USGS

WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: Measurement of Waterborne Pathogens in Wetlands and Wetland Treatment Systems Focus

Categories: MET WQL WW

Keywords: Waterborne Pathogens, Bacteria, E. coli, Giardia, Cryptosporidium, Biomonitoring, Land-Water Interactions, Model Studies, Wastewater, Wetlands, Water Quality, Water Quality Monitoring0

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Federal Funds Requested: $ 22,000

Non-Federal (matching) Funds Pledged: $ 44,862

Principal Investigators: John R. Jones (School of Natural Resources) Jeanne M. Erickson (Biochemistry and Plant Pathology) Mark A. Milanick (Physiology, College of Medicine) University of Missouri, Columbia

Congressional District: 9th Critical

Regional or State Water Problems

Waterborne diseases of significant public-health impact over the past decade have been caused by several microorganisms, including toxin-producing strains of the fecal bacteria E. coli (Craun et al., 1997), and intestinal protozoan parasites such as Giardia and Cryptosporidium (Kramer et al., 1993; Rose, 1997). Such protozoans are readily spread through fecal contamination of water by the protozoan cysts or oocysts. Moreover, cysts and oocysts excreted by animals can infect humans. Any comprehensive examination of water quality should include monitoring for microbial flora, with an emphasis on the pathogens of greatest health concern. Current reservoir research at the University of Missouri - Columbia has the overall objective of determining reservoir water quality within the state (as measured by nutrients, water clarity and algal biomass), and quantifying the major factors controlling present conditions (land use and climate). We propose to expand this research to include measurements of pathogenic microorganisms in surface waters.

Water is a critical resource in Missouri, in all states of the region, and throughout the nation. The proposed research is essential for a complete understanding of the factors that influence water quality, including the incidence and sources of potential pathogens/parasites in water, and the sources of potential long-term changes in water quality independent of climatic induced variations. Land use practices within a given watershed will affect water quality; this is of particular impact, with respect to

waterborne pathogens, in regions where fecal contamination of water by humans, domestic animals and/or wildlife is prevalent. This project is viewed as an initial step towards developing and applying the techniques necessary to conduct widespread monitoring for the occurrence of potential pathogenic microorganisms, and to identify their source of origin in surface waters. As such, this study will provide a model for future programs assessing microbiological and chemical parameters of water quality in wetland treatment systems, environmental wetlands, and drinking water and recreational reservoirs.

Results or Benefits of Research

This research will result in the development of molecular techniques for the measurement of waterborne pathogens, and the ultimate application of that methodology to studies of wetlands and reservoirs in Missouri. We will obtain data on the incidence of total coliforms, fecal coliforms, toxin-producing strains of E. coli, and the protozoan parasites Giardia and Cryptosporidium in the Eagle Bluffs wildlife conservation area and the adjacent wetland treatment system for city wastewater. Measurement of the bacteria and the protozoan cysts and oocysts will be achieved using specific assays based on the polymerase chain reaction (PCR). In addition, bacteria will be measured using standard bacteriological techniques. Our results will allow us to determine the incidence of these microorganisms in the waters surveyed throughout the year, the correlation between the molecular methodology developed and the standard bacteriological methods, and the relationship between the occurrence of these pathogens and other limnological parameters. We will also determine whether the presence of the fecal contaminant E. coli serves as a reliable indicator for the presence of Giardia cysts and/or Cryptosporidium oocysts in these surface waters.

A focus on the nearby Eagle Bluffs region will allow us to refine our ability to monitor for the presence of waterborne fecal bacteria and pathogenic protozoans, and to relate such presence to other parameters of water quality and to land use practices. It will also allow us to assess the efficacy of the wetland treatment system with respect to reducing the density of these microorganisms. Results will be important for the city of Columbia, and may serve as a model for other constructed wetland treatment systems. While we will focus on Missouri waters, the techniques we develop for monitoring waterborne fecal and pathogenic microorganisms --- including the concepts involved in the data acquisition and data analysis --- will be applicable to water quality monitoring elsewhere. Ultimately, we expect that the molecular assays for biomonitoring developed in this project will be incorporated as a regular part of all our programs for monitoring water quality in Missouri reservoirs. This multi-disciplinary approach may serve as a model for other monitoring programs. Moreover, the tools developed in this project will position us for 'source tracking' of pathogens. The Eagle Bluffs Conservation Area is of particular interest in this respect, given that human wastewater treatment cells serve as a primary source of water for the conservation wetlands, and that these wetlands provide habitat for many types of wildlife, including mammals as well as large numbers of migratory waterfowl.

