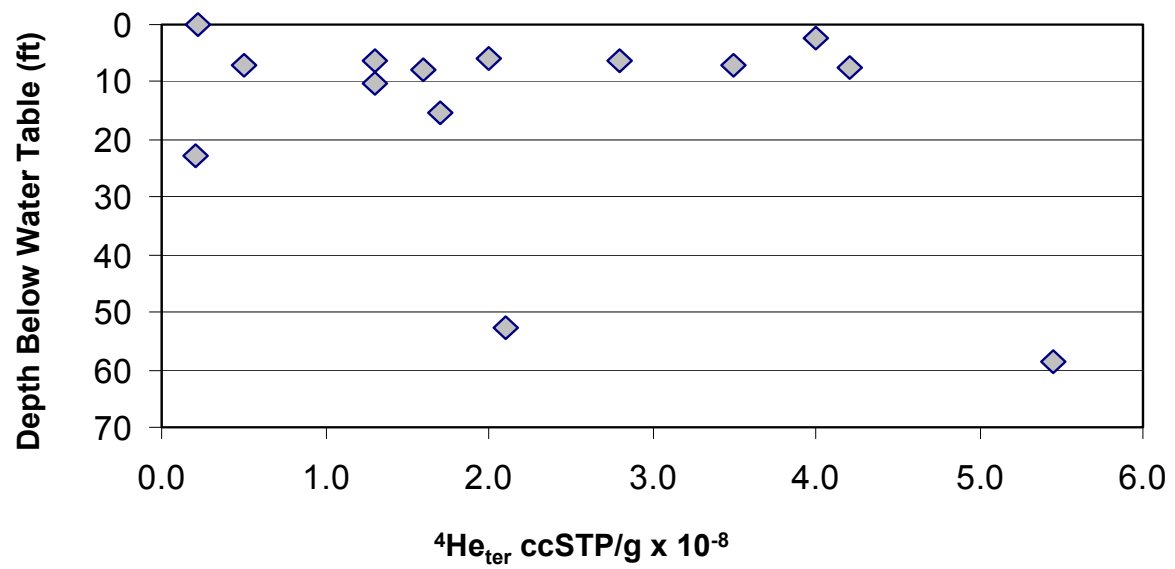
Figure 26. There are no clear trends in the terrigenic ^{4}He concentrations with depth below the water table.
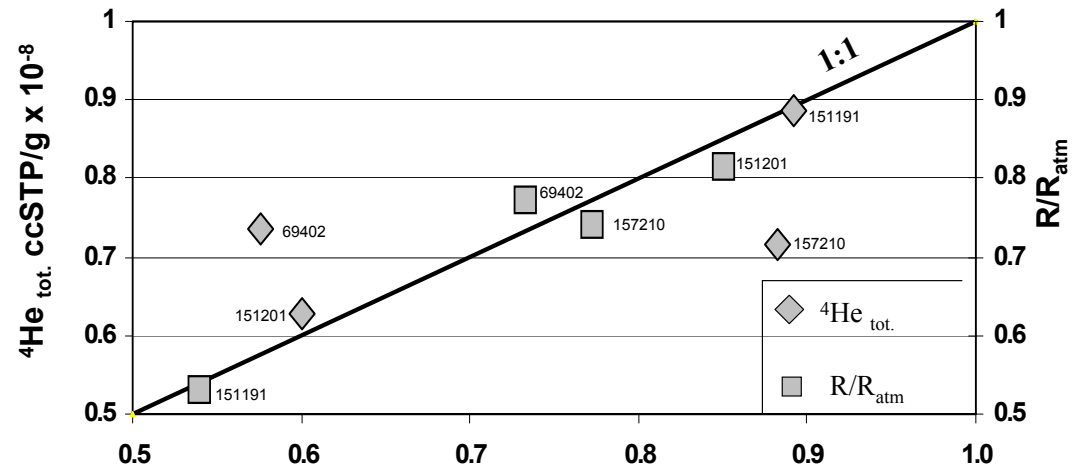
Figure 27. There is good agreement between the results from the four wells that were resampled. Helium isotope ratios are expressed as R/R_a, where R is the $^3He/^4He$ ratio of the sample and R_a is the $^3He/^4He$ ratio of the air standard (1.36×10^{-6}). GWIC identification numbers are shown.

Basic Information

Title:	Enhancement of Fuel Oxygenate (MTBE) Biodegradation Potential in Groundwater
Project Number:	2000MT9G
Start Date:	9/1/1998
End Date:	9/30/2001
Research Category:	Water Quality
Focus Category:	Groundwater, Toxic Substances, Treatment
Descriptors:	Bacteria, Biodegradation, Bioindicators, Contaminant Transport, Groundwater Quality, Natural Attenuation, Petroleum Additives, Solute Transport, Soil Microbiology, Underground Storage Tanks
Principal Investigators:	Al Cunningham , Richard Harold Veeh , Jeffrey A. Kuhn , Paul Sturman

Publication

1. Veeh, Richard and Eric Kern, 2000, Enrichment, isolation, and identification of MTBE-degrading bacteria. Underground Tank Technology Update 14(5):6-8.
2. Kern, E. A., R.H. Veeh, H.W. Langner, R.E. Macur, and A.B. Cunningham. 2002. Characterization of methyl tert-butyl ether (MTBE)-degrading bacteria from a gasolinecontaminated aquifer. Bioremediation Journal 6(2):113-124.
3. Lang,Claus, 2000, Evaluating Transport and Biodegradation of MTBE in a Gasoline Contaminated Aquifer using Groundwater Flow and Transport Modeling, MS, Environmental Engineering, University of Stuttgart, Germany.

Title: Enhancement of Fuel Oxygenate (MTBE) Biodegradation Potential in Groundwater

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    Co-P.I.s Richard Veeh, Paul J. Sturman, MSU/CBE, Jeff Kuhn, MT Dept of Environmental
    Quality, and Stephen Jester, Conoco Inc.

1.0 Abstract

        MTBE is degraded very slowly in the subsurface relative to other gasoline constituents.
Previous research reports indicate that indigenous microbial populations may biodegrade MTBE
and TBA at very low rates and after extended acclimation periods. Therefore, dissolved MTBE
plumes in groundwater may impact larger areas compared to other gasoline constituents. The
relative recalcitrance of MTBE combined with its high water solubility make it the single largest
threat to ground water quality resulting from gasoline releases. Research team members
collected field data and used chemical and environmental parameters to model degradation of
MTBE and plume dynamics at a gasoline release site in Ronan, Montana. All pertinent
historical, hydrogeological, and geochemical data were reviewed, and a limited characterization
of the site microbial population was performed. This characterization focused on enrichment,
isolation, and identification of bacterial strains and consortia that could biodegrade MTBE and
its metabolites (e.g. TBA). This study included an educational component, in-depth scientific
and engineering components, and a political/management component. Under the auspices of a
graduate level class in Environmental Engineering (educational component) and with assistance
and guidance from regulatory professionals within the Montana Department of Environmental
Quality (political/management component), research conducted by this project supports the
following statements:
- A bacterial consortium capable of MTBE biodegradation is present in the subsurface at the
  Ronan, MT gasoline release site.
- MTBE-degrading isolates degraded MTBE at a higher rate in the presence of a co-substrate
  (2-propanol), indicating a possible relationship to MTBE-degrading, propane-oxidizing
  bacteria. MTBE metabolites, TBA and TBF, accumulated as MTBE was biodegraded.
- Genotypic characterization of the MTBE-degrading consortium indicated that at least five
  bacterial species initially contributed to MTBE degradation. However, over time under the

selective pressure of MTBE as the sole carbon source, it appeared that *Rhodoferax fermentans* became the dominant species. Also, no metabolites were observed.

- Although an MTBE-degrading consortium was obtained from the site, the relatively low MTBE degradation rate observed in the laboratory indicates that microbial biodegradation is of minor importance to overall natural attenuation of MTBE in the field.
- Modeling efforts were somewhat successful in predicting MTBE plume dynamics.

Research advances made through this project have provided a mechanism to assess the biodegradation potential at another MTBE release site near Manhattan, MT. Enrichment cultures for MTBE-degradation from this site were initiated using protocols established with samples from the Ronan site. Experimental data suggest that there is also an MTBE-degrading consortium present at the Manhattan release site.

2.0 Research Objectives

The primary goal of this research project was to assess the biodegradation potential of MTBE and to determine what environmental factors may be limiting MTBE degradation at the gasoline release site near Ronan, MT. This work included a review of pertinent hydrogeological and geochemical data and characterization of any MTBE-degrading microbial species that could be identified and/or isolated through enrichment cultures. The objectives of this multi-faceted study included an educational component, in-depth scientific and engineering components, and a political/management component. Educational aspects of the project involved field site assessment, data collection, and transport modeling experience for interdisciplinary students enrolled in an MSU environmental engineering class. The political component of the project was designed to bring students into direct communication with consulting engineers and Montana Dept. of Environmental Quality (MDEQ) personnel both in the field and by presenting their findings in a workshop format of open discussion of logical management options. The MTBE release site was identified through the MDEQ-Underground Storage Tank Program as a threat to a pristine surface water receptor. In order to meet the overall goal, the research was planned to meet the following four general objectives:

I. Site Data Review
- Review of existing data from the Ronan, MT site to establish release history, groundwater hydrogeology, geochemistry, availability of potential electron acceptors, free product occurrence, and evidence of natural attenuation. This information was used to enhance interpretation of laboratory results and to allow site-specific recommendations for remedial action.

II. Field Sampling and Data Collection
- Collection of groundwater and/or soil samples from existing or subsequently installed monitoring well locations. Establishment/confirmation of existing plume dimensions. Samples were indexed and cross-referenced with geochemical conditions present at each sampling location.
- Analysis of site groundwater data for evidence of natural attenuation of MTBE, degradation products, and other petroleum compounds.

III. Laboratory Experimentation and Modeling
- Development and/or substantiation of gas chromatographic methods for analysis of MTBE and its known metabolites for monitoring MTBE degradation in enrichment cultures.
- Development of aerobic MTBE-degrading enrichment cultures using microcosms with minimal growth media and composite soil samples as microbial inocula.
- Identification of possible nutrient limitations (e.g. nitrogen, phosphorus, dissolved oxygen) and environmental limitations (e.g. temperature) to MTBE biodegradation at the field site.
- Identification of possible hydrocarbon co-substrates (e.g. propane) that may enhance MTBE biodegradation.
- Isolation and identification of specific MTBE/metabolite-degrading bacteria.
- Application of BIOSCREEN and MODFLOW models to determine predictive capabilities regarding existing plume dimensions, contaminant concentrations, etc.

IV. Education and Technology Transfer
- Organization of components of the laboratory and field research into a graduate level class in Environmental Engineering.
- Development and delivery of annual workshops/discussions on MTBE fate and transport in groundwater.

3.0 Methodology

Site Characterization

The release site is located at a fuel station east of U.S. Highway 93, one mile south of Ronan, MT. A 16,000-gallon underground storage tank removed in April, 1994 was estimated to have released approximately 10,000 gallons of gasoline over a one-year period. Historical data were retrieved to estimate initial MTBE concentration in the gasoline to aid in subsequent plume modeling efforts by students involved in the engineering class. The Montana Department of Environmental Quality established 20 monitoring wells at the site to identify plume boundaries, and eight additional wells were installed in 1997 for free product removal. A thorough review of reports containing several years of quarterly monitoring well data was performed. These reports originated from consultants (MSE; Butte, MT) for the Montana Dept. of Environmental Quality and contained complete gasoline contaminant concentrations as well as other pertinent chemical analyses, including dissolved oxygen.

The maximum depth to groundwater is approximately 15 ft. at the point of release. The dominant lithology identified from bore holes was silt and fine sand with scattered clay lenses, typical of lakebed deposits common to the area. Active remediation at the site included a combination of passive recovery skimmers, air sparging systems coupled with soil vapor extraction, and an interceptor trench.

Enrichment Study Inocula

Soil and aquifer materials used as inocula for MTBE-degrading bacterial enrichments were collected from two bore holes drilled to a depth of 1 m below the groundwater surface. One bore hole was near the down-gradient edge of the NAPL-phase gasoline plume at M-12 and the second was adjacent to M-19 near Spring Creek (the impacted surface water receptor). Samples were retrieved from above, at the interface with and below the groundwater surface, and were stored at 4ºC for one week prior to inoculation in enrichments. A pristine agricultural soil (Bozeman, MT) was also used as an inoculum in several "control" enrichments.

Enrichment of MTBE-degrading bacteria

Degradation experiments were conducted in closed 125-mL Erlenmeyer flasks under aerobic conditions. The flasks contained 25 mL of a minimal medium (SSE) designed to simulate a "typical" soil solution and contained NH_4NO_3 (1.25 mM), $CaSO_4$ (2 mM), $MgCl_2$ (2 mM), KH_2PO_4 (10 uM), KOH (1.25 mM), $FeCl_2$ (5 uM), supplemented with 100 uL L^{-1} of micronutrient solution. Subsamples of the composite soil/aquifer material were used as inocula (1% w/v) for the MTBE-degrading microcosms. The slurries were spiked with 10 mg L^{-1} MTBE

and ~ 100,000 dpm flask^{-1} [^{14}C]-MTBE. Radiolabeled $^{14}CO_2$ evolved from the slurries was captured and used to track MTBE degradation. Solutions of 0.5 M NaOH (0.3 mL) were utilized as $^{14}CO_2$-traps and were placed in cups suspended from the stoppers. The enrichment traps were analyzed weekly for $^{14}CO_2$ (scintillation analysis), and the aqueous phase was monitored for MTBE disappearance using gas chromatography (GC). Treatments were tested in triplicate and compared to autoclaved controls that contained 250 mg L^{-1} HgCl$_2$.

Due to headspace losses of MTBE by volatilization during sampling of the base traps, subsequent kinetic degradation experiments with the consortium RS24 were performed in 120-mL serum bottles with sealed teflon-coated septa. MTBE was introduced into 40 mL of SSE medium at concentrations ranging from ~10-70 mg L^{-1} and 2-mL samples were aseptically taken for GC monitoring of MTBE degradation. At each sampling time, 3 mL of sterile air was also injected into the serum bottle headspace to maintain aerobic conditions.

