

# **Water Resources Center**

## **Annual Technical Report**

**FY 2000**

### **Introduction**

None

### **Research Program**

Research priorities are: groundwater quality; potential insufficiencies in water resources, including the management of water resources among competing uses; controlling pollution from nonpoint sources; recovering water-based fish and wildlife habitat; developing and evaluating means for formulating policies that are based on limited data; and emerging issues, including other innovative research topics that are not included in the five priorities above. In addition, in 1998, the Illinois Water Resources Center (IWRC) and the Illinois State Water Survey successfully competed in a national USEPA competition to establish regional centers that would provide research and other forms of technical assistance to drinking water systems in small communities, including native American communities. The Midwest Technology Assistance Center (MTAC) started in November 1998 and is a collaborative effort of the IWRC and nine other water resources research institutes in the Midwest and the Illinois Water Survey. In 2000, MTAC-funded projects in four states were completed, including the following research: (1) a survey of the technical and management needs of 200 small systems in ten states; (2) a Benchmark Economic Study to assist small systems with their financial and managerial problems and ensure they will have adequate financial resources to meet new treatment requirements; and (3) a pipe corrosion study that will help communities protect their pipe systems while complying with federal statutes that require disinfection (some disinfection processes accelerate corrosion to such an extent that lead and copper standards for drinking water are exceeded).

The IWRC base research program operates on a biennial schedule: every two years researchers at universities throughout the state are invited to submit proposals for up to two years of funding. In 2000, 9 preproposals were received by the IWRC under the Water Resources Research Institutes (WRRI) program. Based on review by the Executive Committee, 6 full proposals were invited. The full proposals were distributed for review by peers outside the state of Illinois. Based on the peer reviews and the IWRCs announced priorities, the Executive Committee selected two projects for funding.

Synopses of six individual research projects which continued in 2000 appear in the following part of this report. One project (Analysis of Water Use Trends in the United States, 1950-1995) was funded through a national WRRI competition in 1999 and several projects that continued into 2000 were funded through WRRI competitions prior to 2000.

The Center takes a special interest in helping young scientists establish a track record in water resources research. The Water Resources Center both encourages new scientists to submit proposals and gives their proposals extra consideration. The proposals, however, must be of significant scientific merit (as determined by the reviewers and the Executive Committee) and have relevance to the total water program of Illinois to be judged worthy of funding. Virtually all projects supported by the

IWRC contribute significantly to the education of students, both graduate and undergraduate, who participate in the research projects. The Student Support table lists students supported in both the internship program with the Illinois District Office of USGS and on the individual grants to faculty researchers.

The Illinois Water Resources Center (IWRC) also helps administer the research component of the Illinois-Indiana Sea Grant College program in partnership with the National Oceanic and Atmospheric Administration (NOAA). IWRCs involvement in this program has increased the Centers opportunities for coordinating research activities with other water-related programs in the Midwest.

## Basic Information

<b>Title:</b>	Genetic Algorithms for the Control of Sedimentation in River-Reservoir Networks
<b>Project Number:</b>	IL-99-2
<b>Start Date:</b>	3/1/1999
<b>End Date:</b>	2/28/2001
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Sediments, Management and Planning, Methods
<b>Descriptors:</b>	Sediment, optimization, decision support, resevoir management, genetic algorithms
<b>Lead Institute:</b>	Southern Illinois University Carbondale
<b>Principal Investigators:</b>	John William Nicklow

## Publication

1. Nicklow, J.W., and Bringer, J.A. (2001). Optimal control of sedimentation in multi-reservoir river systems using genetic algorithms. In Proceedings of the 2001 Conference of the Environmental and Water Resources Institute, ASCE. Orlando, FL, May 20-24.
2. Nicklow, J.W. (2000). Discrete-time optimal control for water resources engineering and management. *Water International*, International Water Resources Assoc. 25(1): 89-95.

### **Problem and Research Objectives:**

Sediment transport is a natural dynamic of alluvial river-reservoir systems. If left uncontrolled, however, excess scour of a streambed induces major shifts in geometry and threatens stability of bridges, hydraulic control structures, and underground utilities. Similarly, continued deposition of bed material reduces conveyance capacity of a channel and can lead to chronic inundation of adjacent property. In reservoirs, deposition reduces storage capacity and diminishes benefits associated with activities such as hydropower generation, navigation and water supply. While restoration efforts such as dredging, and flushing and filling have been effective in many cases, sediment is difficult and very costly to remove from or restore to drainageways. As a result, focus should be directed towards control methods that prevent excessive scour and deposition, before problems are permitted to occur.

The objective of this research project was to develop a methodology and computational model for determining short- or long-term reservoir operations that limit the rate and occurrence of bed material movement in multiple-reservoir river networks. A secondary objective involved the investigation of uncertainty within the overall model. The methodology is based upon a unique discrete-time optimal control approach that interfaces a sediment transport simulation model with a probabilistic optimization method known as genetic algorithms. The approach goes far beyond mere sediment transport prediction and has practical decision-making utility at the water resources managerial level.

### **Methodology:**

Submitted along with this synopsis is a manuscript that will serve as a final report for the IWRC-funded project. The manuscript, which is currently under review for publication by *Water Resources Management*, Kluwer Academic Publishers, further details the methodology and major findings.

The sediment control problem has been formulated as follows;

- Minimize  $\Rightarrow$  The maximum change in bed elevation occurring over any discrete time interval  
and at predefined river or reservoir cross sections for a simulated storm event(s).
- Subject To  $\Rightarrow$  (1) Physical laws governing hydraulic and sediment transport dynamics;  
(2) operating bounds on reservoir releases, and; (3) bounds on reservoir storage.

A sediment transport simulation model, HEC-6, has been interfaced with an immune genetic algorithm within the framework of discrete-time optimal control. The simulator is used to solve the governing hydraulic and sediment constraints, thus revealing the behavior of a network in response to an imposed reservoir operating policy. Constraints on state variables are accommodated using a bracket penalty function, while bounds on decision variables are handled implicitly by the optimization module. The genetic

algorithm is used to solve the overall control problem, thus yielding an optimal release schedule that satisfies the formulation.

It was originally thought that a chance constraint formulation should be used. However, after preliminary study, it was decided that such a formulation should not be incorporated. This decision was based on several factors. First, a large number of data sets, which rarely exist for most applications, would be required to quantify the probability distribution associated with sediment parameters. If the distribution could not be adequately quantified, added uncertainty would be induced in model application. Second, extensive modification of the original HEC-6 code would be required to incorporate a chance-constraint for sediment gradation, load, and all ten available sediment transport functions offered. The modification would consequently compromise the integrity of the original simulation model, as well as the goal to maintain practical utility of the new model and to minimize additional training required for those already familiar with HEC-6. Finally, sensitivity analyses have shown that different transport functions and load have little effect on the generation of optimal policies, even though numerical results for extent of sedimentation are affected. This fact follows the reasoning that discharge tends to be the dominant factor, regardless of the particular transport function chosen. Uncertainty, however, has been investigated in several ways. First, uncertainty of genetic algorithm parameters was explored through sensitivity analyses and an evaluation of the effects of different parameters such as mutation rates, penalty weights, and population size. Second, uncertainty associated with hydrologic constraints was evaluated through sensitivity analyses and an evaluation of the effects of bound constraint magnitudes. Finally, modifications made to the model allow the user to enter multiple data sets for bed material gradation and upstream sediment load, from which a mean gradation and load will be computed and used for all computations.

### **Principal Findings and Significance:**

Principal tasks that have been completed under this project include:

- Mathematical formulation of the sedimentation control problem
- Development of an immune genetic algorithm and associated computer software module
- Evaluation of suitable sediment transport simulators for inclusion in the overall model
- Selection and modification of the U.S. Army Corps of Engineer's *HEC-6* source code
- Construction of the computational interface between *HEC-6* and the genetic algorithm
- Testing and evaluation of parameter sensitivity through application to a hypothetical system
- Collection and quality control review of existing data from the Illinois State Water Survey and U.S. Army Corps of Engineers for application to the Kaskaskia River, Illinois
- Application of the optimal control model to the Kaskaskia River in Central Illinois

- Investigation of uncertainty and sensitivity of model parameters through repeated application

Major findings of the work include:

- The discrete time optimal control methodology, along with an embedded genetic algorithm, represents an economical and efficient method for controlling excess sedimentation, before problems are permitted to occur. This conclusion is demonstrated through practical applications to a hypothetical 3-reservoir system and the Kaskaskia River system in Central Illinois.
- The methodology is significantly improved over traditional decision-making techniques that require a repeated user interface and excessive CPU time.
- The work firmly demonstrates that for the sedimentation problem, genetic algorithms are superior to gradient-based optimization methods that tend to converge to local optima and have excess derivative requirements.
- Sensitivity analyses have revealed that penalty weights have the most impact on time required for convergence of the genetic algorithm to a global or near-global optima, followed by specification of the mutation parameter.
- Sensitivity analyses indicate the target storage constraint tends to guide solution results since this constraint is required to be “tight” for all applications. Bound constraints on discharge and incremental storage also guide results, but to a smaller extent.

## Basic Information

<b>Title:</b>	Dentrification Rates and Cntrols in Sediments of Illinois Surface Waters
<b>Project Number:</b>	IL-99-3
<b>Start Date:</b>	7/1/1999
<b>End Date:</b>	6/30/2001
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Water Quality, Sediments, Non Point Pollution
<b>Descriptors:</b>	Agriculture, Dentrification, Nitrogen, Water Quality, Streams
<b>Lead Institute:</b>	University of Illinois
<b>Principal Investigators:</b>	Mark B. David

## Publication

### Problem and research objectives:

In agriculturally-impacted streams of the midwestern United States, the dissolved nitrogen (N) pool is dominated by nitrate ( $\text{NO}_3^-$ ), which can approach  $20 \text{ mg NO}_3^- \cdot \text{N L}^{-1}$  following precipitation events (e.g., David et al. 1997). The agricultural Midwest has been identified as a major source of N to the Gulf of Mexico where coastal eutrophication and hypoxia have become problematic (Turner and Rabalais 1994, Alexander et al. 2000). In east-central Illinois, the dominant land use is row-crop agriculture (corn and soybeans) involving the widespread application of commercial, N-based fertilizer. Denitrification, the microbial reduction of  $\text{NO}_3^-$  to  $\text{N}_2\text{O}$  or  $\text{N}_2$ , has been suggested as a potentially major sink for  $\text{NO}_3^-$  in agricultural streams (Hill 1983). Land use in many east-central Illinois watersheds is  $>90\%$  row-crop agriculture, providing an opportunity to examine the role of denitrification in intensively farmed areas. However, measurements of denitrification in agricultural streams of the Midwest are few and no measurements have been reported for east-central Illinois.

