USGS

WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: Evaluation of the Fluorescent Amplified-Fragment Length Polymoprhism Method for Identifying Sources of Fecal Coliform in Grazed Watersheds.

Focus Categories: WQL, AG, NPP

Keywords: fecal coliform, livestock grazing, water quality, watershed monitoring

Duration: 14 months (1 March 2000- 30 May 2001)

Federal Funds Requested: $15,580

Nonfederal Match: $36,397

Principal Investigator: Clayton B. Marlow, Animal and Range Sciences, MSU-Bozeman

Co-Investigator: Donald E. Burgess, Veterinary and Molecular Biology, MSU-Bozeman

Congressional District: Montana-at-Large

Statement of Need

An ongoing study supported, in part, by the Montana Water Center has produced information describing substantial reduction in overland sediment from pastures grazed to a 7 to 8cm (3in) plant stubble height. While this finding appears to have promise for protecting surface waters from sediment pollution in grazed watersheds, corollary data from a companion study using the same stubble height along stream banks revealed a significant increase in waterborne fecal bacteria. This suggests that managing grazing specifically for sediment pollution reduction would do little to curtail fecal coliform levels in surface waters. Confirmation of this relationship would mean that municipal and county water boards would be faced with an ugly decision either buy-out private grazing rights within the watershed or pay more for water treatment. However, there is other information related to fecal coliform contamination of surface water that suggests additional investigations are needed before recommendations are finalized.

The need for detailed investigation of grazing livestock contribution to stream fecal coliform levels is apparent upon review of published reports and preliminary data from current research. Stuart et al (1976) reported that the primary source of fecal streptococci in streams of alpine recreational areas was rodents and moose rather than human visitors. Results of our monitoring efforts on Cottonwood Creek near Ovando, Montana indicated excessive levels of fecal coliform in February 1998 and May 1999 *when no cattle where present along the stream.* Furthermore, disturbance of Cottonwood Creek sediments

increased fecal coliform levels 1.5 to 5 times the pre-disturbance levels and this even occurred in areas that hadn't been grazed by cattle for over 40 years. Brett et al. (1988) reported similar results from grazed and ungrazed Oregon stream reaches. This raises the question, as whether or not the high coliform levels are resuspended cattle fecal coliform or an accumulation of bacteria from all other species in the watershed.

In our studies fecal coliform levels "spike" when cattle first enter a pasture but then *decline until cattle are moved to the next pasture*. If cattle were solely responsible for the high initial levels, then it follows that these levels should remain elevated until cattle leave. The fact that fecal coliform levels go down with cattle still in the pasture suggests that current grazing pressure isn't the only source of fecal coliform contamination. Should streambed sediments harbor fecal coliform from multiple sources removal of cattle may do little to stop or dampen fecal coliform "spikes" during high flow events. This suggests that the host source of stream borne fecal coliform must be identified prior to remediation and/or changes in watershed use. Unfortunately, current water quality analysis procedures (APHA 1998) cannot differentiate among animal sources of fecal coliform species and strains. Thus, with the present state of knowledge, it is difficult to judge whether or not livestock grazing within the watershed is the primary source of fecal coliform levels or if the levels are a composite of these and other host species.
This proposal describes an effort to evaluate a methodology that has the potential to identify the various sources of fecal coliforms in a water sample. Should the methodology prove adequate for the task further studies will be under taken to describe the populations of fecal coliform in the water column and stream sediments.

Results and Outcomes

Limited research indicates that different species or classes of fecal coliform can be identified using a DNA or RNA marker. Based on Dr. Burgess' experience it is quite likely that Fluorescent Amplified- Fragment Length Polymoprhism (FAFLP) can be used successfully to differentiate strains of *E. coli* under controlled conditions. However, the methodology has not been used to differentiate fecal bacteria from water samples taken under field conditions. There is also reason to believe that the efficacy of the method could be compromised by differences in water chemistry between one stream and the next. Thus, the direct outcome of this project would be to verify the utility of FAFLP for differentiating fecal coliform bacteria from field samples. Should the methodology prove unreliable for samples taken from the field the next step would be to use that information to generate grant proposals to funding programs such as the *Water Environment Research Foundation* and *National Research Initiative* for increased funding to either "fine tune" the FAFLP procedure or evaluate another procedure using an RNA "tag". Ultimately, the identification of a procedure to identify the host source of fecal coliform will give researchers and water quality personnel throughout the northern Rockies a way to objectively address grazing animal impacts to water quality.