Gas chromatography

Gas chromatographic analysis of 2-propanol, MTBE, TBA, and TBF was conducted using a Hewlett-Packard 5890 Series II gas chromatograph with a FID detector. A Porapak PS 80/100 mesh packed column (2 mm I.D. x 1.22 m glass; Supelco, Bellefonte, PA) was used with a He carrier gas flow rate of 18 mL min^{-1} at 100ºC and a head pressure of 28 psi. The injector temperature was set at 190ºC and the detector temperature was 250ºC. The initial oven temperature was set at 100ºC for 4 min. and ramped at 10ºC min^{-1} to a final temperature of 200ºC. 2-propanol, TBA, MTBE, and TBF peaks showed retention times of approximately 2.7, 4.5, 6.3, 8.3 min., respectively.

Microbial cell counts

0.5-mL samples of MTBE-degrading cultures were placed above a 0.2-μm, 25 mm-dia. black polycarbonate membrane (Poretics Products; Livermore, CA) clamped in a filter chimney assembly and vacuum manifold apparatus. A 100-μL aliquot of 10 mg L^{-1} DAPI was added, allowed to stain the cells for 2 min. and rinsed 2-3 times to remove any unbound stain. The direct counts were conducted using a Nikon Plan Apo100x/1.40 oil lens (DIC H, WD 0.13, ∞/0.17) and a Nikon Eclipse E800 epifluorescence microscope with a mercury lamp UV source. The filter block used had an excitation bandwidth of 340-380 nm and an emission bandwidth of 435-495 nm. Total cell counts were based on a calibrated ocular grid, calculated total membrane area, and an average of 20 enumerated grid fields.

Isolation of MTBE-degrading bacteria

Soil-derived, MTBE-degrading enrichments were sampled and spread on both R2A and Noble agar (with basal salts) plates. Half of the Noble agar plates also received surface-applied treatments of 50 μL of 20 mg L^{-1} 2-propanol prior to inoculation with bacteria. All of the Noble agar plates were incubated in closed metal containers and exposed to MTBE vapors by diffusion from MTBE-saturated filter paper. Single colonies from both types of plates were purified through repeated transfer under identical cultivation conditions. Colonies chosen for isolation were based primarily on differences in colony color and size. Isolated colonies were used to

inoculate 120-mL serum bottles containing 40 mL of SSE amended with 10 mg L^{-1} MTBE. 2-propanol (20 mg L^{-1}) was added as a cosubstrate in selected treatments. Gas chromatography was used to monitor MTBE disappearance and accumulation of degradation products.

Nucleic acid extraction, polyerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), and sequence analysis

Cell lysis and DNA extraction protocols were based on established methods. Briefly, cells were lysed by mechanical disruption at 6.5 m s^{-1} for 45 s using a beadbeater (Savant Instruments, Qbiogene; Carlsbad, CA). The crude cell lysates were precipitated with 12M ammonium acetate, and the DNA in the resulting supernatant was precipitated with isopropanol. PCR was performed using primers shown to be highly conserved for numerous bacterial lineages, including the proteobacterial, Gram-positive, cyanobacterial, green nonsulfur, and *Cytophagales-Flavobacterium-Bacteriodes* lineages; and they amplify a hypervariable-containing rDNA region from nucleotide positions 1070 to 1392, based on the 16S rRNA sequence in *E. coli*. DGGE was performed using a 6-11% concentration gradient of acrylamide in addition to a urea/formamide gradient. DGGE bands were purified for sequence analysis, and band purity was confirmed by comparing the PCR products on a DGGE gel to the parent community. Sequencing of isolate DNA and DGGE bands was performed on an ABI Prism 310 Genetic Analyzer using a 47-cm capillary (PE Applied Biosystems, Foster City, CA) and Prism BigDye terminator cycle sequencing reaction kits (PE Applied Biosystems, Foster City, CA).

MTBE plume modeling

MTBE plume modeling efforts by engineering students involved the use of two groundwater models, BIOSCREEN and MODFLOW. Inputs to the models included parameters such as initial MTBE gasoline concentration, partitioning coefficients, groundwater flow rates, contaminant solubility, biodegradation rates, and amounts of free product associated with the site. Although some of these parameter values were based on laboratory experimental data, some of the parameter values were estimated.

4.0 Research Results

Soil-derived enrichments

A 12-treatment matrix of enrichment cultures amended with 10 mg L^{-1} MTBE (cold) and ^{14}C-MTBE as described was established to assay the presence of MTBE-degrading bacteria at the gasoline-contaminated site. The treatment matrix consisted of three inoculum types (M-12, M-19 and pristine) and two levels of nutrient amendment (1x and 10x SSE) (6 treatments). In addition, 2-propanol was added to an additional set of 10x SSE-treated inocula (3 treatments), and there was one set of killed controls (3 treatments). One flask (RS24) inoculated with soil from M-12 and amended with 2-propanol displayed significant MTBE degradation compared to sterile controls and other treatments. After an initial lag time of approximately 15 days, ^{14}CO$_2$ analyses indicated a maximum of 33% MTBE degradation after 35 days. Other treatments displayed only minimal degradation over the 35-day trial relative to the sterile controls. Analysis of aqueous phase ^{14}C-MTBE revealed significant losses from all enrichments, apparently due to

6

headspace losses of volatilized MTBE while sampling the base traps. To minimize these losses, all subsequent experiments were performed in sealed serum bottles.

MTBE degradation by consortium RS24

During a subsequent 146-day period, the initial MTBE-degrading enrichment was enriched further (10% v/v inoculum transfer) with 14 mg L^{-1} MTBE and approximately 20 mg L^{-1} 2-propanol. Interestingly, the 2-propanol had been completely utilized after 7 days and small TBA and TBF peaks were evident. At the end of the second week, the TBA and TBF peaks were still evident; however, by the end of the third week no metabolite peaks were observed. Over this 3-week period, the MTBE concentration had decreased to 12 mg L^{-1} and continued to decrease steadily at a rate of about 0.1 mg L^{-1} d^{-1}. After this 146-day trial, another transfer (10% v/v) was made and amended with 6 mg L^{-1} MTBE without the addition of 2-propanol. An extended lag time (~18 days) was again observed before significant degradation occurred. However, with subsequent addition of MTBE on days 40, 47 and 53 the onset of degradation became increasingly rapid. As spiked amendments of MTBE increased from 10.3 mg L^{-1} to 14.7 mg L^{-1} to 66.4 mg L^{-1}, no significant decrease in degradation rate was observed. Beginning on day 47, MTBE disappearance was monitored daily and MTBE degradation rates of 0.15 mg d^{-1} and 0.66 mg d^{-1} were calculated from days 47-53 and days 53-57, respectively. Again, no metabolite peaks were observed in any of the chromatograms. In addition, the culture became noticeably more turbid over this time period suggesting microbial growth was coupled to MTBE degradation. To correlate MTBE disappearance with microbial growth, bacterial cell density was calculated for each sampling time from direct microscopic counts as described above. Increasing bacterial cell density during MTBE degradation in duplicate transfer cultures (10% v/v) of RS24 was observed. A gross estimate of biomass yield based on maximum MTBE utilization rates represented in the graphs was calculated. Assuming an approximate dry mass of 2×10^{-10} mg $cell^{-1}$, a yield of 0.25 ± 0.02 mg dry biomass mg $MTBE^{-1}$ was observed.

A significant lag phase was repeatedly observed upon transfer of active MTBE-degrading cultures of RS24 to fresh medium. However, in re-spiking active cultures with MTBE no lag time was observed. To determine if a growth-related factor in the medium may be involved in the degradation of MTBE by RS24, degradation experiments were conducted with spent culture medium and fresh SSE mineral medium. Two actively growing duplicate MTBE-degrading cultures of RS24 were centrifuged at 8,000 rpm for 10 min at 4ºC. The supernatant (i.e. spent medium) was decanted and saved and the cell pellets were re-suspended in 3 mL of 1x PBS. Aliquots (1 mL) from each cell suspension were used as inocula for duplicate cultures with 40 mL spent growth medium, and 2-mL aliquots were used as inocula for duplicate cultures with 40 mL of fresh SSE medium. The four new cultures were amended with MTBE to a final concentration of 32 ± 6 mg L^{-1}. MTBE degradation with corresponding increases in cell density for the cultures with spent growth medium was observed. In contrast, the cultures with fresh SSE both show minimal MTBE degradation and much lower cell densities over the 60-day trial period. It should be noted that the average initial bacterial counts for duplicate cultures with fresh SSE (2.85×10^{6} cells mL^{-1}) were 2-fold greater than for duplicate cultures with spent media (1.39×10^{6} cells mL^{-1}).

Isolation of MTBE-degrading bacteria

Thirteen isolates were cultivated from the original MTBE-degrading enrichment. MTBE degradation kinetics were determined for each isolate in the presence and absence of 2-propanol. Degradation of MTBE was more rapid and complete in the presence of 2-propanol for all isolates tested. Since many isolates had similar colony morphologies and MTBE degradation kinetics, nucleotide sequence analysis of a 340-bp region of the 16S rRNA gene was performed to identify unique isolates. Of 9 isolates characterized, four isolates were 100% identical to *Pseudomonas* sp. Ant9 and five isolates were 100% similar to *Rhodococcus koreensis*. Figure 6 shows degradation kinetics of MTBE in the presence and absence of 2-propanol for two of the isolates identified as *Pseudomonas* sp. Ant9 and *Rhodococcus koreensis*. MTBE degradation by both isolates was minimal in the absence of 2-propanol as a cosubstrate. GC monitoring of isolates grown with 20 mg L^{-1} 2-propanol revealed an initial decrease in 2-propanol concentration prior to the onset of MTBE disappearance, indicating a probable cometabolic induction of MTBE degradation. Two metabolites observed during MTBE degradation with both isolates in the presence of 2-propanol were identified by GC/MS analysis as TBA and TBF.

Molecular characterization of MTBE-degrading consortia

DGGE profiles of RS24 and two successive subcultures taken from this consortium were generated. Sequence analysis of the DGGE bands revealed a phylogenetically diverse group of bacteria present in the consortium. A decrease in the total number of bands in the profiles was observed with successive subculturing. DGGE bands identified as *Pseudomonas* sp. Ant9 and *Rhodococcus koreensis* showed 100% sequence similarity to the two cultivated MTBE-degrading isolates identified above. Interestingly, the *Rhodococcus koreensis* band disappeared completely by the second subculture, while the band corresponding to *Pseudomonas* sp. Ant9 decreased in intensity with subculturing. However, due to bias inherent in the PCR, differences in DGGE band intensity may not reflect population abundance in the consortium. The band corresponding to *Rhodoferax fermentans* was present in the original consortium and appeared stable in both subcultures. After numerous attempts, bands A and B could not be sufficiently purified for sequence analysis. Bands H1 and H2 are believed to be heteroduplex molecules formed during the PCR reaction, as purification and re-amplification of both bands always yielded H1 and H2 in addition to band A and the band corresponding to *Rhodoferax fermentans*.

MTBE plume modeling

MTBE plume modeling using BIOSCREEN and MODFLOW were somewhat successful in describing the plume dynamics observed at the site. Value uncertainty for many of the input parameters to the models no doubt resulted in sub-optimal performance. Nevertheless, this study did show that transport modeling was a valuable tool for describing general plume behavior. Results from the modeling effort are contained in Lang, 2000, which is a diploma thesis submitted to the University of Stuttgart, Stuttgart Germany.

Annual seminars and discussions

In December of each year for the duration of the project, meetings were held either in Bozeman, MT or Helena, MT in which students presented the results of their work to site consultants and Montana DEQ personnel in a seminar format. These seminars ranged from topics including hydrogeology, biodegradation, modeling, toxicity, etc. Following the presentations, discussions were held among the students and other official representatives to clarify results and to receive input in guiding further research efforts.

5.0 Discussion

Enrichments designed to simulate MTBE and nutrient concentrations present in the aquifer revealed a diverse group of bacteria capable of degrading MTBE. Initial screening of gasoline/MTBE-contaminated soils from two monitoring well locations yielded a single enrichment capable of degrading MTBE in the presence of 2-propanol. Maintenance and transfer of this enrichment resulted in a bacterial consortium that could degrade MTBE at relatively high rates in the absence of 2-propanol as a cosubstrate. In this ultimate enrichment, cell growth coupled to MTBE degradation as the sole carbon source and no appearance of MTBE metabolites indicated the bacterial consortium derived energy from the complete catabolism of MTBE. Calculated gross biomass yields for this latter consortium of RS24 (~0.25 mg dry biomass mg MTBE^{-1}) were generally similar to the estimated biomass yield of 0.18 mg cells mg MTBE^{-1} and 0.21-0.28 mg dry biomass mg MTBE^{-1} reported by other researchers. A previously reported bacterial strain capable of MTBE degradation was identified as a member of the Beta subgroup of Proteobacteria by 16S rRNA analysis. In our study DNA sequencing of a gel band of 16S rDNA amplified by PCR and separated by DGGE identified *Rhodoferax fermentans* as the organism probably most responsible for MTBE degradation in consortium RS24. Interestingly, the genus *Rhodoferax* also belongs to the Beta subgroup of Proteobacteria. Although in latter cultural transfers of RS24 without 2-propanol no TBA or TBF metabolites were observed during MTBE degradation, earlier cultures of RS24 displayed both TBA and TBF metabolites in the presence of 2-propanol. Also, accumulation of TBA was observed early in this study when one RS24-transferred culture was grown in the presence of 10 mg L^{-1} benzene and 20 mg L^{-1} 2-propanol. Because both TBA and TBF were always observed during MTBE degradation by the isolates, *Pseudomonas* sp. Ant9 and *Rhodococcus koreensis*, in the presence of 2-propanol and because these isolates seemed to be more prominent in DGGE gels of the earlier RS24 consortia, we believe that these two isolates are probably limited to cometabolic MTBE-degradation and eventually became subordinate to *Rhodoferax fermentans* under our experimental conditions. However, others have reported degradation of 28-29% of 200 mg L^{-1} MTBE as the sole carbon source by three pure culture isolates over a two-week period (degradation rate ≈ 0.2 mg d^{-1}); and one of these isolates was identified as *Rhodococcus* sp. Also, they observed a reduction in MTBE degradation by this bacterium in the presence of butyl formate, *t*-butanol, and several other simple organic compounds. These results and results from our study showing an accumulation of TBA and TBF by *Rhodococcus koreensis* may indicate an inhibitory effect caused by accumulation of these metabolites.