Our primary objective in this study was to examine denitrification rates determined with and without chloramphenicol in three  $\text{NO}_3^-$ -rich streams in east-central Illinois. Chloramphenicol is an antibiotic that prohibits the *de novo* synthesis of proteins but does not, except at extremely high concentrations, inhibit the action of existing enzymes. In the presence of chloramphenicol, denitrifying bacteria cannot produce additional enzymes in response to the conditions of the assay and, therefore, assays conducted with the antibiotic provide a more accurate measure of rates of denitrification than those performed without it. Smith and Tiedje (1979) used chloramphenicol in conjunction with the acetylene block method in microcosm studies of soil denitrification and identified two phases of  $\text{N}_2\text{O}$  production (i.e., denitrification). In assays performed without chloramphenicol, Phase I was a short-term, linear rate of denitrification and was followed by an increased, often non-linear rate, termed Phase II. In the presence of chloramphenicol, the difference between Phase I and Phase II was slight or nonexistent. Smith and Tiedje (1979) concluded that Phase I was representative of actual in-field rates of denitrification whereas Phase II reflected the new synthesis of denitrifying enzymes and microbial growth subsequent to the reduced oxygen tension. We were interested in whether Phase I and Phase II patterns, such as Smith and Tiedje (1979) observed in soils, could be discerned in stream sediments and, if present, if the patterns were consistent during the course of summer and early autumn when  $\text{NO}_3^-$  concentrations change dramatically.

### Methodology:

Our study streams included the Embarras River near Camargo, Illinois; Black Slough, a first-order tributary to the Embarras River; and Big Ditch, a third-order tributary to the Sangamon River. We used the  $\text{C}_2\text{H}_2$  inhibition method (e.g., Knowles 1990, Tiedje et al. 1989) to measure rates of denitrification in slurries of sediment from each of the study streams. In the laboratory, 25-30 mL of sediment slurry was placed in a 150 mL media bottle with a butyl septum in the lid. Unfiltered stream water was added to bring the total sediment-water volume to 75 mL. Eight media bottles were used for each assay: 4 of which had chloramphenicol added to achieve a final concentration of 5 mM and 4 of

which received no chloramphenicol. For the routine assays, no  $\text{NO}_3^-$  or DOC was added beyond that in the unfiltered stream water. The ambient  $\text{NO}_3^-$  and DOC concentrations for each assay were determined with an ion chromatograph (Dionex DX-120) and carbon analyzer (Dohrmann DC-80), respectively.

Oxygen in the headspace and slurry was removed by purging the media bottles with ultra pure Helium (He) for 5-7 minutes with periodic shaking. Using gas-tight syringes, approximately 10% of the He atmosphere in the media bottles was removed and replaced with  $\text{C}_2\text{H}_2$ . Gas samples from the headspace of each media bottle were analyzed for  $\text{N}_2\text{O}$  (the end-product of denitrification in the presence of  $\text{C}_2\text{H}_2$ ) on a Varian 2600 gas chromatograph equipped with a Porapak Q column and a  $^{63}\text{Ni}$  electron-capture detector. Following removal of the final gas sample, the sediment in each bottle was collected and the dry mass and ash-free dry mass (AFDM) determined. This was done by obtaining the initial mass after drying the sediment at  $60^\circ\text{C}$ , combusting the organics at  $550^\circ\text{C}$ , re-wetting the sediment, drying at  $60^\circ\text{C}$ , and obtaining the final mass (difference between pre- and post-combustion mass = AFDM). The amount of  $\text{N}_2\text{O}$  produced and the rates of denitrification were then calculated with standard equations.

#### Principal findings and significance:

The assays during July 2000 were conducted when in-stream  $\text{NO}_3^-$  concentrations were high ( $>8 \text{ mg N L}^{-1}$ ) and water temperatures were  $20^\circ\text{C}$  or more. The three sites showed a consistent pattern in that assays without chloramphenicol produced  $\text{N}_2\text{O}$  exponentially during the duration of the 3-4 hr period (Fig. 1). Conversely, when the sediment slurries contained a 5 mM concentration of chloramphenicol the production of  $\text{N}_2\text{O}$  was linear and considerably lower than assays without chloramphenicol. The linear denitrification rates were 0.08, 1.52, and  $14.3 \mu\text{g N}_2\text{O g AFDM}^{-1} \text{ hr}^{-1}$  and the exponential rate coefficients were 0.2412, 0.8151, and  $0.7659 \text{ hr}^{-1}$  in Black Slough, the Embarras River, and Big Ditch, respectively.

By mid-August,  $\text{NO}_3^-$  concentrations in the streams had fallen 10-fold or more from those in July but water temperatures remained similar. Regardless of whether or not chloramphenicol was used, no assay during August showed an exponential production of  $\text{N}_2\text{O}$  (Fig. 2). Big Ditch, the stream with the greatest denitrification rate in July, had the lowest rates in August and displayed no substantial difference between assays with or without chloramphenicol ( $0.11$  and  $0.07 \mu\text{g N}_2\text{O g AFDM}^{-1} \text{ hr}^{-1}$ , respectively). In the Embarras River and Black Slough, denitrification rates from assays without chloramphenicol were, respectively, 4.3 and 1.7 times greater than assays with chloramphenicol (Fig. 2). Denitrification rates from assays with chloramphenicol for Black Slough and the Embarras River were  $16.1$  and  $2.4 \mu\text{g N}_2\text{O g AFDM}^{-1} \text{ hr}^{-1}$ , respectively – an increase from values in July despite the lower nitrate concentrations. Assuming the chloramphenicol-amended assays estimated the actual benthic denitrification rates, failure to use the antibiotic would have overestimated denitrification by approximately 3 % in the Embarras River and 76 % in Black Slough.

Our results show that the two phases of denitrification observed in soil by Smith and Tiedje (1979) also can occur in stream sediments. It is not likely, however, that denitrification in benthic sediments occurs in an exponential fashion – if this was the case the supply of electron acceptors quickly would be depleted, even in the  $\text{NO}_3^-$ -rich streams



of the agricultural Midwest. As suggested by Tiedje and Smith (1979), we believe the first phase to be representative of actual in-stream denitrification rates whereas the exponential, second phase is representative of the microbial response to the anoxic conditions of the assay. Chloramphenicol prevented the onset of the exponential phase and it appears that assays conducted without chloramphenicol might overestimate in-stream rates of denitrification, particularly in  $\text{NO}_3^-$ -rich streams. Further work is needed to determine how chloramphenicol affects estimates of denitrification from other locations and stream types.

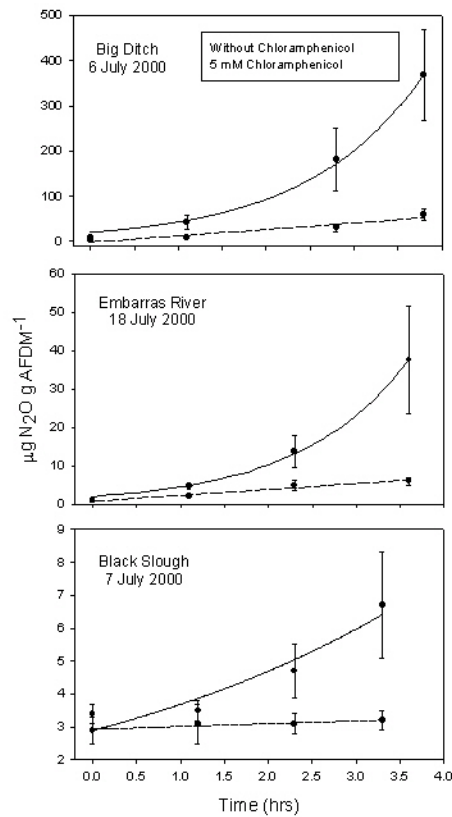


Figure 1. Denitrification measured with and without chloramphenicol in July 2000.

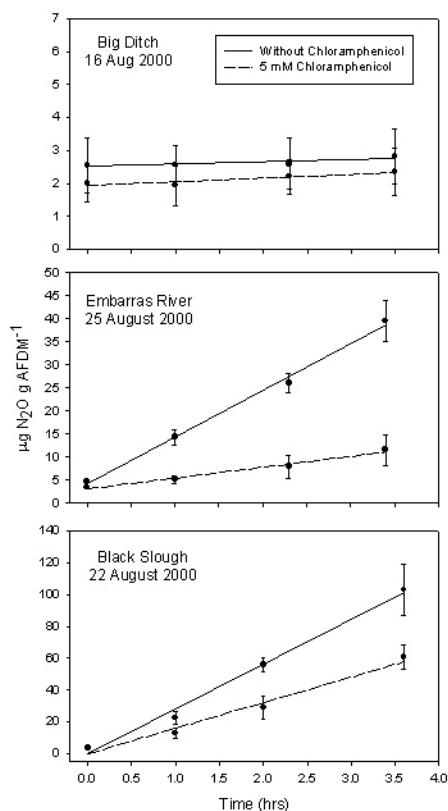


Figure 2. Denitrification measured with and without chloramphenicol in August 2000.

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- Alexander, R.B. R.A. Smith, and G.E. Schwarz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* 403:758-761.
- David, M.B., L.E. Gentry, D.A. Kovacic, and K.M. Smith. 1997. Nitrogen balance in and export from an agricultural watershed. *J. Environ. Qual.* 26:1038-1048.
- Hill, A.R. 1983. Denitrification: its importance in a river draining an intensively cropped watershed. *Agriculture, Ecosystems and Environment* 10:47-62.
- Knowles, R. 1990. Acetylene inhibition technique: development, advantages, and potential problems. Pages 151-166 in (N.P. Revsbech and J. Sorensen, editors) *Denitrification in soil and sediment*, Plenum Press, New York.
- Smith, M.S. and J.M. Tiedje. 1979. Phases of denitrification following oxygen depletion in soil. *Soil Biol. Biochem.* 11:261-267.

Tiedje, J.M., S. Simkins, and P.M. Groffman. 1989. Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. Pages 217-240 in (M. Clarholm and L. Bergstrom, editors.) Ecology of arable land, Kluwer Academic Publishers, Dordrecht, the Netherlands.

Turner, R.E. and N.N. Rabalais. 1994. Coastal eutrophication near the Mississippi river delta. Nature 368:619-621.