Nature, scope and objectives of the research

We plan to develop efficient and cost effective molecular techniques to measure the presence of coliform bacteria and parasites in wetlands. One of these techniques is based on the polymerase chain reaction (PCR), an extremely powerful enzymatic reaction used to produce relatively large amounts of a specific DNA fragment from vanishingly small amounts of initial genetic material. This molecular technique is commonly used for the identification of organisms through "DNA fingerprinting", and is often employed in forensics and medical diagnostics. We will develop a sensitive, specific, reproducible and efficient PCR-based assay for the detection and eventual quantitation of fecal coliforms, including toxic strains of Escherichi coli. The sensitivity of the PCR-based approach will provide greatly enhanced opportunities for the rapid identification of specific strains of microorganisms from environmental water samples. Moreover, this technique will facilitate studies by allowing for the long-term archiving of microorganisms collected from water samples onto membrane filters and available for future molecular assays in the laboratory. These studies will allow us to determine whether E. coli is a reliable water quality surrogate for the parasitic protozoans Giardia and Cryptosporidium in Missouri wetlands and reservoirs. They will also allow us to determine the relationship between the presence of fecal and pathogenic microorganisms, and other standard limnological parameters of water quality. As such, this project is a natural extension of our study of factors influencing lake fertility. Simply stated, our working hypothesis is that factors favoring the growth of algae in lakes (nutrient inputs from farmland and urban/suburban areas in the catchments, and certain climate patterns) may likewise correlate with the presence (abundance) of pathogenic organisms.

Reservoir research at the University of Missouri - Columbia has the overall objective of determining reservoir water quality within the state (as measured by nutrients, water clarity and algal biomass), and quantifying the major factors controlling present conditions (land use and climate). Ultimately, we will determine whether our lake resources are stable or changing with time; where conditions are degrading, we will recommend remedial action based on lake models specific to conditions in our state. This regional study of lake eutrophication (enrichment) assumes that excessive nutrient inputs to lakes produce changes that are detrimental to their function and use. Past studies have resulted in publications on the major features of our reservoirs and empirical models which describe composite patterns in lake water quality. Work in progress includes using remote sensing techniques to quantify influences of watershed characteristics on lake fertility. Also, we are developing a model to distinguish long-term trends from short-term fluctuations caused by climate.

With this proposal, we hope to expand our approach to include measurements of fecal microorganisms, including pathogens. Numerous outbreaks of waterborne disease in drinking water and recreational lakes, caused by microorganisms such as toxin-producing strains of the fecal bacteria E. coli and the protozoan parasites Giardia and Cryptosporidium, highlight the need for an understanding of the incidence and sources of these microorganisms in surface waters. As obligate intestinal parasites, such protozoans are present in feces as cysts or oocysts, and are readily spread through fecal

contamination. Cysts and oocysts excreted by domestic animals can infect humans. Any comprehensive examination of water quality should include monitoring for microbial flora with an emphasis on the pathogens of greatest public health concern. Our approach will be to quantify waterborne microbes in Missouri wetlands and relate their occurrence among the various water bodies to measurements of water quality and land use practices within the watershed. This will expand our studies beyond eutrophication to include basic information on public health. We will also use collected data to determine whether the presence (abundance) of Escherichia coli is an effective water quality index. E. coli has long been used as an indicator of fecal contamination (Whitman et al., 1995), but its use in predicting the presence of pathogenic protozoans in water supplies is not clear. By including this organism in our monitoring program, we can determine whether the presence of the fecal E. coli correlates with the presence of Giardia cysts and/or Cryptosporidium oocysts. We will test the merit of using E. coli as a surrogate indicator for these pathogens.

Our molecular biomonitoring program will focus on the Eagle Bluffs Conservation Area in mid-Missouri, including the adjacent wetland treatment system for city wastewater. This large area (> 4000 acres) is located 6 miles southeast of Columbia, Missouri, in the flood plain of the Missouri River. Water flows from the city treatment system into the conservation area wetlands. Water samples will be collected weekly at multiple sites in the treatment and conservation wetlands, and samples will be assayed for microorganisms and other aspects of water quality as described above. The proposed project is multidiciplinary, combining the respective expertise of three principal investigators. Dr. J. Jones (UM, Natural Resources) will be responsible for the limnological aspects of the project, and Drs. J. Erickson (UM, Biochemistry and Plant Pathology) and M. Milanick (UM, Medical Physiology) will be responsible for the molecular assays for pathogen detection and quantitation.

In summary, during the 12 months of this proposed project we will collect water samples from conservation wetlands and the adjacent wetland treatment system, and measure a variety of water quality parameters, as well as determine the incidence of total coliforms, fecal coliforms, E. coli, and the protozoan pathogens Giardia and Cryptosporidium. These data will allow us to determine the quality of water in the wetlands, the correlation between microbiological and standard limnological parameters, and the efficacy of using fecal E. coli as an indicator of the protozoan pathogens. Ultimately, we expect to incorporate the techniques developed in this project into our studies of water quality in Missouri resevoirs. A review of the literature suggests this expanded effort will be one of the first large-scale, comparative lake studies to include measurement of waterborne pathogens in a routine, water quality monitoring.