The production of TBA and TBF support a previously proposed MTBE degradation pathway in which the enzyme, cytochrome P-450, oxidizes MTBE to *tert*-butoxymethanol, which then may be converted to TBF via an alcohol dehydrogenase. The TBF may then undergo hydrolysis to TBA. This latter hydrolysis step was also proposed by other researchers; however,

they suggested that the initial formation of TBF may simply be a strict chemical oxidation by atmospheric oxygen. We believe our results do not support chemical oxidation as a major MTBE degradation step in that no TBA or TBF peaks were observed in our sterile controls in the presence or absence of 2-propanol.

The fact that no lag phase in MTBE degradation was observed for transferred cultures grown in spent supernatant suggests the presence of a factor in the medium that allowed uninterrupted MTBE degradation to occur. We propose that this mechanism may possibly involve either a repressor-regulated transcriptional system similar to that of the *lac* operon or activator-dependent regulation. In the first case, a constitutively expressed repressor protein would prevent transcription of genes involved in MTBE degradation. Presumably, production or activation of an inducer protein could be initiated by the presence of MTBE or an effector protein. Release of the inducer over time would increase its concentration and effect more binding to and release of the repressor protein and, thus, allow transcription of the MTBE degradation genes. This type of mechanism has already been proposed for other genes involved in contaminant biodegradation. For example, a model has been proposed for dichloromethane (DCM) degradation by *Methylobacterium* sp. in which production of the protein DcmA (responsible for DCM dehalogenase production) is normally repressed by binding of the regulatory protein DcmR to the *dcmA* promoter. De-repression (i.e. activation) of *dcmA* occurs by putative substrate-induced release of DcmR. Also, in another study research showed that the XylR protein has both repressor and activator roles in the control of toluene biodegradation by *Pseudomonas putida*. Undoubtedly, some of these regulatory mechanisms are strictly intracellular in nature. However, we speculate that extracellular release of regulatory proteins acting as autoinducers such as those already identified in other types of quorum sensing and cell-cell communication would confer a selective advantage. For example, other researchers have suggested that quorum sensing may prevent unnecessary gene induction, allow a rapid sensing of changes in the environment, and serve to coordinate consortial metabolism among bacterial species. At the very least, we believe that this type of concentration-dependent phenomena could result in observations of MTBE degradation such those documented in this study.

Although several MTBE-degrading bacterial species were cultured from the Ronan aquifer, MTBE biodegradation in the aquifer is difficult to assess in relation to other processes of natural attenuation. However, because of the generally low quarterly dissolved oxygen (DO) measurements and low temperatures (4-12ºC), we concluded that biodegradation is relatively slow at the site. This conclusion is based on another study in which a low concentration of dissolved oxygen and low temperatures severely slowed MTBE degradation rate and growth rate of the mixed, MTBE-degrading bacterial culture. The low DO levels are likely due to seasonal fluctuations in the groundwater of the region as well as to oxygen utilization coupled to contaminant degradation. For example, DO measurements from the first to the fourth quarters of 1999 were consistently less than 0.5 mg L^{-1} for MW-10. However, for the first quarter of 2000 a DO measurement of 3.3 mg L^{-1} was recorded. Over this same time period for the same monitoring well, MTBE measurements first increased over an order of magnitude to 3,580 μg L^{-1} and then gradually decreased again to < 300 μg L^{-1}. Likewise, TPH measurements increased almost two orders of magnitude to 10,900 μg L^{-1} and then gradually decreased to < 900 μg L^{-1}. Because background DO levels obtained from MW-11 upgradient of the contaminant plume as well as from MW-6 and MW-16 (adjacent to MW-12 but outside the contaminant plume) were

consistently between 4-6 mg L^{-1}, contaminant biodegradation inside the plume is indicated. However, specific evidence of MTBE biodegradation is confounded by the presence of other potential gasoline-related substrates; and no detection of MTBE metabolites has been recorded. Because measured DO levels indicate that oxygen may be limiting biodegradation at this site, we recommend that any applied treatment technology should investigate and/or include oxygen addition, although other important inorganic nutrients such as nitrogen and phosphorus may also be limiting the activity of MTBE-degrading bacteria. One report of enhanced MTBE degradation by injection of pure oxygen into an impacted aquifer supports the above recommendation.

This study has established the presence of bacteria capable of degrading MTBE in the gasoline-contaminated aquifer near Ronan, MT. Other studies targeting the identification of native bacterial populations with the capacity to degrade MTBE in natural environments will help to establish the ubiquity of these organisms and allow better prediction of the efficacy of biodegradation as a means to remediate MTBE-contaminated aquifers. In addition, further laboratory studies designed to elucidate factors that influence MTBE degradation, such as co-substrates or the concentration-dependent factor proposed in this study, may provide insight into more efficient methods of stimulating MTBE degradation by naturally occurring bacteria.

6.0 Publications

Veeh, Richard and Eric Kern. 2000. Enrichment, isolation, and identification of MTBE-degrading bacteria. Underground Tank Technology Update 14(5):6-8.

Kern, E. A., R.H. Veeh, H.W. Langner, R.E. Macur, and A.B. Cunningham. 2002. Characterization of methyl *tert*-butyl ether (MTBE)-degrading bacteria from a gasoline-contaminated aquifer. Accepted for publication in the July, 2002 issue of The Bioremediation Journal.

Lang, Claus. 2000. Evaluating Transport and Biodegradation of MTBE in a Gasoline Contaminated Aquifer Using Groundwater Flow and Transport Modeling. Diploma Thesis, University of Stuttgart, Stuttgart Germany, 28, July, 2000.

7.0 Student Support

Funding for this research project supported all or part of the following students/degrees:

- Claus Lang, PhD, University of Stuttgart, Germany, July 2000
- Elsa Meiser, MS Environmental Engineering, Montana State University
- Approximately 20 students were involved in Dr. Al Cunningham's Environmental Engineering Investigations class (ENVE 534) at Montana State University over a 3-year period. These students received travel reimbursement for visits to the Ronan site for sampling and other data collection. As part of the course requirements, students generated reports on topics related to the site and its contaminants including site characterization, transport modeling, partitioning, biodegradation, natural attenuation, and health risk assessment. These students interacted with consulting engineers from MSE (Butte, MT) and

with Montana Dept. of Environmental Quality personnel in the field and also presented their academic reports at annual meetings involving these and other state personnel.

8.0 Acknowledgments

Basic Information

Title:	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"
Project Number:	2001MT241B
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Descriptors:	stamina, westslope cutthroat trout,radio telemetry
Principal Investigators:	Alexander V. Zale

Publication

DETERMINATION OF THE MAXIMUM-WEIGHT TELEMETRY TRANSMITTER
THAT CAN BE IMPLANTED IN WESTSLOPE CUTTHROAT TROUT WITHOUT
AFFECTING SWIMMING PERFORMANCE: A CHALLENGE TO THE "2% RULE"

FINAL REPORT

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Abstract

We empirically determined the relationship between radio transmitter weight and swimming stamina in telemetered westslope cutthroat trout in the laboratory to facilitate field studies of movements and behavior of this rare native fish. Telemetry studies of small fish are limited primarily by transmitter battery life, which is a function of battery weight. Untested dogma holds that transmitter weight should not exceed 2% of the total body weight of a telemetered fish. We found that telemetry transmitters comprising up to about 5% of total body weight had only minor effects on swimming stamina and growth of westslope cutthroat trout. No threshold beyond which performance deteriorated markedly was observed. Researchers need not be bound strictly by the old "2% rule-of-thumb" but should bear in mind that any increase in transmitter weight will impair physiological performance of telemetered fish, albeit perhaps only slightly. Any benefits of increased transmitter weight should be balanced against such physiological impairments.

Problem and Research Objective

Westslope cutthroat trout *Oncorhynchus clarki lewisi* historically inhabited streams of the upper Missouri River Basin, including the Gallatin and Madison river drainages. Westslope cutthroat trout currently occupy only about 27% of their historical range in Montana. Competition with introduced trout species has contributed to this decline, but the specific mechanisms responsible for displacement are not well understood. Loss of genetic integrity through hybridization is a major problem where westslope cutthroat trout and introduced rainbow trout *O. mykiss* occur sympatrically. Various State and federal agencies are working to maintain and restore westslope cutthroat trout within their native range in Montana.

Genetic sampling of putative westslope cutthroat trout by the National Park Service in streams of the upper Missouri River Basin in the northwest corner of

Yellowstone National Park revealed that only one stream in the Park, the North Fork of Fan Creek in Montana, contains a genetically pure population. The genetic purity of the North Fork Fan Creek population is surprising, considering that the site is not isolated by a physical barrier (e.g., an insurmountable waterfall) preventing invasion by non-native fishes from downstream. This suggests that the population is reproductively isolated either temporally or spatially. That is, the westslope cutthroat trout in this population either spawn in different places or at different times than introduced rainbow and Yellowstone cutthroat trout in this system. Determination of the exact mechanism responsible for this reproductive isolation is essential to maintaining the purity of this population and for duplicating this phenomenon in other streams where pure westslope cutthroat trout are being reintroduced or managed in Montana. Accordingly, we are studying spawning timing and movements of this population.

The primary means by which spawning timing and location are documented is by radio telemetry. Fish are implanted with radio transmitters prior to spawning and followed to their spawning sites. A problem with this technique is that westslope cutthroat trout in small headwater streams are invariably small (≤ 100 g) and therefore cannot be implanted with large transmitters that transmit for long durations; transmitter life is a function of battery size. Conventional wisdom suggests that weight of transmitters should not exceed 2% of the body weight of fish to preclude any effects on swimming performance, survival, and behavior (Winter 1983), but this rule-of-thumb is based on no hard evidence. Trials conducted with very small rainbow trout (5-10 g) suggested that greater transmitter weights may be acceptable (Brown et al. 1999). If we followed the "2% rule," maximum transmitter weights that could be used in Fan Creek westslope cutthroat trout would be 2 g, with maximum transmission durations of about 100 days. However, 4-g transmitters would transmit for about 280 days, and therefore allow earlier implantation, more time for recovery after surgery before spawning, and monitoring of post-spawn movements to determine habitats used during subsequent seasons.

Our objective was to determine empirically the relationship between transmitter weight and swimming stamina in westslope cutthroat trout. Understanding this relationship would allow implantation of the maximum weight transmitter that would not affect the physiological performance of a telemetered fish and thereby gain the maximum amount of telemetry information from that fish. Such information could be used to facilitate restoration and management of this native salmonid.

Methodology

Test fish were obtained from the Montana Fish, Wildlife & Parks Washoe Park Hatchery in Anaconda, which maintains a pure stock of westslope cutthroat trout (Strain M012, Lot M010199Z), and transferred to the Wild Trout Research Laboratory on the Montana State University campus in Bozeman for testing. They were maintained for 2 weeks prior to transmitter implantation in a 465-L fiberglass tank supplied with oxygenated and filtered 13 °C water from by the laboratory's recirculating process system; the water turnover time in the tank was 20 minutes. Experiments were conducted in a swimming stamina tunnel at the laboratory supplied with the same water source. The fish chamber of the tunnel had an inner diameter of 108 mm and a length of 132 cm. It was powered by a 2-horsepower jacuzzi pump. Swimming stamina tests are a convenient and accepted means to assess the physical condition of fish and are a good indicator of stress (Wedemeyer et al. 1990).

The fish were 22 months old at the time of transmitter implantation and averaged 240 mm in total length and 132.8 g in weight (Tables 1 and 2). They were randomly assigned to 7 groups including a control group (no surgery, no transmitter), a group on which surgery was performed but no transmitter was implanted (sham surgery), and 5 groups surgically implanted (into the peritoneal cavity) with transmitters weighing 1, 2, 3, 4, or 5 g. No significant difference existed among mean initial weights of the treatment groups (P=0.7427; Figure 1). Initial transmitter burdens, expressed as percent body weight, ranged from a mean

of 0.77% in the 1-g treatment group to 4.05% in the 5-g treatment group (Table 3). We used standard surgical implantation techniques (Winter 1983) and closed incisions with surgical staples. The transmitters were non-functional facsimiles made of lead shot imbedded in epoxy; their size, shape, and density mimicked those of commercially manufactured radio transmitters with coiled-loop antennae (no trailing external antenna). All fish except controls were injected with a passive integrated transponder (PIT) tag to permit individual identification. Several fish died during or immediately after surgery and were discarded. Numbers of fish in each treatment group one day after implantation were as follows: control 15, sham 14, 1-g 14, 2-g 15, 3-g 14, 4-g 17, and 5-g 15. Following surgery, fish were allowed to recover for 2 weeks in the holding tank prior to initial performance testing. A second trial was conducted 6 weeks after implantation. Fish were fed a standard trout feed (Silver Cup) at a rate of 1% of body weight per day.

Fish were tested in the stamina tunnel individually at a water velocity of 90 cm/second. Preliminary trials indicated that this velocity would be sensitive to differences among individuals (i.e., times to fatigue were not so short that differences were indistinguishable) but also insured that exhaustion would be achieved within 10 minutes. After introduction of the fish to the tunnel, the water velocity was instantaneously elevated to the test velocity and a timer was started. The fish was identified with a PIT-tag reader while in the tunnel. The challenge was terminated when the fish stopped swimming and permanently came to rest against the tail grate of the tunnel. The fish was then expelled from the tunnel by removing the tail grate and weighed and measured. Fish were immediately returned to the holding tank after the 2-week trials and sacrificed and dissected after the 6-week trials to verify transmitter weights and presence.