## Basic Information

<b>Title:</b>	Use of Constructed Wetlands to Improve Water Quality in Lake Bloomington
<b>Project Number:</b>	WRC-97-1
<b>Start Date:</b>	9/1/1997
<b>End Date:</b>	2/28/2001
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Water Quality, Wetlands, Non Point Pollution
<b>Descriptors:</b>	Agriculture, Denitrification, Nitrogen, Nutrients, Pollutants, Subsurface Drainage, Water Quality, Water Quality Management, Wetlands
<b>Lead Institute:</b>	University of Illinois
<b>Principal Investigators:</b>	David Kovacic, Mark B. David, Lowell E. Gentry, Richard A. Cooke

## Publication

1. Hoagland, Curtis R. 1999. MS Thesis, Natural Resources and Environmental Sciences, University of Illinois, Urbana, Illinois, 47pp.
2. Larson, A.C., L.E. Gentry, M.B. David, R.A. Cooke and D.A. Kovacic. 2000. The role of seepage in constructed wetlands receiving agricultural tile drainage. *Ecological Engineering* 15:91-104.

## **Problem and Research Objectives:**

We have shown that drainage tiles are the major source of nitrate entering the Embarras River in central Illinois, causing nitrate concentrations to repeatedly exceed the EPA's MCL (David et al., 1997). Improvements in surface water quality will require the implementation of strategies and management practices that reverse these effects by directly reducing nitrate application through fertilizer management; and, by reducing nitrate output from tile drainage systems to streams using techniques such as constructed wetlands. Constructed wetlands may prove to be a practical, economical, and effective method to reduce surface water nitrate contamination. These "wetlands" are formed by berming an area adjacent to a stream and forming a small detention basin or holding pond that intercepts tile drainage water before it enters the stream. The basin acts to reduce transport of nitrate in drainage water through plant uptake and microbial transformation and degradation. Following wetland "treatment", drainage water is slowly released to the stream, through regulated flow. Research on constructed wetlands addresses the WRRP interest areas of watershed protection, wetlands processes and management, and water quality and supply for small communities; and may provide new methodologies to resolve associated water contaminant problems.

The goal of the proposed research was to determine input/output nitrogen, and water budgets for experimental wetlands constructed in conjunction with two experimental systematically tile-drained sub-basins in the Lake Bloomington watershed. The Bloomington wetlands and their experimental watershed can serve as a valuable case study in determining the efficiency of wetlands for removal of agricultural NPS pollutants. Results from this study will allow us to determine whether or not constructed wetlands have the potential to remove NPS nitrogen from adjacent subsurface tile-drained agricultural fields before they enter the surface waters of Lake Bloomington. In a collaborative study with Illinois State University, the city of Bloomington, the McLean County Natural Resources Conservation Service, the McLean County Soil and Water Conservation Service, and the Nature Conservancy, we will establish potential wetland management criteria that will be used to reduce nitrate levels in surface waters of the Midwest. To achieve this goal, detailed studies on the effectiveness of constructed wetlands were conducted over a multi-year period.

Our overall objective was to study constructed wetlands receiving tile drainage water from two experimental tile drained watersheds (this work will be done in conjunction with the city of Bloomington, who will construct the wetlands, and researchers at Illinois State University, who are studying fertilizer nitrate losses following varied application rates).

The specific objectives of our research component were to:

- 1) Determine nitrogen concentrations and loads entering and leaving constructed wetlands in the Lake Bloomington watershed for a two-year period;
- 2) Determine potential seepage rates from the constructed wetlands;
- 3) Determine the potential effectiveness of constructed wetlands as a tool in reducing nitrate loading to midwestern surface waters;

We are addressing the following hypothesis:

Constructed wetlands can remove a substantial portion of nitrate from subsurface tile drainage water before entering surface waters.

### **Methodology:**

*Wetland study area.* Two replicate wetlands were constructed on city owned land adjacent to an existing experimental agricultural tile drainage system at the edge of Lake Bloomington in McClean County, Illinois. The wetlands intercept drainage waters from experimental tile drained watersheds rather than allowing them to flow directly into Lake Bloomington. Watersheds were planted in a typical corn/soybean rotation. Wetlands were initially planted with barnyard grass to provide rapid cover and an abundant source of carbon for denitrification. Our previous research on constructed wetlands points to the need to construct experimental wetlands large enough to prevent severe rainfall events from breaching the wetland berms. Wetland sizes are approximately 0.2 ha (0.5 acre) for the 2-ha (5-acre) experimental watershed and 0.4 ha (1 acre) for the 4-ha (10-acre) watershed (a 1:10 wetland-to-watershed ratio). Weirs were constructed by the city of Bloomington for detailed chemical budget analysis, including input and output flow measurement. Automated sampling devices and data logging equipment allowed concentrations to be determined through weather events and provided information to create a detailed budget for nitrate leaving the agricultural drainage. Output from the wetland was also measured in a similar fashion to determine if constructed wetlands could reduce nitrate concentrations from drainage water.

Flow measurement (pressure transducers and V-notch weirs) and water sampling equipment (ISCO automated samplers) were installed at each site to measure the quantity and quality of both surface and tile drainage water entering from the agricultural fields. Campbell data loggers were used to record data and to control the ISCO collectors. We measured surface and subsurface flow, surface water in the wetlands, and monitored N chemistry of the subsurface waters entering and leaving the sites. These provided a constant record of water flow and nutrient fluxes into and out of the wetlands and also a record of changes occurring within each wetland.

*Laboratory analysis. Nutrient analysis.* Each solution sample was analyzed for the major forms of N (including  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and organic N).

*Field studies. Data collection and analysis.* Pairs of PVC sampling piezometers were installed at two locations on each wetland site to measure potential wetland seepage through the berm (based on head differential) and to measure potential flow and concentrations leaving the wetlands. They were placed approximately 4 feet below the base of the berm outside the wetland, at the depth where seepage would be expected. Water depth in the wetland was monitored continuously throughout the experiment. Water samples were collected weekly at the weirs (if flowing) between precipitation events, and in the adjacent lake. More intensive sampling occurred during selected precipitation events when flow increased. Samples analyzed for nitrate concentrations along with water quantity data were used to determine nutrient budgets for the wetlands. The collected nutrient data will be used to make the following comparisons; 1)

inflow versus outflow nutrient concentrations and loadings for each wetland, 2) differences between wetlands, and 3) seasonal nutrient differences for each wetland.

**Principal findings and significance:**

*Progress Report.* Preliminary results: Chemical analyses. Inlet and outlet flow and nutrient concentrations for water year 1999 have been monitored since October 1, 1998. Wetland 1 inlet concentration peaked at 17 mg L<sup>-1</sup> on April 20, 1999, outlet concentration peaked at 12 mg L<sup>-1</sup> on March 15, 1999. Wetland 2 inlet concentration peaked at 19 mg L<sup>-1</sup> on May 15, 1999, outlet concentration peaked at 17 mg L<sup>-1</sup> on April 20, 1999. Wetland 1 average inlet and outlet nitrate, ammonium and organic-N concentrations were 13.4, 0.09, 0.08 and 7.1, 0.03 and 0.40 mg L<sup>-1</sup>. Total N removal in wetland 1 during water year 1999 was 44%. Wetland 2 average inlet and outlet nitrate, ammonium and organic-N concentrations were 14.3, 0.03, 0.28 and 10.4, 0.05 and 0.39 mg L<sup>-1</sup>. Total N removal in wetland 2 during water year 1999 was 25% (Tables 1 & 2). Removals are based on concentration data only. Problems were encountered with flow determinations. Flow corrections are now being made; when complete they will be used to determine the actual budgets for N entering and leaving the wetlands.

**Table 1. Wetland 1 average nitrate, ammonium and organic N concentrations for water year 1999.**

	Nitrate	Ammonium	Organic N	Total N
Inlet	13.4	0.09	0.08	13.5
Outlet	7.1	0.03	0.40	7.6
% Retained	47	62	-500	44

**Table 2. Wetland 2 average nitrate, ammonium and organic N concentrations for water year 1999.**

	Nitrate	Ammonium	Organic N	Total N
Inlet	14.3	0.03	0.28	14.6
Outlet	10.4	0.05	0.39	10.9
% Retained	36	-54	-40	25

Organic N was probably a result of algal growth within the wetlands and loss through outlet flow in wetlands 1 and 2. Algae probably assimilated nitrate and ammonium resulting in a greater concentration of organic N leaving than entering the wetlands. The majority of the N retained by the wetlands was probably in the form of nitrate (36% in wetland 1 and 47 % in wetland 2) (Tables 1 & 2).

The role of plant nutrient uptake is important in understanding nutrient cycling in wetlands. In a supporting study, biomass in an existing wetland site was studied to determine the role of wetland vegetation in the overall N budget (Hoagland, 1999). Most plant growth occurred after tile input stopped, therefore plants had a small direct effect on the N budget. However, plants were an important source of carbon for microbial denitrification which was the

main source of N removal from the wetland. Although aquatic vegetation did not play a role in permanent nutrient removal, it did act as a sink for N during periods of active growth. Algae, often the only plants active early in the growing season, may play a role as a sink in the early spring and during wet years when wetlands dry out much later than normal.



## Basic Information

<b>Title:</b>	Analysis of Water Use Trends in the United States, 1950-1995
<b>Project Number:</b>	99HQGR0222
<b>Start Date:</b>	9/1/1999
<b>End Date:</b>	2/28/2002
<b>Research Category:</b>	Not Applicable
<b>Focus Category:</b>	Water Use, Conservation, Models
<b>Descriptors:</b>	Water Demand, Water Use Data, Statistical Models Data Sources
<b>Lead Institute:</b>	Southern Illinois University Carbondale
<b>Principal Investigators:</b>	Ben A. Dziegielewski, Subhash C. Sharma

## Publication

## **Problem and research objectives:**

Improved information about water withdrawals and uses in specific geographical regions and a basic understanding of the factors that influence water use, are a necessary requirement for sound water resources management. The USGS National Water-Use Information Program (NWUIP) has collected and published an extensive inventory of water use information since 1950. This information has provided a basis for the development and evaluation of national water policies as well as regional and local plans for the development and use of water resources. It also offers an excellent opportunity for examining and explaining historical trends in water use, and to improve our understanding of the factors that influence water use.

The research objectives are: (1) to provide important insights into the effects of individual factors that have influenced historical water withdrawals, and uses by various sectors, (2) to estimate the quantitative impact of various efforts aimed at improving the efficiency of water use, and (3) to provide the state coordinators with improved water use models for estimating water use in “nonreporting” categories or counties and verifying the reported data based on values of demographic, economic, and other data, which determine water use.

