

# Water Resources Institute

## Annual Technical Report

### FY 1998

## Introduction

## Research Program

### Basic Project Information

Basic Project Information	
Category	Data
<b>Title</b>	Mercury Speciation and Transport in the Florida Everglades
<b>Project Number</b>	S-01
<b>Start Date</b>	01/01/0095
<b>End Date</b>	09/20/0099
<b>Research Category</b>	Ground-water Flow and Transport
<b>Focus Category #1</b>	Ecology
<b>Focus Category #2</b>	Groundwater
<b>Focus Category #3</b>	Toxic Substances
<b>Lead Institution</b>	The University of Wisconsin

### Principal Investigators

Principal Investigators			
Name	Title During Project Period	Affiliated Organization	Order
David E. Armstrong	Professor	The University of Wisconsin	01
James P. Hurley	Professional Staff	Water Resources Institute	02

### Problem and Research Objectives

The various anthropogenic influences on the Florida Everglades region, such as alteration of hydrology, nutrient enrichment and contaminant inputs are of great concern to the long-term future of this unique ecosystem. Of particular importance is mercury (Hg) cycling, since recent studies have found elevated Hg levels in higher trophic levels, such as largemouth bass and the endangered Florida panther. Mercury bioaccumulation through the food chain is influenced by numerous factors, but it is apparent that factors influencing bioavailability of Hg in the water column and sediments are extremely important for

understanding fate and transport. In conjunction with the U.S. Geological Survey- Middleton, the Wisconsin Department of Natural Resources, and the Water Chemistry Program, University of Wisconsin-Madison, the Water Resources Institute is examining the speciation and transport of dissolved, particulate and gaseous Hg in the upper wetlands of the Florida Everglades system.

## **Methodology**

This research has involved obtaining a better understanding of biogeochemical cycling of total and methyl Hg in the Florida Everglades system. Our group has specifically focused on two major research areas, processes controlling transport of Hg species across interfaces (air-water, sediment-water) and processes controlling bioaccumulation through the food chain. We have developed and instituted new techniques for sampling that integrate classical "trace metal clean techniques" into our sampling protocols. Our group has designed and participated in several diel studies at selected sites in the Everglades to determine the effects of sunlight-induced processes on Hg transport and partitioning. We have cooperated with the South Florida Water Management District and the Florida Game and Freshwater Fish Commission in coordinated research. Furthermore, our multidisciplinary Aquatic Cycling of Mercury in the Florida Everglades (ACME) project has allowed our group to work with several USGS research groups in Middleton WI, Boulder CO, and Menlo Park CA.

## **Principal Findings and Significance**

Our group has recently shown that methylation of Hg, a process typically thought to be limited to surface sediments, actively occurs in floating periphyton in many parts of the Everglades (Cleckner et al. 1999). This finding has potential management applications in that several regulatory agencies in Florida are considering developing stormwater treatment areas for nutrient removal and some designs call for periphyton-based systems. We have been continuing our work on identification and quantification of photosynthetic pigments in suspended particulate matter and periphyton in the Everglades. Pigment composition in the eutrophied northern regions of Water Conservation Area 2A has shown that pigments typical of blue-green and green algae dominate. In the southern regions where calcareous periphyton is the dominant primary producer, pigments typical of diatoms and chrysophytes dominate. We have identified the presence of a UV-blocking pigment compound (with spectral properties similar to scytonemin) in periphyton from southern portions of the Everglades. Additionally, we have observed the presence of bacteriochlorophylls, compounds characteristic of phototrophic sulfur bacteria. This is important because the presence of these microbes is usually in close proximity to sulfate reducing bacteria, principal methylators of Hg. The results of this research will be incorporated by various regulatory agencies in Florida (Florida Department of Environmental Protection, South Florida Water Management District) in a management plan for the Florida Everglades. Concurrent studies on nutrient inputs to the Everglades system will also be incorporated into a system model. The U.S. Geological Survey-sponsored project has provided a mercury cycling model that best synthesizes the results of the research. This model will allow various scenarios that aid in management decisions for Hg in the Everglades. Many of the principles developed during modeling efforts from our related work on northern Wisconsin lakes serve as the basis for the Everglades model. This Wisconsin Department of Natural Resources routinely uses the earlier model and may use the Everglades model to evaluate northern wetland cycling.

## **Descriptors**

Mercury, Contaminant transport, Florida Everglades, Wetlands

## Articles in Refereed Scientific Journals

Krabbenhoft, D.P., J.P. Hurley, M.L. Olson and L.B. Cleckner, 1998, Diel variability of mercury phase and species distributions in the Florida Everglades. *Biogeochemistry*, 40, pp. 311-325. Hurley, J.P., D.P. Krabbenhoft, L.B. Cleckner, M.L. Olson, G. Aiken, and P.J. Rawlik, 1998, System controls on aqueous mercury distribution in the northern Everglades, *Biogeochemistry*, 40, pp. 293-310. Cleckner, L.B. P.J. Garrison, J.P. Hurley, M.L. Olson and D.P. Krabbenhoft, 1998, Trophic transfer of methylmercury in the northern Everglades, *Biogeochemistry*, 40: pp. 347-361. Olson, M.L., L.B. Cleckner, J.P. Hurley, D.P. Krabbenhoft, and T.W. Heelan, 1997, Resolution of matrix effects on analysis of total and methyl mercury in aqueous samples from the Florida Everglades, *Fresenius J. Analytical Chemistry*, 358, p. 392-396. Cleckner, L.B., C.C. Gilmour, J.P. Hurley, and D.P. Krabbenhoft, 1999, Mercury Methylation by Periphyton in the Florida Everglades, *Limnology and Oceanography* 44, pp. 1815-1825. Babiarz, C.L., S.R. Hoffmann, M.M. Shafer, J.P. Hurley, A.W. Andren, D.E. Armstrong. 2000. A critical evaluation of tangential-flow ultrafiltration for trace metal investigations in fresh water systems: Part II Total and Methylmercury. *Environmental Science and Technology* (In Press). Krabbenhoft, D.P., J.P. Hurley, G. Aiken, C.C. Gilmour, M. Marvin-DiPasquale, W.H. Orem and R. Harris. 2000. Mercury cycling in Florida Everglades: A mechanistic field Study. *Verhandlungen Internationale Vereinigung Limnologie*. (In Press).

## Book Chapters

## Dissertations

## Water Resources Research Institute Reports

## Conference Proceedings

Krabbenhoft, D.P., J.P. Hurley, M.L. Olson, and L.B. Cleckner, 1996, Mercury transformation processes in the Everglades: temporal variations in mercury phase and species distribution and controlled exposure experiments, (abs) Proceedings of the Fourth International Conference on Mercury as a Global Pollutant, August 4-8, 1996, Hamburg, Germany. Olson, M.L., D.P. Krabbenhoft, L.B. Cleckner, and J.P. Hurley, 1996, Resolution of matrix effects on analysis of total and methyl mercury in aqueous samples from the Florida Everglades, (abs) Proceedings of the Fourth International Conference on Mercury as a Global Pollutant, August 4-8, 1996, Hamburg, Germany. Hurley, J.P., D.P. Krabbenhoft, G. Aiken, M.L. Olson, and L.B. Cleckner, 1996, System controls on water column total and methyl mercury in the northern Everglades (abs) Proceedings of the Fourth International Conference on Mercury as a Global Pollutant, August 4-8, 1996, Hamburg, Germany. Cleckner, L.B., P.J. Garrison, J.P. Hurley, D.P. Krabbenhoft, M.L. Olson, and T. Heelen, Relations between water chemistry and trophic transfer of mercury in the northern Everglades, (abs) Proceedings of the Fourth International Conference on Mercury as a Global Pollutant, August 4-8, 1996, Hamburg, Germany King, S.A., C.J. Miles, D.P. Krabbenhoft, J.P. Hurley, and L.A. Fink, 1997, Mercury studies in the Everglades Nutrient Removal Area, (abs), Proceedings of the Fourth International Conference on Mercury as a Global Pollutant, August 4-8, 1996, Hamburg, Germany. Krabbenhoft, D.P., J.P. Hurley, M.L. Olson, and L.B. Cleckner, 1996, Sunlight-induced, temporal variations in mercury phase and species distributions in the marshes of the Florida Everglades, (abs) American Chemical Society, national meeting, Program with Abstracts, Orlando, FL, August 23-28, 1996. Hurley, J.P., D.P. Krabbenhoft, L.B. Cleckner, S.A. King and M.L. Olson, 1996, System controls on the aqueous mercury distribution in the northern Everglades, (abs) American Chemical Society, national meeting, Program with Abstracts, Orlando, FL, August 23-