Times to fatigue were compared among treatment groups using analysis of variance. Regression analysis was used to discern if a relationship existed between times to fatigue and relative transmitter burdens. Controls, but not sham-

surgery fish, were excluded from the regression analysis to preclude confounding. Statistical analyses were performed using SAS software.

Principal Findings and Significance

Several fish died during the first 2 weeks after transmitter implantation and before the first stamina trial. These included one control, two sham-surgery, one 3-g treatment, and three 4-g treatment fish. In addition, one 3-g treatment fish expelled its transmitter through its implantation incision, which had failed to heal properly. These fish were excluded from further analyses. Numbers of fish in each treatment group in the 2-week stamina trials were as follows: control 14, sham 12, 1-g 14, 2-g 15, 3-g 12, 4-g 14, and 5-g 15.

No significant difference existed among the mean times to fatigue of the different treatment groups 2 weeks after implantation (P=0.1234; Figure 2). Fish with 2-g or larger transmitters tended to tire faster than fish with 1-g or no transmitters, but the difference was not significant and did not become more pronounced with increasing transmitter burden (Figure 2). Regression analysis of time to fatigue versus transmitter burden 2 weeks after implantation indicated a negative relationship; each percent increase in tag-to-body weight ratio resulted in a 5.0% decrease in time to fatigue. However, the relationship was not significant (P=0.0957) and explained only 3.6% of the variability among individuals (Figure 3). Fish with similar transmitter burdens tired over a wide range of times.

No significant difference in mean weights existed among the treatment groups 2 weeks after implantation (P=0.2992; Table 2, Figure 1). However, a significant negative relationship existed between mean daily individual growth rates during the first 2 weeks after implantation and relative initial transmitter burdens (P=0.0344); the relationship explained 5.6% of the variability among individuals (Figure 4). Each percent increase in tag-to-body weight ratio resulted in a 13.4% decrease in growth rate.

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One control fish died between the 2-week and 6-week stamina trials. Transmitters were lost through ruptured incisions by one 1-g, two 3-g, and one 4-g treatment fish, and single 3-g and 5-g treatment fish expelled their transmitters through their vents. These fish were excluded from further analyses. Numbers of fish in each treatment group in the 6-week stamina trials were as follows: control 13, sham 12, 1-g 13, 2-g 15, 3-g 9, 4-g 13, and 5-g 14.

No significant difference existed among the mean times to fatigue of the different treatment groups 6 weeks after implantation, but the differences approached significance (P=0.0541; Figure 2). Fish with 2, 3, and 4 g transmitters tended to tire faster than fish with no, 1-g or 5-g transmitters. Regression analysis of time to fatigue versus transmitter burden 6 weeks after implantation indicated a negative relationship that approached significance (P=0.0682) but explained only 4.6% of the variability among individuals (Figure 3). Each percent increase in tag-to-body weight ratio resulted in a 5.6% decrease in time to fatigue.

Mean weights of tested fish (Table 2, Figure 1) were not significantly different among treatment groups 6 weeks after implantation (P=0.2639). However, a significant negative relationship existed between mean daily individual growth rates between the two stamina trials and relative initial transmitter burdens (P=0.0098) that explained 8.2% of the variability among individuals (Figure 4). Each percent increase in tag-to-body weight ratio resulted in a 10.3% decrease in growth rate. A similar relationship existed between growth rates during the full 6-week period and initial relative transmitter burdens (P=0.0014, r^2=0.1217); growth decreased 11.6% with each 1% increase in tag-to-body weight ratio.

The principal conclusion of our study is that telemetry transmitters comprising up to about 5% of total body weight had only minor effects on swimming stamina and growth of westslope cutthroat trout. No threshold beyond which performance deteriorated markedly was observed. Researchers can therefore likely double the size of transmitters recommended by the old "2% rule-of-thumb" apparently without

drastically impairing physiological performance of telemetered fish. This increase in transmitter size substantially lengthens the durations that telemetered fish can be tracked, thereby increasing the quantity and utility of data each telemetered fish yields at only slightly increased cost (for larger batteries) and only slightly decreased physical status. In the case of small, headwater-dwelling westslope cutthroat trout, this trade-off may be acceptable and warranted. However, we strongly caution against exceeding 5% of body weight because our study did not evaluate effects beyond this level. Furthermore, our findings suggest that any incremental increases in transmitter-to-body weight ratio, including those below 2%, may have deleterious effects on telemetered fish. Therefore, researchers should always choose the lightest possible transmitters that allow study goals to be achieved.

We failed to determine the maximum weight transmitter that could be implanted in westslope cutthroat trout without eliciting clearly deleterious effects because we did not challenge fish with an excessively high transmitter burden. Whether swimming stamina would decline gradually or drop precipitously beyond some threshold transmitter burden is unknown. Additional trials with larger transmitters or smaller fish are needed therefore. We also recommend using more fish in stamina trials of this type because individual variability in stamina is high; larger sample sizes would enhance detection of genuine effects.

Publications and Citations

None thus far, but we intend to submit a manuscript on this work to a major fisheries science journal shortly.

Student Support

Ms. Carrie Brooke, a M.S. graduate student in the Fish & Wildlife Management Program in the Department of Ecology at Montana State University, conducted the implantations and stamina trials. She thereby gained valuable training in experimental design, laboratory techniques and protocols, and statistical analyses.

In addition, the findings allowed her to implant larger transmitters in the westslope cutthroat trout she is studying for her thesis research.

Notable Achievements and Awards

Our findings have been communicated informally among fisheries scientists in Montana and the region. Researchers are already using heavier transmitters in current telemetry studies based on our results. Our study has thereby already enhanced the ability of fisheries scientists and managers to investigate fishery problems related to fish migration, movements, and behavior.

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Table 1. Mean total lengths (mm) and standard deviations and ranges of total lengths of each treatment group of westslope cutthroat trout at time of transmitter implantation and 2-week and 6-week stamina trials.

Treatment	Implantation	2 Weeks	6 Weeks
Control	240.3 15.6 210-265	242.1 14.1 215-265	250.4 16.0 220-280
Sham	240.0 16.2 210-260	246.2 13.7 225-265	252.5 11.8 235-270
1 gram	244.3 22.0 210-280	244.3 22.6 210-285	251.2 22.2 220-290
2 gram	242.7 14.5 220-275	242.8 14.4 225-270	246.1 16.7 220-275
3 gram	237.1 13.0 220-260	237.5 13.2 220-255	246.1 8.6 230-255
4 gram	238.5 11.7 220-255	237.1 14.0 210-260	242.3 16.3 215-270
5 gram	236.5 14.6 220-265	234.6 13.1 220-265	239.6 16.3 220-270

Table 2. Mean total weights (g) and standard deviations and ranges of total weights of each treatment group of westslope cutthroat trout at time of transmitter implantation and 2-week and 6-week stamina trials.

Treatment	Implantation	2 Weeks	6 Weeks
Control	128.1 26.1 81.3-165.6	134.8 20.5 98.5-167.2	163.7 26.6 107.9-201.8
Sham	135.1 26.0 85.3-181.2	152.8 21.5 114.9-201.0	178.4 27.1 133.8-229.3
1 gram	140.0 41.3 85.0-206.9	149.3 43.2 90.8-217.6	175.6 43.5 112.3-236.3
2 gram	140.4 26.2 110.5-202.7	147.9 32.3 113.2-214.5	163.4 34.8 113.5-234.3
3 gram	125.8 22.9 94.6-165.2	135.4 23.5 104.6-171.6	158.5 23.3 125.3-198.2
4 gram	130.5 21.5 90.2-175.4	136.4 22.1 106.2-186.7	149.8 32.5 100.7-204.7
5 gram	129.8 33.4 93.9-199.9	130.8 25.1 99.1-188.8	155.5 29.4 108.0-210.5

Table 3. Mean ratios (%) of transmitter weights to body weights and standard deviations and ranges of these ratios of each treatment group of westslope cutthroat trout at time of transmitter implantation and 2-week and 6-week stamina trials.