## **Methodology:**

One of the major purposes of this project is to develop a methodology for estimating water use based on routinely collected governmental data on demographic and economic activities within geographical areas. The aggregate analysis of this project is a new approach to modeling sectoral water demands. The important methodological aspects of this research include 1) predictive accuracy of models at multiple levels of sectoral and spatial aggregation of water use, and 2) parsimony of statistical models, in terms of the number of explanatory variables.

## **Principal Findings and Significance**

The *Analysis of Water Use Trends* is a project in progress. Predictive models have been developed for the public, industrial and thermoelectric water use sectors, but the findings are preliminary.

The following sections describe the progress-to-date on project components.

### **Data collection**

Most of the data targeted for collection under the original project plan has been assembled. However, the targeted county-level data has often been unavailable in easily accessible formats, one of the prerequisites for variable selection. Also, collection of county employment data has been hampered by “disclosure” issues. Work is proceeding on testing alternative specification of employment variables for county level models, and alternative data sources are still being investigated.

### **Data cleaning**

It was discovered that there is considerable variance present in all dependent variables (USGS data), in both the cross section and time series data. In order to confirm that this variance was an accurate representation of actual water use rather than an error

in data collection or reporting, USGS water use coordinators were contacted on several occasions. The issues that coordinators were asked to respond to included the accuracy of thermoelectric water withdrawals, and the actual years of data collection. Considerable efforts has also been made to identify and resolve problems with data use to develop independent variables.

### **Models specification**

Preliminary models have been developed for state-level public, industrial, thermoelectric, domestic, and livestock water use. Preliminary county-level models have been developed for the public and industrial water use sectors.

## Basic Information

<b>Title:</b>	Relative Scales of Hydrodynamic and Geomorphologic Influence on the Hydrologic Response in the Illinois River Basin
<b>Project Number:</b>	IL-99-4
<b>Start Date:</b>	6/1/1999
<b>End Date:</b>	5/31/2001
<b>Research Category:</b>	Climate and Hydrologic Processes
<b>Focus Category:</b>	Geomorphological Processes, Hydrology, Floods
<b>Descriptors:</b>	Geomorphology, channels, watershed management, hydrologic models
<b>Lead Institute:</b>	University of Illinois
<b>Principal Investigators:</b>	Praveen Kumar, Ben Yen, Bruce Rhoads

## Publication

### **Problem and Research Objectives:**

The objective of the proposed work was to determine the relative effects of geomorphologic dispersion and hydrodynamic dispersion on the hydrological response of the Illinois River system as scale increases. The specific hypothesis tested was that as basin size increases, the river network structure, as compared to channel hydrodynamic properties, plays an increasingly dominant role in determining the hydrological response. The research also explored the effects of human actions, namely the modification of network structures via land drainage activities, on contemporary hydrologic conditions. The addition of headwater tributaries through land drainage activities in the late 1800s has undoubtedly greatly modified the geomorphologic dispersion, however the influence of this activity is unknown. The results provide important information and predictive capabilities for assessing the influence of future management scenarios on the hydrology of the Illinois River.

The geomorphologic dispersion coefficient,  $D_G$ , is a measure of the tendency of a disturbance to be dispersed by the river network structure. This concept incorporates the idea that raindrops falling on different areas at the same time will not reach the outlet at the same time (Rinaldo et al. 1991). The hydrodynamic dispersion coefficient,  $D_L$ , is a measure of the tendency of a disturbance to disperse longitudinally as it travels downstream. This dispersion is caused by the turbulence induced by the shearing effect of channel boundaries (Henderson, 1966; Mesa and Mifflin, 1986; Rinaldo et al., 1991). The two dispersion coefficients constitute the variance of an instantaneous unit hydrograph, as shown in Figure 1.

### **Methodology:**

**Task 1.** Assess the reliability of the DEM extracted data for the Illinois River Basin.

This research uses 90-m digital elevation model (DEM) data to estimate the properties of the stream network. The aim of this task is to evaluate the consistency of the DEM extracted river network with the surveyed river network. The following activities were performed under this task:

- 1) Obtained the stream network for the Illinois River Basin from the EPA's Reach files (RF3).
- 2) Compared the stream network derived from the DEM to the EPA network. The derived network corresponded to the EPA network well. Figure 2 is an overlay of the two networks. Also note that the EPA network is a fifth order basin, whereas the DEM-extracted network is a sixth order basin.

**Task 2.** Determination and comparison of dispersion coefficients as scale increases.

The geomorphologic and hydrodynamic dispersion coefficients were determined for eight of the major watersheds in the Illinois River Basin (Fox, Des Plaines, Kankakee, Vermilion, Mackinaw, Spoon, La Moine, and Sangamon) as follows:

- 1) The geomorphologic dispersion coefficient,  $D_G$ , was determined as:

$$D_G = \frac{u}{2\bar{L}(\Omega)} \left( \sum_{\gamma \in \Gamma} p(\gamma) [\bar{L}(\gamma)]^2 - \left[ \sum_{\gamma \in \Gamma} p(\gamma) \bar{L}(\gamma) \right]^2 \right)$$

where  $u$  is the kinematic wave celerity ( $u = \alpha u^*$ ,  $\alpha$  is an empirical constant dependent upon the channel cross-section shape, and  $u^*$  is the reference flow velocity),  $\Omega$  is the order of the basin,  $\bar{L}(\Omega) = \sum_{\gamma \in \Gamma} p(\gamma) \bar{L}(\gamma)$ ,  $\gamma$  is the specific path a raindrop follows, i.e., for a third order basin:

$$\begin{aligned} \gamma_1 &\rightarrow O_1 \rightarrow C_1 \rightarrow C_2 \rightarrow C_3 \rightarrow \text{outlet} \\ \gamma_2 &\rightarrow O_1 \rightarrow C_1 \rightarrow C_3 \rightarrow \text{outlet} \\ \gamma_3 &\rightarrow O_2 \rightarrow C_2 \rightarrow C_3 \rightarrow \text{outlet} \\ \gamma_4 &\rightarrow O_3 \rightarrow C_3 \rightarrow \text{outlet} \end{aligned}$$

where  $O_i$  and  $C_i$  represent the hillslope and channel states, respectively,  $i$  is the Horton-Strahler order of the hillslope or channel state,  $\Gamma = \{\gamma_1, \gamma_2, \gamma_3, \gamma_4\}$ ,  $p(\gamma)$  is the probability that a raindrop will follow path  $\gamma_i$ , and  $\bar{L}(\gamma)$  is the mean length of path  $\gamma_i$ .

- 2) The hydrodynamic dispersion coefficient,  $D_L$ , was computed as:

$$D_L \cong \frac{uh}{3\bar{S}}$$

where  $h$  is the flow depth and  $\bar{S}$  is the mean slope of the channel.

- 3) The flow velocities and flow depths, as a function of the frequency of occurrence of a particular discharge  $F$  and the order of the channel  $\omega$ , were calculated using the downstream hydraulic geometry equations developed from discharge data (Stall and Fok, 1968). The geomorphologic data, i.e., channel length, drainage area, slope, etc., were derived from the DEM.
- 4) The two dispersion coefficients were computed for each order of the eight watersheds, averaged by order, and then compared. Note that an increase in order signifies an increase in scale. Figure 3 displays the ratio of  $D_G$  to  $D_L$ ,  $D_R$ , as a function of order  $\omega$  and frequency  $F$ .

**Task 3.** Evaluation of changes in network structure on the hydrological response of the Mackinaw River Basin.

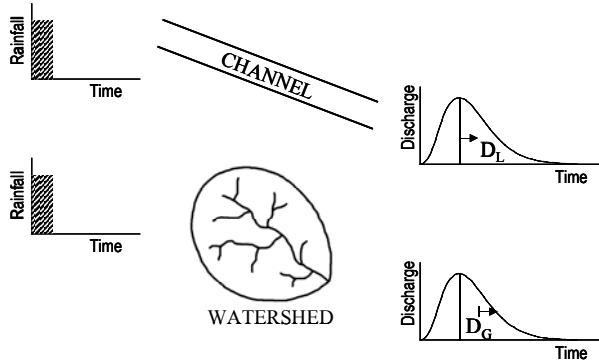
The anthropologic effects on the hydrologic response were determined as follows. To evaluate changes in network structure, the Mackinaw River Basin was chosen as the focus.

- 1) The geomorphologic instantaneous unit hydrograph (GIUH) was determined for the channel portion of the eight basins for each order, representing the change in scale of the hydrologic response. The inverse gaussian formulation of the GIUH (Rinaldo et al., 1991) was used to compute the GIUHs for each order of each basin.
- 2) Plats developed from public and private land surveys performed between 1804 and 1855 were used to establish a “pre-settlement” river network and the DEM-extracted network was considered as the “post-settlement” river network.
- 3) Two orders of tributaries were added upstream of the pre-settlement network, thus the first order of the pre-settlement network corresponds to the third order of the post-settlement network.
- 4) The pre- and post-settlement hillslope response was determined using the kinematic wave model and convolved with the pre- and post-settlement channel response (determined in item 1), respectively, to estimate the hydrologic response of the entire basin.
- 5) The pre- and post-settlement IUHs for equivalent orders, i.e.,  $\omega_{\text{post}} = \omega_{\text{pre}} + 2$ , were compared (Figure 4).