28, 1996. Cleckner, L.B., P.J. Garrison, J.P. Hurley, D.P. Krabbenhoft, M.L. Olson, and T. Heelen, 1996, Relations between water chemistry and trophic transfer of mercury in the northern Everglades, (abs) American Chemical Society, national meeting, Program with Abstracts, Orlando, FL, August 23-28, 1996. Krabbenhoft, D.P., J.P. Hurley, M.L. Olson, and L.B. Cleckner, 1997, The aquatic cycling of mercury in the Everglades (ACME) project: Results from the first two years of study, (abs), American Society of Limnology and Oceanography, national meeting, Santa Fe, NM, February 10-14, 1997. Cleckner, L.B., J.P. Hurley and P.J. Garrison, D.P. Krabbenhoft and M.L. Olson, 1997, The role of periphyton in mercury bioaccumulation in the Florida Everglades, (abs), American Society of Limnology and Oceanography, national meeting, Santa Fe, NM, February 10-14, 1997. Krabbenhoft, D.P., Hurley, J.P., M.L. Olson, and L.B. Cleckner, 1997, System controls on the aqueous mercury distribution in the northern Everglades, in U.S. Geological Survey Program on the South Florida Ecosystem - Proceedings of the Technical Symposium in Ft. Lauderdale, Florida, August 25-27, 1997; U.S. Geological Survey Open-File Report 97-385, p. 62-63. Krabbenhoft, D.P., Hurley, J.P., M.L. Olson, and L.B. Cleckner, 1997, Mercury transformation processes in the Everglades: Temporal variations in mercury phase and species distributions and controlled exposure experiments, (abs) in U.S. Geological Survey Program on the South Florida Ecosystem - Proceedings of the Technical Symposium in Ft. Lauderdale, Florida, August 25-27, 1997; U.S. Geological Survey Open-File Report 97-385, p. 63-64. Cleckner, L.B., J.P. Hurley and P.J. Garrison, D.P. Krabbenhoft and M.L. Olson, 1997, The role of periphyton in mercury bioaccumulation in the Florida Everglades, (abs), in U.S. Geological Survey Program on the South Florida Ecosystem - Proceedings of the Technical Symposium in Ft. Lauderdale, Florida, August 25-27, 1997; U.S. Geological Survey Open-File Report 97-385, p. 64-65. Olson, M.L., D.P. Krabbenhoft, L.B. Cleckner, and J.P. Hurley, 1996, Resolution of matrix effects on analysis of total and methyl mercury in aqueous samples from the Florida Everglades, (abs), in U.S. Geological Survey Program on the South Florida Ecosystem - Proceedings of the Technical Symposium in Ft. Lauderdale, Florida, August 25-27, 1997; U.S. Geological Survey Open-File Report 97-385, p. 65-66. Hurley, J.P., D.P. Krabbenhoft, P. Schuster, L.B. Cleckner, M.L. Olson, and G.R. Aiken, 1998, Development of a Conceptual Model of the Everglades Mercury Cycle: Results from Diel Studies, EOS, Transactions, American Geophysical Union, 1998 Spring Meeting, Program and Abstracts, May 26-29, Boston, MA. Krabbenhoft, D.P., Hurley, J.P., Aiken, G.R., Olson, M.L., Dewild, J.F., Cleckner, L.B., Grimshaw, J., Lindberg, S., 1998, The Influence of Photochemical Processes on the Everglades Mercury Cycle., EOS, Transactions, American Geophysical Union, 1998 Spring Meeting, Program and Abstracts, May 26-29, Boston, MA. Hurley, J.P., 1998, Chlorophyll and Carotenoid Pigments as Indicators of Algal and Phototrophic Bacterial Distributions in The Florida Everglades, EOS, Transactions, American Geophysical Union, 1998 Spring Meeting, Program and Abstracts, May 26-29, Boston, MA. Cleckner, L.B., C.C. Gilmour, J.P. Hurley, and D.P. Krabbenhoft, 1998, Mercury Methylation by Periphyton in the Florida Everglades, EOS, Transactions, American Geophysical Union, 1998 Spring Meeting, Program and Abstracts, May 26-29, Boston, MA. Schuster P, M.M. Reddy, G.R. Aiken, J.P. Hurley, and D.P. Krabbenhoft, 1998, Diel Sulfide and Dissolved Oxygen Concentration Gradients at Two Sites in the Everglades, EOS, Transactions, American Geophysical Union, 1998 Spring Meeting, Program and Abstracts, May 26-29, Boston, MA. Krabbenhoft D.P., Hurley, J.P., Marvin-DiPasquale, M., Orem, W.H., Aiken, G.R., Schuster, P.J., Gilmour, C.C., and Harris, R., 1999, The Aquatic Cycling of Mercury in the Everglades (ACME) Project: A Process-Based Investigation of Mercury Biogeochemistry in a Complex Environmental Setting, Program on the South Florida Ecosystem, Proceeding of the South Florida Restoration Science Forum, May 17-19, 1999, Boca Raton, FL, USGS Open-File Report 99-181, p. 15-16. Krabbenhoft D.P., and Hurley, J.P., 1999, The Sun's Detoxifying Effects on Mercury in the Everglades, Program on the South Florida Ecosystem, Proceeding of South Florida Restoration Science Forum, May 17-19, 1999, Boca Raton, FL, USGS Open-File Report 99-181, p. 17-18. King, S.A., Harvey, J.W., Krabbenhoft, D.P., Hurley, J.P., Armstrong, D.E., DeWild, J.F., and Olson, M.L., 1999, Distribution and Transport Mechanisms of Mercury and Methylmercury in Peat of the Everglades Nutrient Removal Area, Program on the South

Florida Ecosystem, Proceeding of the South Florida Restoration Science Forum, May 17-19, 1999, Boca Raton, FL, USGS Open-File Report 99-181, p. 19-20. Krabbenhoft, D.P., Hurley, J.P., Aiken, G.R., Gilmour, C.C., Mark Marvin-DiPasquale, M.M., William H. Orem, W.H., and Harris, R., 1999, The Aquatic Cycling of Mercury in the Evergaldes (ACME) Project: A Process-Based Investigation of Mercury Biogeochemistry in a Complex Environmental Setting, A&WMA International Specialty Conference on Mercury in the Environment, Minneapolis, Minnesota, September 15-17, 1999 Hurley, J.P., Krabbenhoft, D.P., Schuster, P.J., and George R. Aiken, G.R., 1999, Diel Studies in the Florida Everglades: Physical and Biological Influences on Mercury Dynamics, A&WMA International Specialty Conference on Mercury in the Environment, Minneapolis, Minnesota, September 15-17, 1999.

## Other Publications

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### Basic Project Information

Basic Project Information	
Category	Data
<b>Title</b>	Groundwater Protection by Application of Modern Portfolio Theory to Microbiotests
<b>Project Number</b>	B-01
<b>Start Date</b>	02/01/0096
<b>End Date</b>	02/28/0099
<b>Research Category</b>	Water Quality
<b>Focus Category #1</b>	Groundwater
<b>Focus Category #2</b>	Methods
<b>Focus Category #3</b>	Water Quality
<b>Lead Institution</b>	Water Resources Institute

### Principal Investigators

Principal Investigators			
Name	Title During Project Period	Affiliated Organization	Order
Gordon Chesters	Professor	Water Resources Institute	01
George A. Blondin	Professional Staff	Water Resources Institute	02

### Problem and Research Objectives

The growing impact of environmental regulations on the economy has led International, Federal, Regional, and State policy-makers to search for new cost-effective strategies for productive natural resource management in conformity with regulatory requirements. A specific approach advocated in the

sphere of water quality assessment and management is to use an integrated strategy of biological and chemical methods to monitor toxic substances in water matrices from industrial, agricultural, and municipal point and non-point sources. A major advantage of this tactic according to Dowd (1984) is that "only the aggregate toxicity of all constituents in a complex effluent or non-point source need be measured in screening programs and this aggregate measure becomes the yardstick for mandated toxicity reductions." The expensive analytical chemistry required to translate total toxicity into engineering controls for toxicity reduction would only be applicable to water sources that give rise to toxicity detection during a biological pre-screening stage of water quality assessment. Today, successful implementation of such a strategy is especially feasible because of the large array of new and established microbiotesting (MB) regimes (Microtox™, SMP™, algal, etc.) that incorporate the speed and cost-effectiveness necessary to justify their routine utility. The only major issues which have not been fully addressed are 1) how to select a small subset of the most appropriate MBs for a biomonitoring exercise directed at a particular group of toxicants, and 2) how to evaluate the assumption that the aggregate toxicity of all constituents in a complex water sample is detectable by this subset. The objective of the proposed study is to address these two issues by 1) evaluation of statistical models for their ability to facilitate the assembly of efficient multi-component MB batteries optimized for integrated quantitation of toxicity and 2) appraisal of an existing model (Finney, 1952) for its suitability as a potential predictor of aggregate toxicity in complex mixtures of pernicious chemicals.

## Methodology

The overall methodology encompasses 1) an extensive literature search to assemble toxicity indices for 40 toxic substances for each of 5 commercially available MBs and the computer generation of an additional 10 groups of toxic responses representing hypothetical MBs with unusual response characteristics (high or low sensitivity) relative to one or more of the chemical groups or constituents, 2) evaluation and comparison of 4 separate standard statistical procedures and one adaptation [Sharp (1963) Single Index model] for assembling efficient MB test suites, and 3) testing the suitability of Finney's equitoxic model for predicting the aggregate toxicity of MB test batteries. The 40 toxic substances selected for inclusion in the study are apportioned into 5 different groups of 8 chemical species each, including pesticides, chlorophenols, metal ions, homologous alcohols, and miscellaneous organic chemicals. These will be evaluated by various MB test suites by group, by various combinations of groups, and by the full 40 chemical substance collection ("Full Chemical Model"). The 5 commercially available MBs include 3 different mitochondrial (SMP, RET, and CET) bioassays, the ALG (Micro-Algal) test and the Microtox™ test. Together with the 10 hypothetical tests, these are referred to as the "Full MB Model." The standard statistical procedures for test suite selection comprises forward selection, backward elimination, and stepwise regression modules of SPSS v.7, a commercial high quality statistical software package and the Mallow Cp regression model which is a component of the Minitab™ (Release 11) Best Subset Regression model. The adaptation procedure is based on the Sharp (1963) Single Index Model (SIM) for Asset Allocation which was originally designed to find the weightings of stocks in an efficient portfolio which maximizes the portfolio rate of return for a given level of portfolio risk. Solution of this model involves multiple iterations of a series of calculations that give rise to the desired goal (maximize return) for each of several specified risk levels. The model is easily transferrable for the purpose of determining the best subsets (weightings) of MBs that give rise to the desired goal (maximize R-square). The only changes required are that the following constraints were imposed on the model to accommodate its translation: 1) The model is constrained so that the composition of each MB in a given suite can only be equal to 0 or 1. This constraint is necessary because we need to eliminate fractions of MBs or multiple identical MBs in the same suite; and 2) The final model (SIMTOX) is constrained to encompass a maximum of only 5 individual MBs per test suite. The model described by Finney (1952) is used for validation experiments. The model has

been validated for acute quantal responses by Smyth et al., (1969) and given rigorous statistical attention by Dunnett (1968). The procedure calls for the preparation of group mixtures of toxic substances in which the concentrations of individual components are equitoxic (in a 1:1 ratio of their respective EC-50 values). A dose response curve for the mixture is obtained and an observed EC-50 determined and compared with that provided by the model. The original model is as follows:  $1/PE_m = Pa/Ea + Pb/Eb + \dots + Pi/Ei$  where Pa, Pb, and Pi are the proportions (as a decimal part of 1) of each chemical constituent in the mixture, PE<sub>m</sub> is the predicted EC-50 of the mixture, and Ea, Eb, and Ei are the individual EC-50 concentration values of a, b, and the i<sup>th</sup> components. The model assumes additivity of action of the constituents and with all values on the right hand of the equation known from a given equitoxic mixture, a predicted EC-50 for the mixture can be calculated and compared with the actual experimentally determined value to verify integration of the response. Literature toxicity values for Fathead minnow responses to the 40 chemical list were taken from CLSES (1984) or on-line through ACQUIRE. MB toxicity responses were taken from COMPUTOX, a CD-ROM database of microbioassay toxicity responses available through Microbics Corp. or by computer literature searches. MB analysis to obtain statistical regression values for each of the 15 MBs for input to the various models were performed using Minitab software. The Solver module of EXCEL 5.0 was used to execute the SIMTOX model. The number of iterations required to achieve a solution involving 15 sets of input were determined semi-empirically according to the number of possible combinations of 2, 3, 4, and 5 MBs out of a list of 15. More sophisticated software packages are available to perform these compilations but the EXCEL module is extremely flexible and readily available in most PC installations which will likely simplify distribution of the SIMTOX Code to interested researchers.