Treatment	Implantation	2 Weeks	6 Weeks
Control	0	0	0
	--	--	--
	--	--	--
Sham	0	0	0
	--	--	--
	--	--	--
1 gram	0.77	0.72	0.60
	0.22	0.20	0.16
	0.48-1.18	0.46-1.10	0.42-0.89
2 gram	1.46	1.41	1.27
	0.24	0.28	0.26
	0.99-1.81	0.93-1.77	0.85-1.76
3 gram	2.46	2.28	1.93
	0.44	0.40	0.28
	1.82-3.17	1.75-2.87	1.51-2.39
4 gram	3.15	3.00	2.79
	0.55	0.45	0.62
	2.28-4.44	2.14-3.77	1.95-3.97
5 gram	4.05	3.94	3.32
	0.83	0.68	0.62
	2.50-5.32	2.65-5.04	2.38-4.63
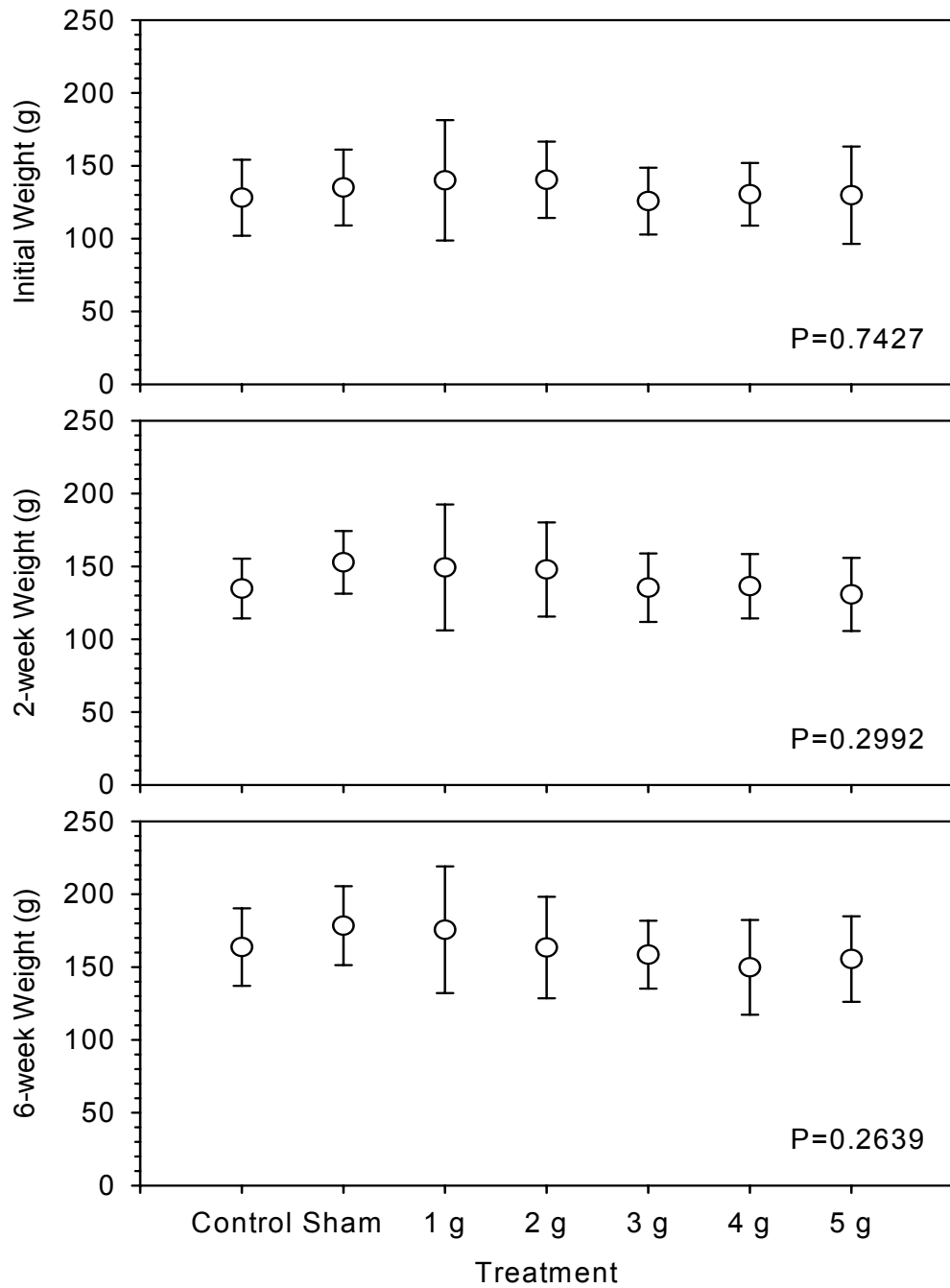
Figure 1. Mean body weights of westslope cutthroat trout implanted with transmitters of various weights (treatments) at time of surgery and 2 and 6 weeks after implantation. Error bars denote ±1 standard deviation.
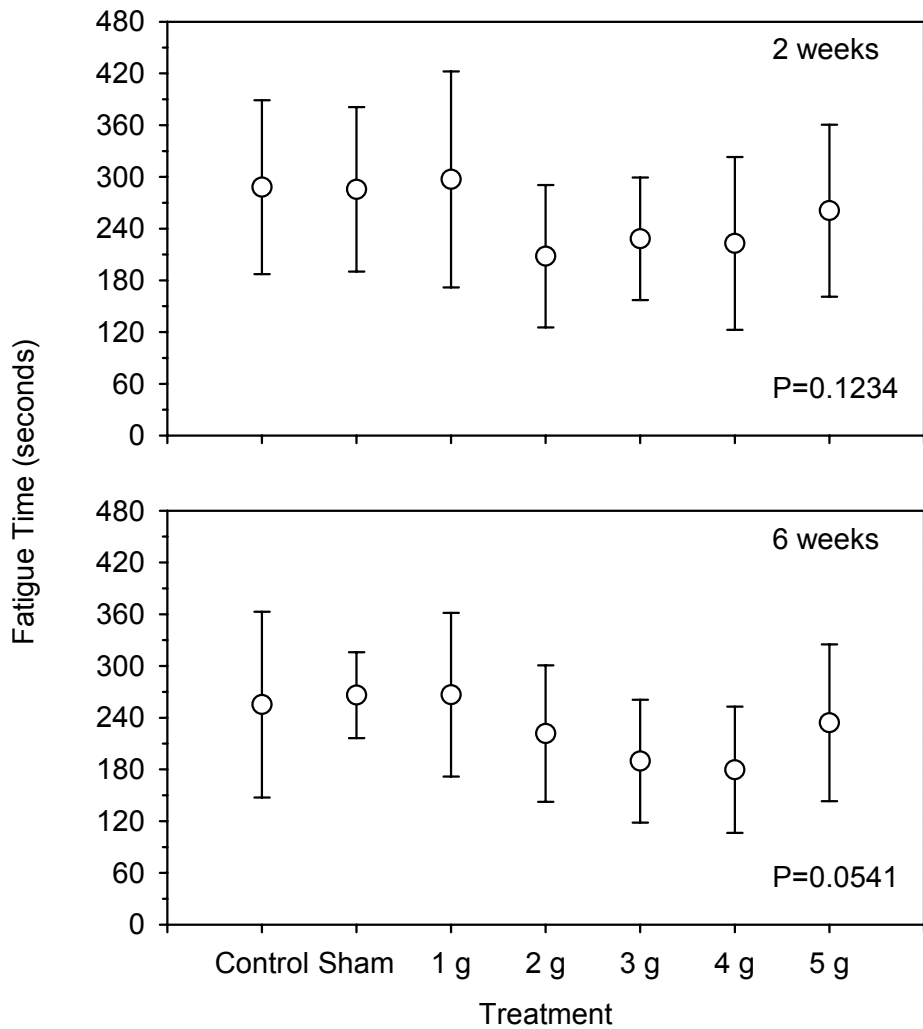
Figure 2. Times to fatigue at 90 cm/second water velocity of westslope cutthroat trout implanted with transmitters of various weights (treatments) at 2 and 6 weeks after implantation. Error bars denote ±1 standard deviation.
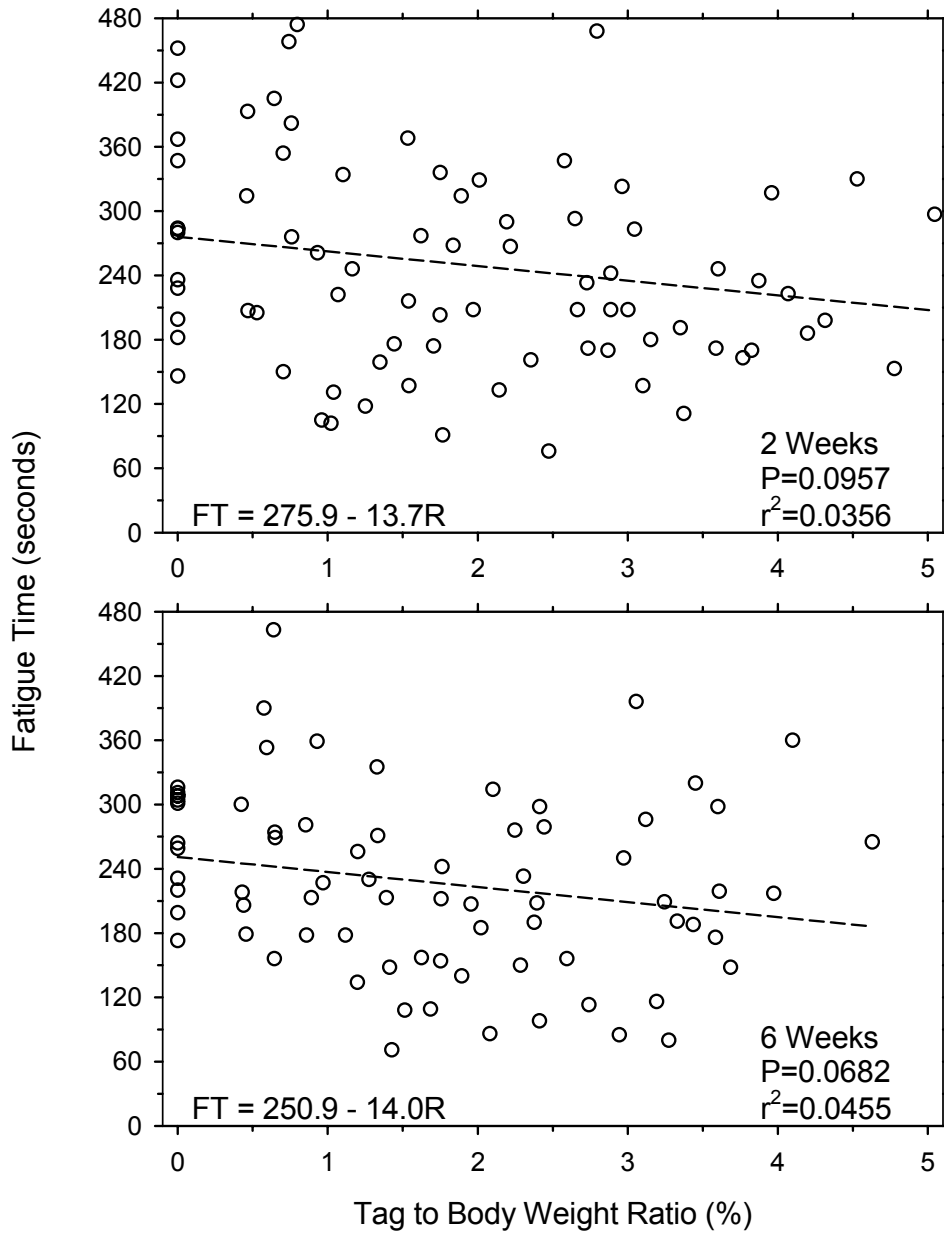
Figure 3. Times to fatigue at 90 cm/second water velocity of individual westslope cutthroat trout implanted with transmitters of various proportions of body weight at 2 and 6 weeks after implantation.
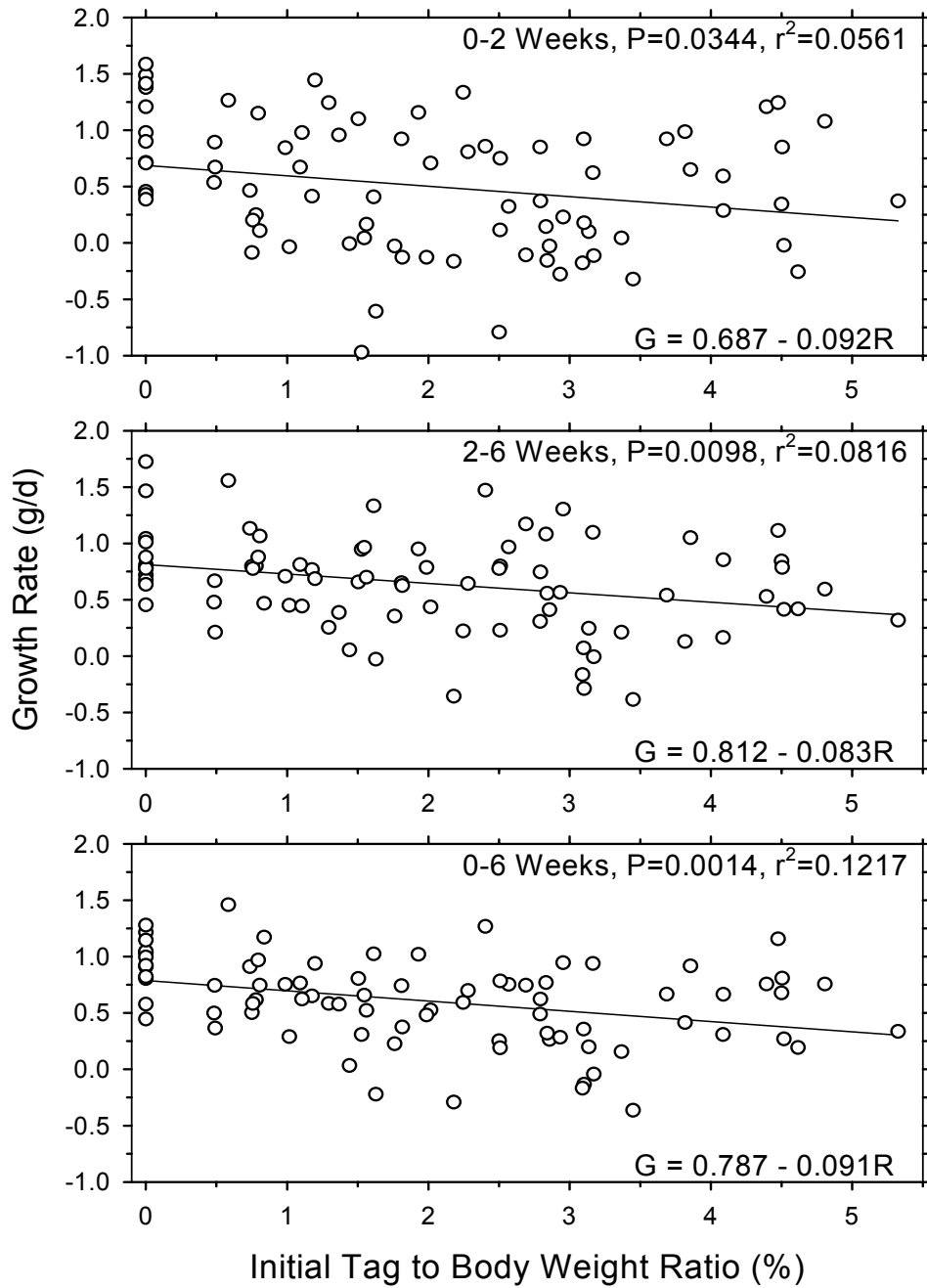
Figure 4. Daily growth rates during the first 2 weeks, 2 to 6 weeks, and all 6 weeks after implantation of individual westslope cutthroat trout implanted with transmitters of various proportions of body weight.

Basic Information

Title:	Enhanced Wet Air Oxidation of Sediment and Soil Contaminated with Recalcitrant Organic Compounds
Project Number:	2001MT261B
Start Date:	3/1/2001
End Date:	2/28/2002
Research Category:	
Focus Category:	Treatment, Toxic Substances, Sediments
Descriptors:	Treatment, Sediment, Wet Oxidation, Organochlorine compounds
Principal Investigators:	James E. Duffy

Publication

Enhanced Wet Air Oxidation of Sediment and Soil Contaminated with Recalcitrant Organic Compounds

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Abstract- Wet air oxidation (WAO) is investigated as a method of treating river sediments contaminated with polychlorinated biphenyls (PCBs). Aqueous slurries containing 2.5% (w/w) sediment were oxidized with oxygen in a one liter, high-pressure, batch reactor at temperatures up to 250^oC. Concentrations of PCBs adsorbed on the sediment and reactor surfaces and dissolved in the water and gas phases after oxidation were determined by high-resolution gas chromatography. Results indicate that no significant wet oxidation of PCBs in sediment slurries occurs for temperatures at or below 250^oC. However, during reactor heat-up, significant degradation of PCBs occurred at high temperature regions near the reactor wall even when bulk fluid temperature was quite low. The addition of a readily degraded organic compound did not result in enhanced degradation of PCBs through a kinetic coupling mechanism as has been observed for other recalcitrant organic compounds.

Introduction

Many coastal areas of the world have sediment that is contaminated with organochlorine compounds including polychlorinated biphenyls (PCBs). In the Great Lakes region alone, U.S. and Canadian environmental agencies have designated 43 areas of concern with heavy sediment contamination and just recently the U.S. EPA has ordered the dredging of 2.65 million cubic yards of sediment from the Hudson River that is contaminated with PCBs. Because of the hydrophobic nature of these compounds, they tend to bioaccumulate throughout the food web and, therefore, slow release of these contaminants to surface and groundwater provides a route for animal and human exposure. Many of these compounds (e.g. PCBs, DDT, pentachlorophenol, dioxins, and furans) are classified as hormonally active agents and significant prenatal exposure to these compounds from consumption of contaminated fish, meats, and dairy products can cause low birth weight, shorter gestation periods, IQ and memory deficits, and delayed neuromuscular development.[1]

Currently, approved methods for treating these sediments involve dredging, de-watering, and either depositing the sediment in an approved chemical waste landfill or incinerating the sediment. There is widespread public opposition to both of these approaches. Moreover, incineration of sediments contaminated with PCBs can be very costly. Other treatment methods may be approved on a case-by-case basis.

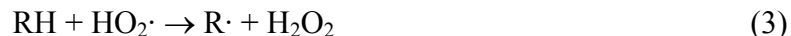Wet air oxidation (WAO) is a commercial process used to remediate aqueous waste streams containing organic solutes and to regenerate powdered activated carbon (US Filter/Zimpro PACT Process). Conventional wet air oxidation utilizes air or oxygen in conjunction with elevated temperatures and pressures to oxidize moderately contaminated waste streams. Fairly simple modifications to commercially available wet oxidation systems would

allow for the treatment of excavated sediment and soil following an initial screening to remove

large debris and a separation step (e.g. hydrocyclone) to remove sand particles. These larger

particles are not amenable to wet oxidation treatment but also are not expected to be

contaminated with high concentrations of adsorbed organic compounds. The resulting fine silt

and clay fractions, which are expected to contain the majority of contaminants, are amenable to

wet air oxidation. However, as determined in several studies, many of these contaminants

(including PCBs) are not effectively degraded by conventional wet oxidation except at

temperatures in excess of $300^{\circ}C$ and pressures in excess of 1500 psi.[2,3] These high temperatures

and pressures necessitate the use of very expensive materials, pumps, and air compressors.