### **Principal Findings and Significance:**

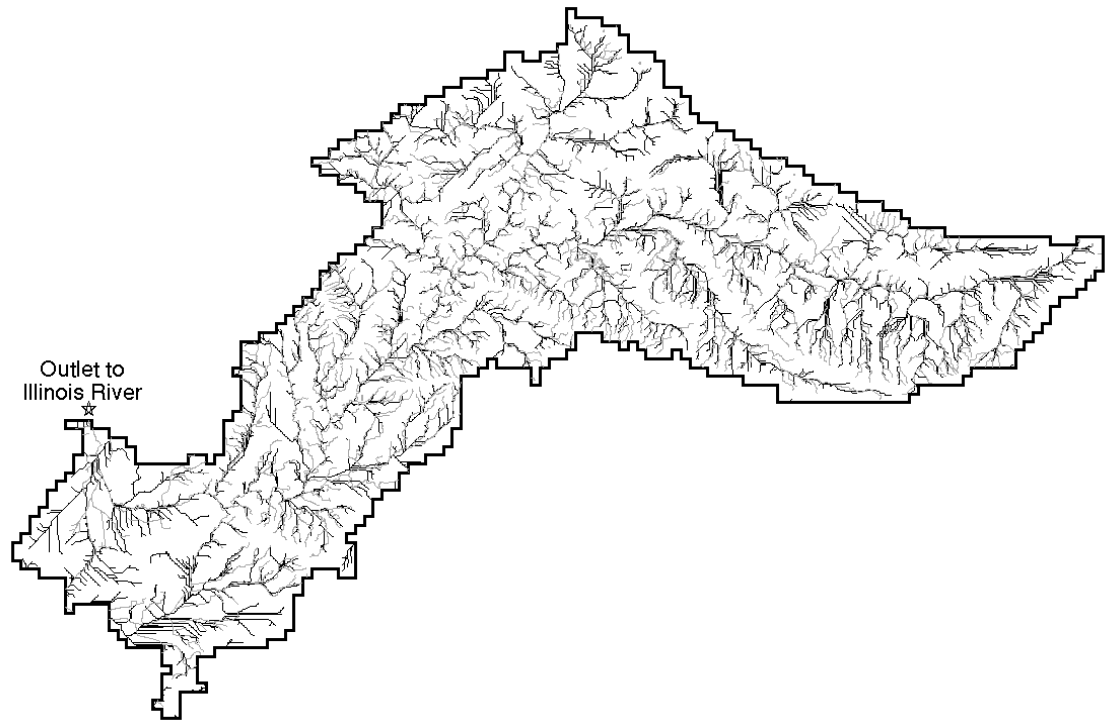
- 1) A river network derived using DEM data adequately reflects the actual river network (Figure 2); hence the reliability of information from the DEM-extracted network was confirmed.
- 2) Both  $D_G$  and  $D_L$  increase with increasing order and decreasing flow frequency, however  $D_L$  generally increases at a faster rate with respect to order than  $D_G$ . With respect to frequency, the ratio of  $D_G$  to  $D_L$ ,  $D_R$ , increases exponentially (Figure 3a). With respect to order,  $D_R$  increases for third through fifth order subbasins, decreases for sixth and seventh order subbasins, then increases for eighth order subbasins (Figure 3b). As frequency decreases, the crests and troughs of the curves of the ratio become less pronounced. The form of these curves indicate that the relative influence of geomorphologic dispersion on hydrologic response is greatest in low-order watersheds at high flow frequencies. In high-order watersheds, hydrodynamic dispersion plays an increasingly important role with respect to the hydrologic response; however, geomorphologic dispersion is still the governing influence.
- 3) These results have important implications in hydrologic modeling. The focus of modeling efforts in the past has been on measuring and surveying channel characteristics such as flow depth, top width, discharge, channel bed roughness, channel cross-sectional area, etc., to determine the hydrologic response of a river basin. These results show that the hydrologic response tends to be governed by geomorphologic dispersion at a variety of spatial scales, hence, the network structure plays a dominant role. Current models have substantial data requirements that are challenging and expensive for large or ungauged watersheds, but the network structure parameters are relatively simple to extract from DEMs, creating an environment in which the hydrologic response could be reasonably approximated without elaborate, expensive fieldwork.

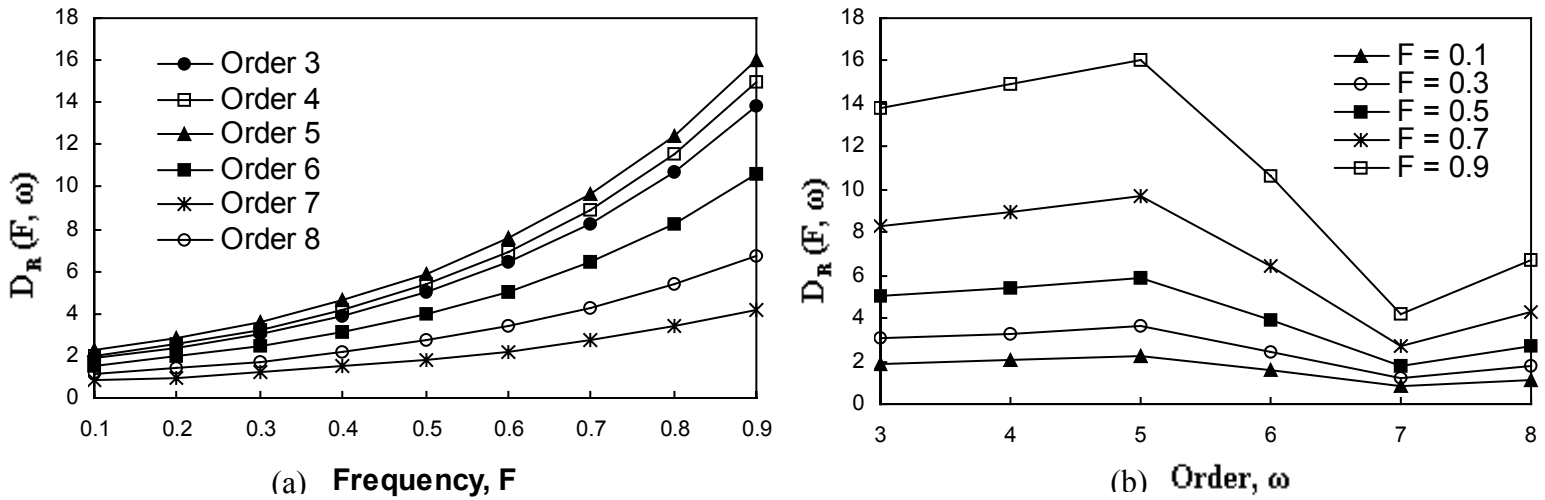
- 4) The effects of European settlement on the Mackinaw River Basin's hydrologic response are consistent with those seen in urban watersheds, i.e., increases in peak discharges and decreases in the time to peak (Figure 4). For a frequency of occurrence of a particular discharge,  $F$ , of 0.1, these changes are more prominent in low-order watersheds (29.41% increase in the peak discharge for  $\omega_{\text{post}} = 3$ ) and the less prominent in high-order watersheds (2.74% increase in the peak discharge for  $\omega_{\text{post}} = 6$ ).



**Figure 1.** Hydrodynamic ( $D_L$ ) versus geomorphologic ( $D_G$ ) dispersion with respect to the variance of the instantaneous unit hydrograph.

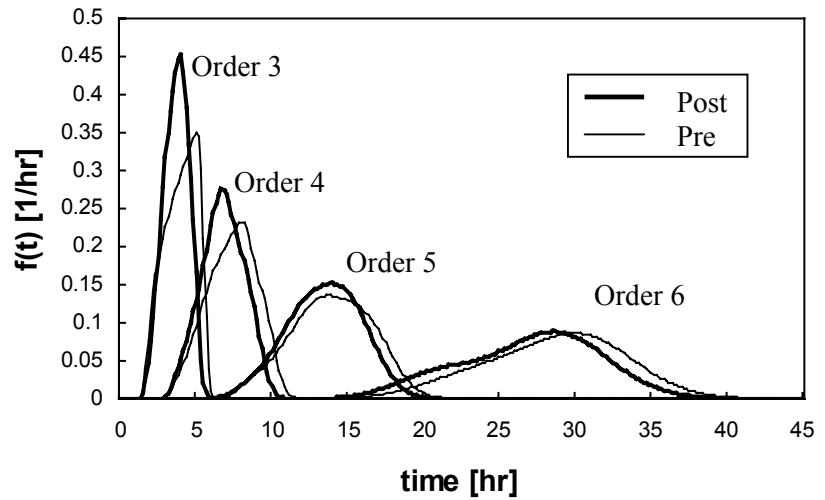
**Figure 2.** Overlay of the EPA river network (dashed lines) with the DEM-extracted river network (solid lines).





**Figure 3.** Ratio of  $D_G$  to  $D_L$ ,  $D_R$ , (a) as a function of frequency  $F$  for various orders  $\omega$  and (b) as a function of order  $\omega$  for various frequencies  $F$ .

**Figure 4.** Comparison of pre- and post-settlement IUHs where the order represents the post-settlement river network configuration ( $F=0.1$ ).



**References:**

[1] Henderson, F. M., 1966: *Open Channel Flow*, Macmillan, New York.

[2] Mesa, O. J., and E. R. Mifflin, 1986: On the relative role of hillslope and network geometry in hydrologic response. *Scale Problems in Hydrology*, edited by V. K. Gupta, I. Rodríguez-Iturbe, and E. R. Wood, D. Reidel, Normwell, Massachusetts, pp 1-17.

[3] Rinaldo, Andrea, Alessandro Marani, and Riccardo Rigon, April 1991: Geomorphological dispersion. *Water Resources Research*, Vol. 27, No. 4, pp 513-525.

[4] Stall, John B. and Yu-si Fok, July 1968: Hydraulic geometry of Illinois streams. *University of Illinois Water Resources Center Research Report No. 15*, Illinois State Water Survey, Urbana, Illinois.



## Basic Information

<b>Title:</b>	The Cricket Frog ( <i>Acris crepitans</i> ) as a Biological Indicator of Watershed Health
<b>Project Number:</b>	IL-97-3, IL-99-5
<b>Start Date:</b>	2/20/1999
<b>End Date:</b>	4/30/2001
<b>Research Category:</b>	Biological Sciences
<b>Focus Category:</b>	Ecology, Water Quality, Non Point Pollution
<b>Descriptors:</b>	Amphibian, anuran, frog, tadpole, herbicides, pesticides, pollutants, ponds, streams, wetlands, water quality, runoff, indirect effects, atrazine, mutagenicity, conservation, ecosystems, health effects, ecotoxicology
<b>Lead Institute:</b>	University of Illinois
<b>Principal Investigators:</b>	Val Richard Beasley, David Bunick

## Publication

1. Cope, Rhian., Craig Miller, Margaret Post, Nohra Mateus-Pinilla, Joseph Murphy, and Val Beasley. 2000. Use of synthetic human luteinizing hormone releasing hormone for induction of breeding in the cricket frog (*Acris crepitans*). *Journal of Herpetological Medicine and Surgery*. 10:7-8.
2. Miller, Craig., David Bunick, and Val Beasley (2000). Identifying a genetic marker of sex in cricket frogs (*Acris crepitans*), a declining amphibian species, in *Environmental Horizons 2000*, The Environmental Council, University of Illinois at Urbana-Champaign, IL, p 44. (Abstract).

### **Problem and Research Objectives:**

There is a global consensus that amphibian populations are declining at rates exceeding other wild vertebrates (Phillips, 1990; Vitt et al., 1990; Wake, 1990; Dunson et al., 1992). Suspected causes include habitat destruction, UV exposure, infectious diseases, and pollutants, including those that alter sexual development. Amphibians, which have lives dependent upon water and land, fulfill essential roles in the environment. They are important converters of algae and periphyton to animal tissues (as tadpoles); they serve as important prey species; and they are predators on insects, and a host of other small and often prolific animal species. In addition, amphibians rely on both aquatic environments and the terrestrial landscape. Accordingly, amphibians are unique sentinels of ecosystem health.