## Principal Findings and Significance

The principal findings are: 1. R-square values, which are a measure of the degree and ordering of the toxicity responses of an individual MB test relative to the response of the standard fathead minnow test, ranged from 93.3% to 59.6% for the full chemical model (40 chemicals) challenge to each of the 15 different MBs. Full Chemical model challenge to the Full MB model gave rise to an R-square of 98.4%. 2. The R-square range of values for individual chemical group challenges to each MB test are shown as follows. Chemical Groups Highest R-Square Lowest R-Square Group Description R2 (%) Test I. D. R2 (%) Test I. D. 1 n-Alcohols 99.7 Mitochondrial CET 95.6 Hypothetical "G" 2 Heavy Metals 84.2 Micro Algal 9.6 Hypothetical "I" 3 Chlorophenols 88.9 Mitochondrial RET 0.3 Hypothetical "I" 4 Pesticides 91.6 Mitochondrial SMP 4.7 Microtox 5 Misc. Organics 97.4 Hypothetical "C" 64.1 Microtox 3. Standard statistical procedures (SPSS and Minitab) and the SIM model often choose the same two MBs for inclusion in the best 2 component test suite, whereas 3, 4, and 5 component test suites are invariably different with respect to the identity of components 3, 4 and 5 that are chosen by SPSS/Minitab or SIMTOX modeling. 4. Predictions of relative potency for the various test suites versus chemical groupings is strongly affected by the presence of regression outliers. 5. The following table is illustrative of findings 3 and 4 above. TEST BATTERY SELECTION RESULTS WITH FULL 40 CHEMICAL DATASET Test Battery Components R-square Mallows Cp Potency Selected by SIMTOX

Test Battery	Components	R-square	Mallows	Cp	Potency	Selected by SIMTOX
ALG 1	93.30%	68.41	82.90%	ALG SMP 2	94.74%	65.52 99.90%
ALG SMP J 3	94.87%	44.40	118.7%	ALG SMP J E 4	94.88%	23.51 134.1%
ALG SMP J E F 5	94.91%	13.64	138.8%	Selected by SPSS Forward Selection Regression		
ALG 1	93.30%	68.41	82.90%	ALG SMP 2	94.74%	65.52 99.90%
ALG SMP B 3	95.50%	36.50	93.80%	ALG SMP B H 4	96.20%	28.10 82.70%
ALG SMP B H C 5	96.90%	19.30	71.50%	R-SQUARE is adjusted; Bold Test Battery components have at least 1 significant outlier. The first and second regressors inserted by either model in the above table are those with the largest simple correlation with the response variable (fathead minnow LC-50). From this point on, the two models diverge in their selection process and results. The SIMTOX model continues to add regressors on the basis of which has the next largest simple correlation with the		

response variable while the SPSS Forward Selection procedure adds regressors by choosing the regressor that has the largest correlation with the response variable after adjusting for the effect of the second regressor entered, i.e., a new R-square and F-statistic is calculated for the ALG:SMP subset and the new regressor is added to this virtual regressor. In the SPSS Forward Selection procedure these partial correlations go on until the partial F-statistic does not exceed the F-in level. At this point, adding additional regressors is not likely to noticeably enhance the correlation of the subset (shown here as a 5 component subset) with the full 15 component model. The Minitab Best Subset regression procedure gives rise to the same five component subset as the SPSS Forward Selection procedure but it utilizes an entirely different criteria for determining the insertion termination point. If you look at the "Mallow's Cp" column adjacent to the SPSS section you will observe that termination occurred when Cp was reduced to 19.3. According to this procedure, regressors are added until the Cp statistic is close to the Cp statistic of the Full MB model which is equal to 16.0. As this work began to unfold, we soon recognized that we do not have a termination mechanism for the SIMTOX model. The Cp statistic was chosen to fulfill this function and its calculation was incorporated into the SIMTOX model. The last column in the table contains relative potency data. The data suggest that the predicted toxicity responses of the subsets are serially increasing relative to that of the fathead minnow as regressors are added by the SIMTOX model, but that they decrease as the regressors are added by the SPSS model. It is important to note that these features are not invariable characteristics of either model. For example, as regressors are added by the SPSS model, potency rises with the first two regressors, but serially declines by addition of the final 3. This behavior is probably related to and coincides with additions of MB tests "B" and "H" which have single major outliers (DDT and diethyl-hexyl phthalate, respectively) that are poorly detected by the respective test regimes. By contrast, the potency of the subsets generated by the SIMTOX model becomes progressively greater as regressors are added. But this behavior is likely to be partly due to the effect of the outlier, zinc, to which the "E" test is significantly more responsive. The significance of this work is that 1) diverse statistical procedures can be applied to the task of assembling microbioassay batteries that are likely to be more efficient than selection of test components on the basis of intuition; 2) incorporation of the potency statistic into the various models provides a response prediction for each subset which can be validated in the laboratory to give a unique level of insight to the utility of the various subsets; 3) some of the potential pitfalls to these procedures, notably the magnification of outlier effects, have been identified; and 4) a basic strategic difference between the SIMTOX and standard statistical procedure methods for assembling test batteries has been highlighted and needs to be further tested to determine the advantages or disadvantages viz a viz either method.

## **Descriptors**

Biomonitoring, Decision models, Groundwater quality, Pollutants, Toxic substances

## **Articles in Refereed Scientific Journals**

Dowd, R. M. 1984. Water quality based on toxicity testing. *Environ. Sci. Technol.* 18:281a. Sharpe, W. S. 1963. A simplified model for portfolio analysis. *Mgmt. Sci.* 9:277-293. Smyth, H. F., C. S. Weil, J. S. West, and C. P. Carpenter. 1969. An exploration of joint toxic action: twenty-seven industrial chemicals intubated in rats in all possible pairs. *Toxicol. Appl. Pharmacol.* 14:340-347.

## **Book Chapters**

CSLES. 1984. Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*). L. T. Brooke, D. J. Call, and D. L. Geiger (Eds.). Vols. 1-5. Center for Lake Superior Studies, Superior, WI.

Dunnett, C. W. 1968. Biostatistics in pharmacological testing. pp. 7-49. In: A. Burger (ed.). Selected pharmacological testing methods. Marcel-Dekker, Inc., New York. Finney, D. J. 1952. Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge University Press, London. pp. 1-318.

## Dissertations

## Water Resources Research Institute Reports

## Conference Proceedings

## Other Publications

## Basic Project Information

Basic Project Information	
Category	Data
Title	Molecular Probes for Human Pathogens in Water and Soils
Project Number	C-01
Start Date	09/01/0096
End Date	01/31/0099
Research Category	Water Quality
Focus Category #1	Water Supply
Focus Category #2	Waste Water
Focus Category #3	Water Quantity
Lead Institution	The University of Wisconsin

## Principal Investigators

Principal Investigators			
Name	Title During Project Period	Affiliated Organization	Order
William J. Hickey	Professor	The University of Wisconsin	01
John M. Harkin	Professor	The University of Wisconsin	02
John M. Harkin	Professor	The University of Wisconsin	02

## Problem and Research Objectives

Human pathogens (mainly enteric bacteria, protozoa and viruses) introduced into soil through septic system effluent and land-applied sewage sludge can be conveyed to surface and groundwater used as sources of drinking water and therefore pose significant public health risks on a nationwide scale, but especially in rural and agricultural areas such as the North Central States. Traditional techniques used to examine water for the presence of pathogens rely mainly on the culturing of non-pathogenic indicator organisms for detection by inference. The methods used are slow, are unable to distinguish between closely related pernicious or benign strains, and fail to detect viable but nonculturable bacteria. Thus,

when fecal contamination is suspected based on indicator-organism tests, the presence of pathogens, and the source of contamination (i.e., human v. animal excreta) remain uncertain. Alternatively, fecal contamination may go undetected if indicator bacteria are in a viable but non-culturable condition. To resolve these inadequacies of existing tests, this project focuses on developing a rapid molecular method using the polymerase chain reaction (PCR) coupled to an enzyme immunoassay (EIA) to test soils for the presence of specific sewage-borne pathogens. The new protocol would obviate the need to culture organisms for detection, and could remedy shortcomings of traditional techniques by allowing rapid, sensitive, and specific identification of the pathogens of concern rather than indicator organisms. To establish the validity and efficacy of the approach, a model PCR-EIA protocol will be developed to detect *Escherichia coli* and *Shigella dysenteriae* based on unique ribosomal RNA (rRNA) sequences, and will be used to examine questions regarding relationships between survival/occurrence of indicator organisms and pathogens in soil. This research was designed to develop a useful test for specific detection of two typical waterborne human enteric bacteria utilizing tailor-made molecular probes and demonstrate its utility for tracking pathogens through septic systems and soils treated with sewage, and for testing water from private wells, streams and swimming beaches in areas vulnerable to contamination by fecal or sewage wastes. The project objectives are to: 1. Design and validate PCR primers for specifically amplifying DNA from *E. Coli* and *S. dysenteriae* by targeting regions of 16S or other rRNA genes encoding regions unique to these organisms. 2. Optimize an FIA method for detecting DNA amplified by the PCR protocol and determine its precision and sensitivity. 3. Determine the performance of the PCR-EIA method for assaying the target organisms' DNA in nucleic acid extracts prepared from soils inoculated with live cells, dead cells, or purified nucleic acids. For the live cell treatments, PCR-EIA results will be compared to those of standard culture techniques. 4. Test the applicability of the PCR-EIA for investigating relationships between fecal coliforms and *S. dysenteriae* in soil impacted by on-site waste water treatment systems and sludge spreading. The PCR-EIA results will be compared to those of standard culture techniques for cost, processing time, and sensitivity.