Wet air oxidation of organic compounds proceeds via a free radical mechanism initiated

by the thermal reaction of the organic substrate with oxygen.[4]

$$RH + O_2 \rightarrow R\cdot + HO_2\cdot \tag{1}$$

This step, which is believed to be rate limiting for most compounds, is extremely slow for

chlorinated aromatics (e.g. PCBs and chlorinated benzenes) that do not contain other functional

groups. The free radicals formed by this initiation step subsequently react with oxygen and other

organic and inorganic compounds forming a variety of radical species including organic radicals

and hydroxyl radicals.

$$R\cdot + O_2 \rightarrow ROO\cdot \tag{2}$$

$$RH + HO_2\cdot \rightarrow R\cdot + H_2O_2 \tag{3}$$

$$RH + ROO\cdot \rightarrow R\cdot + ROOH \tag{4}$$

$$H_2O_2 \rightarrow 2HO\cdot \tag{5}$$

$$ROOH \rightarrow RO\cdot + HO\cdot \tag{6}$$

Hydroxyl radicals are highly reactive with PCBs with measured rate constants being close to those corresponding to the diffusion-limited reaction.[5,6]

Therefore, in wet oxidation processes, the degradation of recalcitrant compounds such as PCBs may be closely linked to the production of active radical species generated by the degradation of readily oxidized organic compounds or hydrogen peroxide. These "kinetic coupling" phenomena have been observed in the wet air oxidation of acetic acid and m-xylene in the presence of phenol and simple alcohols such as ethanol.[7-9] For example, in experiments reported by Willms et al., the rate of degradation of m-xylene in the presence of an equimolar concentration of phenol was more than one order of magnitude greater than the rate for m-xylene alone.[7] Birchmeier et. al. described a similar effect in studies of the oxidation of recalcitrant wet oxidation products (low molecular weight carboxylic acids) in the presence of phenol.[8] Initially rapid oxidation rates became much slower as unreactive products were generated. The oxidation rates of the recalcitrant compounds increased again when more phenol was added.

In this paper, we report the effects of various operational parameters and additives on the wet oxidation of sediments contaminated with PCBs from the Hudson River (NIST SRM 1939a). The hypothesis that we are testing is that the destruction of PCBs during wet oxidation is dependant on the generation of radical species, specifically hydroxyl radicals, either in solution or at the sediment/water interface.

Experimental Methods

All experimental systems and procedures were the same as reported previously by Duffy et al.[10] Briefly, a slurry of sediment in water (2.5 % w/w) was oxidized in a 1-L high-pressure reactor (Parr Scientific) using pure oxygen as the headspace gas as depicted in Figure 1. The

contaminated sediment (NIST SRM 1939a – PCBs in River Sediment) was originally obtained

from the Hudson River and contains 11.4% (w/w) organic matter and approximately 75 mg/kg

PCBs. Sulfuric acid was added to the slurry to achieve the desired initial pH of 2.6. Following

acidification of the slurry, the reactor was sealed, pressurized with oxygen, and heated to the

desired temperature (usually $250^{o}C$). The reactor was maintained at this temperature for the

desired period of time (usually 1 hour) and then rapidly cooled to room temperature. The

duration of a typical experiment including heat-up and cool-down was 110 minutes. Following

cool-down, volatilized PCBs in the reactor headspace were captured by sparging the headspace

gas through two bottles of acetone in series. An absence of PCBs collected in the second bottle

indicated that all PCBs were collected in the first bottle. The effluent slurry was then filtered and

PCBs in the water and sediment were extracted using liquid-liquid extraction with

dichloromethane and sonication extraction with acetone/hexane, respectively. All internal

reactor surfaces were rinsed twice with acetone. No PCBs were evident in the second rinsing

indicating that all PCBs on the reactor surfaces were collected with one rinse. All samples were

analyzed using high resolution gas chromatography. Details of the extraction and analysis

procedures were reported previously.[10]

Results and Discussion

Validation of Experimental and Analytical Methods: The experimental protocols for

extraction of the sediment were validated by comparing average recoveries from duplicate

extractions to concentrations of PCBs certified by NIST. Results of these experiments have been

previously reported and indicate that the methods utilized in these experiments resulted in

quantitative extraction of the analytes of interest as compared to the certified values.[10]

Control Experiments: For wet air oxidation to occur, both elevated temperature and an oxidant (usually oxygen) are required. Control experiments were performed to verify that PCB losses were not occurring through avenues other than by oxidation. Results of these experiments have been previously reported and indicate that losses of PCBs by routes other than oxidation were negligible.[10]

Effect of Stir Rate: The possibility that the rate of oxygen transfer may limit the reaction rate in wet oxidation systems has been reported.[11,12] The transfer of oxygen in a batch reactor system pressurized with oxygen is governed by the stir rate. Experiments were performed at different stir rates to determine any effects or limitations imposed by the mixing of the system. Multiple experiments were conducted at 1000 rpm to determine variability which is indicated by the error bars which represent one standard deviation. From these results (Figure 2), the system does not appear to be limited by mixing for stir rates of 500 rpm and above. All subsequent experiments were conducted at or above 700 rpm.

Partitioning of PCBs following Oxidation: The distribution of PCBs between the gaseous phase, aqueous phase, and sediment and reactor surfaces following cool-down is depicted in Figure 3. The individual congeners were chosen so as to provide one representative from each of the homologue groups in the range from two to seven chlorine atoms. For congeners containing only a few chlorine atoms, a substantial fraction partitioned to the aqueous and gaseous phases. This fraction decreased significantly for the more highly chlorinated congeners. In fact, congeners containing five or more chlorine atoms were not detectable in the gas phase. The distribution of congeners between the solid surfaces (sediment and reactor) and aqueous phase is consistent with published solid/water distribution ratio trends for individual congeners. In

6

subsequent experiments, PCBs collected from the gas and water phases, the sediment, and the reactor rinse were combined into one sample for analysis.

Effect of Temperature on the Rates of Destruction of PCBs: Temperature is an extremely important variable in the wet oxidation process. System pressure, reaction rates, the solubilities of organic compounds and oxygen, and the rates of desorption of organic compounds from particulate matter are all appreciably affected by temperature.[2-4,13-16]

The present studies of wet air oxidation of sediment contaminated with PCBs were conducted at temperatures ranging from ambient to 250°C. The percentages of selected individual congeners remaining after each experiment are depicted in Figure 4. For reaction temperatures above ambient, a significant fraction of the PCBs were destroyed. Since PCBs are recalcitrant to wet air oxidation at temperatures up to 300°C,[2] this result was initially very surprising. However, a study of PCB degradation as a function of reaction time at 250°C (Figure 5) indicates that all of the degradation occurs during reactor heat-up. Subsequent measurement of reactor wall temperature during heat-up (Figure 6) indicates localized wall temperatures well in excess of 400°C near the heating elements. After heat-up, wall temperatures stabilized at levels near that of the bulk fluid and no further degradation was observed. Therefore, we conclude that PCB degradation in all experiments was occurring only in very high temperature regions at or near the reactor wall and that oxidation of PCBs in regions with temperatures at or below 250°C is insignificant. The extensive degradation of PCBs during reactor heat-up does however indicate that the potential exists for significantly accelerating the wet oxidation of recalcitrant compounds at low bulk fluid temperatures and pressures by passing the solution or slurry over very hot surfaces.

Figure 4 also shows that di and tri-chlorinated congeners were much more effectively degraded as compared to their more highly chlorinated homologues. This behavior is likely the result of faster desorption and/or faster reaction of the less chlorinated congeners. Yang et al. investigated the extraction of PCBs from a heavily contaminated industrial soil. Analysis of their kinetic data indicates that in subcritical water at 250°C and 50 atm, congeners containing only a few chlorine atoms are rapidly extracted in less than 10 minutes whereas the more highly chlorinated congeners require up to 1 hour for complete extraction.[17] The PCB congeners containing only a few chlorine atoms also react more readily with hydroxyl radicals via a hydrogen abstraction mechanism. Sedlak et al. investigated the aqueous phase oxidation of PCBs using Fenton's reagent.[6] In these studies, relative reaction rates for the oxidation of individual congeners in an Aroclor 1242 solution were determined by competition experiments. These researchers demonstrated the existence of a linear relationship between the number of non-halogenated sites and the average reaction rate. For each chlorine atom added to the biphenyl structure, there is a 10% reduction in the reaction rate.

Efforts to Enhance Wet Oxidation Rates through Generation of Active Radical Species: As stated earlier, several authors have demonstrated that kinetic coupling can result in the accelerated degradation of a recalcitrant species when it is oxidized in the presence of a readily degraded species. An example of this kinetic coupling is the enhanced wet oxidation of organic species in the presence of hydrogen peroxide. Hydrogen peroxide readily decomposes at elevated temperature to form two hydroxyl radicals which can subsequently react with organic species present in solution or at solid/solution interfaces. The effect of hydrogen peroxide addition during wet oxidation of sediments contaminated with PCBs has been reported previously.[10] These experiments indicate that oxidation of PCBs is significantly accelerated in

the presence of hydrogen peroxide (Figure 7). In these experiments, hydrogen peroxide was added following heat-up to the designated temperature. For experiments without peroxide addition, all PCB degradation occurred during reactor heat-up as previously described. We believe that the increased oxidation is primarily the result of hydroxyl radical attack initiated by thermal decomposition of hydrogen peroxide.

Active radical species are also generated by wet oxidation of organic species. To test the hypothesis that readily degraded organic matter may generate the active radical species necessary for PCB destruction, aqueous solutions of phenol were added to the sediment/water slurry with a high-pressure pump following heat-up to 250°C. Phenol was chosen because it readily degrades at 250°C and has been shown to accelerate oxidation of other recalcitrant compounds.[7-9] Twenty milliliters of phenol solution with a concentration of 50 gm/L was pumped in at 10 mL/min for 2 minutes (total added phenol = 1 gm). No additional degradation of PCBs (beyond that occurring during heat-up) was observed in these experiments. Therefore, we infer that wet oxidation of phenol at 250°C does not produce highly active radical species (hydroxyl radicals) in concentrations sufficient to result in measurable co-oxidation of PCBs.

Transition metal catalysts (iron and copper salts) have also been shown in a number of studies to accelerate the oxidation of recalcitrant organic species. In a single experiment, copper sulfate was added to the sediment slurry at a concentration of 5 mM prior to wet oxidation at 250°C. A copper salt was chosen to test the effect of a homogenous catalyst because it has been shown to be catalytically active in a variety of situations.[3,13,18,19] No significant degradation of PCBs (beyond that occurring without catalyst during heat-up) was observed.

Conclusions

1. No significant degradation of PCBs occurs during wet air oxidation of contaminated sediments at temperatures at or below 250°C.

2. Significant degradation of PCBs was observed during heat-up periods in which the reactor wall reached temperatures well above 300°C in regions near the heating elements. This degradation is believed to occur on or near the wall surface.

3. Addition of hydrogen peroxide during wet oxidation of sediments results in significant degradation of PCBs even at the relatively low temperature of 125°C. This enhanced degradation is believed to occur via hydroxyl radical attack initiated by thermal decomposition of hydrogen peroxide.

4. Addition of phenol during wet oxidation of sediments at 250°C does not result in degradation of PCBs through kinetic coupling as has been observed for other recalcitrant organic species.

5. Addition of homogeneous copper catalyst does not accelerate the wet oxidation of PCBs at 250°C.

Student Support: Funding for this research was used to support Justin Ray who received a master's degree in chemical engineering based on the experimental work presented in this report.

Publications and Presentations: This work has not yet been published; however a similar version of this report will soon be submitted. The journal has yet to be determined. This work was presented at the 2002 Montana AWRA conference.

Other Notable Achievements: None.

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Figure 1. Schematic diagram of the reactor system.

Figure 2. Effect of reactor stir rate on PCB destruction. Conditions: temperature = 250°C; partial pressure of oxygen = 300 psi; initial pH = 2.6.

**Figure 3.** Distribution of PCBs between gas, liquid, and solid phases following WAO. Conditions: temperature = 225°C; initial pH = 2.6; partial pressure of oxygen = 300 psi; stirring rate = 720 rpm.

Figure 4. WAO of sediment slurries at temperatures of 25, 125, and 250°C. Conditions: stirring rate = 720 rpm; partial pressure of oxygen = 300 psi; initial pH = 2.6.

Figure 5. Effect of reaction time on PCB destruction. Conditions: temperature = 250°C; stirring rate = 1000 rpm; partial pressure of oxygen = 300 psi; initial pH = 2.6.

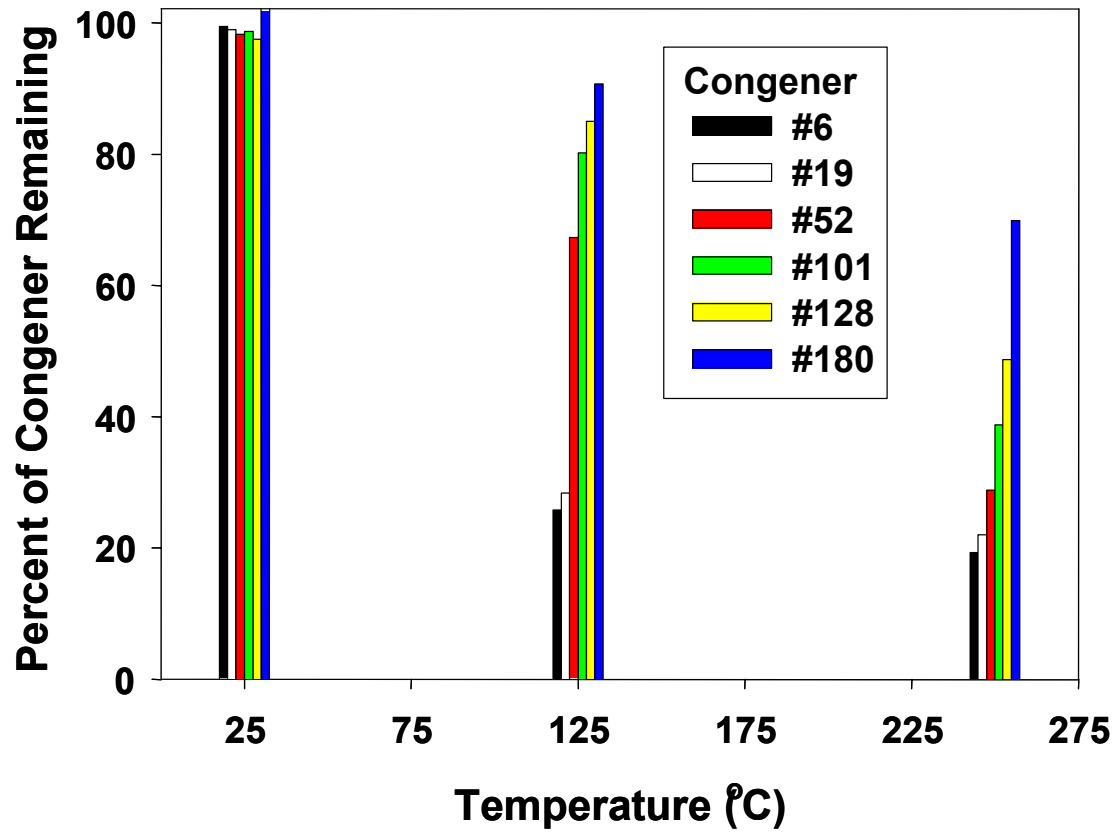**Figure 6.** Bulk fluid and heating band temperatures during WAO experiments.

Figure 7. PCB destruction during WAO with and without hydrogen peroxide addition.

Metering Pump

Reactor

O₂ Supply

Heating Band

Temperature Controller

Hotplate/stirrer
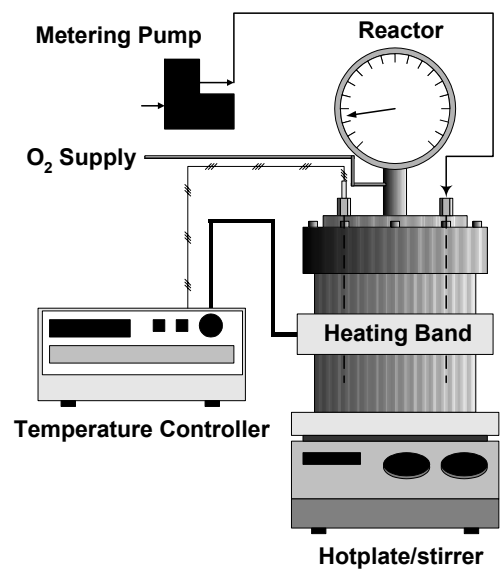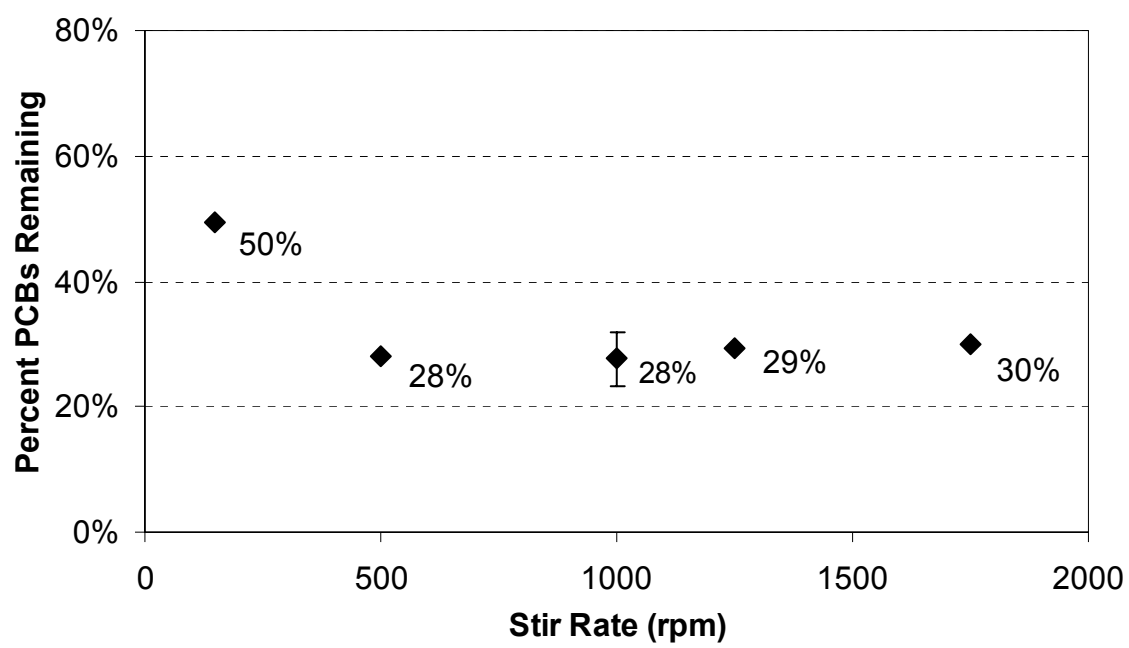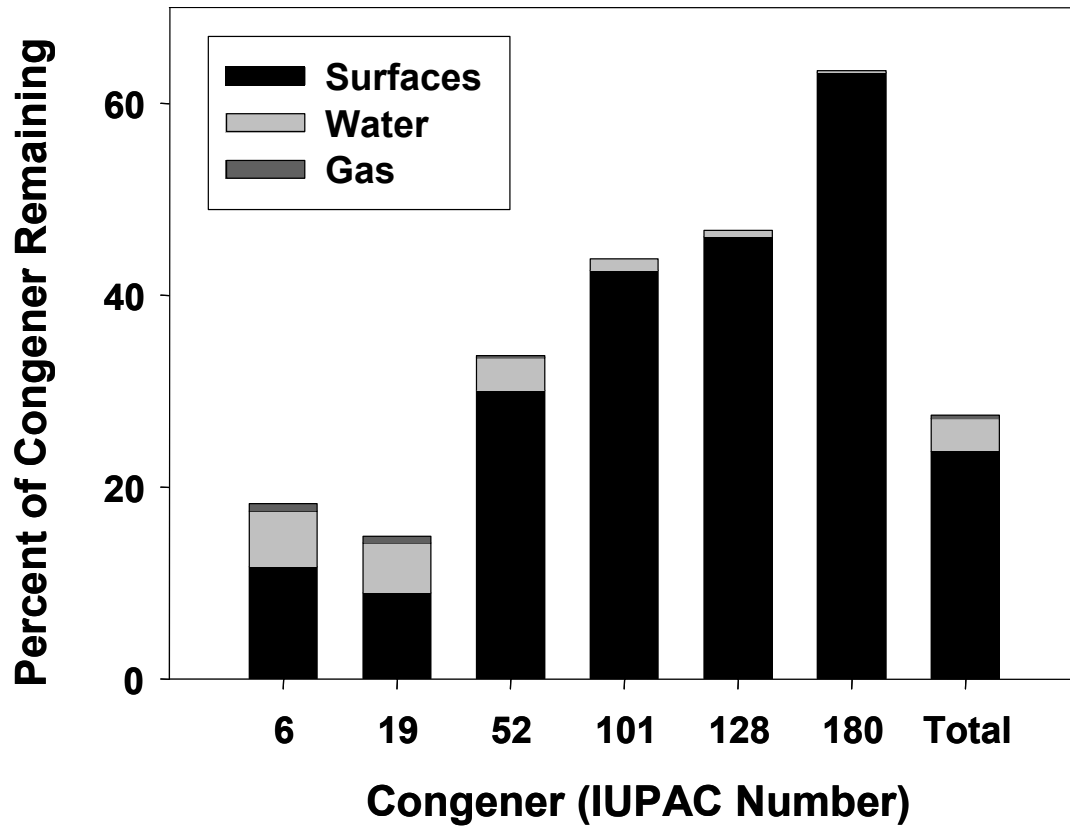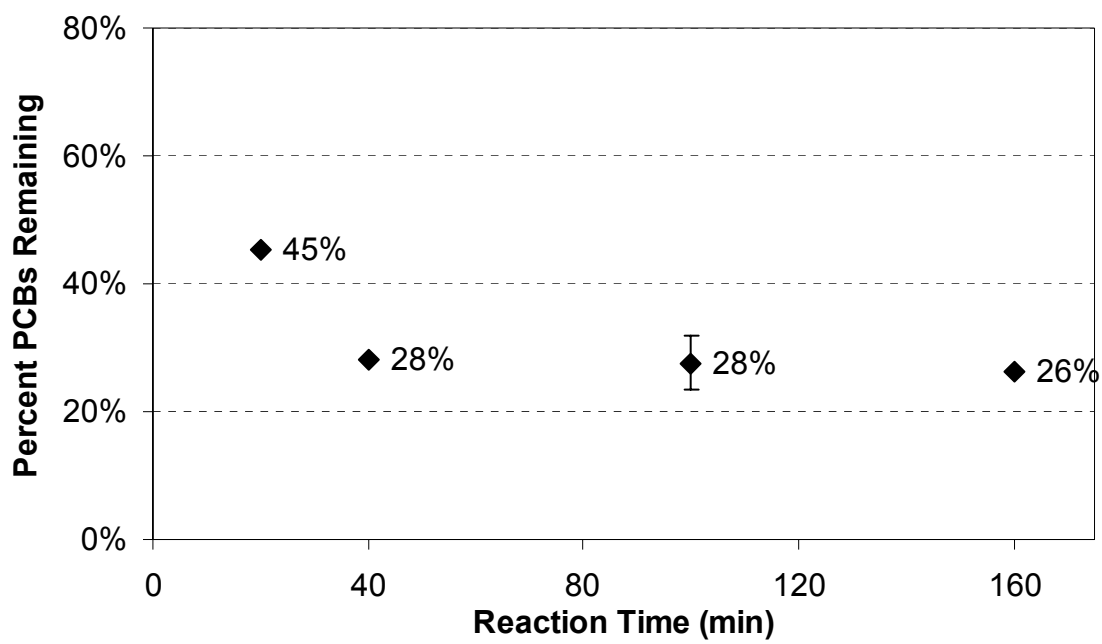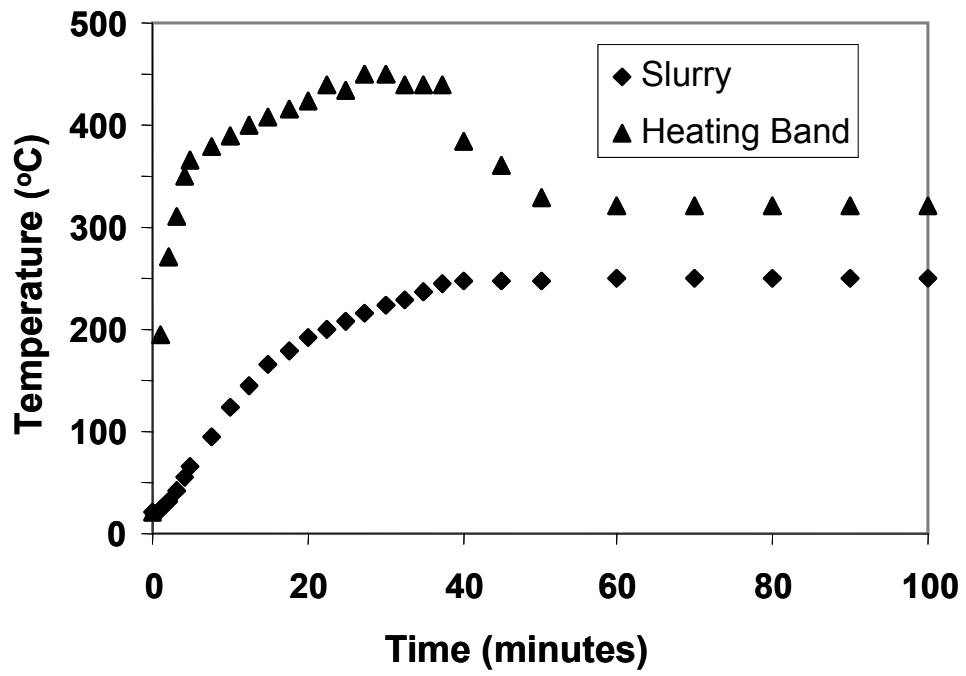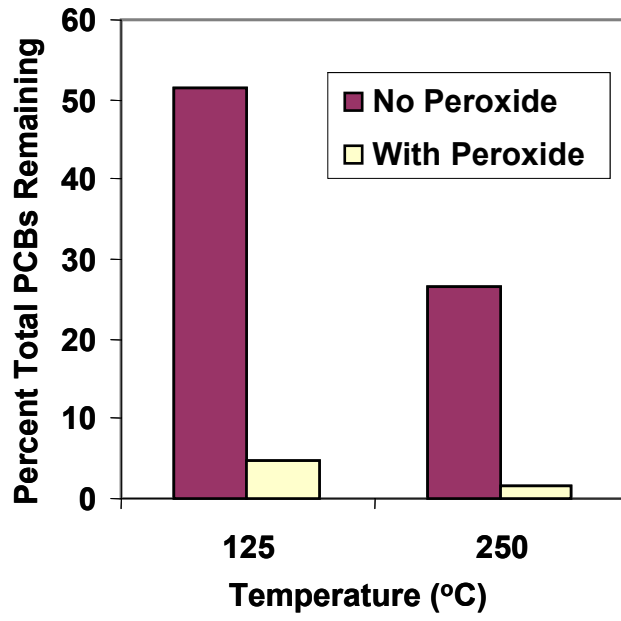
Basic Information

Title:	MTBE Biobarriers in Stream Sediments
Project Number:	2001MT281B
Start Date:	3/1/2001
End Date:	2/28/2002
Research Category:	
Focus Category:	Treatment, Water Quality, Groundwater
Descriptors:	biobarriers, groundwater,gasoline
Principal Investigators:	Joel E. Cahoon , Al Cunningham , Clain Jones

Publication

1. Meiser, Elsa Emilie. 2002. The oxygen dependence of the degradation rate of Methyl tert-Butyl Ether by a bacterial isolate. Master's Thesis, Montana State University Department of Civil Engineering.