Cricket frogs (*Acris crepitans*) have experienced a severe decline in recent decades in the upper Midwest. Two of our studies have suggested that the widely used herbicide, atrazine, may cause intersex gonads in cricket frogs, and a mixture of polychlorinated biphenyls and polychlorinated dibenzofurans was associated with a marked sex ratio reversal (Reeder et al., 1998; Reeder et al., in preparation). Also, there are concerns about atrazine acting as a mutagen, especially after prolonged exposure. However, genetic markers of sex in the frogs remain to be identified, whether temperature extremes can alter sex determination in cricket frogs creating a confusion factor is unknown, and the properties of atrazine as a mutagen in amphibians remains to be adequately studied. The objective of this study was to gain background knowledge essential to understanding cricket frog declines. Our research specifically **1)** searched for a genetic marker of sex in the cricket frog; **2)** examined the influence of atrazine and temperature on sexual development in cricket frogs; and **3)** examined the role of atrazine as a mutagen in frogs.

### **Methods:**

#### **Methods for Objective 1: Search for a Genetic Marker of Sex in Cricket Frogs**

Cricket frogs (n=145) were collected across Illinois to search for a sex-linked genetic marker. Frogs were sexed based on gross and histologic appearance of gonads. We employed random screens for sex-linked genes using PCR and RAPD analysis. DNA was obtained from leg muscle of individual frogs using the DNeasy Tissue Kit (Qiagen, Valencia, CA) following the DNA isolation protocol for rat tail. DNA was quantified based on absorbance at 260 nm using a GeneQuant DNA/RNA calculator (Pharmacia LKB Biochem Ltd., Cambridge, England). To ensure the DNA was not degraded, it was electrophoresed (120 volts for 2 hours in 0.8% agarose gel). To optimize the assay, different DNA concentrations were tested (50, 100, 200 and 500 ng) with both 5 and 25 picomoles of a 10-mer primer. Later, DNA (50, 100 and 500 ng) was tested with 5, 25, and 50 picomoles of a 10-mer primer. While individual stocks of cricket frog DNA were maintained, DNA also was pooled into four groups (two male and two female) with DNA from 5 individuals in each pool for both Clinton and Fayette Counties. The four DNA pools were then screened with random 10-mer primers in Random Amplified Polymorphic DNA analyses to search for a primer that would produce a different banding pattern between the sexes. While non-sex-specific bands would be expected to occur in both male and female pools, band(s) unique to one sex or the other would suggest the presence of a sex-linked gene. The PCR reaction conditions employed are listed in Table 1.

**Table 1: PCR Reaction Conditions**

Reagent	Concentration	Volume
DNA	10 ng/μL	5 μL
dNTP	1.25 mM	2 μL
10x Buffer	1x	2.5 μL
MgCl <sub>2</sub>	25 mM	2 μL
Water	100%	10.5 μL
Primer	25 pmoles/μL	2 μL
Taq	0.5 U/μL	1 μL

Protocols for amplification reactions were followed according to Operon Technologies (Alameda, CA). Seakem ME agarose (2%) (FMC Bioproducts, Rockland, ME) gels were made, and 15 µL of amplification products were electrophoresed at 40 mV for 6.5 hours to separate the DNA by size and allow photographic imaging. Once a promising primer was determined by comparison of male and female pool banding patterns, individual male and female DNA was screened using the same protocol. The DNA band of interest was isolated, amplified using a TOPO Kit, and sequenced. PCR data were confirmed via Southern blotting.

### **Methods for Objective 2: Examine the Influence of Atrazine and Temperature on Sexual Development in Cricket Frogs**

To examine the influence of atrazine and temperature on sexual development, it was necessary to breed cricket frog tadpoles in order to begin with subjects that had not been exposed to atrazine and/or unusual temperature extremes. In these studies cricket frogs, were induced to breed via hormonal treatment for the first time (Cope et al., 2000). Embryos were placed individually in aquaria with 6 different treatments consisting of room temperature (19-22°C), high temperature (30°C), low temperature (16°C), room temperature with 20ppb atrazine, room temperature with 200 ppb atrazine, and a positive control (ethynlestradiol). A single tadpole was placed in a one-gallon glass mason jar for a total of sixty jars/treatment. All jars were placed randomly within the experimental layout. At the conclusion of the study, tadpoles were anesthetized in MS-222 (tricaine methane sulfonate) before blood was collected for analysis in the mutagenicity component of the study. Tadpoles were then preserved in 10% neutral buffered formalin in preparation for gross and histopathologic analysis of sexual development.

### **Methods for Objective 3: Examine the Role of Atrazine as a Mutagen**

Animals and Exposures: As noted below, in Year 1, our cricket frog microcosm studies were unsuccessful due to high mortality in all groups. Also, the limited availability of the appropriate cricket frog life stages from other sources, and the small size of tadpoles made this species poorly suited for this research.

To compensate for the loss of laboratory-based exposure data on cricket frogs, blood was collected from metamorphic leopard frogs captured from 36 ponds in Minnesota as part of an EPA funded project to examine leopard frog health in relation to spatial, biotic and abiotic parameters. Included among these parameters was an analysis of water and sediment from each pond for the presence of pesticides as well as water quality measurements including temperature, dissolved oxygen and pH. Analysis of leopard frog health included tissue screens for toxicology, a parasitology survey of each animal, and histopathologic examinations of selected organs. We believed that analysis of the blood of metamorphic leopard frogs, as part of this EPA study, would provide a similar spectrum of exposure to both endocrine disrupting chemicals as well as developmental temperatures under real environmental conditions. Further, it would provide the opportunity for more detailed analysis with the larger database of leopard frog and pond data. This work is ongoing.

Also, we undertook laboratory exposures to atrazine on tadpoles of the American toad (*Bufo americanus*), bullfrog (*Rana catesbeiana*), wood frog (*Rana sylvatica*) and the gray tree frog complex (*Hyla versicolor/chrysoscelis*). In addition, since *Xenopus laevis* is a well-studied laboratory amphibian species and tadpoles can be readily obtained commercially at all times of the year, they served as a reference model in this study. Tadpoles of the American toad (*Bufo americanus*), a native North American amphibian species, were used in experiments in the spring when egg clutches were readily available in the wild.

In a pilot study to assess toxicity, tadpoles of both *X. laevis* and *B. americanus* were exposed to atrazine at a range of concentrations up to 1000 ppb. To assess mutagenicity, tadpoles were exposed to atrazine at 3, 30, 300 and 3000 ppb for 1- and 4-week periods. The concentrations were selected to represent either regulatory or environmental exposure levels. Thus, 3 ppb represents the current maximum contaminant level (MCL) for drinking water as set by the EPA. Thirty ppb is a likely concentration that may be proposed as a new MCL. Three hundred ppb reflects concentrations found in watersheds adjacent to agricultural lands. And 3000 ppb represents atrazine concentrations that can be found running through field tiles. The exposure regimen also was selected to approximate what occurs during the growing season.

Given the typical spring/summer rainfall patterns, the 4-week exposure period was designed to simulate the period during which a developing aquatic animal may be exposed to a pulse of agricultural runoff containing atrazine.

**Mutagenicity Assays:** Anurans have nucleated blood cells and so it is possible to collect a DNA sample for analysis by collecting the blood. Blood samples were obtained from tadpoles, anesthetized in MS222, via heart puncture or exsanguination. Blood was placed in Storage Buffer containing DMSO and cryoprotectant and flash frozen in liquid nitrogen. Following standard procedures for analysis via laser beam flow cytometry, DNA was extracted from the samples, tagged with propidium iodide (a dye that will fluoresce from excitation by the laser beam) and run through a Coulter XL laser beam flow cytometer. A summary of DNA fragmentation, a measure of DNA strand breakage by mutagens and clastogens, is expressed as a coefficient of variation (CV). A comparison of the CV from treated vs control animals provides an assessment of the mutagenic potential of a toxicant.

### **Principal Findings and Significance**

#### **Findings and Significance, Objective 1: Search for a Genetic Marker of Sex in Cricket Frogs**

One RAPD PCR primer (B1) initially seemed to show great promise in differentiating males from females when Clinton Counter frogs were examined. When individuals from additional sites were examined, it identified a unique band in nearly all phenotypic males, nearly all intersex individuals, and some phenotypic females as shown in Table 2.

**Table 2: Cricket Frog DNA Exhibiting Band with Primer B1**

County of Origin	Males (ratio bands/total)	Females (ratio bands/total)	Intersex (ratio bands/total)
Cass	4/4	5/5 *	1/1
Clinton	5/5	0/5	--
Fayette	5/5	3/5	--
McDonough	2/3	2/2	--
Schlyer	5/6	3/3	--
Champaign (6-99)	14/14	4/8	3/3
Champaign	0/0	2/4	0/0
Platt	8/8	4/6	1/2
Unknown	--	1/1	2/2
<b>TOTAL</b>	<b>43/45 (95.5%)</b>	<b>24/39 (61.5%)</b>	<b>7/8 (87.5%)</b>

There is more than one possible explanation as to why some phenotypic females have the putative “male” sex-linked band identified by marker B1. While this band in females may represent a gene(s) of a similar size with sufficient homology to bind the marker, we believe it is more likely that, in cricket frogs as in certain races of *Rana temporaria* and *Rana catesbeiana*, all individuals develop initially as phenotypic females regardless of whether they are genetically male or female. Later in those species/races, sex-determining genes of genetic males are expressed, leading to the development of the male gonad and regression of the female gonad. Under this hypothesis, adult phenotypic female cricket frogs with the “male” band may represent sex-reversed males. In actuality, they failed to express their male phenotype. This hypothesis would also explain why we have found intersex cricket frogs that possess this “male” band. They may represent genetically male individuals in the transition phase from female to male phenotype. While it seems highly likely that endocrine disrupting chemicals would have an effect on this developmental process, it has been difficult to study this without a means for reliable differentiation of the genetic sex from the phenotypic sex. We have isolated DNA from the male band, sequenced it, and compared it to known sequences in Genbank. We were unable to find a match, suggesting this sequence may be part of a previously unknown gene on a region of the sex chromosome in cricket frogs. Southern blotting also failed to confirm that it was related to a known sex-linked gene. This study sets the stage for an ongoing analysis.