Nature, Scope and Objectives of the Research

This project will be a laboratory trial involving samples analyzed under controlled (laboratory) conditions. Phase 1 will involve the use of FAFLP to differentiate between a laboratory strain of *E. coli* and fecal coliform collected from a "laboratory" cow housed at the Montana Agricultural Experiment Station's Livestock Nutrition Center. During this phase we will be able to adapt methodologies to livestock sources (to date FAFLP has only been used on human samples) and determine the reliability of the test. If we can segregate cattle *E. coli* from the laboratory strain with 95% confidence, we will move to Phase 2.

Through discussions with Dr. E.R. Atwill in the University of California-Davis School of Veterinary Medicine we have learned that the fecal coliform populations within the gut of cattle may differ significantly from herd to herd within a relatively small area. Furthermore, these herd differences have produced correspondingly dissimilar fecal coliform levels in neighboring waters. Thus, if the FAFLP methodology can segregate cattle *E. coli* from laboratory strains of the same bacterium, it would be useful to learn whether or not this procedure can also differentiate between herds. After determining the reliability of FAFLP to segregate different strains of *E. coli* we will begin a second trial (Phase 2) to determine the utility of this method for identifying fecal coliform from separate herds of cattle. To accomplish this goal Phase 2 will involve collection of fecal material from herds at three separate locations across western and central Montana. These samples will be returned to the MSU Veterinary and Molecular Biology laboratory for FAFLP testing. Because of the potential for overlap in bacterial populations in cattle, the confidence level for this test will be set at 70%.

The final series of tests run in this project will be to test water samples that have been inoculated with fecal material from elk, deer, sheep and bison. All four species are ruminants like cattle and often graze the same watersheds so it is important to learn if FAFLP can segregate cattle *E. coli* from that of other domestic and wild ruminants. Samples from all four species will be collected from the Flying D (elk and bison) and Red Bluff Ranches (deer and sheep) near Bozeman. The same protocol will be followed as in Phase 2 except a higher level of detection (95%) will be the standard.

Project Objectives

I. Phase 1 - Determine the utility of FAFLP to segregate cattle *E. coli* from a known laboratory strain with 95% confidence. If successful, we will initiate Phases 2 and 3.

II. Phase 2 – Determine the utility of FAFLP to segregate *E. coli* from different herds of cattle.

III. Phase 3 – Determine the utility of FAFLP to segregate cattle *E. coli* from elk, deer, sheep and bison fecal coliform.

IV. Should Phase 1 be unsuccessful (no segregation or a confidence of 90% or less) we will contact researchers at the University of Washington for information on their use of RNA tags to differentiate fecal coliform strains and species.

Methods, Procedures and Facilities

A. Evaluate a biotechnological technique referred to as *Fluorescent Amplified-Fragment Length Polymorphism Analysis* (Arnold et al. 1999) for its ability to identify fecal coliform strains from different host sources. This portion of the project will be carried out in collaboration with Dr. Donald Burgess, Veterinary and Molecular Biology Department, Montana State University-Bozeman.

1. An artificial stream environment will be created in the laboratory to facilitate testing of the *FAFLP* method. Twenty pvc tubes will be filled with a sterilized mixture of silt, sand and gravel to approximate a streambed. The cylinder will then be filled with sterilized water.

2. Fecal coliform drawn from cattle on the Montana Agricultural Experiment Station and a laboratory population of *E. coli* will be used to seed ten of the columns with fecal coliform; the remaining columns will serve as a negative or control group. Equal proportions of the two *E. coli* sources will be added to each column. During the trial columns will be held at 20 C to approximate Montana stream temperature.

3. After 48h incubation period 1000ml will be withdrawn from each column and split into two 500 ml subsamples. One subsample will be analyzed for *E. coli* with the FAFLP methodology and the second will be analyzed for *E. coli* via standard water quality procedures (APHA 1998).

4. A paired T statistic will be used to test for differences between the FAFLP detected levels and those measured through the standard water quality procedure. If there is no significant difference in total *E. coli* at the alpha 0.05 level, further analysis will be performed.

B. Cattle fecal samples from three different geographic areas of Montana will be collected to determine if FAFLP can distinguish fecal coliform from different cattle herds.

1. Fecal samples will be taken from grazed ranges at 5 localities (herds) in western (2) and central (1) Montana. A

composite sample of 10 fresh pats will be made at each locality.