Oxygen Dependence of the Degradation Rate of Methyl *tert*-Butyl Ether by a Bacterial

Isolate

By Elsa Meiser

Abstract

Methyl *tert*-Butyl Ether (MTBE) is a fuel oxygenate added to gasoline to boost octane

and reduce carbon monoxide emissions. Studies indicate that MTBE is cometabolically

oxidized by monooxygenase (MO) enzyme activity that is induced by the oxidation of n-

alkanes under aerobic conditions. The MO enzyme requires molecular oxygen to oxidize

the target chemical. The Michaelis-Menton model is a mathematical model that

describes the enzyme-mediated dependence of reaction rate on a given substrate. The

Hill model further describes enzyme activity when cooperativity among enzymes has

been introduced. This study investigated the Michaelis-Menton and Hill models as

appropriate models to describe the dependence of MTBE disappearance rate on dissolved

oxygen concentration by a bacterial isolate. It was found that the Hill model represents a

better model than the Michaelis-Menton model for the given data as determined by

regression analysis. Furthermore, MTBE disappearance rate also has a dependence on

MTBE concentration that must be incorporated into a mathematical model to predict the

behavior of this system.

Research Objectives

        The purpose of this research was to determine the dependence of MTBE disappearance rate on oxygen by a particular bacterial isolate, PM1. Two mathematical models describing reaction rate dependence on a substrate in the presence of enzyme activity were analyzed to determine which provided the best model fit for the data. Regression analysis were performed and compared between the two models.

Methodology

        Experiments were run in microcosm batch format. The microcosms were adjusted to different levels of dissolved oxygen and given MTBE as a sole carbon and energy source. The bacterial isolate used in these experiments was PM1, a culture known to be capable of utilizing MTBE.

        In each experiment, the microcosms were monitored over the course of seven days, the time that it took to fully deplete approximately 14 mg/L of MTBE. Samples were removed and analyzed every other day. Measurements were made of MTBE and TBA concentration, optical density, and headspace concentrations of oxygen, carbon dioxide and nitrogen.

        The data were used to calculate MTBE disappearance rates in the microcosms. MTBE disappearance rates were regressed against dissolved oxygen concentrations. Model fits were performed using an algorithm to minimize the sum of the squares of the residuals in a non-linear regression scheme. The goodness of fit was determined by R^2 value. R^2 values were compared between the Michaelis-Menton and Hill models, both with and without taking MTBE concentration into account.

Principal Findings and Significance

The Hill model accounting for MTBE concentration was found to have the best fit for the data. Recall that the Hill model takes cooperativity between enzymes into account. Cooperativity refers to the phenomenon in which initial enzyme activity increases the activity of subsequent enzymes. This model is characterized by regions in which MTBE disappearance rate increases rapidly over short increases in dissolved oxygen. Above a given level of dissolved oxygen, which ranges from 0.5 to 2 mg/L for the given data, MTBE disappearance rate reaches a maximum. Thus, above this level of dissolved oxygen, MTBE disappearance rate is independent of the dissolved oxygen concentration.

It was found that the data adjusted for initial MTBE concentration produced better model fits than data in which the MTBE concentration was not taken into account. Apparently, the behavior of the MTBE disappearance rate is more complex than can be described by either the Hill or the Michaelis-Menton model, which in this case take only the substrate oxygen into account.

These findings suggest that oxygen application schemes for the purpose of enhancing natural attenuation or bioaugmentation would greatly assist the ability of the microorganisms to degrade MTBE. Furthermore, increasing dissolved oxygen to above approximately 2 mg/L would optimize the MTBE degradation rate. Increases in dissolved oxygen above this level would not be effective in increasing the MTBE degradation rate.

Publications/Citations

A Master's thesis was prepared from these findings.

This project has not yet been submitted elsewhere for publication or presentation.

Student Support

Master's candidate, Civil Engineering.

Notable Achievements and Awards

I will let you know after I present my finding publicly!

Basic Information

Title:	Development of an internet mapping application to allow spatial queries and data extraction from the MBMG Abandoned/Inactive Mine database
Project Number:	2001MT2001B
Start Date:	3/1/2001
End Date:	2/28/2002
Research Category:	
Focus Category:	Water Quality, Acid Deposition, Groundwater
Descriptors:	data access, water quality, internet map server,abandoned and inactive mines
Principal Investigators:	Patrick J. Kennelly

Publication

Development of an internet mapping application to allow spatial queries and data extraction from the MBMG Abandoned/Inactive Mine database
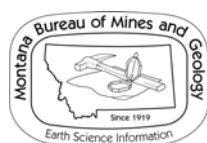
Report No. 215
Montana University System Water Center

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Abstract

The Montana Bureau of Mines and Geology (MBMG) has constructed an internet mapping application to access its Abandoned/Inactive Mines (AIM) database <http://mbmggis/website/mines>. The AIM database includes information on more than 4,000 sites inventoried by MBMG for the US Forest Service (USFS) and the Bureau of Land Management (BLM). This study used ESRI ArcIMS software to create an online map interface where the user can make spatial or attribute queries.

Problem and Research Objective

The quality of water associated with abandoned and inactive mines (AIM) is an important environmental issue in the state of Montana. The Montana Bureau of Mines and Geology (MBMG) has inventoried nearly 4,000 abandoned and inactive mine sites for the U.S. Forest Service (USFS) and the Bureau of Land Management (BLM).

Previous to this project, however, there was no easy method for the public and other government agencies to access this data. The internet mapping application created for this study provides a map-based interface to MBMG's AIM database covering the entire state <http://mbmggis/website/mines>. It includes all AIM sites on or influencing federal land which have the potential to influence the water quality in many of Montana's streams.

Methodology

This internet mapping application was developed using Environmental Systems Research Institute (ESRI) ArcIMS software on a PC (900 MHz AMD processor, 256 Mb RAM, ASUS motherboard) dedicated as the internet map server.

Currently, all of the sites in the AIM database is converted to an ArcView shapefile. This point theme is then included in the ArcIMS project and displayed with other geographic base layers. In the future, MBMG hopes to enhance this ArcIMS application so that locations will be displayed and can be queried directly from the AIM database.

Results

The resulting internet application allows both spatial and attribute queries of data from the MBMG AIM database. Examples of spatial queries would be all sites within the Gallatin National Forest or within Park County. Examples of attribute queries would be all sites with water pH values less than 3. This service will provide a readily available and easily updated tool that can be used with all sorts of environmental applications in areas near abandoned and inactive mines associated with USFS and BLM property.

Information Transfer Program

Basic Information

Title:	Information Transfer Program
Start Date:	3/1/2001
End Date:	2/28/2002
Descriptors:	
Principal Investigators:	Kathleen Tohe Stephens

Publication

INFORMATION TRANSFER PROGRAM

BACKGROUND

Research, education, and information transfer (IT) have been the key components of the Montana University System Water Center's mission to promote problem-solving partnerships among university, government, and private sector participants since 1964. Prior to the advent of the World Wide Web (WWW), small budgets assigned to the IT effort were often applied to copying and postage costs. In 1996, the IT concept at the Montana Water Center was redefined when hosting a website became an affordable reality. As with most fledgling websites, the Center's first attempts at online information were minimal, including reports, notices, directories, and links highlighting Center activities only.   However, we soon realized that the Center's web-based information transfer efforts could be better utilized by developing and maintaining an impartial clearinghouse of Montana water information. A unified and affordable structure through which scientists, agency personnel, and watershed group participants could access information and resources and exchange experiences did not yet exist. A timely EPA appropriation for watershed development in 1997 allowed the Montana Water Center to seize the opportunity to construct a significant website titled *Montana Water* (http://water.montana.edu). At the same time, the Montana Watershed Coordination Council (MWCC) provided guidance (and a few small grants) on watershed group materials, since many isolated watershed projects were moving vigorously forward throughout the Northern Rocky Mountain region.

PURPOSE

Create and maintain a website to centralize communication and data-sharing efforts among agencies, organizations, and individuals engaged in Montana water issues i.e., watershed modeling and planning, water quality efforts, water-related events, grants and funding opportunities, policy and legislation information, etc.

PARTNERS

EPA Montana Office, Bureau of Reclamation through the Montana Watershed Coordination Council, U.S.G.S. - Water Institute Program base grant (funding). Natural Resources Information System (NRIS) of the Montana State Library, Montana Bureau of Mines & Geology, MT Department of Environmental Quality, MT Department of Natural Resources and Conservation, MT Association of Conservation Districts, USDA Natural Resources & Conservation Service, MT Department of Fish, Wildlife & Parks (others).

OTHER ACTIVITIES

Montana Watershed WEB - a component of *Montana Water* - this site serves as a bulletin board and resource for a variety of Montana Watershed groups as well as a central switchboard for several ongoing agency activities (http://water.montana.edu/watersheds2).

AWRA-MT Web - In addition to providing the necessary organizational and logistic support to the annual water conference, the Montana Water Center also maintains the AWRA-MT Web site hosted by AWRA National at http://www.awra.org/state/montana/.

Outreach and Information Referral - Using the web sites as tools, the Montana Water Center's Communication/Outreach Coordinator continues to provide information to the general public on a daily basis in the form of website updates, email announcements, and responses to requests for information.

Student Support

Student Support					
Category	Section 104 Base Grant	Section 104 RCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	1	0	2	0	4
Masters	20	0	1	0	21
Ph.D.	3	0	0	0	3
Post-Doc.	0	0	0	0	0
Total	0	0	0	0	0

Notable Awards and Achievements

1. Technology Assistance Center for Small Systems: EPA Office of Ground Water and Drinking Water, EPA Region 8, and Indian Health Service The Operator Basics training course for operators of small public water systems has proved to be popular nationwide. The course covers all aspects of operations, from source water protection to water quality to cross-connection control, at an introductory level. Development of this interactive, computer-based training tool is funded by a US Environmental Protection Agency grant to the Small Systems Technical Assistance Center operated by the Montana Water Center. Operators can take their training online or from a CD-ROM. The state agencies that certify operators are in the process of approving the tool for training, so operators in most states can now receive continuing-education credit for the time they spend with Operator Basics.

2. Whirling Disease Initiative: U.S. Fish & Wildlife Service, state agencies and universities a. Field Diagnostic Apparatus: The purpose of this project was to develop an apparatus for collecting triactinomyxons quantitatively from natural waters, so the degree of infection of a stream can be readily assessed. A treatment sequence using rotating-drum and packed-bed filters performed well with known concentration of TAMs in the laboratory. Coupled with pre-filtration and postconcentration steps, it was then demonstrated successfully in the field. The tamometer is now ready for use in assessing parasite loads in streams.

b. Stream Characteristics vs Infection Rates: Age-0 brown, cutthroat, rainbow, and brook trout were collected in streams of all sizes throughout the Salt River drainage, Wyoming. Trout not infected with whirling disease were only found in the headwaters of four mountain tributaries, all upstream from barriers to fish movement. Disease infection rates and histological evidence of whirling disease were highest at sites with large amounts of fine sediments and low channel slopes. Such sites were found in some spring streams with very low channel slopes, some spring streams with higher channel slopes and habitat improvements that resulted in numerous deep pools with abundant aquatic vegetation, and mountain tributaries downstream from complexes of beaver dams.

c. Temperature Modeling: The seasonal pattern of stream temperatures is a key determinant of the distribution, spread, and intensity of the whirling disease epizootic. The investigators used a validation database of Montana stream temperatures to develop a model and database to calculate the mean daily water temperature for any location on any stream in Montana, for any day in the past 21 years. For a particular stream of interest, one week of calibration data is needed. This model will allow statistical examination of the disease epidemiology as it relates to water temperature.

d. Worm Infection Assays: The investigators developed a rapid technique for sectioning the worm host of whirling disease to examine the course of infection within individual worms. Three strains of worms with varying susceptibility to infection were examined. The new technique allowed the investigators to trace the differing courses of infection they exhibited. It also allowed them to identify infected worms at an earlier stage than was previously possible. This should permit researchers to rapidly determine if worms taken from streams are or will shortly be releasing triactinomyxons.

3. USGS 104B: U.S. Geological Survey and universities a. Paul Sturman, Coordinator of Industrial Development with Montana State Universitys Center for Biofilm Engineering, sought to answer the specific question: Can we engineer the addition of dissolved organic carbon to mine tailings to mitigate acidity on a range of scales and for an extended period of time?

MSE Technology Applications, a Butte-based company, will be implementing multi-acre plot treatments based on Sturmans research at the currently active Golden Sunlight gold mine. The mining industry is also interested in this treatment protocol as a cost-efficient and labor-saving means to remediate acid rock drainage with a minimum of infrastructure investment.

Recommendations and needs for future investigations include a better understanding of the complex microbial ecology of mine tailings. This research supported one PhD student and partially supported an undergraduate. In the future, Sturman would like to be able to support a masters student on acid rock drainage projects.

b. Pat Kennelly and Teresa Donato recently completed the USGS Section104(B) project Development of a GIS for analyzing and mapping ground-water elements related to coalbed methane production.

This research produced two maps: Montana Bureau of Mines and Geology (MBMG) Open File Report (OFR) No. 448, Hydrologic features of the potential coalbed methane development area of the Powder River Basin, Montana, (2002) and MBMG Geologic Map Series (GM) No. 60, Anderson and Knobloch coal horizons and potential for methane development, Powder River Basin, Montana, (2001).

These maps, especially OFR 448, which provides an inventory of wells and springs in the Powder River Basin, have received positive reviews from a variety of stakeholders. Many copies of this map have been distributed to state agencies, industry groups, and landowners. MBMG staff members frequently use the map both in-house and on the road.

Further research suggested by the investigators includes a field inventory of the hydrological features shown on the map. Monte Smith, a hydrogeologist with the MBMG, and Donato are also in the process of writing a funding proposal for work to determine whether the cause of decreased spring output in the Powder River Basin is due to drought conditions or coalbed methane withdrawals.

c. Montana Water A Water Information Network This web site (http://water.montana.edu) continues to be a comprehensive source of up to date information for water resources professionals as well as the general public. New to Montana Water this year was the Web Catalog of Watershed Projects, which lists projects completed under the 319 Non Point Source Program, the MT Department of Fish, Wildlife & Parks Future Fisheries Program, and the NRCS Environmental Quality Incentive Program beginning in 1990.

Publications from Prior Projects