Understanding genetic programming of sex determination in cricket frogs will enable mechanistic studies of sex determination in this species and probably other amphibians. After a sex-linked gene is identified, the probe could be used in studies to establish whether contaminants or temperature disrupt endocrine

function. This would enable determination of concentrations of contaminants at which expression of sex-determining genes are disturbed, facilitating regulation of pesticides to protect amphibians. Furthermore, the RAPD-PCR technique explored in this study may be of benefit to other species. It should be possible to identify and characterize a sex-linked sequence in any species that possess heterogeneous chromosomes.

### **Findings and Significance for Objective 2 - Influence of Atrazine and Temperature on Sexual Development**

This component of the study was unsuccessful due to high mortality in all treatment groups. This high mortality was associated with infections of the liver and kidneys as determined via histopathology. The unexpected high mortality and inability to rear animals through metamorphosis left us with too few individuals for a statistically meaningful analysis of cricket frogs. In an effort to compensate for the situation, we examined the gonads of gray tree frog (*Hyla versicolor*) metamorphs from an earlier study in which mesocosms containing the tadpoles had been treated with atrazine at 0, 20, 200, or 2000 ug/L. Unfortunately, gonads in these recently metamorphosed hylids were just beginning to differentiate, making it impossible to interpret the effect of atrazine exposure with regard to altering male/female gonadal development as well as the presence/absence of intersex. Of controls that were clearly in the process of differentiation, 10 were consistent with differentiation into males and 5 appeared to be intersex. No normal females were found.

Stages of tadpole development and metamorphosis encompass a period of rapid and dramatic change in the development of all organ systems. Establishing a baseline of normal development in a number of target anuran species (including cricket frogs and gray tree frogs) by conducting a sequential histologic examination of gonad morphogenesis from early tadpole stages through completion of metamorphosis and maturation would aid in interpretation of the potential toxic effects of endocrine disrupting chemicals.

### **Findings and Significance for Objective 3 – Role of Atrazine as a Mutagen**

Due to the high mortality in the cricket frog treatments and the relationship of this mortality to disease as described above, there were too few surviving animals from which to draw blood to provide a statistically meaningful analysis.

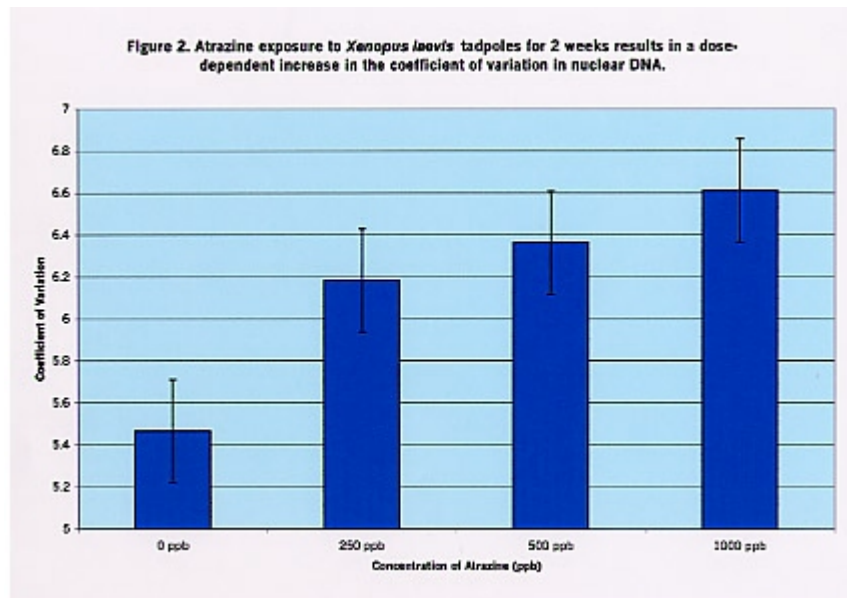
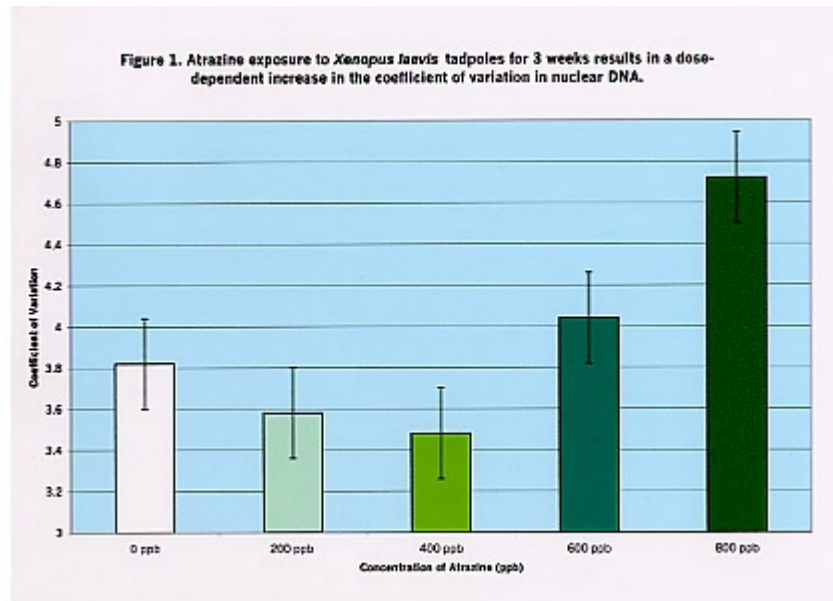
Our preliminary studies indicate that high levels of atrazine do adversely affect the tadpoles of both *X. laevis* and *B. americanus* species. At levels above 1000 ppb, a significant increase of mortality occurs. The tadpoles begin to die at one-week exposure and at two weeks, many are dead. Beyond two weeks, few animals are left in the treated tanks. *Xenopus* tadpoles seem to be more susceptible than *B. americanus* to this toxicity.

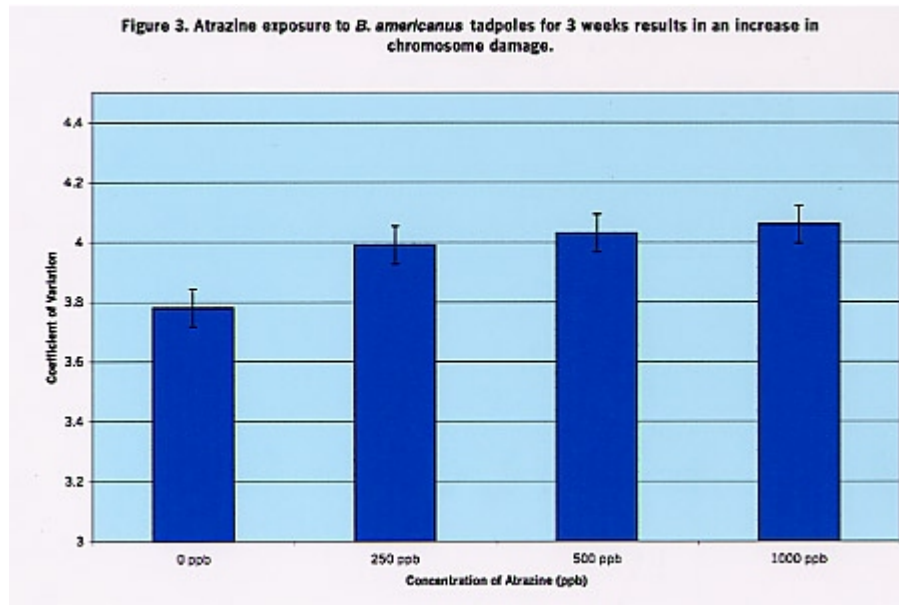
To date, analysis of leopard frog DNA has been completed from 16 of the 36 sites in Minnesota for a total of 240 samples. The average CV of these samples was 2.04 with a standard deviation of 0.2. In general, the CVs of leopard frogs from most sites examined appeared normal. In nearly all sites, the CVs from leopard frogs collected within a site had CVs similar to those collected among sites. While analysis of sediment samples is still pending, these results compare with the water quality results. No significant concentrations of pesticides were detected at these sites. By contrast, at one highly contaminated site presenting, the CV was much wider, indicating likely DNA damage.

Further work with the gray treefrog complex has proven especially interesting. The two-species in this complex, *Hyla chrysoscelis* and *Hyla versicolor* are phenotypically indistinguishable from one another. Identification of each species, for purposes of establishing distribution ranges, is currently accomplished employing a call survey. The mating calls of males of each species are quite distinguishable and can be identified at night during the breeding season. Cytogenetic analysis of chromosomes in the lab (a time consuming and labor intensive process) was the only other means by which to distinguish the two species. The chromosome number of the *H. chrysoscelis* is diploid while that of its sibling species, the gray treefrog *H. versicolor* is tetraploid. Using laser beam flow cytometry and correlating this with call survey data from the 36 ponds in our EPA study sites, we have shown that it is possible to demonstrate the presence of either or both species at a site based on identifying gray treefrog tadpoles that are diploid and tetraploid. This technique correlated well with our field-based call survey data and in cases where these species were not heard calling by our field personnel, but tadpoles were collected, it became possible to identify the species

and fill in missing data. In addition to facilitating species distribution efforts, this technique will augment provide a means determine whether these closely related species hybridize.

Atrazine appears to be genotoxic in both species. In *Xenopus laevis*, at both three week and two-week exposure, tadpoles at the higher levels of atrazine have increased chromosome data as measured by flow cytometry. The increase appears to be in a dose dependent fashion (Figures 1 and 2). A similar phenomenon is also seen in the American toad. At high concentrations of atrazine, an increase in chromosome damage is occurring at three weeks (Figure 3).





Although these results are preliminary, they do indicate that atrazine does have a genotoxic affect on tadpoles. Interestingly, the effect seems greater on *Xenopus* tadpoles. Since the American toad tadpoles were collected in areas that may have atrazine exposure it may be hypothesized that they have developed a tolerance to atrazine. In addition, it appears that the most genotoxicity occurs at concentrations of atrazine over 500 ppb.

### References

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# Information Transfer Program

The major functions of the Illinois Water Resources Center are to oversee a research program and convey the results of research and development within the water resources field to specialists and the interested public. Information transfer is accomplished through workshops, conferences, published proceedings, a web site, and maintenance of a lending library of Illinois Water Resources Center reports and videotapes. In addition, the Center Director and staff serve on state advisory committees and consult with government agencies.