2. Fecal material will be stabilized and shipped according to experience gained in the first trial.

3. An ANOVA test at the 0.05 alpha level will be used to test for differences in FC species composition within locality (between herds) and between regions (western and central Montana).

C. If we are successful in developing a cattle FC profile that holds for all cattle across the western and central portions of the state, the applicability of the method as a water sampling method will be further tested under laboratory conditions at Montana State University-Bozeman.

1. A laboratory study will be conducted on the MSU campus to determine whether or not the profile(s) can be used to identify cattle FC in water and sediment samples contaminated with FC from other sources.

2. 20 pvc cylinders filled a third of their depth with a sterilized gravel (30% by weight) clay loam (70% by weight) will be used too represent stream sediment. Sterilized water will be used to fill each cylinder.

3. After a 48h equilibration period, each cylinder will be inoculated with equal amounts of sheep, deer, bison, elk and cattle fecal matter (all five species are common to watersheds in western and central Montana). The water columns and sediments will be held at a constant temperature (20 degrees C in the first trial) for 48 hrs. After an incubation period of 36h a 1000ml water sample will be withdrawn, the sediment agitated for 60 seconds and a second water sample taken.

4. The disturbed and undisturbed samples will be tested for total FC using standard water quality procedures and the FAFLP profile.

5. Results will be evaluated to determine if cattle FC could be isolated and if it was in the same proportion to the total fecal coliform (TFC) content as in the original inoculation.

6. Following the initial test, the cylinders will be emptied and sterilized and the entire process repeated two more

times. Each trial will be considered a replication for purposes of statistical analysis.

7. Because we will be working with relative proportion we will use chi-square analyses to test for the level of confidence we can place in the methodology.

Related Research

In 1996 the Principle Investigator received funding from the Montana Department of Natural Resources to conduct a demonstration of the newly adopted Grazing Best Management Practices (BMPs) on the Forestry Experimental Station/Agricultural Experiment Station Bandy Research Ranch. A major portion of the demonstration effort was to monitor changes in water quality under each of the new grazing practices.

After a year of monitoring (July 1997) FC levels recorded in streams on and near the ranch were not fitting patterns expected under livestock grazing. The commonly accepted pattern is that elevated stream FC counts persist after livestock are removed from a pasture (Jawson, et al. 1982 and Stepheson and Street 1978 as reported in Quigley et al. 1989). However, on the Bandy Ranch FC levels rose rapidly when cattle entered the pasture but *declined* while cattle were still present (Fig. 1).

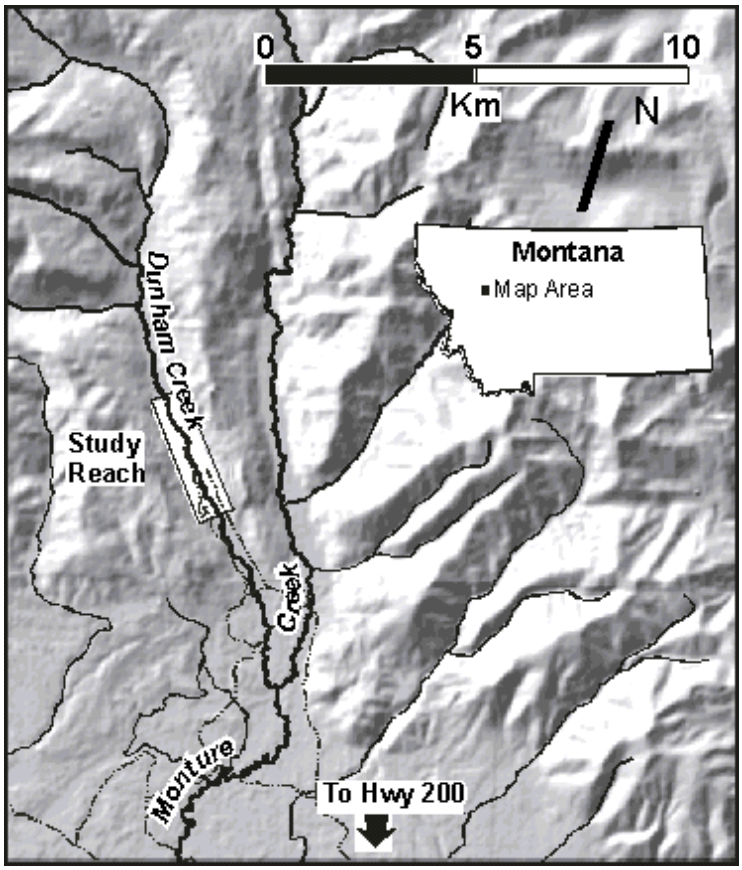

Figure 1: Study reach location.

Fig. 1. Fecal coliform levels in Cottonwood Creek on the Forestry Conservation Experiment Station/Agricultural Experiment Station Bandy Research Ranch.

Because of earlier work by Sherer et al. (1988) indicating that FC levels could be significantly elevated when stream bottom sediments were disturbed, we undertook a short-term project during the fall 1998 to find out if cattle watering along the stream might cause resuspension of stream sediment. Following the methods described by Sherer et al. (1988) we made our scheduled water samples, raked a 2 ft^2 spot on the streambed and collected a second water sample at each monitoring station. As in the Sherer et al. (1988) study we recorded FC levels 1.5 to 5 times greater following disturbance than that in the previous undisturbed sample (Fig. 2). Our data and that of Quigley et al. (1989) suggests that livestock contribution to the degradation of water quality could be the result of accumulated FC in streambed sediments over several years of livestock grazing. However, the very high levels recorded in a small stream on the Blackfoot/Clearwater game range (Fig. 2) indicate that livestock may not be the only contributors to the sediment FC pool. The sample from the game range stream, which hadn't been grazed by livestock for 40+ years, had the highest level of FC following streambed disturbance. These levels could have been produced by wildlife as reported by Stuart et al. (1976)

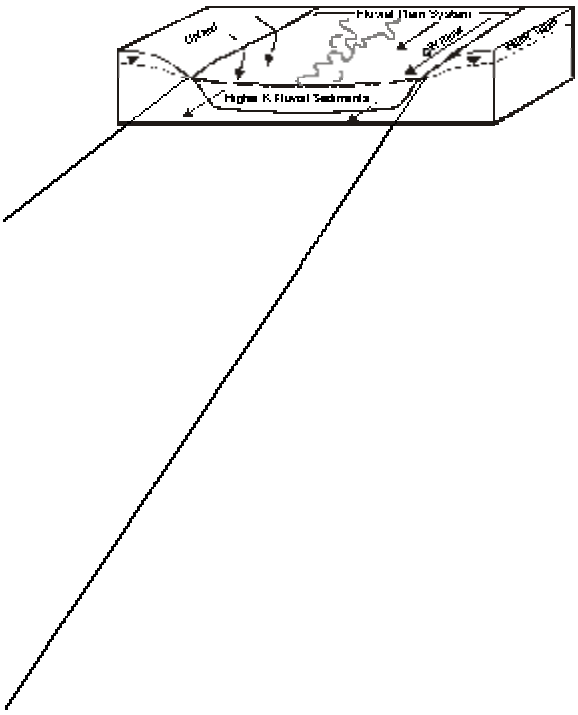

Fig. 2. Fecal coliform levels prior to and following disturbance of a streambed on the Forestry Conservation Experiment Station/Agricultural Experiment Station Bandy Research Ranch.

or long-term survival of FC deposited by livestock grazing the area in the 1940's. Davies et al. (1995) reported the survival of *culturable* levels of *Escherichia coli* in marine and freshwater sediments for 68 days. The same authors went on to report that longer periods of survival in stream sediments could be possible. An earlier report by Davenport et al. (1976) adds credence to the long-term survival of FC when they found high survival rates of fecal coliform and fecal streptococcus under the ice of streams at 0 degree C. This raises the question as to whether the FC loads noted during high flow events are due to immediate contamination by wildlife and livestock or from resuspension of bacteria surviving in streambed sediments. However, to address this question researchers must be able to differentiate between potential host sources of fecal coliform in streams and rivers.

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Information Transfer Plan

Because the results from this project center on the utility of a particular methodology the outcomes will be of most interest to other scientists and water quality personnel rather than ranchers and the general public. Consequently, our plan is to disseminate the information through:

1. Presentation by both students at the Annual Montana State University Undergraduate Research Symposium
2. Presentations at the Society for Range Management Annual Meeting and the American Microbiological Meeting.

3. The results of the laboratory tests will be submitted to the *Journal of Environmental Management* or *Journal of Applied Environmental Microbiology* for publication.