## Methodology

The methods contemplated are summarized by the objective addressed. Objective 1. PCR primers will be designed by aligning sequences obtained either through ribosomal RNA sequence databases or by direct analysis of type species. Specificity will be verified by PCR tests with type cultures of the target organisms, closely related species, and non-coliform (negative control) bacteria. Amplification primers developed by previous investigators targeting the *uidR* gene (coding for  $\beta$ -D-glucuronidase) in *E. coli* and *S. dysenteriae* will be used as a positive control. Objective 2. The EIA detection method will use the PCR stage to incorporate a detectable label in the target sequence, which is then captured by hybridization to an immobilized oligonucleotide probe. EIAs will be done in 96-well microtiter plates analyzed colorimetrically by an automated plate reader. Objective 3. Soil will be inoculated with known densities of the target organisms, and nucleic acids directly extracted, and analyzed by PCR-EIA. To evaluate the potential for PCR-EIA to detect coliform DNA recovered from non-viable sources, dead cells and pathogen DNA will be added to soil and their persistence over time determined. Objective 4. The distribution and occurrence of the pathogens in soil samples obtained from existing, previously characterized septic systems and sludge disposal sites will be determined by using the PCR-EIA method; results will be compared to the traditional plate and membrane filter techniques for cost, processing time and sensitivity.

## Principal Findings and Significance

Objective 1. We have developed two classes of PCR primers that specifically detect *Escherichia coli*. One class targets the 16S ribosomal RNA gene (rDNA) while the other is directed against *phoR*, a gene

that regulates bacterial survival under conditions of phosphate-starvation. Design of rDNA PCR primers for *E. coli* was facilitated by the availability of a relatively large number of rDNA sequences for this organism. The primers were tested for amplification of rDNA from a collection of 30 different bacteria, which included common Gram-positive and Gram-negative soil bacteria, as well as *S. dysenteriae* and a variety of enteric bacteria closely related to *E. coli*. We found that to obtain the desired specificity (i.e., to distinguish *E. coli* from *Shigella* or other enterics) the PCR annealing temperature needed to be elevated to 70 C, which is 10 C above optimal. We were able to retain sensitivity by decreasing the cycle time. The optimized PCR has a total reaction time of 2 hours, and can detect as little as 10 fg of *E. coli* rDNA (approximately two genomic equivalents). Development of rDNA PCR primers for *S. dysenteriae* has been hampered by the paucity of rDNA sequence data for this organism; two sequences are available through databases and we determined a third. However, these three sequences differ significantly in the rDNA region that would allow differentiation between these organisms, and on which the *E. coli* 16S primers are based. Two sets of *phoR* primers were developed, one for *E. coli* and one for *S. dysenteriae*. These were tested against the culture collection described above, and against 10 clinical *E. coli* isolates and 11 mixed-cultures derived from water samples positive for *E. coli* by Colilert™. This allowed the primers to be tested for false negatives. The *E. coli* *phoR* primers amplified DNA from all *E. coli* cultures tested and there was no amplification for non-target organisms. The primers allowed detection of target DNA at the 5 fg level (one genome-equivalent). However, *S. dysenteriae* *phoR* primers cross-reacted with two clinical *E. coli* strains and 9 of 11 mixed water cultures. The absence of *Shigella* in the environmental samples was confirmed by using primers directed against a key pathogenicity gene that is present even in avirulent strains. Two types of PCR primers have been validated for specifically amplifying DNA from *E. coli*. Because these primers target different genes they are essentially independent, mutually complementary assays. The design of analogous PCR primers for *S. dysenteriae* has been hindered by the lack of available gene sequence data from *S. dysenteriae*.

Objective 2. Improvements in labeling technology have allowed us to substitute fluorescein for biotin as the label. Fluorescein-tagging of the *E. coli* rDNA primers did not alter their performance in PCR and gave detection limits as good as the non-labeled primers. The performance of the biotin-labeled primers, however was severely impaired. Synthesis of the capture probe, which spans a region of rDNA sequence that is highly conserved among bacteria, was also initiated. To create the probe, two complementary oligonucleotides were synthesized, phosphorylated, and annealed. This double-stranded version of the probe was then cloned into the filled-in *Sma*I site in the pBluescript II SK-. In the next stages, the cloned oligonucleotide will be released by *Pst*I digestion and then ligated together to create a linear array of ca. 50 copies of the probe. This will be ready for single-stranded expression from the *f1* phage origin of replication present on the vector, the single stranded form would be immobilized on the 96-well plate. Fluorescein-tagged PCR primers give the same performance as non-tagged primers under PCR conditions optimized for the latter. This means that a second round of tedious optimization experiments is unnecessary and that efforts can now be focused on development of the capture probe, which is the last stage of method development.

Objective 3. Two sets of experiments were done. The first set of experiments compared culture- and PCR (*E. coli* rDNA primers)-based methods for tracking *E. coli* in sterile- or non-sterile soil (an agricultural silt loam). Six different densities of stationary-phase *E. coli* (from 1,200 to 1.2 billion colony-forming units CFU/g) were inoculated into the soil. Then, after one day of incubation DNA was extracted from the soil and plate counts of culturable *E. coli* made using EMB medium (allows differentiation of *E. coli* from other bacteria by colony coloration). The same analyses was done after four or five days incubation. For the sterile soil incubated one day, plate counts showed *E. coli* numbers declined 5- to > 400-fold and were below detection limits (< 280 CFU/g) for soils inoculated with 1,200 CFU/g. PCR, however, was positive for all inoculated samples and negative for all non-inoculated controls. In non-sterile soil incubated one day, *E. coli* were not detectable by plate counts if the initial inoculum density were 100,000 CFU/g. Again, PCR was positive for all inoculated samples and negative for the non-inoculated controls. After five days incubation, there was no change in the plate count or PCR results. In the second set of experiments, the DNA extracted

from the soil used in the first group of experiments was spiked with varying amounts of purified *E. coli* DNA and then analyzed by PCR (*E. coli* rDNA primers). As a positive control, PCR was also done with universal rDNA primers that should amplify DNA from all organisms. With the universal primers, all samples amplified successfully. When *E. coli* rDNA primers were tested using the optimized PCR method, only reactions spiked with *E. coli* genomic DNA were positive. PCR was successful even with the smallest spike tested, which was 100 fg *E. coli* DNA (20 genomic equivalents) present in 100 ng of a heterogenous soil DNA extract. The PCR method can effectively track *E. coli* even in cases where the organism is non-detectable by standard plate-count methods. The apparent detection limit for the PCR method is at least 20 genomic equivalents. Objective 4. Analysis of drainfield soils at existing on-site waste disposal systems began late last summer (1998) in conjunction with an ongoing study in the Department of Biological Systems Engineering, University of Wisconsin-Madison. Drainfield soils beneath the conduit of drip irrigation systems were cored in 6-inch segments to a depth of 3.5 feet. After processing for total and fecal coliforms analysis by traditional culture-based methods, the core segments were subjected to DNA analysis. It is expected that samples taken at the level of the pipe will contain most of the fecal bacteria; the biomat which forms at this level acts as a filter and removes bacteria. Four groups of cores were analyzed during the fall of 1998. In the third group, coliform bacteria in the effluent had been severely attenuated through the use of an acidic drain cleaner; neither culture nor PCR showed any positive results. In the fourth group, PCR results were positive for samples which contained >500 CFU/gram wet weight of fecal coliforms, with PCR product yield correlating with coliform population. Comparison of PCR results with culture-based methods was hampered because: (1) the two analyses used two different subsamples of relatively inhomogeneous soil cores and therefore assessed two different samples, (2) the culture-based method counts fecal coliforms, most but not all of which are *E. coli*, (3) there was not an appropriate internal standard, and (4) the number of samples analyzed was quite large. These shortcomings are being addressed in the current sampling season.

## **Descriptors**

Bacteria, Bioindicators, Biomonitoring, Environmental sanitation, Groundwater quality, Health effects, Pollution control, Public health, Pollutants, Runoff, Septic tanks, Sludge, Soil microbiology, Viruses, Waste disposal, Wastewater, Water quality

## **Articles in Refereed Scientific Journals**

## **Book Chapters**

## **Dissertations**

## **Water Resources Research Institute Reports**

## **Conference Proceedings**

## **Other Publications**

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## Basic Project Information

Basic Project Information	
Category	Data
<b>Title</b>	Use of Specific Sorbents and Rapid Bioassays for Groundwater Monitoring
<b>Project Number</b>	C-02
<b>Start Date</b>	09/01/0097
<b>End Date</b>	06/30/0099
<b>Research Category</b>	Water Quality
<b>Focus Category #1</b>	Groundwater
<b>Focus Category #2</b>	Toxic Substances
<b>Focus Category #3</b>	Methods
<b>Lead Institution</b>	The University of Wisconsin

## Principal Investigators

Principal Investigators			
Name	Title During Project Period	Affiliated Organization	Order
John M. Harkin	Professor	The University of Wisconsin	01
John M. Harkin	Professor	The University of Wisconsin	01
Ronald Crunkilton	Professor	The University of Wisconsin	02

## Problem and Research Objectives

Groundwater contamination by pesticides and other organic compounds is a widespread problem in rural midwestern states, especially in areas of intensive agriculture and shallow or sandy soils. Detection of groundwater pollution using analytical methods is well documented, but its effects are not well known. To assess the acceptability of well water for human consumption, scientists usually analyze samples for chemicals such as hydrocarbons and fertilizer and pesticide residues and review toxicity testing information -- mostly on laboratory rodent species -- to estimate aggregate risks. This approach is slow, expensive and unable to account for additive, offsetting, synergistic, or antagonistic effects of mixtures of trace chemicals commonly encountered in drinking water. This problem is compounded by the fact that most contaminants exist in groundwater at concentrations below those detectable by inexpensive analytical methods. Analytical scans with state-of-the-art instrumentation needed for low level analysis are cost prohibitive for the typical groundwater user. There is a need to employ new cost effective assessment tools that directly measure potential health effects of ambient levels of contaminants in groundwater. This approach has been successfully applied to detect toxins found in surface water and written into permit compliance monitoring protocols in many states. Biomonitoring or toxicity testing is an established screening tool that can be used directly to assess the outcome of complex contaminant interactions in water samples, but this approach has yet to be applied in a systematic manner to the identification of potential hazards from exposure to groundwater pollution. Measurements of toxicity are not intended to replace chemical quantification methods. However, they can make better use of analytical time by screening samples for biological effects and using that information to prioritize samples for more expensive chemical testing.

## Methodology

Sample collection: Extensive groundwater monitoring will be accomplished at existing sites with known groundwater flow patterns and already containing numerous groundwater monitoring wells. These include a former manufactured gas plant contaminated by polynuclear aromatic hydrocarbons, two landfills where leachate has entered surrounding groundwater, and a pesticide production facility with known groundwater contamination by organochlorine, organophosphate, and carbamate pesticides. Appropriate sorbents with which we have prior experience include semipermeable membrane devices (SPMDs), C18 Sep-pak cartridges, and blue rayon. Of these, SPMDs were deemed the most useful and became the focus of subsequent studies. SPMDs were deployed directly within each monitoring well. Semipermeable membrane devices (SPMDs) have been used extensively as monitors of hydrophobic contaminants in surface water systems. They effectively concentrate non-ionic hydrophobic compounds from large volumes of water via passive hydrophobic partitioning into the membrane and its lipid contents from the surrounding water. These devices, consisting of lay-flat, low-density polyethylene (LDPE) tubing encapsulating 1 mL of a model lipid (triolein), are deployed in aquatic environments for extended periods and have been shown to highly concentrate trace levels of many classes of environmental pollutants. Chemical classes sampled include polynuclear aromatic hydrocarbons (PAHs), organochlorine and organophosphate pesticides, dioxins and polychlorinated biphenyls. Following removal of the SPMD from the well, they are extracted to isolate sorbed contaminants. These extracts are then split for concomitant analysis via GC-MS and in-vitro bioassay protocols. Water concentrations of contaminants can be back-calculated from residues accumulated in the triolein, using the formula  $CL = CW * RS * t / VL$  where  $CL$  = concentration in lipid,  $CW$  = concentration in water,  $RS$  = sampling rate,  $t$  = time, and  $VL$  = volume of lipid in SPMD exposure in days. Another back-calculation method involves determining the SPMD exposure volume, i.e. the volume of water that the SPMD has been exposed to during the deployment period. The mass of analyte accumulated by the SPMD is then divided by the exposure volume to obtain average water concentrations. The volume of water to which the in-situ samplers (SPMDs) will be exposed, and sampled, will be determined by calculating the rate of groundwater flow through the fixed volume of the sampling device that allows for lateral flow, but not vertical flow. Since monitoring wells are in areas of well-established groundwater flow, the flow velocity within the aquifer is known through the use of bromide tracer studies or estimated from knowledge of the hydraulic conductivity and gradient. Therefore, the rate of flow through the fixed volume of the sampler over time provides the total amount of water sampled. Toxicity testing: Rapid, in-vitro submitochondrial particle (SMP) tests were conducted at UW-Madison using protocols developed for use with an automated 96-well microtiter-plate reader. SMP bioassays assess the capacity of the inner membrane of mitochondria for electron transport and energy coupling as monitors of toxicity. SMP are added to buffer, reaction substrates, and SPMD extracts (dissolved in DMSO) and reactions are monitored spectrophotometrically at 340 nm in kinetic assays that assess the rate of reduction/oxidation of NADH. Reaction rates of toxicant trials are compared to rates with vehicle controls to determine the extent of redox-activity inhibition, which occurs in a reproducible dose-response fashion. With the microplate method, multiple variants of the SMP test can be run simultaneously. Since these afford different sensitivities reliant upon various idiosyncrasies of mitochondrial respiration, their use increases the likelihood of detecting toxicity compared to any single assay, especially when substance mixtures are being tested. Because of the small volume required per test, only tiny amounts of extracted toxicant are needed, so that many replicates can be conducted with any sample. Commercially available photoluminescent bacterial bioassays (Microtox/Mutatox) will be employed to assess the toxicity/mutagenicity of sample extract at UW-SP for comparison with the SMP bioassays. Both tests correlate well with whole-organism and in-vitro assays.

## **Principal Findings and Significance**

Water samples obtained from impacted groundwater wells at the various study sites were collected, extracted, and underwent chemical analysis and bioassay procedures. In general the SMP bioassay results were predictive of contamination identified in analytic scans especially at sites that were highly impacted, while the Microtox assay was less predictive. In part, this results from the Microtox bacteria's sensitivity to elemental sulfur present at toxic concentrations in some groundwater well extracts. This result emphasizes the importance of removing sulfur via a copper cleanup method prior to testing extracts using the Microtox assay. The SMP assay is far less sensitive to sulfur toxicity and its presence is of less concern. The bioassays, however, were not sensitive to low levels of contamination – reinforcing the argument for using SPMDs. SPMDs performed well in this study; SPMD-derived PAH concentrations in the groundwater at the former manufactured gas plant were similar to values obtained by the conventional techniques. Furthermore, they allowed detection of some PAHs not detected by conventional approaches. A primary goal in this research is to conduct toxicity testing on extracts from SPMDs deployed in groundwater wells. A major obstacle to adapting the SPMD dialysates to toxicity testing is removing co-extracted triolein impurities and LDPE oligomers prior to solvent exchange to DMSO and toxicity testing. The most prevalent contaminant, methyl oleate, is typically removed using gel-permeation chromatography (GPC) conducted on HPLC. However, this method is costly, time-consuming, and not practical in many laboratory settings. An SPE cleanup method based on normal-phase separation using a silica-based restricted-access sorbent has been devised to selectively remove methyl oleate from extracts while not affecting PAH concentrations. This allows for a simple, one-step removal of co-extracted interferences, a significant advance in adapting SPMDs for use with bioassays. This advance will allow for the processing of SPMDs for use in both bioassay and analytical procedures. Results will be used to determine the suitability of the SPMDs as in-situ samplers in groundwater monitoring protocols and the use of the toxicity tests to provide a measure of chemical contamination and the potential impact of these contaminants on organism and human health.

## **Descriptors**

Biomonitoring, Groundwater quality, Land-water interactions, Toxic substances, Trace organics, Water Chemistry, Water quality, Water quality monitoring

## **Articles in Refereed Scientific Journals**

Gustavson, K. E., A. Svenson, and J. M. Harkin. 1998. Comparison of toxicities and mechanism of action of n-alkanols in the submitochondrial particle (SMP) and the *Vibrio fischeri* bioluminescence (Microtox) bioassay. *Environ. Toxicol. Chem.* 17:10:1917-1921.

## **Book Chapters**

Read, H. W., J. M. Harkin, and K. E. Gustavson. 1998. Environmental applications with submitochondrial particles. Pp. 31-52. In: P. G. Wells, K. Lee, and C. Blaise (eds.). *Microscale testing in aquatic toxicology: Advances, techniques and practice*. CRC Lewis Publishers, Boca Raton, Florida.

## **Dissertations**

## **Water Resources Research Institute Reports**

## Conference Proceedings

## Other Publications

Sonsthagen, S., K. Gustavson, and R. Crunkilton. 1999. Use of in vitro and whole-animal assays to assess groundwater contamination. Abstract. American Water Resources Association-Wisconsin Section and Society of Environmental Toxicology and Chemistry- Midwest Chapter, Joint Annual Meeting, La Crosse, Wisconsin. p. 27.

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## Basic Project Information

Basic Project Information	
Category	Data
<b>Title</b>	Monitoring and Research of Streamflow, Sediment Transport, and Water Quality in the Colorado River, Glen Canyon Dam to Lake Mead
<b>Project Number</b>	S-02
<b>Start Date</b>	05/01/0098
<b>End Date</b>	12/31/0099
<b>Research Category</b>	Water Quality
<b>Focus Category #1</b>	Sediments
<b>Focus Category #2</b>	Surface Water
<b>Focus Category #3</b>	Water Quality
<b>Lead Institution</b>	The University of Wisconsin

## Principal Investigators

Principal Investigators			
Name	Title During Project Period	Affiliated Organization	Order
Carl J. Bowser	Professor	The University of Wisconsin	01
Mark T. Harris	Professional Staff	US Geological Survey	02

## Problem and Research Objectives

The ecosystem along the Colorado River through Grand Canyon National Park and Glen Canyon National Recreation Area has been altered by the construction of Glen Canyon Dam and the subsequent manipulation of flow to generate hydroelectricity. When Lake Powell formed behind Glen Canyon Dam, three major environmental changes occurred in the downstream river: regulation of flow has eliminated

annual flooding of the river; sediment load of the main channel is now deposited in the lake, creating clear water releases from the dam; and temperature of the downstream river is colder because water released from the dam can only be drawn from the lower depths of Lake Powell. These changes have, in turn, given rise to others. The physical processes of flow, sediment transport, and water quality were identified as key factors linking dam operations to changes in the ecosystem below the dam. In cooperation with the U.S. Geological Survey (USGS), we are conducting a program of monitoring and research that is designed to detect change in key environmental variables over time. This program is designed to sufficiently understand sediment transport processes so that the system can be manipulated to more efficiently manage the limited sediment supply below the dam. The sediment supply is currently valued most for maintenance of camping beaches and backwater habitats for native fish. Specific objectives of the project are to: • Develop a monitoring and research approach that over time will differentiate short-term variation from long-term trends in sand storage in the most sediment limited reach; • Examine the role that sediment grain size has on the measurement and prediction of sand transport through the Grand Canyon; • Compare the means of indicating the sand-storage condition of the channel and whether a small network of cross-section measurements is as reliable as other more extensive measurements; • Better understand the source of clay- and silt-sized particulates in the mainstem, their role in reducing light penetration, and, if possible, link this work to the work of other scientists who are conducting research on native fish; • Develop and implement a plan for monitoring appropriate water quality parameters in the Colorado River to assess the effects of river management on primary production in the river below the Glen Canyon Dam; and • Link variations in river production to processes operating within the Lake Powell Reservoir and the effects of management operations on these processes.

## **Methodology**

Hydrolaboratory stations have been established for five recording stations in the Glen Canyon tailwater reach extending from Lees Ferry to River mile-11. Monitoring for pH, dissolved oxygen, temperature, and specific conductivity is done for four 48-hour periods coinciding with the two equinoxes and two solstices of the solar year. Measurements are made at 5-min. intervals. Calibration of the instruments were conducted before and after the 2-day measurements to assess instrument drift. Data is downloaded to a spreadsheet form and shared among the data collection group for evaluation. During the river measurement periods, similar water parameters in the forebay of Lake Powell were measured using a "Searbird@" instrument.

## **Principal Findings and Significance**

To date, four sampling periods have been completed for water year 1998-1999. In June 1998 a set of stations was established in the Glen Canyon tailwater reach. Monitoring stations were set up at river miles -3, -6, -9, -11, and at the Lees Ferry water gauge. Hydrolab instruments were deployed on four occasions (June, September, and December 1998, and March 1999). Preliminary evaluations of the data have been ongoing. An analysis of the results is premature at this time since the purpose of the measurements was to conduct a full year's observations before synthesizing any results.

## **Descriptors**

Dams, Ecosystems, Flow, Rivers, Sediment transport, Surface water, Water quality

## **Articles in Refereed Scientific Journals**

**Book Chapters**

**Dissertations**

**Water Resources Research Institute Reports**

**Conference Proceedings**

**Other Publications**

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**Basic Project Information**

<b>Basic Project Information</b>	
<b>Category</b>	<b>Data</b>
<b>Title</b>	The Use of Subsurface Irrigation to Restore Degraded Groundwater-fed Wetlands
<b>Project Number</b>	S-03
<b>Start Date</b>	09/01/0098
<b>End Date</b>	02/28/0001
<b>Research Category</b>	Water Quality
<b>Focus Category #1</b>	Groundwater
<b>Focus Category #2</b>	Wetlands
<b>Focus Category #3</b>	None
<b>Lead Institution</b>	National Science Foundation

**Principal Investigators**

<b>Principal Investigators</b>			
<b>Name</b>	<b>Title During Project Period</b>	<b>Affiliated Organization</b>	<b>Order</b>
Kenneth W. Potter	Professor	The University of Wisconsin	01
Jean M. Bahr	Professor	The University of Wisconsin	02

**Problem and Research Objectives**

Development can cause significant degradation of aquatic ecosystems, desiccating wetlands and springs by reducing recharge (increased impervious area) and lowering water table levels (groundwater extraction or drainage), or increasing flooding due to urban stormwater. Wetland restoration efforts have been undertaken more frequently in recent years as society has recognized the value of wetland functions lost due to human impact. Unfortunately, these projects have often been less than successful in restoring the conditions conducive to the development of natural wetland communities. This is especially true for groundwater-fed wetland systems because of the difficulty in establishing an

appropriate hydrologic regime. Because of this difficulty, most groundwater-fed wetlands harmed by the effects of development have either been replaced with systems fed by surface water, which often results in completely different wetland communities, or have not been replaced at all. The net result is that the groundwater-fed wetlands that were major constituents of the pre-settlement lowlands in Wisconsin and much of the rest of the Midwest are steadily declining. Research into the mitigation of development impacts on groundwater-fed wetlands has applications across most areas of water resources. Resource agencies are charged with permitting and enforcement responsibilities with regard to wetlands impacts and restoration, and perform wetland restorations to enhance natural resources within their jurisdictions. Water utilities aim to meet increased demands for groundwater resources while reducing the environmental degradation caused by water table drawdown. Wastewater and stormwater utilities look to meet their mandated responsibilities while facing greater pressure to reuse their effluents and not shunt flows out of their watersheds. Finally, landowners such as private citizens or park agencies are interested in controlling the invasive species accompanying wetland desiccation, specifically reed canarygrass (*Phalaris arundinacea*), and restoring natural communities for habitat and aesthetic purposes. By developing a means to restore groundwater-fed wetlands using water sources such as stormwater or wastewater effluent, future wetland degradation may be minimized, historical degradation reversed, and water that would otherwise be quickly flushed out of the watershed would be used to maintain important biological communities. We are testing a new scheme for wetland restoration: reintroducing water into a desiccated wetland via subsurface irrigation. In our test, highly treated effluent flows from the Madison Metropolitan Sewerage District (MMSD) pipeline to the Sugar River would be introduced into the subsurface of a degraded sedge meadow via perforated pipes inserted beneath the ground surface. The experimental site once supported a sedge meadow but now maintains a monospecific reed canarygrass community. This project will replicate a hydrologic regime similar to that occurring in sedge meadows elsewhere in the Nine Springs system. An ongoing research project being conducted by the same investigators in a relatively undisturbed sedge meadow approximately 2.5 kilometers southwest of the project site will be used to define such a regime. By raising the water table to the surface during the growing season the project will provide conditions more favorable to desirable sedge meadow species and less favorable to invasive plants such as reed canarygrass. The area will be subdivided into 12 experimental plots, four of which will serve as controls. The water will enter the experimental plots through a "reverse drain tile" which would reintroduce water until the water table approaches the surface. Plant growth in the plots will be monitored through several growing seasons to determine whether this restoration technique will allow more desirable wetland communities to develop. The main objectives of this project are to:

- Design and implement an experimental system for using treated effluent from MMSD to offset the impacts of reduced groundwater levels on a portion of degraded wetlands in the Nine Springs E-way.
- Determine the changes in plant community which result from the subsurface application of the treated effluent.
- Determine the changes in plant community which result from the subsurface application of treated wastewater which has undergone additional phosphorus removal.
- Determine whether water table manipulation is an effective control technique for reed canarygrass.

## **Methodology**

Twelve 25 m<sup>2</sup> plots will be fabricated to test the effects of reintroducing water into the subsurface of a degraded wetland in the Nine Springs E-way. Impervious barriers extending into the underlying silt layer will isolate each experimental plot from the others and from the rest of the site. Eight of these plots will be treated and four will act as controls. Treatment will consist of mowing and tilling the existing reed canarygrass and installation of lengths of 4-inch perforated PVC drain tile. During the growing season (spring thaw through first freeze) return water will be fed to the drain tiles. Return flows from the MMSD line will be introduced to the plots through a simple hydraulic system. A rubber

hose will carry water from a valve installed in the return flow pipe approximately 1,000 feet to the experimental site. This hose will enter an equalizing chamber consisting of a large (50 to 100-gallon) open trough. The water level in this trough will be maintained by a float connected to a butterfly valve on the inflow; flow will be allowed into the trough until the water level nears overflow, at which point the flow will be shut off. Two hoses will drain this trough and take flows to the experimental plots. One of these hoses will supply two of the experimental plots directly while the other will pass flows through a phosphorus reduction chamber before supplying the other two experimental plots. Flows entering each plot will be controlled, probably using a solenoid valve, based on the groundwater level in that plot and an electronic flowmeter will be used to monitor flow into each plot. Hourly output from the flowmeter and the control system will be stored in a data logger for later analysis. Groundwater levels will be monitored at several points within and around the experimental plots using a number of mini-piezometers. The groundwater level in each plot will be used to control the inflow of water into that plot; water will be added until the groundwater level reaches the desired level in the plot at which point the water will be turned off until more is needed. Control levels will be set based on the levels observed in a nearby existing sedge meadow. The flow of water will be discontinued following the first hard freeze in the fall and be restarted following the spring thaw. Two types of experimental control will be included in this experiment. The first control type will test the effects of the initial treatment alone; a plot will be installed, mowed, tilled and isolated but no subsurface water will be introduced into it. The second, no-action control will be sites set aside to monitor the dynamics of the reed canarygrass community on the site independent of any treatment effects. Among the data collected for this experiment will be that assessing the hydraulic function of the experimental system, the state of the groundwater on the site, the chemical and physical quality of the groundwater and the biological response of the wetland community. Hydraulic function will be physically assessed every 2-3 days and hourly inflow and control groundwater data will be electronically collected for each experimental plot. During the growing season weekly sampling trips will be used to measure groundwater levels and to download data logger data. Groundwater quality will be sampled once or twice a month and standard parameters will include temperature, conductivity, dissolved oxygen, pH, chloride and nutrients. Biological response will be intensively evaluated by annual or semiannual plant surveys, and biweekly photographs will be used for more subjective evaluation.

## **Principal Findings and Significance**

In the few months since this project began, we have designed the experimental system and are in the process of installing the wetland cells. The wetland cells should be installed by the end of June 1999 and at that time we will begin artificially flooding the cells.

## **Descriptors**

Groundwater, Plant-water relationships, Urban hydrology, Wetlands

## **Articles in Refereed Scientific Journals**

## **Book Chapters**

## **Dissertations**

## **Water Resources Research Institute Reports**

## **Conference Proceedings**

# Information Transfer Program

Library Collection and Facilities The Water Resources Institute Library (WRIL) maintains a specialized collection of over 21,000 water-related publications in hard copy and microfiche, more than 35 journals, and more than 135 newsletters. The collection covers all major topics in water resources, including the water cycle, water conservation, water management, water quality and quantity, point and nonpoint water pollution sources, water law, and aquatic life. The collection is particularly strong in Wisconsin and Great Lakes water resources issues, groundwater protection, wetlands issues, and the impacts of agricultural chemicals. The Water Resources Institute Library serves Institute staff, University of Wisconsin faculty, staff, and students, Wisconsin state government, businesses, and industry, and other Wisconsin residents. The Library will lend documents to non-Wisconsin residents, but provides other services only as time and resources permit. The Library is staffed and open to the public 9 a.m. to 4:30 p.m. Monday through Friday. The entire collection is now included in Madcat, the University of Wisconsin online catalog and can easily be searched by author, title, or subject heading by anyone on campus and by anyone in the world over the Internet. The collection is also available online in the CIC (Committee on Institutional Cooperation) Virtual Catalog and the Online Computer Library Center's (OCLC) WorldCat. Access to water resources indices and databases is gained through the University of Wisconsin Electronic Library, Dialog, and the many sources of information on the Internet. This unique Wisconsin resource of water-related information is one of only two such libraries established under the State Water Resources Research Institutes Program. The Library collection, electronic resources and services are built upon and the result of long-term cooperation and coordination with other University of Wisconsin and area libraries. Library staff participate in campus library groups, the Wisconsin Chapter of the Special Libraries Association, the Wisconsin Library Association, and other library organizations. Through this coordination, the librarian builds a unique collection that does not duplicate the collections of other area libraries. WRI Library contributions help make Water Resources Abstracts available online to the UW-Madison campus. In turn, the Library depends on the UW Electronic Library for access to other online water resources indices, databases and full text journals and documents. The UW General Library System partially supports WRI Library participation in MadCat, the University online catalog. The Library also cooperates with the Wisconsin Department of Natural Resources (WDNR) Library which depends on the library to collect water-related technical reports. To this end, the WDNR contributes funds each year to support the purchase of such materials. New Activities: During FY 1999, the Library expanded its Web site at <http://wri.wisc.edu/library> and increased the scope of its monthly "Recent Acquisitions" list to include new and noteworthy Web sites. In addition to redesigning the Web site, new features include: . "A Guide to Finding Water-Related Information on the Web" . "A Guide to Finding a Water-Related Job on the Web" . "A Guide to Searching MadCat for WRIL Documents" The librarian has expanded the monthly "Recent Acquisitions" list to include "Web Sites of Interest" to water resources professionals and students. The list is now primarily sent out through email, but library staff also provide print copies for those who request it. Staff also provide back issues on the Web. In addition to the URL, an annotation is provided for each Web site. Library Services: The Water Resources Institute Library offers the following services: . Loan of documents and journals through interlibrary loan, the mail, or in person. . Distribution of the monthly library newsletter, "Recent Acquisitions and Web Sites of Interest", to approximately 300 university personnel, state agency staff, researchers, consultants, libraries, private organizations and interested citizens. The newsletter is distributed by mail, email, and put on the Library Web site. . Subject searches of MadCat, Water Resources Abstracts, UW Electronic Library databases, Dialog databases, and Internet resources. . Ready reference and referral, e.g., short answers to questions. Examples include addresses, telephone numbers, bibliographic citations, etc. The Library has also instituted an e-mail reference service, AskWater. . Making the WRIL collection available in MadCat, the CIC Virtual Catalog and OCLC WorldCat. The collection can now be

searched by anyone over the Internet. . A WRI Library web site at <http://wri.wisc.edu/library> includes information about the library and library policies, links to renew and request books, an electronic reference service (AskWater), backfiles of "Recent Acquisitions and Web Sites of Interest", links to the UW online catalog and databases, and guides to finding water-related information and jobs on the Web. . Provision of two terminals with access to all of the resources of the UW Electronic Library, Dialog and the Internet for use by faculty, staff, students and others who come to the library. The Library also provides free printing and photocopying. . Since August, 1990 the Library has circulated and served as a depository for the reports of the WDNR Groundwater Management Program Monitoring Project. The Library has added these reports to Madcat, listed them in the Recent Acquisitions list, and provided staff to put summaries of these projects on the world wide web. Reference Services Use of the WRI Library by faculty, students, federal agencies, private consulting firms, and others interested in water has grown greatly over the last several years. Book circulation has increased more than 51% and requests for information more than 63% from FY 98 to FY 99. Since adding the collection to MadCat, the University of Wisconsin online catalog, in the summer of 1995, student use has increased significantly. During FY99, the library staff responded to 1238 requests for individual titles and subject searches. More than 691 UW-Madison faculty, staff, and students, WDNR staff, private consulting organizations, and members of the public contacted the WRIL last year. Water Resources Institute Publications Results of WRI-supported research are published in a variety of forums. Much of the WRI research ultimately appears in refereed professional journals, although results of WRI research can also be accessed as technical reports, conference proceedings and abstracts, book chapters, or as dissertations and theses. A list of all publications resulting from WRI-supported research is maintained and will be added to the WRI web site over the next year. Copies of the publications housed at the WRI are distributed upon request. A highlight during the past year was the production of the Wisconsin Water Resources Institute Program Directory. This directory provides a brief history of the Wisconsin WRI program, briefly describes all research projects supported through the WRI, and gives a general overview of the WRI program. During the next fiscal year the WRI will begin publishing a newsletter. Each issue will feature a noteworthy research project, announce recently funded research, post new and noteworthy water-related web sites, list significant acquisitions to the WRI Library, and provide a forum for announcing relevant local, regional or national water-related news. Water Resources Institute Web Site The WRI has maintained a Web site since 1995 to provide an efficient means for the transfer of water-related information. The web site was originally developed by the WRI Library on the the University of Wisconsin General Library System server; but the Web site has been greatly expanded and transferred to WRI's own on-site server. The site provides information about WRI programs and staff, water resources funding opportunities, conference information, project summaries, links to other water-related sources, and extensive information about the WRI Library. In addition, The Directory of Water Resources Personnel in Wisconsin, a comprehensive listing of more than 800 water professionals in Wisconsin, has been added to the WRI web site and is searchable by last name of expert, area of expertise, and/or research interest. The address of the WRI web site is <http://www.wri.wisc.edu>. Conferences, Meetings and Presentations The Wisconsin Water Resources Institute co-sponsored the American Water Resources Association -- Wisconsin Section Annual Meeting on March 23 and 24, 2000 in Green Bay, Wisconsin. This meeting provided a forum for nearly 50 papers that covered a variety of water-related subjects and were presented during six technical sessions and a poster session. This meeting is unique because it especially encourages students to present papers or posters describing their original research. Awards are presented to the student papers judged to be "best." At the meeting, the WRI librarian in cooperation with Steenbock Agricultural Library staff made a presentation on "Finding a Water-Related Job on the Web". The librarian was the 1999 Chair of the Wisconsin Library Association's Association of Special Librarians (AWSL) and involved in program planning for the annual WLA conference. In 1999, AWSL sponsored four programs and two special library tours - all well-attended and enthusiastically received - but particularly popular were the tours of the Fish Lab (USGS's Upper Midwest Environmental Sciences Center) and its library and the UW-LaCrosse Steamboat Archives.

## **USGS Internship Program**

## Student Support

Student Support					
Category	Section 104 Base Grant	Section 104 RCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	N/A	1	N/A	N/A	1
Masters	2	1	N/A	N/A	3
Ph.D.	2	4	1	N/A	7
Post-Doc.	N/A	N/A	N/A	1	1
<b>Total</b>	4	6	1	1	12

## Awards & Achievements

The University of Wisconsin System, through the Water Resources Institute, has coordinated and conducted a wide-ranging program of priority groundwater research. With nearly 75% of its base budget targeted for research, during FY 99 the WRI supported 28 individual projects, including short-term and long-term studies of either a fundamental or an applied nature. These have provided a balanced program of laboratory, field, and computer modeling studies and applications aimed at preserving or improving groundwater and surface water quality. In addition, the projects have provided training in several disciplines for post-doctoral research associates, graduate student research assistants, and undergraduate students at the University of Wisconsin-Madison, University of Wisconsin-Milwaukee, University of Wisconsin-Parkside, and University of Wisconsin-Stevens Point. Researchers working on the project "Mercury Speciation and Transport in the Florida Everglades" have recently shown that methylation of Hg, a process typically thought to be limited to surface sediments, actively occurs in floating periphyton in many parts of the Everglades. This finding has potential management applications in that several regulatory agencies in Florida are considering developing stormwater treatment areas for nutrient removal and some designs call for periphyton-based systems. The WRI has maintained a web site since 1995 to provide an efficient means for transfer of water-related information. The web site was originally developed through the University of Wisconsin General Library System, but recently has been greatly expanded to and transferred to WRI's own on-site server. The current address for the WRI web site is <http://wri.wisc.edu>. Two WRI-supported students were selected to receive U.S. Environmental Protection Agency STAR (Science to Achieve Results) Graduate Fellowships. The selection process, in part, considered the students' work on the projects, "Use of Specific Sorbents and Rapid Bioassays for Groundwater Monitoring" and "Mercury Speciation and Transport in the Florida Everglades." The Ph.D. Dissertator supported by the U.S. Geological Survey-funded project, "Use of Specific Sorbents and Rapid Bioassays for Groundwater Monitoring," won the Promising Young Scientist Award at the Ninth International Symposium on Toxicity Assessment, Pretoria, South Africa, September 26 - October 1, 1999. This student also won the Outstanding Platform Presentation Award sponsored by 3M Corporation at the Society of Environmental Toxicology and Chemistry - Midwest Chapter meeting in Bloomington, April 13-14, 2000. The highly competitive and prestigious Horton Research Grant, awarded annually by the Hydrology Section of the American Geophysical Union, was won by a WRI-supported Ph.D. candidate this year. The student was selected, in part, for his work on the project, "The Spatial Variability of Natural Groundwater Recharge." Current water-related information is made easily accessible to researchers, public officials, state administrators, university students and the public through the WRI information transfer program. A list of all publications resulting from WRI-supported research is maintained and will be added to the WRI web site over the next year. Copies of the publications housed at the WRI are distributed upon request. In addition, the WRI serves as a repository for reports on all projects carried out through the Wisconsin Groundwater

Coordinating Council. A highlight during the past year was the production of the Wisconsin Water Resources Institute Program Directory. This directory provides a brief history of the Wisconsin WRI program, briefly describes all research projects supported through the WRI, and gives a general overview of the WRI program. During the next fiscal year the WRI will begin publishing a newsletter. Each issue will feature a noteworthy research project, announce recently funded research, post new and noteworthy water-related web sites, list significant acquisitions to the WRI Library, and provide a forum for announcing relevant local, regional or national water-related news. During FY 1999, the WRI librarian redesigned and expanded the Library Web site at <http://wri.wisc.edu/library>. The following new features were added to the Web site: · "A Guide to Finding Water-Related Information on the Web" · "A Guide to Finding a Water-Related Job on the Web" · "A Guide to Searching MadCat for WRIL Documents" Last fiscal year, the librarian also increased the scope of the monthly "Recent Acquisitions" list to include new "Web Sites of Interest" to water resources professionals and students. In addition to the URL, a descriptive annotation and evaluation is provided for each Web site.

## Publications from Prior Projects

### Articles in Refereed Scientific Journals

Cheng, Y. S., J. L. Halsey, K. A. Fode, C. C. Remsen, and M. L. P. Collins. 1999. Detection of methanotrophs in groundwater by PCR. *Appl. Environ. Microbiol.* 65:648-651. Cleckner, L. B., C. C. Gilmour, J. P. Hurley, and D. P. Krabbenhoft. 1998. Mercury methylation by periphyton in the Florida Everglades. *Nature*. (In press.) Elder, C. R., and C. H. Benson. 1999. Air channel formation, size, spacing, and tortuosity during air sparging. *Ground Water Monitor. Remed.* 19(3):171-181. Gustavson, K. E., A. Svenson, and J. M. Harkin. 1998. Comparison of toxicities and mechanism of action of n-alkanols in the submitochondrial particle (SMP) and the *Vibrio fischeri* bioluminescence (Microtox) bioassay. *Environ. Toxicol. Chem.* Hurley, J. P., D. P. Krabbenhoft, L. B. Cleckner, M. L. Olson, G. Aiken, and P. J. Rawlik. 1998. System controls on aqueous mercury distribution in the northern Everglades. *Biogeochemistry* 40:293-311. Keating, E. H., and J. M. Bahr. 1998. Using reactive solutes to constrain groundwater flow models at a site in northern Wisconsin. *Water Resour. Res.* 34(12):3561-3571. Keating, E. H., and J. M. Bahr. 1998. Reactive transport modeling of redox chemistry: Approaches to chemical disequilibrium and reaction rate estimation at a site in northern Wisconsin. *Water Resour. Res.* 34(12):3573-3584. Krabbenhoft, D. P., J. P. Hurley, G. Aiken, C. C. Gilmour, M. Marvin-DiPasquale, W. H. Orem, and R. Harris. 1999. Mercury cycling in the Florida Everglades: A mechanistic field study. *Verhandlungen Internationale Vereinigung Limnologie*. (In review.) Miller, L. W., and M. Anderson. 1998. Fiber-mediated titanium dioxide photocatalysis. *J. Adv. Oxidation Technol.* (In press.) Miller, L. W., M. Anderson, and I. Tejedor-Anderson. 1998. Titanium dioxide-coated quartz waveguides for the photocatalytic oxidation of formic acid in water. *Environ. Sci. Technol.* (In review.) Rosenshield, M. L., M. B. Jofre, and W. H. Karasov. 1999. Effects of polychlorinated biphenyl 126 on green frog (*Rana clamitans*) and leopard frog (*Rana pipiens*) hatching success, development, and metamorphosis. *Environ. Tox. Chem.* 18(11):2478-2486. Schreiber, M. E., and J. M. Bahr. 1999. Special electron acceptor variability: Implications for assessing bioremediation potential. *Bioremed. J.* 3(4):363-378. Schulze-Makuch, D., and D. S. Cherkauer. 1998. Variations in hydraulic conductivity with scale of measurement during aquifer tests in heterogeneous, porous carbonate rocks. *Hydrogeol. J.* 6(2):In press. Shi, Y., M. D. Zwolinski, M. E. Schreiber, J. M. Bahr, G. W. Sewell, and W. J. Hickey. 1999. Molecular analysis of microbial community structure in pristine and contaminated aquifers: Field and laboratory microcosm studies. *Appl. Environ. Microbiol.* 65(5):2143-2150. Tsay, T.-S., and J. A. Hoopes. 1998. Numerical simulation of ground water mounding and its verification by Hele-Shaw model. *Comp. Geosci.* 24(10):979-990. Yuan, H., J. L. P. Collins, and W. E. Antholine. 1998. Concentration of Cu, EPR-detectable Cu, and formation of cupric-ferrocyanide in membranes with pMMO. *J. Inorganic Biochem.* 72:179-185. Yuan, H., J. L. P. Collins, and W. E.

Antholine. 1999. Type 2 Cu<sup>2+</sup> in pMMO from *Methylobacterium album* BG8. *Biophys. J.* 76:2223-2229.

### **Book Chapters**

Schreiber, M. E., J. M. Bahr, M. Zwolinski, Y. Shi, W. J. Hickey, and K. A. Brownell. 1997. Field and laboratory studies of BTEX bioremediation under denitrifying conditions. pp. 13-18. In: *In situ and on-site bioremediation*. Battelle Press, Columbus, Ohio.

### **Dissertations**

Champion, G. 1998. Transient and steady-state flow models of a groundwater and lake system: Trout Lake basin, northern Wisconsin. M.S. Thesis. Department of Geology and Geophysics, University of Wisconsin-Madison. 105 pp. + appendices. Cook, R. C. 2000. Relationship between private well water, stream base flow, and land use in the Tomorrow-Waupaca River Watershed. M.S. Thesis. College of Natural Resources, University of Wisconsin-Stevens Point. Halsey, J. L. 1998. Development of methodology for the detection of methanotrophs in groundwater. M.S. Thesis. Department of Biological Sciences, University of Wisconsin-Milwaukee. Miller, L. W. 1998. Photocatalytic oxidation of organic compounds via waveguide-supported titanium dioxide films. Ph.D. Dissertation. Water Chemistry Program, University of Wisconsin-Madison. 103 pp. Schreiber, M. E. 1999. Experimental and modeling approaches to evaluating anaerobic biodegradation of petroleum-contaminated groundwater. 1999. Ph.D. Dissertation. Department of Geology and Geophysics, University of Wisconsin-Madison. 226 pp. + appendices.

### **Water Resources Research Institute Reports**

Allran, J. W., and W. H. Karasov. 2000. Chronic effects of atrazine and nitrate on northern leopard frog (*Rana pipiens*) larvae exposed in the laboratory from post-hatch through metamorphosis. WRI GRR 00-01. Water Resources Institute, University of Wisconsin-Madison. 17 pp. Allran, J. W., and W. H. Karasov. 2000. Sub-acute effects of atrazine on embryos, larvae, and adults of anuran amphibians. WRI GRR 00-02. Water Resources Institute, University of Wisconsin-Madison. 17 pp. Foose, G. L., C. H. Benson, and T. B. Edil. 1999. Methods for evaluating the effectiveness of landfill liners. WRI GRR 99-02. Water Resources Institute, University of Wisconsin-Madison. 42 pp. Hickey, W. J., J. M. Harkin, G. Sabat, and P. Rose. 2000. Molecular methods for detection of sewage-borne human pathogens in soil and water. WRI GRR 00-01. Water Resources Institute, University of Wisconsin-Madison. 37 pp. Hickey, W. J., and B. N. Moran. 1999. Biostimulation of trichloroethylene degradation in contaminated aquifers. WRI GRR 99-05. Water Resources Center, University of Wisconsin-Madison. 22 pp. Hoopes, J. A., S. Rashad, Y. Majali, and T.-S. Tsay. 1999. Field evaluation of near source transport of contaminants in heterogeneous media: Estimation of flow parameters and simulation of water table elevations. WRI GRR 99-03. Water Resources Institute, University of Wisconsin-Madison. 26 pp. Hoopes, J. A., S. Rashad, Y. Majali, and T.-S. Tsay. 1999. Field evaluation of near source transport of contaminants in heterogeneous media: Numerical simulation of groundwater mounding. WRI GRR 99-04. Water Resources Center, University of Wisconsin-Madison. 22 pp. Klima, J. S., T. B. Edil, and C. H. Benson. 1999. Field assessment of monitoring and water supply well seals. WRI GRR 99-01. Water Resources Institute, University of Wisconsin-Madison. 48 pp.

### **Conference Proceedings**

Ansari, S., and D. Cherkauer. 1998. Relation of groundwater recharge rates in southern Washington

County to land use and soil type. p. 38. In: Water resources management: Changes as we approach the 21st century. American Water Resources Association - Wisconsin Section 22nd Annual Meeting. Water Resources Center, University of Wisconsin-Madison, Madison, Wisconsin. Cheng, Y. S., J. L. Halsey, P. D. Anderson, C. C. Remsen, and M. L. P. Collins. 1998. Use of PCR to detect particulate methane monooxygenase in groundwater. p. 377. In: Abstracts of the General Meeting of the American Society for Microbiology. Davis, T. S., and R. W. Taylor. 1998. An application of electrical anisotropy in hydrogeological modeling. In: Proceedings of Symposium on the Application of Geophysics to Engineering and Environmental Problems. Society for Engineering and Environmental Geophysics. Fode, K. A., C. Wimpee, C. C. Remsen, and M. L. P. Collins. 1999. Use of competitive PCR for quantification of methanotrophs in groundwater. p. 486. In: Abstracts: General Meeting of the American Society for Microbiology. Fode, K. A., C. Wimpee, C. C. Remsen, and M. L. P. Collins. 1999. Direct application of PCR to environmental samples. p. 572. In: Abstracts: General Meeting of the American Society for Microbiology. Krabbenhoft, D. P., J. P. Hurley, G. Aiken, C. C. Gilmour, M. Marvin-DiPasquale, W. H. Orem, and R. Harris. 1998. Mercury cycling in the Florida Everglades: A mechanistic field study. XXVII Congress, Societas Internationalis Limnologiae, Dublin, Ireland. Simo, J. A., M. T. Harris, and M. A. Muldoon. 1998. Stratigraphy and sedimentology of the Silurian dolostones, Door County, Wisconsin. pp. 3-15. In: Fluid flow in carbonates: Inter-disciplinary approaches. SEPM Research Conference, September 20-24, 1998, Door County, Wisconsin. Water Resources Institute. 2000. Abstracts: Water Resources 2000 - Challenges for the new Century. American Water Resources Association Wisconsin Section 24th Annual Meeting, Green Bay, Wisconsin. Water Resources Institute, University of Wisconsin-Madison. 48 pp.

## **Other Publications**

Cheng, Y. W., J. L. Halsey, K. A. Fode, C. C. Remsen, and M. L. P. Collins. 1999. Detection of methanotrophs in groundwater by the polymerase chain reaction. Department of Biological Sciences, University of Wisconsin-Milwaukee. Harris, M. T., J. J. Kuglitsch, R. Watkins, D. P. Hegrenes, and K. R. Waldhuetter. 1998. Early Silurian stratigraphic sequences of eastern Wisconsin, Midcontinent U.S.A. pp. 39-49. In: E. Laning, and M. Johnson (eds.). Silurian cycles: Linking of Dynamic Processes in the Atmosphere and Oceans. New York State Museum Bulletin. Harvey, R. G. 1998. A simple technique for predicting future weed problems and choosing the best management practices. pp. 37-43. In: Report of the Expert Consultation on Weed Ecology and Management. Plant Production and Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy. Muldoon, M. A., and K. R. Bradbury. 1998. Tracer study for the characterization of groundwater movement and contaminant transport in fractured dolomite. Open File Report 98-02. Wisconsin Geological and Natural History Survey, Madison, Wisconsin. 85. pp.