## Basic Information

<b>Title:</b>	Watershed Academy
<b>Start Date:</b>	3/1/1999
<b>End Date:</b>	6/30/2003
<b>Descriptors:</b>	Watershed, planning, management, water quality
<b>Lead Institute:</b>	Illinois Water Resources Center
<b>Principal Investigators:</b>	Richard E. Sparks, L. Chris Johns, Doug Austen

## Publication

1. Illinois Water Resources Center, University of Illinois, Illinois Department of Natural Resources, and Illinois Environmental Protection Agency. 2001. Organizing for Success: Workbook. 200p.



A top-down regulatory approach has worked in the control of pollution from point sources (industrial and municipal sources). The same cannot be said of problems originating from nonpoint sources (excessive nutrients, pesticides, excessive sediment, water flow extremes), partly because of the diffuse nature of the sources and complexity of the problems and partly because of socio-political concerns about retaining local control and protecting private property rights. Watershed management addresses the complexity issue and watershed partnerships provide a bottom-up, collaborative alternative to top-down control. However, the partnerships will not succeed and water quality will not improve unless some degree of technical competence and organizational skill can be transferred to the local partnerships. Connections must be made between the partnerships, delivery systems (e.g., extension), and the expanding knowledge base in both nonpoint pollution control and local governance. A Watershed Academy developed by the Illinois Water Resources Center provides those connections.

Following the first curriculum in basic watershed science (1999), a second curriculum on effective leadership was developed by a team from the University of Illinois, U of I Extension, Institute of Government and Public Affairs, regulatory agency representatives, Natural Resources Conservation Service in Illinois, The Conservation Foundation, and a veteran watershed group (2000-2001). A 200-page loose-leaf resource manual was prepared and tested at the second Watershed Academy workshop “Organizing for Success” in Champaign-Urbana on January 31-February 2, 2001.

## Basic Information

<b>Title:</b>	Governor's Illinois River Conference 2001
<b>Start Date:</b>	3/1/2000
<b>End Date:</b>	10/31/2001
<b>Descriptors:</b>	Outreach, conference, river, management, restoration, basin
<b>Lead Institute:</b>	Heartland Water Resources Council
<b>Principal Investigators:</b>	Robert Frazee, Stephen P. Havera

## Publication

Both the IWRC Director and the Editor served on the planning committee for the 2001 *Governor's Conference on the Management of the Illinois River System*, which will be held 2-4 October 2001 in Peoria, Illinois. The Center will set up a display in the exhibition area of the Governor's Conference, and the Center Editor will produce the Conference program, abstracts, and proceedings.

## Basic Information

<b>Title:</b>	Midwest Technology Assistance Center (MTAC)
<b>Start Date:</b>	11/1/1998
<b>End Date:</b>	6/30/2003
<b>Descriptors:</b>	Drinking water, water treatment, small systems
<b>Lead Institute:</b>	Illinois Water Resources Center
<b>Principal Investigators:</b>	Richard E. Sparks, Kent Smothers, John Braden

## Publication

1. Beck, Roger, Ben Dziegielewski, and Tom Bik. Final report 2000. Benchmark investigation of small public water system economics. Published by Midwest Technology Assistance Center.
2. Park, Jae K. and Abigail Cantor. Final report November 2000. Study of corrosion control in small public water systems. Published by Midwest Technology Assistance Center.
3. Papanos, Lauri. CD version 1.0. 2000. Public water system emergency planning interactive guide. Published by Midwest Technology Assistance Center.

MTAC is a cooperative effort of the ten states of the Midwest (congruent with USEPA regions 5 and 7), led by the Illinois State Water Survey and the Illinois Water Resources Center. The participation of each state in MTAC is led by the Director of their Water Resources Institute. Following a national competition, the Midwest Technology Assistance Center (MTAC) was established 1 October 1 1998 to provide assistance to small public water systems throughout the Midwest with funding from the United States Environmental Protection Agency (USEPA) under the 1996 amendments to the Safe Drinking Water Act.

The mission of MTAC is to assist small community water systems in the assessment, evaluation, and implementation of technological solutions to their drinking water problems.

In 2001, the Midwest Technology Assistance Center produced a CD-ROM-based Emergency Planning Guide for small systems, so small communities anywhere in the nation can handle droughts, power outages, and other emergencies that threaten water supplies. MTAC also created a web site and held a workshop to disseminate information to the small systems. MTAC and the Missouri Technology Center co-sponsored the workshop for small public water systems on 29 February 2000 in St. Louis, Missouri.

## Basic Information

<b>Title:</b>	Water 2000
<b>Start Date:</b>	12/1/1999
<b>End Date:</b>	2/28/2001
<b>Descriptors:</b>	Water science, technology, policy, education, drought, stormwater, groundwater
<b>Lead Institute:</b>	Illinois Water Resources Center
<b>Principal Investigators:</b>	Richard E. Sparks, Tim Feather, Vernon Knapp

## Publication

1. Conference Proceedings of the 2nd Biennial Conference, Water 2000, held at the Holiday Inn, Urbana, Illinois, 13-14 November 2000. Edited by P. Mehrbani and N. Koeneman. 63p.

The Center held the second statewide biennial conference on water issues (Water 2000) on 13-14 November 2000. The conference addressed science, technology, and policy developments in water resources and engaged citizens, researchers, and groups interested in water issues. Specific issues addressed were water conservation and drought preparedness, non-point source pollution, inland lakes, and urban stormwater.

## Basic Information

<b>Title:</b>	IWRC Web Site
<b>Start Date:</b>	3/1/1999
<b>End Date:</b>	2/28/2003
<b>Descriptors:</b>	Web site, education, water, information
<b>Lead Institute:</b>	Illinois Water Resources Center
<b>Principal Investigators:</b>	L. Chris Johns

## Publication



The Illinois Water Resource Center web site ([www.viron.uiuc.edu/iwrc](http://www.viron.uiuc.edu/iwrc)) provides direct links to IWRC publications, news, funding sources, and a calendar of events. Links to current topic resources include TMDL's, Gulf hypoxia, and aquatic invasive species. The IWRC homepage links to the National Institutes for Water Resources ([wrrri.nmsu.edu/niwr/](http://wrrri.nmsu.edu/niwr/)) where all 54 State Water Research Institute's home pages are listed, to the Universities Council on Water Resources ([www.uwin.siu.edu/ucowr/index.html](http://www.uwin.siu.edu/ucowr/index.html)), and the Illinois-Indiana Sea Grant home page ([www.iisgcp.org/](http://www.iisgcp.org/)). Links to over 50 government and non-government web sites about water resource related topics can be accessed from the IWRC main page link to Watershed Science on the Web ([www.viron.uiuc.edu/iwrc/watershed\\_science\\_on\\_the\\_web.htm](http://www.viron.uiuc.edu/iwrc/watershed_science_on_the_web.htm)).

The Illinois Watershed Academy web site ([www.viron.uiuc.edu/iwrc/WatershedAcademy/](http://www.viron.uiuc.edu/iwrc/WatershedAcademy/)) provides information about upcoming Academy workshops, registration information and forms, and materials that are part of the developing curricula.

## Basic Information

<b>Title:</b>	the Link
<b>Start Date:</b>	3/1/2000
<b>End Date:</b>	2/28/2001
<b>Descriptors:</b>	Newsletter
<b>Lead Institute:</b>	Illinois Water Resources Center
<b>Principal Investigators:</b>	Nancy Koeneman, Parna Mehrbani

## Publication

1. the Link. Fall 2000 and Spring 2001 Newsletters. Environmental Council and Illinois Water Resources Center, University of Illinois at Urbana-Champaign.

**the Link** is a publication of the Environmental Council and the Illinois Water Resources Center at the University of Illinois at Urbana-Champaign. **the Link** is published bi-annually and distributed to faculty members, departments, and other persons interested in the efforts of the Environmental Council and the Illinois Water Resources Center. The publication highlights outstanding efforts and research of faculty members and students and brings together the efforts and achievements of environmentally-related programs, groups, and events on campus. **the Link** also promotes conferences and other outreach activities and provides program descriptions of environmentally-related undergraduate and graduate programs.

**USGS Summer Intern Program**

## Student Support

Student Support					
Category	Section 104 Base Grant	Section 104 RCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	2	1	18	1	22
Masters	5	1	0	0	6
Ph.D.	4	4	0	2	10
Post-Doc.	0	0	0	1	1
Total	11	6	18	4	39

## Notable Awards and Achievements

Project B-03. Use of Constructed Wetlands to Improve Water Quality in Lake Bloomington. Data from this IWRC project were used in the preparation of a grant proposal to the USDA/CSREES National Research Initiative which was successfully funded for \$315,000. Title of proposal is Factors controlling stream denitrification in an agricultural landscape. PIs: Jennifer L. Tank, University of Notre Dame, and Mark B. David and Todd V. Royer, University of Illinois.

Project C-06. Analysis of Water Use Trends in the U.S., 1950-1995. This project is in progress. Predictive use models have been developed for the public, industrial, and thermoelectric water use sectors, but the findings are preliminary.

A Watershed Academy was established to provide technical assistance to leaders in more than 100 watershed partnerships that now exist in Illinois. The curricula and workshops developed by the Academy should facilitate local planning and implementation of nonpoint source management and ultimately help improve the quality of rivers and streams throughout the state.

MTAC is a cooperative effort of the ten states of the Midwest (congruent with USEPA regions 5 and 7), led by the Illinois State Water Survey and the Illinois Water Resources Center. The participation of each state in MTAC is led by the Director of their Water Resources Institute. Following a national competition, the Midwest Technology Assistance Center (MTAC) was established 1 October 1 1998 to provide assistance to small public water systems throughout the Midwest with funding from the United States Environmental Protection Agency (USEPA) under the 1996 amendments to the Safe Drinking Water Act.

## Publications from Prior Projects

1. Belander, S., M.H. Keefe, Jennifer L. Welch, J.T. Hupp, "Rapid derivatization of mesoporous thin-film materials based on Re(I) zinc-porphyrin 'molecular squares': selective modification of mesoporous size and shape by binding of aromatic nitrogen donor ligands." *Coordination Chemistry Review*. 190-192 (1999) pp 29-45.
2. Reed, Patrick, Barbara Minsker, and Albet Valocchi, "Cost effective long-term groundwater monitoring design using a genetic algorithm and global mass interpolation." *Water Resources Research*, Vol. 36:12, December 2000, pp. 3731-3741.

3. Reed, Patrick, Barbara Minsker, and Albert Valocchi, "Designing a competent simple genetic algorithm for search and optimization." *Water Resources Research*, Vol. 36:12, December 2000, pp. 3757-3761.
4. Zhao, Ping 2001. Relationship between nom fluorescence characteristics and brominated DBPs. MS Dissertation. Dept. of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign.