Water Resources Research Institute
Annual Technical Report
FY 2003

Introduction

The FY 2003 Annual Technical Report of the Mississippi Water Resources Research - GeoResources Institute provides a summary of USGS-supported research, education, and information/technology transfer activities. Descriptions of four research projects, which completed their first year of funding in February 2004 are included in this summary along with two projects that were funded from previous years and granted an extension.

Research Program

Screening of Environmental Contaminants Detected in Mississippi Sediments as Inducers and/or Inhibitors of CYP1B1 Expression in Channel Catfish - Continuation

Basic Information

Title:	Screening of Environmental Contaminants Detected in Mississippi Sediments as Inducers and/or Inhibitors of CYP1B1 Expression in Channel Catfish - Continuation
Project Number:	2002MS2B
Start Date:	3/1/2003
End Date:	5/30/2005
Funding Source:	104B
Congressional District:	First
Research Category:	Water Quality
Focus Category:	Toxic Substances, Sediments, Agriculture
Descriptors:	pesticides, toxic substances, bioindicator
Principal Investigators:	Kristine L. Willett

Publication

1. Butala, H., C. Metzger, J. Rimoldi, and K.L. Willett, 2004, Microsomal Estrogen Metabolism in Channel Catfish. Marine Environmental Research (Accepted).
2. Patel, M., H. Butala, C. Metzger, J. Rimoldi, and K.L. Willett, 2003, "CYP1B mRNA Expression and Estrogen Metabolism in Channel Catfish Collected from the Mississippi Delta" in 2003 Proceedings of the Mississippi Water Resources Conference, MS Water Resources - GeoResources Institute, Mississippi State, MS, page 36.

B. Research Proposal

1). Title: Screening of Environmental Contaminants Detected in Mississippi Sediments as Inducers and/or Inhibitors of CYP1B1 Expression in Channel Catfish"- Continuation.

2). Focus Categories: TS, SED, AG

3). Keywords: Pesticides, Toxic Substances, Bioindicator

4). Duration: March 1, 2003 through February 28, 2004

5). Federal Funds Requested: $16,800

6). Non-Federal Funds Pledged: $33,600

7). Principal Investigator: Kristine L. Willett, University of Mississippi, University MS

8). Congressional District: Number 1

9). Statement of critical regional water problems:

Sediments associated with Mississippi rivers and lakes contain significant concentrations of environmental contaminants including pesticides and industrial by-products. Chemical characterization of these complex mixtures is often expensive and incomplete. Certain cytochrome P450 enzymes such as CYP1A have been developed as biomarkers of exposure in fish and wildlife. These physiological endpoints integrate exposure to several types of contaminant, are cheaper than analytical analyses, and are indicative of bioavailable contaminants. Biomarker methodologies are critical in order to detect toxic insult at sublethal exposures so that individuals, population and community structure are not affected by contamination of Mississippi waterways. This project is specifically aimed at characterizing the utility of a recently discovered cytochrome, CYP1B1, as a marker of exposure to contaminants that have been reported by the USGS NAWQA and BEST programs in Mississippi sediments and fish samples. Because channel catfish are such an abundant and economically significant species in Mississippi, they will be used as the test organism in these studies.

10). Statement of the expected results:

Using primary cultured channel catfish liver hepatocytes and gill cells to screen a series of diverse contaminants including polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, polycyclic aromatic hydrocarbons and organochlorine pesticides, we will continue to characterize the inducibility and/or inhibition of CYP1B1 RNA. To do this, we use a highly sensitive new technology, quantitative real time reverse transcription PCR. We have shown that benzo(a)pyrene *in vitro* inducibility predicted CYP1B1 induction following *in vivo* exposures. Furthermore, we have measured CYP1B1 from channel catfish collected from three Mississippi Delta lakes. While we will continue to test other sediment contaminants singly in the catfish cell systems, in this final year we will also test chemical extracts of the Delta sediments. To establish relevancy of a new bioassay, it is important to understand how the bioassay responds to both single compounds *and* complex mixtures. Sediment extracts will be tested in both the catfish cells and the more established rat hepatoma cell-line so that bioassay utility and sensitivity can be compared. This project has the potential to develop an entirely new, more representative physiological endpoint of contamination in fish. Because of its role in carcinogenesis, insight into the mechanisms of CYP1B1 induction across taxa will be a significant advance toward applications of CYP1B1 status as a marker for environmental contaminants and potentially cancer.

11). Nature, scope and objectives of the research.

The Mississippi River is over 2,350 miles in length. Its basin encompasses 30 states and two provinces making it the world's second largest drainage basin (Mississippi River Basin Alliance). Because much of this land is for agricultural use, pesticides and herbicides applied great distances from the Mississippi delta can be carried along the river and deposited in this region. In fact, data being collected by the USGS NAWQA indicates that there are at least 30 different pesticides and industrial chemicals detected in fish tissues collected in Mississippi (B.A. Kleiss unpublished data; Schmitt et al., 1999). Additionally, there are at least 14 rivers and lakes in Mississippi where fishing advisories are in effect due to environmental contamination. Some of these contaminants include: polychlorinated biphenyls (PCBs), dioxin, DDT, toxaphene, and mercury.

Halogenated aromatic compounds, typified by the polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and PCBs are industrial compounds or byproducts that have been widely identified in the environment. PCBs were extensively used as heat transfer fluids, plasticizers, fire retardants and in dielectric fluids for capacitors and transformers (Safe, 1990). PCDDs and PCDFs were never used industrially, but instead are byproducts formed during the synthesis of industrial halogenated aromatics or byproducts of combustion. Toxaphene and DDT (1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane) are chlorinated pesticides which were extensively used before they were banned in the United States in the 1980s and 1970s, respectively. The primary use of toxaphene was to treat cotton pests and to a lesser extent for soybeans and peanut crops. The majority of toxaphene use in the United States was in the southeastern states including Mississippi (Glassmeyer et al., 1997). Technical grade DDT contained both p,p'-DDT and o,p'-DDT. These compounds can be metabolized *in vivo* to p,p'-DDD, o,p'-DDD, and the highly stable p,p'- and o,p'-DDE.

Because of the differential chlorine substitutions and metabolites possible, these organochlorine compounds often exist in highly complex environmental mixtures where toxicity varies depending on the particular congeners present. These compounds are highly lipophilic where lipophilicity generally increases with increasing chlorination. They are resistant to breakdown, hence their environmental stability. Because of this environmental persistence, these compounds, especially PCBs and DDTs, can be detected in nearly any environmental matrix tested. Organochlorine concentrations particularly relevant to this proposal include soil samples collected in southern Mississippi that contained 0.16 to 22.9 ppt dry mass toxic equivalents of PCDD/Fs (Fiedler et al., 1995). Additionally, Cooper et al. (1995) collected 38 samples of various food items from grocery stores and local fish markets in southern Mississippi. All 38 samples had detectable levels of PCDD/Fs, and levels in fish and shellfish were higher than levels in meat and dairy products. Farm-raised catfish had the highest toxic equivalencies of all the food types analyzed (Cooper et al., 1995). Dioxin-like PCBs have also been reported in catfish fillets and nuggets from Mississippi (Fiedler, et al. 1998). Additionally, the Yazoo National Wildlife Refuge is closed to fishing in all waters because of high toxaphene and DDT concentrations (Schmitt et al., 1990).

Polycyclic aromatic hydrocarbons are also ubiquitous environmental contaminants because they are byproducts of incomplete combustion. Benzo(a)pyrene (BaP), a model carcinogenic PAH, is a component of tobacco smoke, automobile and boat exhaust, and a byproduct of residential heating. In contrast to the more persistent organochlorine compounds previously discussed, PAHs are more quickly metabolized by vertebrate animals. However, the metabolism of higher molecular weight PAHs can lead to reactive metabolites that can bind to DNA and are implicated in carcinogenesis. For example, in the Black River, Ohio, 39% of brown bullhead collected from near a PAH-contaminated USX coking facility had liver cancer (Baumann and Harshbarger, 1995). The Mississippi Sound, Biloxi Bay was one of only six sites along the Gulf of Mexico to have PAH concentrations greater than 1000 ng/g in oysters (Jackson et al., 1994).

Chemical analysis of these classes of compounds is relatively expensive because these compounds are invariably in complex mixtures in the environment. Furthermore, extraction methods must be developed and confirmed for each sample matrix. Even if concentrations of contaminants are measured in both fish and sediments, it is still unclear what the physiological significance of the contaminant concentrations is to the organisms. For this reason, biomarker approaches have been developed to more quickly and cheaply determine if an organism has been exposed to contaminants. A biomarker is defined as "a xenobiotically induced variation in cellular or biochemical components or processes, structures, or functions that is measurable in a biological system or sample" (Shugart et al., 1992). A biomarker that has been extensively used to characterize exposure to PAHs, PCBs, and PCDD/DFs is induction of the CYP1A gene.

The cytochrome P450 superfamily of genes (including CYP1A and CYP1B1) are involved in the oxidation, metabolism and excretion of both endogenous and exogenous nonpolar compounds in the body. The mechanism of action of the most toxic of the PCDD/DF, PCB, and PAH isomers is related to the binding affinity of compound to the aryl hydrocarbon receptor (AhR). In AhR-responsive systems 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, the most potent congener; strongest AhR agonist) can induce drug metabolizing enzymes including CYP1A1, CYP1A2, CYP1B1, glutathione S-transferase, and glucuronyl transferase. Quantitating induction of any of these genes can be useful biomarkers of exposure to AhR ligands. As previously mentioned, induction of the CYP1A gene has been extensively used for this purpose. CYP1A induction can be measured at the mRNA level by northern blots (Kloepper-Sams and Stegeman, 1989; Haasch et al., 1993), the protein level by western blots (Stegeman et al., 1987) or by the enzyme activity of ethoxyresorufin-O-deethylase (EROD) (reviewed in Bucheli and Fent, 1995). These endpoints have been extensively characterized in many fish and other wildlife systems. Additionally, mammalian cell bioassay systems such as the H4IIE rat hepatoma bioassay have been utilized to test environmental samples for induction of EROD (CYP1A). We have used this assay in our laboratory to characterize environmental contamination in Mobile Bay and Miami River sediments and Galveston Bay oyster extracts (Annavarapu et al., 2002; Willett et al., 1997a).

In contrast to CYP1A, which was first identified as a biomarker in 1975 (Payne and Penrose, 1975), the CYP1B1 gene was only discovered in 1994 (Sutter et al., 1994).

It has since been cloned from tissues of humans, mice, rats, and most recently fish (Tang et al., 1996; Savas et al., 1994; Walker et al., 1995; Godard et al., 1999; and Leaver and George, 2000, respectively). In mammalian systems, CYP1B1 is induced by PAHs and TCDD (Larsen et al., 1998; Walker et al., 1999). Recombinant human CYP1B1 is highly active in oxidizing the potent carcinogenic PAHs, BaP and 7,12-dimethyl-benzanthracene (DMBA), to their respective carcinogenic metabolites. In fact, Shimada and coworkers (1999) found that CYP1B1 was more active than CYP1A1 in metabolizing BaP to the proximate toxicant BaP-7,8-diol. Their study suggests that species with less CYP1B1 may be less likely to form DNA reactive PAH metabolites, and thereby be more resistant to carcinogenesis. This finding is supported by studies with CYP1B1-null mice. Seventy per cent of DMBA-treated wild type mice developed highly malignant lymphomas whereas the CYP1B1-null mice only had 7.5% cancer incidence (Buters et al., 1999). Because CYP1B1 was only recently cloned in fish (plaice), its expression, tissue distribution, inducers, and inhibitors all remain to be characterized in non-mammalian species.

Channel catfish (*Ictalurus punctatus*) are freshwater fish, native to the central and eastern United States and southern Canada. Aquaculture of channel catfish in ponds in the lower Mississippi River Valley is the largest aquaculture industry in the United States (Tucker and Hargreaves, 1998). Because of their abundance and their economic importance, channel catfish are the ideal species to characterize with respect to their responses to environmental contaminants detected in Mississippi.

For these reasons, the **original objectives** of this study were:

1). Use primary cultured channel catfish liver hepatocytes to determine the inducibility and/or inhibition of CYP1B1 by a series of environmental contaminants detected in Mississippi sediments.

2). For compounds that indicate *in vitro* CYP1B1 inducibility, conduct *in vivo* exposures to characterize the *in vivo* time course and dose response relationships in channel catfish.

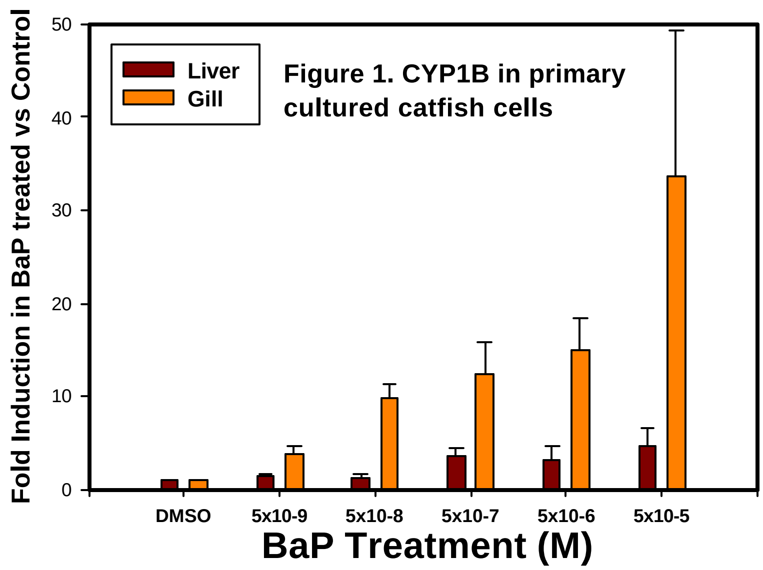
3). Characterize the utility of CYP1B1 as a biomarker of exposure compared to CYP1A and sediment concentrations in fish collected in Mississippi lakes and rivers.

Based on mammalian research, we hypothesize that TCDD (Walker et al., 1999), certain PCBs, such as PCB 126 (3,3',4,4',5-pentachlorobiphenyl), PCB 81 (3,4,4',5-tetrachlorobiphenyl) and PCB 169 (3,3',4,4',5,5'-hexachlorobiphenyl) (Pang et al., 1999), and higher molecular PAHs such as BaP and dibenzo(a,l)pyrene (Kim et al., 1998, Luch et al., 1999) will be potent inducers of catfish CYP1B1. Because other organochlorine pesticides such as DDT and toxaphene do not operate through the aryl hydrocarbon receptor, they likely will not be CYP1B1 inducers. However, they may be significant inhibitors of CYP1B1 which would confound biomarker studies if not tested and well characterized.

Progress to Date on Objectives 1-3 (Funding received May 8, 2001 = 17 months).

Objective 1). In order to completely describe CYP1B1 in channel catfish we have been performing RACE (Random Amplification of cDNA Ends) to clone the gene. To date we have cloned an 861 nucleotide sequence to the polyA tail which encodes 183 amino acids prior to the stop codon. This sequence is 67% similar to the human (Sutter et al., 1994) and plaice (Leaver and George, 2000) CYP1B1 protein sequences with 104 of the 183 residues shared by all three species. Experiments are in progress to continue cloning in the 5'-direction.

Using the sequence information, CYP1B1 and 18S rRNA primers were designed and optimized for SYBR green quantitative real-time reverse transcription PCR. The advantage of qRT/RT-PCR is that it is capable of detecting PCR products as they accumulate during exponential phase of the PCR run and thus enable the accurate and reproducible quantitation of product over a wide dynamic range (Bustin 2000). In the cell experiments, twenty-four hr after isolation and primary culture, cells were treated with DMSO or $5x10^{-9}$ to $5x10^{-5}$ M BaP. RNA was isolated from the cells 24 hr after dosing. Figure 1 shows relative fold induction of CYP1B message in liver and gill cells exposed to increasing concentrations of BaP. We have completed this study with cells from three fish and found that at all BaP concentrations ($5x10^{-9}$ to $5x10^{-5}$ M) CYP1B was significantly ($p< 0.01$) induced in gill cells compared to DMSO treated control cells. Furthermore the CYP1B message was inducible by BaP in a dose-dependent manner. In contrast to the primary cultured gill cells, there was no significant CYP1B induction in liver hepatocytes.



Using a similar approach we are now testing other environmental contaminants that have been reported in Mississippi Delta sediments. At this time we only have an n = 1, but we have tested PCB 77, PCB 126, PCB 153, p,p'-DDT, and TCDD. This work will continue and is supported by year 2 funding.

Objective 2). To determine whether the induction of CYP1B that we saw *in vitro* by BaP also occurred following *in vivo* exposures, male channel catfish were exposed to 20 mg/kg of BaP or corn oil control. RNA was isolated from whole blood, kidney, gill, liver and gonad four days after the *i.p.* exposure. qRT/RT-PCR results are shown in Figure 2. In all tissues except the gill BaP caused statistically significant CYP1B induction *in vivo*. There are several possible explanations that the gill was not significantly induced as would have been predicted by the cell studies. First, there were fairly large inter-individual differences in gill CYP1B induction, and the variability decreased the statistical power to distinguish differences. Second, constitutively the gill has much more CYP1B than the other tissues in the fish. Because of these already high levels of CYP1B, it may be harder to detect induction. Finally, the catfish were exposed *i.p.* so the gill exposure to BaP is potentially much less than would be expected in wild fish who take in contaminants across their gills. Overall, induction in the *in vitro* gill cell system predicted *in vivo* induction by BaP.

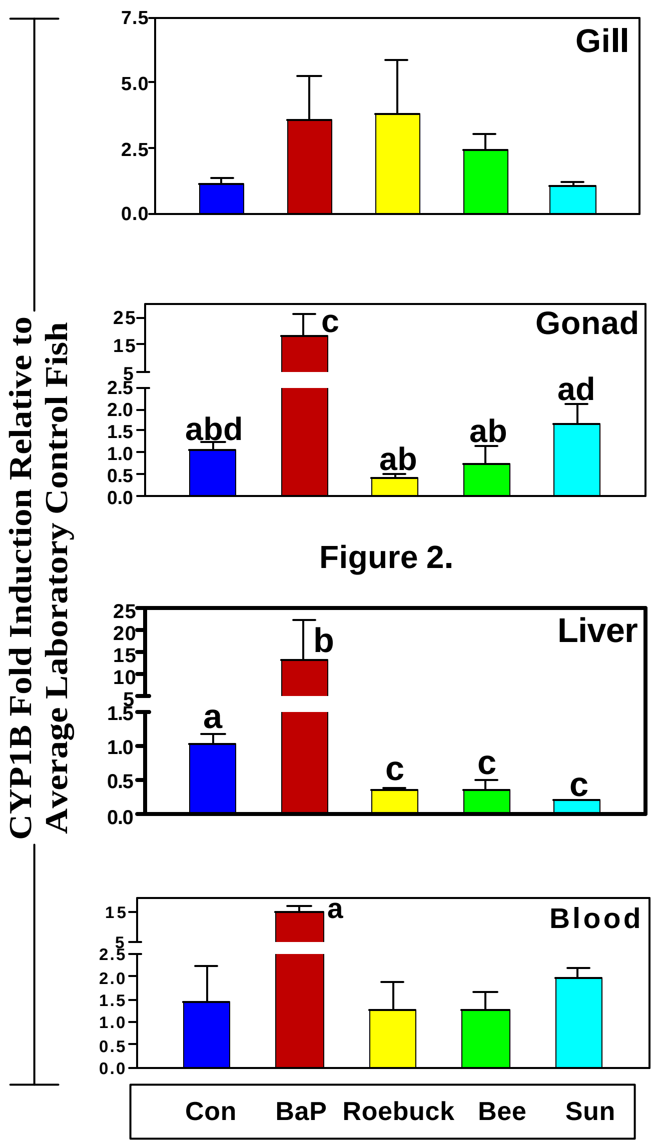


Figure 2.

A second aspect of the *in vivo* experiments was to determine how CYP1B levels in wild fish collected from the Mississippi Delta compared to both laboratory and BaP exposed fish. RNA was isolated from male fish from three Delta lakes. The number of samples extracted and analyzed is shown in the table below. Results from the Delta fish are also shown in the Figure 2. The data for all samples is expressed as the fold induction of CYP1B relative to the response in the average of the laboratory control fish. All the responses of CYP1B were also standardized to the amount of 18S rRNA in the same sample. Bars with the same letter are not statistically different from each other by ANOVA ($p < 0.05$).

# Male Fish Tissues from Delta Sites	*Liver*	*Blood*	*Gill*	*Gonad*	*Size (cm)*
Lake Roebuck	5	5	4	5	29 - 37
Bee Lake	4	4	4	4	24 - 33
Sunflower River	3	2	3	3	15 - 40

In the wild fish again there was a lot of variability and none of the gill samples were significantly different compared to the control or dosed animals. Note, however that the animals from Lake Roebuck (the most contaminated site) had the highest gill CYP1B and averaged higher than the BaP-dosed fish. Blood and gonad CYP1B levels from wild fish were not different than control channel catfish. Interestingly, the amount of CYP1B in wild fish liver was statistically lower than CYP1B in the laboratory control fish livers. This could be because some of the environmental contaminants cause CYP1B inhibition (as we hypothesized with the DDT compounds). Alternatively, some of these fish had relatively severe parasite infestations in their liver which may also be responsible for P450 inhibition.

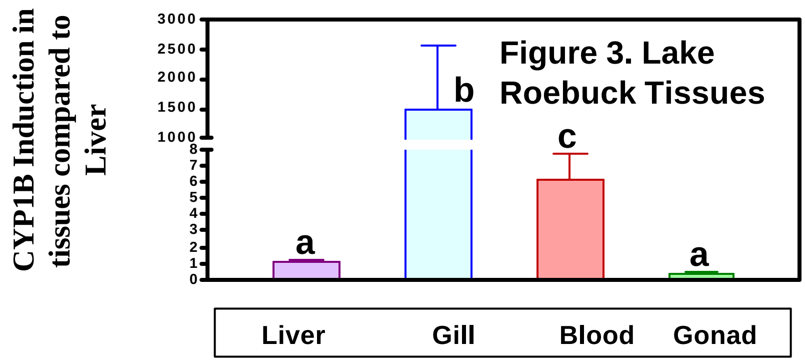


Figure 3. Lake Roebuck Tissues

All of the PCR data in the previous figures has been normalized to the laboratory control fish within tissue. From the previous figures one has no insight into relative tissue levels of CYP1B in the various tissues from the same fish. Figure 3, however, shows that when all the tissues from the Lake Roebuck fish are normalized to the Lake Roebuck liver responses, the amount of CYP1B mRNA is not different between liver and gonad. However, gill and blood have roughly 1000 and 6 times more CYP1B RNA, respectively, compared to liver. This is the basis for our previous statement regarding the high amounts of CYP1B in gill. The tissue distribution of CYP1B in fish was unknown prior to this study.

Objective 3. The aim of this third objective is ultimately to bring together the CYP1B, CYP1A and analytical sediment data to assess the relative utility of CYP1B as a biomarker. Results from PAH, PCB, and pesticide analysis of two sediment samples from each of the initial six sites were provided previously. Analytical results indicated Bee Lake was the least contaminated with no detectable organochlorine pesticides or PCBs in the sediments and relatively low PAH concentrations. In all samples, PAHs were the dominant contaminant. Lake Roebuck was the most contaminated site having the highest concentrations of all the classes of contaminants. o,p-DDE was the predominant organochlorine pesticide detected and was highest at Cassidy Bayou and Lake Roebuck. The majority of the PAHs detected were low molecular weight compounds. BaP was only detected in Cassidy Bayou and Wolf Lake. The CYP1B qRT/RT-PCR results shown in Figure 2 for the Delta fish are consistent with the relatively low sediment concentrations detected thus far.

In order to compare the utility of CYP1B to CYP1A as a biomarker of environmental contamination, we performed EROD assays on liver and gill microsomes from laboratory control and dosed fish and the wild-caught animals. The EROD assay is generally recognized to represent CYP1A activity. Catfish have CYP1A in their livers which is inducible by BaP (Willett et al., 2000 and Figure 4). Liver and gill microsomes have been prepared from all fish collected to date, and the results are shown in Figures 4A and B. The EROD activities from the Delta fish are plotted with laboratory control and BaP-treated animals (n = 3–5). The field-collected fish were intermediate between the control and treated fish and liver EROD activities were much higher than those of the gill. The treated animals had statistically significant higher activities compared to the wild-caught fish ($p < 0.005$ by ANOVA with SNK). The majority of our year 3 effort will be spent on Objective 3 and establishing a quantitative relationship between CYP1B and CYP1A responses and sediment contaminants. This is the information that is most needed for risk assessors in order to use CYP1 as a tool to characterize a contaminated waterway.

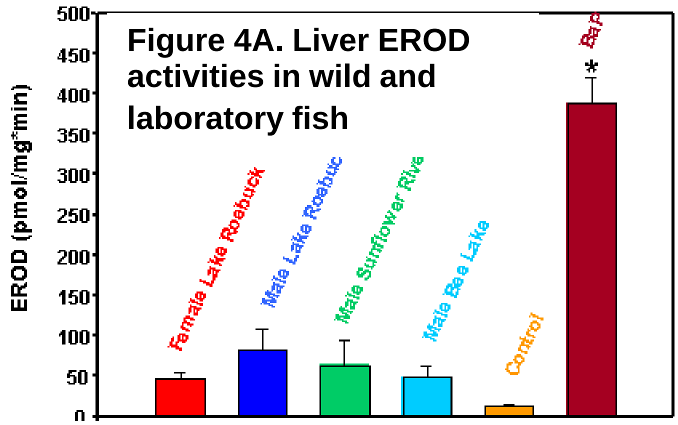


Figure 4A. Liver EROD activities in wild and laboratory fish

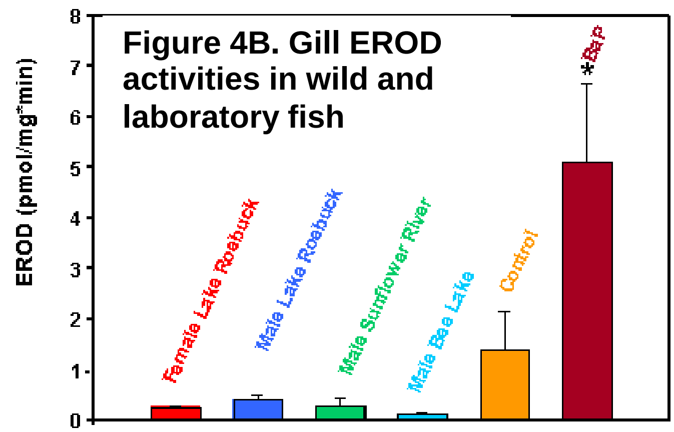


Figure 4B. Gill EROD activities in wild and laboratory fish

Training and Information Transfer: Six students have worked on this project in various roles. Beiming Sun, graduate student, performed some of the initial cloning studies before she left the University. Rooha Contractor and Monali Patel, both graduate students in Pharmacology, replaced Beiming on the project. Harshala Butala, a chemical engineering graduate student, has helped with the *in vivo* exposures and does the microsomal assays. A high school student from Mississippi School for Science and Math worked in my laboratory and accompanied us on two sample collection trips (Diana Kahle). For two years, Kate Argote an undergraduate psychology major has worked in the lab and maintained all of our catfish cultures. My technician, Christine Metzger, also contributed a significant amount of time to this project, and Jimmy Allgood performed the sediment extractions and chemical quantitations. A fifth graduate student, Srinivas Annavarapu, will help with the sediment bioassays proposed for this third year.

Results of these studies have been presented by myself or students at the following nine scientific meetings:

Willett, K.L., Metzger, C., Sun, B., Di Giulio, R.T., and Alworth, W.L. Environmental contaminants that affect CYP1B gene expression in two fish species. Signals and Sensors Meeting, Jackson, MS June 14, 2001. Platform.

Sun, B., Lienesch, L., Di Giulio, R.T., and Willett, K.L. Identification and distribution of a CYP1B-like message in channel catfish and brown bullhead. Pollutant Responses in Marine Organisms Meeting, Plymouth England July 2001. Platform.

Metzger, C. and Willett, K.L. Environmental contaminants that affect CYP1B gene expression in channel catfish (*Ictalurus punctatus*). South Central Society of Toxicology Meeting, Oxford, MS October 2001. Poster.

Sun, B., Metzger, C, Lienesch, L. Di Giulio, R.T. and Willett, K.L. Identification and distribution of a CYP1B-like message in two fish species. 22nd National Society of Environmental Toxicology and Chemistry, Nov. 2001. Poster.

Metzger, C, Di Giulio, R., and Willett, K.L., CYP1B mRNA expression in two related catfish species. Society of Toxicology Meeting, Nashville, TN. March 2002. Poster.

Willett, K.L. Environmental contaminants that affect CYP1B gene expression in channel catfish (*Ictalurus punctatus*). 32nd Annual Mississippi Water Resources Conference, Raymond, MS April 2002. Poster.

Butala, H., Metzger, C., Rimoldi, J., and Willett, K.L. Comparison of (*in vitro*) estrogen metabolism in two species of catfish. Mid-South Society of Environmental Toxicology and Chemistry Meeting, Vicksburg, MS June 2002. Platform. (3rd Place Student Presentation).

Patel, M.R., Metzger, C., and Willett, K.L. Effects of environmental contaminants on CYP1B induction / inhibition in channel catfish collected from different Mississippi lakes. South Central Society of Toxicology Meeting, Jefferson, AR October 2002. Poster.

Butala, H., Metzger, C., Rimoldi, J., and Willett, K.L. CYP1B mRNA expression and estrogen metabolism in channel catfish. 23rd National Society of Environmental Toxicology and Chemistry Meeting, Salt Lake City, UT November 2002. Poster.

12) Methods, Procedures, and Facilities
Year 1 Goals with Year 3 Additions and Modifications:

1). Use primary cultured channel catfish liver hepatocytes and gill cells to determine the inducibility and/or inhibition of CYP1B1 by a series of environmental contaminants detected in Mississippi sediments.

As suggested last year all current and future *in vitro* work will compare how contaminants affect CYP1B1 in *both* liver and gill cells. We have begun testing several PCB congeners, TCDD, and DDT but we still have to do these experiments in triplicate for statistical analysis. The qRT/RT-PCR method is working very well and the results are very repeatable.

Additionally, 5'-RACE experiments are still underway so that we can characterize the entire gene and compare the full-length sequences. The gene appears to have secondary structure that has made cloning in the 5' direction very difficult. We just received a new protocol that includes a high temperature reverse transcriptase step using a CYP1B specific primer. We are hopeful that with this new technique we will finish our cloning. Following cloning the full-length sequence, we will submit a manuscript for publication which will include much of the data included in this proposal.

2). For compounds that indicate *in vitro* CYP1B1 inducibility, conduct *in vivo* exposures to characterize the *in vivo* time course and dose response relationships in channel catfish.

The *in vivo* BaP experiments were very successful, and we found that BaP caused significant induction in liver, gonad, and blood. If PCB congeners or DDT isomers have interesting induction or inhibition profiles in the gill and liver cell *in vitro* experiments, then those compounds will also be tested *in vivo*.

3). Characterize the utility of CYP1B1 as a biomarker of exposure compared to CYP1A and sediment concentrations in fish collected in Mississippi lakes and rivers.

It is this objective that we want to spend the most time in this third and final year of the proposal. This is the objective that will critically analyze the benefits of using both a new P450 (CYP1B) and a species specific bioassay (channel catfish). In years one and two of this project, we have been testing individual compounds that have been previously detected in Mississippi Delta sediments. We will use the same channel catfish cell systems that we have optimized, but this year we are going to analyze the complex mixtures that we extract from Delta sediments.

To establish relevancy of a new bioassay, it is important to understand how the bioassay will respond to complex mixtures in addition to single compounds. We will return to Lake Roebuck, Wolf Lake, Bee Lake, Cassidy Bayou and the Sunflower River to collect fresh sediments. (We collaborate with the USDA-Sedimentation Laboratory for field collection trips). The sediment samples will be chemically extracted as described by Gardinali and coworkers (1996), and then these extracts will be used to dose the channel catfish liver and gill cells. We will use the channel catfish cells to perform both EROD and CYP1B mRNA analyses to determine how the sediment extract results compare to our prior studies with single compounds. Simultaneously, we will analyze these same sediment extracts in the H4IIE rat hepatoma bioassay. The rat cell assay is very sensitive to EROD induction in the presence of environmental contaminants. We have used this assay to characterize PAH contamination in oyster extracts from Galveston Bay. Currently, we use this assay to compare sediment contamination in Mobile Bay compared to estuaries in Florida. In addition to our own work with the H4IIE bioassay and PAHs, this bioassay has been extensively used by other scientists including the USGS to study induction potency of various halogenated hydrocarbons including PCBs (Villeneuve et al., 2001), polychlorinated dibenzo-p-dioxins and furans (Tillit et al. 1991;USGS 2000).

By testing the Delta sediment extracts in both the channel catfish cells and the rat cells, we can make conclusions as to the importance of using fish specific bioassays in risk assessment. Additionally, by using assays that predominantly measure CYP1A (EROD) and the PCR method that is specific for CYP1B, we will be able to directly compare the sensitivity of the two P450s in response to the complex mixtures that organisms in the Mississippi Delta waterways may be exposed.

When the Delta sediments are chemically extracted, one half of the samples will be used in the cell studies described above while the other half will be used for additional analytical contaminant quantitation. Our year one analytical data is from sediment samples collected right next to the bank. In contrast, year three samples will be collected from the middle of the water bodies and should be more representative of the contaminant load. Quantitative analysis of samples used in bioassays provides more insight into the utility and sensitivity of the bioassays. However, as described in the introduction, the advantage of the bioassays is that they integrate the total response of the sediment extract and not just the quantitatively measured compounds.

Facilities Basic laboratories are equipped with analytical and microbalances, scintillation counter, centrifuges, refrigerators, water baths, and an ultracold freezer. Two laminar-flow hoods are available to provide a sterile environment for *in vitro* cell culture. In addition, microscopes (Nikon TS-100 Inverted; Olympus B-Max 40; Olympus MEIJI) and a digital image analyzer system (Kodak Catseye DKC- 5000 with Image Pro Plus version 3.03 software) are available. A TECAN SLT Rainbow UV-VIS scanning microplate spectrophotometer with WinSelect version 2.0 software is utilized for biochemical measurements. There are several desktop and notebook computers available for word processing and data handling and analysis. A Perkin Elmer HTS 7000 BioAssay Reader performs both absorbance and florescence plate reading and is used for EROD assays. A Robocycler PCR machine and related molecular biology equipment (gel boxes, power supplies, centrifuges) are available for molecular analyses. The GeneAmp 5700 qRT/RT-PCR machine is located in the adjacent National Center for Natural Products Research.

The Aquatic Toxicology Laboratory is equipped for specialized research with aquatic vertebrates and invertebrates. The Laboratory currently has five rooms within a larger animal facility. The rooms are temperature controlled and supplied by Gast Regenair blowers for water aeration. High quality water is supplied by a Model 2952 organic bed service carbon filter for chlorine and chloramines removal (U.S. Filter Systems) and delivery to numerous exposure systems (30, 80, and 400L aquaria and Frigid Unit Living streams).

The Environmental Toxicology Analytical equipment consists of a Hewlett-Packard 5973 Mass Spectrometer, Hewlett-Packard Model 8452A diode array UV-VIS spectrophotometer with autosampler and kinetics software, two Hewlett-Packard Model 5890 Series II gas chromatographs (GCs) with dual electron-capture detectors, a Hewlett-Packard Model 5890 Series II GC with flame photometric and flame ionization detectors, a Hewlett-Packard Model 6890 GC with flame ionization and nitrogen-phosphorous detectors. The GCs are linked with a Hewlett-Packard Vectra 25 GC data station with Hewlett-Packard Chemstation software. Also included is a Waters Model 600E HPLC system with Model 484 UV Absorbance Detector, Model 474 Scanning Fluorescence Detector, Model 717 autosampler, a fraction collector and Millennium 2010 chromatography software. A CEM Model MDS-2100 Microwave Digestion System as well as Varian SpectrAA-20 and SpectrAA-400 Zeeman atomic absorption spectrometers are available. Through the 1997 National Research Council of Canada/National Oceanic and Atmospheric Administration Intercomparison Studies (NOAA/10) the analytical laboratory has earned a rating of Very Good for accuracy evaluation of sediments and Superior for accuracy evaluation of biological tissues.

13). Related research.

I am not familiar with WRSIC and SSIE information systems, but this proposal does not represent a duplication of work based on MedLine and SciFinder literature searches. With respect to the related work being conducted in my laboratory, for the past five years I have been investigating the mechanistic reasons for differential sensitivities in polycyclic aromatic hydrocarbon (PAH) induced liver carcinomas in two related species of Ictalurid catfish (Willett et al., 2000 and Willett et al., 2001). The objectives of my current research aim to characterize CYP1B1 gene expression and its associated estradiol-4-hydroxylase activity as appropriate markers of PAH-induced carcinogenesis in a non-mammalian system. Comparison of responses in the two fish species, channel catfish (resistant) and brown bullhead (*Ameriurus nebulosus)* (sensitive), will allow for an *in vivo* and *in vitro* assessment of the mechanistic relationship between CYP1B1, estrogen metabolism, oxidative stress and PAH-mediated cancer sensitivity. Our hypothesis is that brown bullhead, the more sensitive species will show more constitutive and/or inducible CYP1B1 activity. The two fish species may prove to be a good model for human CYP1B1 polymorphisms which may be related to cancer sensitivity in people exposed to PAHs environmentally or through smoking. Furthermore, potential therapeutic approaches may be derived from our research on the relative roles of CYP1A1 and CYP1B1 in generating toxic metabolites of PAHs and estrogen.

While the emphasis on CYP1B1 is relatively new to my laboratory, I have had extensive experience using CYP1A as a biomarker of environmental contamination in fish. Together with coworkers, I have characterized the relative utility of EROD enzyme activity, CYP1A messenger RNA expression, PAH biliary metabolites and PAH-DNA adduct formation in BaP-exposed killifish (Willett et al., 1995). Additionally, we have used CYP1A endpoints to assess environmental contamination in croakers and catfish in Galveston Bay, TX (Willett et al., 1997b) and in various species collected from around production platforms in the Gulf of Mexico (McDonald et al., 1996). In the rat hepatoma cell culture system, we characterized the CYP1A induction potency of a series of environmentally relevant PAHs and compared bioassay results from contaminated mussel extracts to theoretical results calculated from analytically determined PAH concentrations (Willett et al., 1997a). Finally, we investigated immunoreactive CYP1A protein levels in pond-raised catfish from the southeast United States (Fiedler et al., 1998).

14). Investigators' Qualifications.

Kristine Willett, Principal Investigator, will train and supervise all personnel throughout all aspects of this project including animal maintenance, cell culture techniques, experimental design of dosing regimes, field sampling, and tissue collections. She will also be responsible for preparing all reports and manuscripts. Dr. Willett joined the Environmental Toxicology Research Program as an Assistant Professor of Pharmacology just over two years ago. She has extensive experience not only working with environmental contaminants and their affects on CYP genes but also with channel catfish specifically.

Monali Patel, Graduate Student, will be responsible for assisting in all aspects of this study as part of her M.S. research project in the Department of Pharmacology.

Srinivas Annavarapu, Graduate Student, will be responsible for assisting in sediment bioassay aspects of this study as part of his M.S. research project in the Department of Pharmacology.

TBA, Senior Research Technician, will be responsible for maintaining the laboratory on a day to day basis. Her responsibilities will include ordering supplies, preparing tissue culture buffers and media, maintaining catfish cultures, and assisting in field sampling.

Kate Argote, Undergraduate Psychology Major, will be responsible for helping maintain fish cultures in the laboratory in addition to routine laboratory tasks associated with this project.

15). Training potential. As described above this research provides the funds to train one graduate student and one undergraduate student. Other students supported by other sources also contribute to this research. The graduate students will learn concepts of cell culture, *in vitro* and *in vivo* dose-response and time-course experiments, field sampling, and tissue/sediment extractions for organic contaminants. They are already performing tissue extractions, PCR reactions, and sediment bioassays associated with their current research. The undergraduate student continues to appreciate the responsibilities associated with fish care and maintenance. She has also learned the techniques associated with microsome preparation and is gaining general laboratory experience.

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C. Information Transfer Plan

1). Subject matter and problems to be addressed: Sediments associated with Mississippi rivers and lakes contain significant concentrations of environmental contaminants including pesticides and industrial by-products. Chemical characterization of these complex mixtures is often expensive and incomplete. Using primary cultured channel catfish liver hepatocytes and gill cells to screen a series of diverse contaminants including polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, polycyclic aromatic hydrocarbons and organochlorine pesticides, the inducibility and/or inhibition of CYP1B1 will be tested. A highly sensitive new technology, quantitative real time reverse transcription PCR, is used to detect differences across contaminant dose responses and cell systems. For compounds that indicate *in vitro* inducibility, we will conduct *in vivo* exposures to characterize the *in vivo* time course and dose response relationships in channel catfish. Ultimately, we will characterize the *in situ* utility of CYP1B1 as a biomarker of exposure to contaminated sediment in channel catfish collected from Mississippi lakes and rivers. This project has the potential to develop an entirely new physiological endpoint of contamination in fish.

2). Target audience: The utility of biomarker approaches to environmental contamination is that results associated with exposure and effect can be relatively quickly and cheaply determined. Results from this study may indicate that CYP1B1 is a very sensitive and useful biomarker for certain classes of environmental contaminants. Additionally, these studies will add insight into the physiological significance of CYP1B1 in fish. By comparing the fish cell responses to sediment extracts to responses in rat liver cells, insight will be provided into the need for species-specific risk assessment. CYP1B1 could be another tool that environmental risk assessors can use to characterize a contaminated waterway in Mississippi and beyond. It will be important to provide these results to the environmental and toxicological scientific communities.

3). Strategies: The results of this research will continue to be presented at regional and national scientific conferences (nine to date). Additionally, results will be published in peer-reviewed journals such as *Environmental Toxicology and Chemistry*, *Toxicological Sciences*, and/or *Toxicology and Applied Pharmacology*. We anticipate submitting three peer-reviewed publications by the conclusion of this project. Within our department and university there are various forums and seminars where we will present our results throughout the project. In this way, we will get on-going feed-back and suggestions from our peers.

4). Cooperators: The University of Mississippi will assist us in dissemination of the results of this research. Field collections will be done in collaboration with the USDA Sedimentation Laboratory. Quantitative real time RT-PCR will be done with the USDA's instrument located in the National Center for Natural Product Research.

ABSTRACT

Title: Screening of Environmental Contaminants Detected in Mississippi Sediments as Inducers and/or Inhibitors of CYP1B1 Expression in Channel Catfish- Continuation.

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Focus Categories: TS, SED, AG
Keywords: Pesticides, Toxic Substances, Bioindicator

Sediments associated with Mississippi rivers and lakes contain significant concentrations of environmental contaminants including pesticides and industrial by-products. Chemical characterization of these complex mixtures is often expensive and incomplete. Certain cytochrome P450 enzymes such as CYP1A have been developed as biomarkers of exposure in fish and wildlife. These physiological endpoints integrate exposure to several types of contaminant, are cheaper than analytical analyses, and are indicative of bioavailable contaminants. Biomarker methodologies are critical in order to detect toxic insult at sublethal exposures so that individuals, population and community structure are not affected by contamination of Mississippi waterways. This project is specifically aimed at characterizing the utility of a recently discovered cytochrome, CYP1B1, as a marker of exposure to contaminants that have been reported by the USGS NAWQA and BEST programs in Mississippi sediments and fish samples. Because channel catfish are such an abundant and economically significant species in Mississippi, they will be used as the test organism in these studies.

Using primary cultured channel catfish liver hepatocytes and gill cells to screen a series of diverse contaminants including polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, polycyclic aromatic hydrocarbons and organochlorine pesticides, we will continue to characterize the inducibility and/or inhibition of CYP1B1 RNA. To do this, we use a highly sensitive new technology, quantitative real time reverse transcription PCR. We have shown that benzo(a)pyrene *in vitro* inducibility predicted CYP1B1 induction following *in vivo* exposures. Furthermore, we have measured CYP1B1 from channel catfish collected from three Mississippi Delta lakes. While we will continue to test other sediment contaminants singly in the catfish cell systems, in this final year we will also test chemical extracts of the Delta sediments. To establish relevancy of a new bioassay, it is important to understand how the bioassay responds to both single compounds *and* complex mixtures. Sediment extracts will be tested in both the catfish cells and the more established rat hepatoma cell-line so that bioassay utility and sensitivity can be compared. This project has the potential to develop an entirely new, more representative physiological endpoint of contamination in fish. Because of its role in carcinogenesis, insight into the mechanisms of CYP1B1 induction across taxa will be a significant advance toward applications of CYP1B1 status as a marker for environmental contaminants and potentially cancer.

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Hydrologic Controls on Wetland Tree Growth: Determining the Origin, Residence Time and Water Quality of Groundwater in the Root Zone

Basic Information

Title:	Hydrologic Controls on Wetland Tree Growth: Determining the Origin, Residence Time and Water Quality of Groundwater in the Root Zone
Project Number:	2002MS3B
Start Date:	3/1/2003
End Date:	8/29/2004
Funding Source:	104B
Congressional District:	First
Research Category:	Biological Sciences
Focus Category:	Wetlands, Groundwater, Ecology
Descriptors:	surface-groundwater relationships, isotopes, plant-water relationships, wetlands
Principal Investigators:	Gregg R. Davidson

Publication

1. Davidson, Gregg, Brian Laine, Stanley Galicki, and Stephen Threlkeld, 2003, "Hydrologic Controls on Bald Cypress Growth in Seasonally Inundated Wetlands" in 2003 Proceedings of the Mississippi Water Resources Conference, MS Water Resources Research - GeoResources Institute, Mississippi State, MS, p. 161.
2. Long, N., B. Laine, and G.R. Davidson, 2003, "Origin, Isotopic Composition, and Residence Time of Water in the Root Zone of a Bald-Cypress Dominated Wetland", SE/SC GSA Conference, Memphis, TN, March 13-14,2003, GSA Abstracts with Programs, Vol. 35, No. 1, p.26.

1. Title: **Hydrologic controls on wetland tree growth: determining the origin, residence time and water quality of groundwater in the root zone – Year 2**

2. Focus Categories: Wetlands, Ground Water, Ecology

3. Keywords: Wetlands (293), Surface-Groundwater Relationships (241), Isotopes (133), Plant-Water Relationships (179)

4. Duration: March 1, 2003 through February 28, 2004

5. Federal funds requested: $14,445

6. Non-Federal funds pledged: $28,890

7. Principal investigator: Gregg R. Davidson, The University of Mississippi

8. Congressional district: 1st

9. Statement of critical regional water problems

Mississippi Water Research Priorities addressed: Wetlands and Ecosystems

Preservation of natural wetlands is a national priority, but there is still much to be learned concerning the impact of human activity on wetland systems. In areas of intensive agricultural production, nearby wetlands often serve as collection points for agricultural chemicals and sediments eroded from fields. Construction of roads and drainages alter the hydrologic regime in ways that can enhance or diminish the water supply in the wetland. The response of wetland organisms to these alterations are likely caused by multiple factors, not all of which may be recognized. The end result is that the response of an organism to changes in wetland dynamics is often *observed* rather than *understood*. Effective management of natural wetlands requires an understanding of how changes in the wetland influence specific organisms.

The bald cypress (*Taxodium distichum*) is a dominant wetland tree species throughout the southeastern United States, and is prolific in the wetlands of Mississippi. The primary control on growth has often been linked to precipitation, but the link is poorly understood. Trees growing in continuously flooded soils still appear to respond to precipitation with higher rates of growth, which means precipitation may often be an indirect cause of growth. The true stimulus may be the flushing of nutrient-rich or oxidizing water through the root zone, or the delivery of nutrients via transported sediments during precipitation runoff events. Proactive management of bald cypress dominated wetlands requires a more thorough understanding of the hydrologic variables that potentially impact this population.

10. Statement of the results, benefits, and information gained

This proposal represents a continuation of research funded through the WRRI that began in May, 2002. A summary of results from year 1 funding is provided at the end of Section 12.

Completion of this project will yield two end products. First, the results will enhance our understanding of the hydrologic controls on the health of a dominant wetland tree species: the bald cypress. This will in turn lead to an improved ability to manage the natural wetlands of the southeastern United States, particularly regarding issues of sediment accumulation and alterations to the hydrologic regime. Information gained from the project for a specific wetland will include characterization of nutrient availability associated with precipitation events, identification of the origin of shallow subsurface water, and quantification of shallow groundwater residence time at variable depths.

The second product will be a general characterization of wetland hydrology for a type of wetland in a geographical area that is under-represented in the published literature. The study site is in the Delta region of Mississippi, characterized by low relief and many oxbow-lake wetlands. These wetlands are unique in that they often lie near streams or other lakes that can fill quickly during high stream flows, sometimes reversing shallow groundwater gradients.

Data and information gained during the second year of this study will be used to pursue funding from the National Science Foundation (NSF) for a large-scale study that will include additional wetlands.

11. Nature, scope, and objectives of the research

Introduction

Effective management of natural wetlands relies on a thorough understanding of the processes that affect wetland health. It is well understood that hydrologic controls play a preeminent role in these environments, but the exact nature of that role is often only partially known. The relationship between wetland tree growth and the source or availability of water is a good example. The bald cypress (*Taxodium distichum*) is a widespread tree species that grows in wetland environments throughout the southeastern United States. These trees often grow in continuously flooded environments, yet a number of studies have documented a correlation between precipitation and tree growth (Stahle et al., 1985; Stahle and Cleaveland, 1992 and 1996; Latimer et al., 1996). For trees growing in already saturated sediments, the cause of increased growth following precipitation must be linked to a secondary phenomenon.

The observed correlation is most likely a result of an increase in the availability of nutrients following precipitation, or changes in water quality parameters conducive to tree growth, such as pH and Eh, that accompany an influx of new water (Dickson and Broyer, 1972; Dickson et al., 1972; Haynes, 1990; Pezeshki and Santos, 1998). The delivery mechanism of water or nutrients is not clear, however. In a typical wetland in the Delta region of northern Mississippi, precipitation may deliver nutrients to the wetland by several different pathways including offsite recharge to a shallow groundwater followed by subsurface flow into the wetland, by backflow from an adjacent river or lake into the wetland, or by precipitation-driven sediment influx to the wetland.

Many of the wetlands have formed in abandoned stream channels and remain close to streams. The low relief in the region results in relatively small gradients between rivers and shallow groundwater systems that can reverse during periods of high river stage (Anderson and Munter, 1981; Adams and Davidson, 2001). Subsurface water flowing into the wetland can thus potentially come from surrounding fields or from an adjacent river system. If groundwater flow is the primary source of nutrient-rich water, subsurface flow rates must be fairly rapid to produce the high correlation observed between precipitation and tree growth in the same season.

Precipitation events also introduce nutrients to wetlands via sediment eroded from the surrounding drainage area. Intensive agricultural production in the Delta region generally results in an increase in the rate of sediment accumulation and associated agrochemicals. In this case, nutrients may reach wetland flora in a useable form without any replacement of shallow groundwater. Preliminary data collected from the current study site supports a hypothesis that the correlation between tree growth and precipitation can be explained in part by precipitation-driven influxes of nutrient-rich sediment (Galicki et al., 2002). Tree cores collected from two populations of bald cypress growing in areas with substantially different rates of sediment accumulation showed higher growth rates in the area of higher sedimentation. Further investigation of this relationship is not the focus of the proposed project, but it is being pursued concurrently as part of the larger investigation.

Objectives

The objectives of the project are aimed at identifying hydrologic responses to precipitation events that could lead to a correlation between tree growth and precipitation, particularly for trees growing in saturated soils where more water is of no apparent benefit. This requires knowledge of where water in the root zone is coming from, how long it takes for water to be flushed through the root zone, and what chemical changes take place in root-zone water in the days following precipitation.

Specific objectives are:

1) Identify the source of water in the shallow subsurface through multiple seasons in a selected Mississippi Delta wetland.
2) Determine if nutrient concentrations increase in subsurface waters following precipitation events.
3) Determine the residence time of water at different depths within the top 3 m of sediment.

These objectives are being met using a series of nested piezometers completed at different depths along a transect starting near the high-water line, and ending at the extreme edge of the vegetated fringe near the low-water line. The piezometers are being used to track lateral and vertical changes in hydraulic head, and to collect samples for chemical and isotopic analyses for comparison with lake, stream and precipitation samples. A detailed description of how the samples and analyses are being used is provided in section 12.

Study Site

The study site is situated at the northern end of Sky Lake, located in Humphreys County in the Delta region of Mississippi (Fig. 1). Sky Lake is an oxbow of the ancient Mississippi River, and is believed to have formed between 7,500 and 10,000 years ago (Saucier, 1994). The lake currently sits between the Yazoo River and the Big Sunflower River. The lake exhibits the classical morphology of a lake-fringe wetland with the vegetation forming a buffer that surrounds all but the western edge of the lake. The canopy dominant species in Sky Lake include bald cypress (*Taxodium distichum*), water tupelo (*Nyssa aquatica*), and waterlocust (*Gleditsia aquatica*).

Sky Lake receives runoff from approximately 1,900 ha of predominantly agricultural land through several drainage systems and by direct overland flow. During high stage in the Yazoo River, water will flood Wasp Lake to the east which in turn will overflow into Sky Lake resulting in periodic fluctuations in water level of over 3 m within a single week. The vegetated fringe at the northern end of the lake is fully inundated when the lake level is high. When the lake level falls, the exposed vegetated land ranges from approximately 100 to 800 m in width.

Sky Lake was selected as the study site to meet the needs of a multi-faceted investigation that has included studies of element cycling and sequestration, hydrologic controls on tree growth, the influence of agriculture on sedimentation rates, and the reliability of bald-cypress dendrochemistry. Attributes of Sky Lake that make it a favorable study site include the presence of a large population of century-old bald cypress, some tree-ring data was already available from studies at Sky Lake by the University of Arkansas Tree Ring Laboratory, areas of high and low sedimentation rate can be found along the northern boundary, the sediments have not been reworked so it is possible to date sedimentation rates using ^{210}Pb and ^{137}Cs, groundwater gradient reversals are likely when river water backs into the lake, the wetland is surrounded by agricultural lands, and the site is easily accessible from a nearby county road.

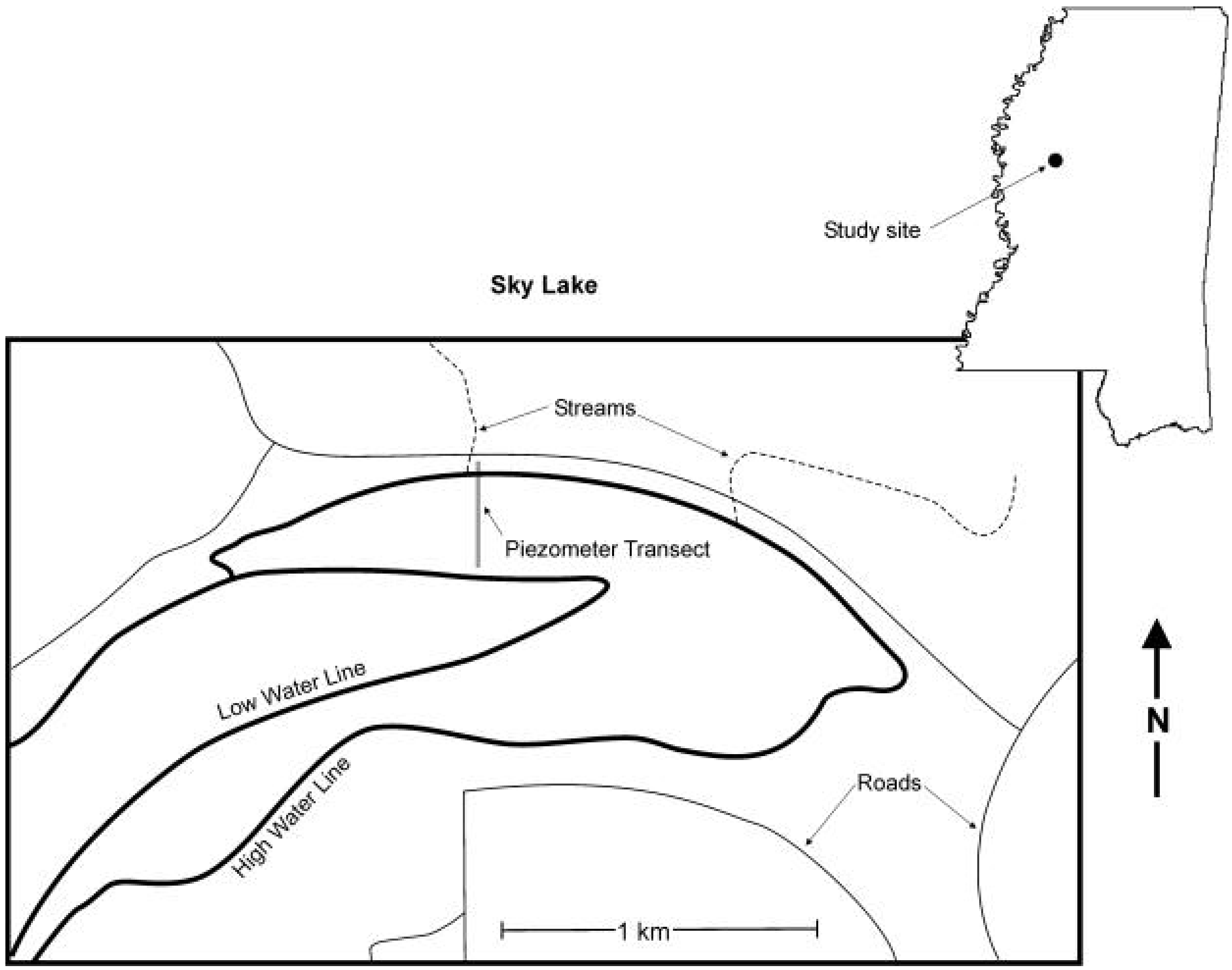

Figure 1. Site map of the northern end of Sky Lake in Humphreys County, Mississippi. Bald cypress trees grow as far as the low water line.

12. Methods, procedures, and facilities

Overview

All three objectives mentioned in section 11 are being met using hydraulic head measurements and water samples collected from precipitation, from the lake and streams, and from a series of nested piezometers. Three sets of nested piezometers have been placed along a north-south transect that begins near the edge of the low water line and extends to near the top of the high water line (Fig. 1). The piezometers were completed at depths of 3.0 m, 1.8 m and 0.6 m (10, 6 and 2 ft) at each of the three sites. Slow recovery rates following water sampling (discussed in more detail at the end of this section) will necessitate installation of additional piezometers so the current piezometers may be devoted solely to monitoring hydraulic head.

Objective 1: Identify the source of water in the shallow subsurface in a characteristic Mississippi Delta wetland.

The origin of water in the shallow subsurface at Sky Lake is being determined using a combination of hydraulic head and water chemistry measurements. Continuous hydraulic head measurements from the piezometers will allow determination of the direction of subsurface flow, both vertically and horizontally, in relation to changes in surface water elevation. Data obtained during the previous year and at other lakes suggest that strong vertical gradients can exist, with flow direction varying with season.

Aqueous chemistry measurements for common ions will be made on samples collected from the piezometers and from the lake to identify any trends in water chemistry that suggest a lake-water or groundwater origin. Oxygen isotope measurements provide a powerful tool for tracking water movement in these systems. Evaporitive enrichment of ^{18}O in water in shallow lakes during the summer gives it a unique isotopic signature relative to precipitation and stream inflow. The origin of shallow groundwater can then be deduced by comparing the $\delta^{18}O$ of groundwater samples with the $\delta^{18}O$ of stream inflow and lake water.

Objective 2: Determine if nutrient concentrations increase in subsurface waters following precipitation events.

Water samples collected from the piezometers will be measured for nutrient concentrations (NO_3^-, NO_2^-, NH_4^+ and ortho-P) to determine if nutrient-rich water is being flushed through the root zone following precipitation events. Preliminary data collected from core samples at Sky Lake have shown that the redox potential of saturated sediments is typically reducing. Eh and pH will be measured in the piezometers to determine if oxidizing waters are introduced following precipitation events.

Objective 3: Determine the residence time of water at different depths within the top 3 m of sediment.

Residence time will be calculated for water at each of the three depths using a combination of tritium and $\delta^{18}O$ measurements. Tritium measurements will provide a rough estimate of how long water has been isolated from the atmosphere at each depth. The current concentration of tritium in surface waters of northern Mississippi are anticipated to fall in the range of 5 to 10 TU based on preliminary data. Analytical methods at the Center for Applied Isotope Studies at the University of Georgia have a lower limit of detection of approximately 0.3 TU. The half-life of tritium is 12.7 years, making it possible to date the age of water recharged within the last 40 to 50 years. If residence times are very short, the tritium results will reflect modern values.

Water samples for $\delta^{18}O$ analysis will be collected from the piezometers, a stream, the lake and precipitation every other week. Trends in the $\delta^{18}O$ value of wetland piezometer samples over time will be plotted against trends in the data for the other sources to determine if any correlations can be found. If water in the piezometers comes predominantly from a single source, the $\delta^{18}O$ data should follow a similar trend as found in the source water, with a possible time lag that reflects the transit time. If two or more sources contribute to the shallow groundwater, the $\delta^{18}O$ data should fall between the values for the two source waters. Given the inherent heterogeneity of flow in wetland systems (Hunt et al., 1996), analyses will be performed on samples at different depths to determine if residence time varies significantly over short vertical distances in the wetland.

Piezometers

The piezometers are made from 2.5 cm (1 in) I.D. PVC with flush-shoulder threads that form a water-tight seal at junctions. The bottom 2 cm of each piezometer is screened. A solid conical plug sits at the bottom that will not trap water if the water level drops below the bottom of the screened interval. The piezometers were manually pushed into the sediment to the desired depth, eliminating the need for drilling and packing. The piezometers extend 6 m above the ground surface to prevent submergence during high water. The piezometers are constructed using 1.5 m sections that can be unscrewed to allow sampling when lake level is low. The piezometers are capped at the top with a breathing hole drilled into the side.

Hydraulic head measurement

Two piezometers at each nest have data-logging pressure transducers installed recording hourly head data. The hydraulic head in the shallowest piezometers at each nest will be measured manually during water sampling every two weeks. Differences in surface elevation between the piezometer nests was determined by marking the exterior of the piezometers at each nest during high water, and measuring the height of the mark above the ground surface during low water.

Lake, Stream and Groundwater sampling

Lake, stream and groundwater samples are being collected every two weeks for chemical and isotopic analyses. Normally, water standing in the piezometers is pumped off before sampling in order to recover water that has not been exposed to the atmosphere. The hydraulic conductivity of these wetland sediments is too low to follow this practice. Based on observations during the previous six months, the piezometers do not fully recovered within two weeks of sampling. Dedicated piezometers will be installed for continued water sampling and will be pumped dry each time. Minor exposure to the atmosphere will occur between sampling. Of the chemical parameters measured, Eh and $\delta^{18}O$ are the most sensitive to atmospheric interactions. This potential problem will be addressed by drawing all piezometer samples from the bottom and dedicating the first aliquots to $\delta^{18}O$ and Eh measurement.

Samples for chemical analysis will be filtered in the field, placed in two 100 mL plastic bottles and stored on ice for transport back to the laboratory. One bottle will be used for cation analysis and will be acidified in the field to a pH of approximately 2 using high-purity nitric acid. The second bottle will be used for anion and nutrient analysis and will remain unacidified. Samples for $\delta^{18}O$ measurement will be placed in 250 mL glass bottles with a conical cap-insert to ensure an air-tight seal. A much smaller volume is required for isotopic analysis, but larger volumes minimize the risk of fractionation during handling. Smaller samples will be collected if water levels in the piezometers are low.

A single round of sampling for tritium is currently planned during the second half of the project currently underway (Year 1). The same protocol used for $\delta^{18}O$ samples will be used for tritium samples. Samples will be analyzed at the Center for Stable Isotope Studies at the University of Georgia.

Precipitation sampling

Weekly precipitation depth will be monitored both locally with a rain gage, and regionally using data from the Belzoni Weather Station No. 660, 23 km west of Sky Lake and from the National Ocean and Atmospheric Administration (NOAA) for Mississippi Region 4. Samples for $\delta^{18}O$ measurement will be collected weekly using a "funnel-and-jug" type sampler that will allow samples to sit for up to a week without being compromised (Clark and Fritz, 1997). The collection system consists of a glass bottle placed in an enclosure beneath a funnel. Silicon oil is placed in the bottom of the bottle which will float on top of water as it collects. The oil will minimize evaporation and contact with the atmosphere, both of which can alter the $\delta^{18}O$ signature. A local high school student will assist with collection during the weeks between water sampling.

Chemical and isotopic analyses

All instrumentation described below is available for use in laboratories under the full or partial direction of the principle investigator. This instrumentation includes a Dionex dual column ion chromatograph (IC), a Perkin Elmer inductively coupled plasma optical emission spectrometer (ICP-OES), a ThermoQuest (Finnigan) isotope ratio mass spectrometer (IR-MS) with a dual inlet and element analyzer, and a custom built high-vacuum sample processing line for isotope sample preparation. The IC and ICP-OES are equipped with autosamplers. The IR-MS has ports for loading 10 samples at a time via the dual inlet.

Cation analysis will be performed using the ICP-OES. At a minimum, analyses will be performed for Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}. Potential also exists for simultaneous measurement of many trace metals.

Anion and nutrient analysis will be performed using the IC. At a minimum, analyses will be performed for Cl^{-}, SO_4^{2-}, ortho-P, NO_3^{-}, NO_2^{-} and NH_4^{+}. Potential also exists for simultaneous measurement of Br^{-} and F^{-}.

Oxygen isotope measurements will be made using the CO_2-equilibration technique (Epstein and Mayeda, 1953). A known mass of water is transferred to the sample preparation line where it is exposed to a mass of purified CO_2. The CO_2 is allowed to equilibrate isotopically with the water at a constant temperature. Following equilibration, the CO_2 is extracted and analyzed for its $\delta^{18}O$ signature using an IR-MS. The fractionation between CO_2 and H_2O is well characterized and can be used to calculate the $\delta^{18}O$ value of the water sample.

Results from Year 1

Funding for the first year of this project was provided in May, 2002 (6 months prior to the submission deadline for this proposal). At that time, three sets of nested piezometers were placed in Sky Lake to monitor hydraulic head and water chemistry changes over time. Shortly after placement, unseasonably high water levels in the lake rose above the tops of the piezometers which compromised future water sampling at these sites. Water levels soon dropped below the tops of the piezometers, but remained unseasonably high through June. As soon as the water level returned to its normal summer low, the piezometers were pulled and moved to new locations. The top of each piezometer was extended to ensure that they will not be submerged again. Data-logging pressure transducers were placed in two piezometers at each nest and set to record water levels every hour. An additional transducer was placed in the lake to record lake level. Continuous monitoring of hydraulic head in the newly placed piezometers began in August. Time was allowed for water levels in the piezometers to equilibrate before sampling for chemical and isotopic analyses, which began the following month.

An example of the hydraulic head data is provided in Figure 2. During the Fall, a substantial downward gradient existed at all three nests. The horizontal gradient was more complex. The hydraulic head in the piezometers completed at 1.8 m consistently declined toward the south suggesting flow toward the lake. The deeper piezometers showed a hydraulic high in the middle nest. This apparent anomaly was persistent throughout the period of monitoring. Figure 2 also demonstrates that the hydraulic conductivity of the sediments in the root zone is very low. Water levels do not have time to recover between the water-quality sampling intervals. A second set of piezometers will be installed for water sampling so that the current piezometers can be devoted solely to monitoring hydraulic head.

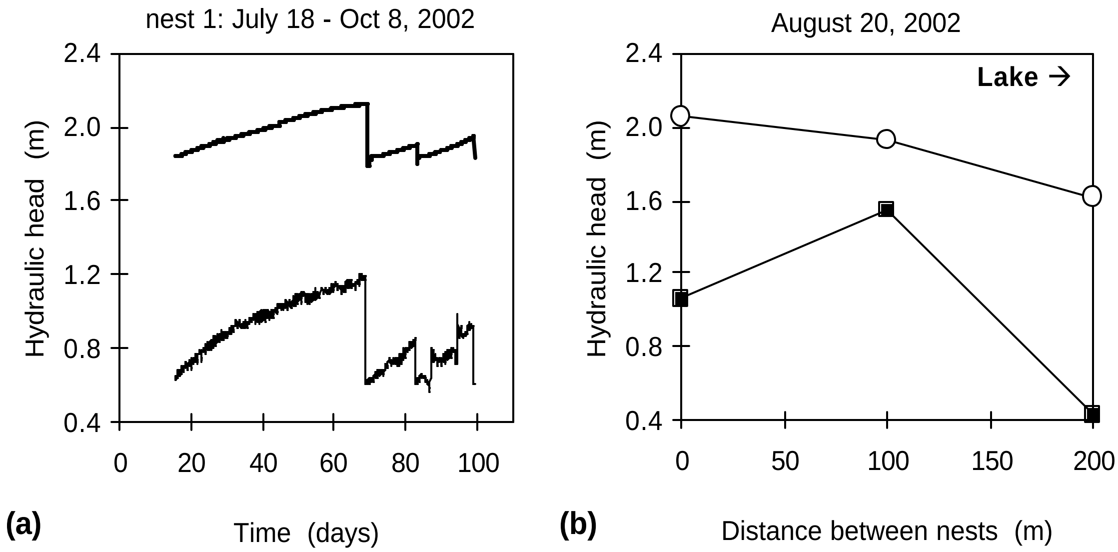

Figure 2. (a) Continuous hydraulic head measurement in piezometer nest 1 (highest elevation). Upper curve is for 1.8 m depth, lower curve is for 3.0 m depth. Sudden drops represent water removal for water-quality sampling. (b) Hydraulic head in the 1.8 m piezometers (open circles) and 3.0 m piezometers (filled squares) in all three nests on August 20, 2002.

Oxygen isotope data are now available for two sampling rounds. This is not yet enough data to make assessments of residence time, but several processes are already clear from the limited data set. First, substantial evaporation from the lake is evident from the elevated summer $\delta^{18}O$ values for lake water (+3‰ VSMOW) relative to stream inflow values (-1.3 to -4.9‰). Second, the influence of Hurricane Lili (Sept. 21, 2002) was apparent from a significant drop in the precipitation $\delta^{18}O$ value. Normal values during this time of year have been in the range of –3 to –6‰. Hurricane Lili delivered water with a $\delta^{18}O$ of –11‰. Lastly, the $\delta^{18}O$ of groundwater in the root zone is more similar to inflowing stream water than to the enriched summer lake-water, which is consistent with the hydraulic gradient observed in the 1.8 m piezometers. Changes will be monitored with time to determine if the isotopic signature approaches lake values during the winter months when the lake level is high.

13. Related research

Bald cypress growth

Several studies have investigated the growth response of bald cypress to environmental conditions, including addition of nutrient rich wastewater (Lemlich and Ewel, 1984; Straub, 1984), cypress dome size (Ewel and Wickenheiser, 1988), changes in wetland hydroperiod (Stahle et al., 1992; Young et al., 1995), and precipitation or stream discharge (Stahle et al., 1985; Stahle and Cleaveland, 1992 and 1996; Latimer et al., 1996; Cleaveland and Stahle, 1989; Valentine et al., 1997). Most of the latter studies observed a positive correlation between tree growth and precipitation (Stahle et al., 1985; Stahle and Cleaveland, 1992 and 1996; Latimer et al., 1996) or between tree growth and stream discharge (Cleaveland and Stahle, 1989). The only exception was Valentine et al. (1997) who found no apparent response to either precipitation or stream discharge in a region where the trees had been cut off from the floodplain by stream incision. To my knowledge, no studies specific to wetland tree growth have been conducted investigating the mechanisms by which water or nutrients reach the root zone.

Wetland Hydrology

The tools proposed in this study to investigate the hydrology of a wetland are not new. Several studies have been published in recent years using hydraulic head measurements in nested piezometers and utilizing water chemistry and isotopic signatures to identify the origin and residence time of water. Several papers have come from an ongoing investigation of a natural wetland in Wisconsin that has been artificially expanded to include a constructed wetland. In this wetland, Hunt et al. (1996 and 1998) used $\delta^{18}O$ measurements to determine that water in the upper 30 cm of the saturated zone contained a mixture of precipitation and upwelling groundwater, while deeper water contained little evidence of precipitation. Residence time of water in the root zone of this system, based on hydraulic gradients, evapotranspiration estimates, and $\delta^{18}O$ data was on the order of a few months (Hunt et al., 1996; Lott, 1997). A separate study also noted the importance of sampling at different depths because of inherent heterogeneities within the wetland (Hunt et al., 1997).

Similar techniques have been applied to determine the origin and fate of groundwater-wetland interactions in Minnesota (Komar, 1994), groundwater-lake interaction in Kenya (Ojiambo et al., 2001), and groundwater-river interaction in Germany (Maloszewski et al., 1987). The wetland study of Komar (1994) utilized both head measurements and $\delta^{18}O$ data to determine the proportions of shallow groundwater and upwelling deep groundwater present in the wetland subsurface.

The uniqueness of the proposed study:

1) The selected wetland is unique because of large fluctuations in water level due to its proximity to the Yazoo River, and the potential for seasonal reversals in subsurface flow.

2) The study will provide information about the hydrology of wetlands in a geographical region for which there is very little data.

3) The study will provide results that will be used to evaluate the hydrologic controls on wetland tree growth.

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14. Investigator's qualifications

The proposed project fits within a larger study that deals with the interface between biological and hydrological systems with an emphasis on the hydrological controls that may effect wetland flora. A biologist, Dr. Stephen Threlkeld, is part of that research team (note publication by Valentine et al., 1997). For the proposed project, only hydrologic and geochemical expertise is required. Dr. Davidson received training at both the M.S. and Ph.D. level from the internationally recognized Hydrology and Water Resources program at the University of Arizona, and has demonstrated an ability to complete and publish research projects in diverse fields associated with hydrogeology and aqueous chemistry. Dr. Davidson has also instructed senior and graduate level courses in Hydrogeology, Environmental Geochemistry, and Isotope Hydrogeology at the University of Mississippi for the last seven years.

An oral presentation of the Year-1 results will be presented by Brian Lain at the combined Southeastern & South-Central GSA meeting in Memphis in March, 2003, and at the WRRI conference in April, 2003. Completion of the Year-2 study is expected to result in published papers in journals such as *Wetlands*.

15. Training potential

A graduate student and an undergraduate student from the Department of Geology and Geological Engineering at the University of Mississippi are currently involved in the project. Brian Laine, a first-year graduate student, is working on the investigation as part of his thesis project. Nathan Long, a senior in Geological Engineering, is working on the investigation as part of an undergraduate research project sponsored by NASA. Brian Laine will continue working through Year 2 of the proposed work. Nathan Long will finish his portion of the work at the end of Year 1, and another undergraduate student will be recruited for the Year-2 work.

Brian Laine began working on the project while still an undergraduate student, and was an author on a published abstract for the combined Southeastern & North-Central GSA meeting in Lexington, Kentucky in April, 2002. Nathan Long and Brian Laine are both authors on an abstract that will be published following the combined Southeastern & South-Central GSA meeting in Memphis, Tennessee in March, 2003.

Information Transfer Plan

Those most likely to benefit from the information gained from this project will be scientists, managers and regulators with interests or responsibilities in wetland ecosystems or wetland hydrology. This is a fairly broad audience. The most effective means of reaching this audience is through publications in applied journals such as *Wetlands*. It is anticipated that the students working on this project will join me in writing one or more manuscripts for submission to a peer-reviewed journal.

Dissemination will also be accomplished through presentations of ongoing work at both regional and national meetings. I am a member of the Hydrology Division of the Geologic Society of America (GSA) and regularly attend and present at the national meetings. I am also the technical program chair for the 2003 Southeast & South-Central GSA meeting to be held in Memphis, Tennessee, where there will be several sessions related to wetland hydrology.

Intermittent dissemination of results will be accomplished through speaking engagements. I am periodically invited to give scientific presentations to other academic programs and professional organizations.

On an individual level, I hope to open a window of knowledge and opportunity for a local high school student in the Mississippi Delta by direct involvement in the work, and participation in the 2003 regional GSA meeting in Memphis.

Improved Estimation of Nutrient and Pesticide Runoff Losses from Golf Courses and Residential Lawns in the South Atlantic-Gulf Region

Basic Information

Title:	Improved Estimation of Nutrient and Pesticide Runoff Losses from Golf Courses and Residential Lawns in the South Atlantic-Gulf Region
Project Number:	2003MS16B
Start Date:	3/1/2003
End Date:	8/29/2004
Funding Source:	104B
Congressional District:	Third
Research Category:	Ground-water Flow and Transport
Focus Category:	Non Point Pollution, Water Quality, Surface Water
Descriptors:	rainfall-runoff processes, fertilizers, pesticides, nutrients, water quality
Principal Investigators:	Joseph H. Massey

Publication

1. Massey, J.H., E.F. Scherder, R.E. Talbert, R.M. Zablotowicz, M.A. Locke, M.A. Weaver, M.C. Smith, and R.W. Steinriede, 2003, "Reduced Water Use and Methane Emissions from Rice Grown Using Intermittent Irrigation" in 2003 Proceedings of the Mississippi Water Resources Conference, MS Water Resources Research - GeoResources Institute, Mississippi State, MS, pp. 27-35.

A Research Proposal Submitted to WRRI

(1) Title: Improved Estimation of Nutrient and Pesticide Runoff Losses from Golf Courses and Residential Lawns in the South Atlantic-Gulf Region

(2) Focus Categories: NPP, WQL, SW

(3) Keywords: water quality, rainfall-runoff processes; fertilizers, pesticides, nutrients

(4) Duration: March 1, 2003 through February 28, 2004

(5) FY 2003 Federal Funds Requested:	$15,000 Total	($15,000) Direct	($0) Indirect
(6) Non-Federal (Matching) Funds Pledged:	$30,034 Total	($20,423) Direct	($9,611) Indirect

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Collaborators: Mary Nett[4], Don Wacuhope[5], Rusty McCulley[6], and Frankie L. Boykin[7].

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(8) Congressional District: 3rd Congressional District

(9) Statement of Critical Regional Water Problems:

The ***Mississippi Water Research*** and ***South Atlantic-Gulf Region Water*** priorities addressed by this project are: the measurement and protection of surface water quality from nutrient and pesticide contamination (***Water Quality***), and predicting the rates of movement and concentrations of nutrients and pesticides to surface waters (***Contaminant Transport Mechanisms***).

Turfgrass is the most intensively managed biological system in metropolitan areas. Currently, over 40 million acres of turf are estimated to be growing in the U.S. If the areas of the approximately 15,0000 golf course were combined, they would encompass an area larger than Delaware and Rhode Island. An average of 350 new or expanded golf courses have opened each year since 1990, each averaging 150 acres. Following the national trend, turf acreage in Mississippi is expanding at a steady pace. Mississippi currently has an estimated 800,000 residential lawns comprising 300,000 acres and over 2,500 athletic fields. These figures do not include turf maintained at city parks, schools, churches, cemeteries, airports and industrial/commercial sites. An estimated 170 golf courses (ca. 15,000 A) and

175 sod farms (ca. 5000 A) are currently in operation in MS. In addition, about 2 million A of highway roadsides are maintained in Mississippi, a significant portion of which are treated with one or more herbicides each year. Turf-related agrochemical spending is expected to continue growing at 5.5% per year to 6.2 billion dollars by 2006. Unlike turf professionals, homeowners tend to apply more chemical than is necessary for effective results. As a result, the use of pesticides by homeowners may be as high as 5 to 10 lbs. per acre, almost ten times more chemical per acre than is used by farmers. The intensity of pesticide and nutrient use, coupled with the anticipated continued growth in turf acreage, suggests that concerns over the impacts of turf chemicals on surface water quality will likely increase over time.

Unfortunately, current models used to estimate nutrient and pesticide runoff from managed turf are not accurate, making it difficult to allocate between different sources of agricultural and non-agricultural contamination and to assess overall turf impacts on water quality. This project is designed to improve the estimation of nutrient and pesticide runoff from warm-season turf managed according to conditions found on golf course fairways and residential lawns.

(10) Statement of the Results, Benefits and Information Expected:

The expected results of our project are:

- Direct comparisons of the hydrology and nutrient and pesticide transport rates from warm-season turf grown to simulate golf course fairways (the largest treated areas on golf courses) and residential lawns (the largest segment of managed turf receiving pesticide and nutrient inputs).

- Determination of the scalability of runoff events from large and small treated areas for both residential and intensively managed turf.

- Improved simulation models used to estimate agrochemical runoff from warm-season turf.

By advancing the science of runoff estimation, our project will benefit surface water quality in the South-Atlantic-Gulf region as follows:

- Improved runoff estimation will allow the impacts of different turf maintenance regimes to be compared, greatly aiding in the development and targeting of practical, effective BMPs to reduce environmental impacts of agrochemical runoff from turf.

- Improved runoff estimation will enhance the ability of regulatory agencies to better allocate between agricultural and non-agricultural NPS loads, a key step in TMDL development.

- Improved hydrological models of warm-season grasses can be used to better predict the fates of oils and various inorganic contaminants washed onto grass from roadways, parking lots, etc.

- Improved runoff estimation will allow "what if" analysis and provide quantitative results to support rules/regulations for turf maintenance practices devised for a given watershed district.

- We anticipate that the real-world runoff scenarios generated by this project could be incorporated as sub-routines in BASINS and other watershed management programs to increase the accuracy of turf runoff estimations for mixed land-use watersheds.

(11) Nature, Scope and Objectives of Research

Turf Acreage & Agrochemical Use

Turfgrass is the largest, most intensively managed biological system in most metropolitan areas. In 1985, an estimated 3.6 million acres of turf were treated annually with agrochemicals[1] in the U.S. (Lin and Graney, 1992). More recently, a total of 30 to 40 million acres of turf were estimated to be growing in the U.S. (Hull et al., 1994; Emmons, 1995). Stuller (1997) reported that over 15,000 golf courses exist in the U.S., which if added together would encompass an area larger than Delaware and Rhode Island combined. An average of 350 new or expanded golf courses have opened each year since 1990, averaging 150 acres each (Stuller, 1997). Of this area, fairways comprise by far the largest percentage of intensively managed turf associated with golf course designs (Beard, 2000).

Following the national trend, turf acreage in Mississippi is expanding at a steady pace. Mississippi currently has an estimated 800,000 residential lawns comprising 300,000 acres and over 2,500 athletic fields (Wells, 2002). These figures do not include turf maintained at city parks, schools, churches, cemeteries, airports and industrial/commercial sites. The golf and sod-production industries, in particular, represent growth industries for the state. An estimated 170 golf courses comprising about 15,000 acres and 175 sod farms comprising about 5000 acres are currently in operation in Mississippi. In addition, the state of Mississippi maintains about 2 million acres of highway roadsides, a portion of which are treated each year with one or more herbicides (Wells, 2002).

Turf maintenance represents an important and growing market for pesticides and fertilizers. Turf-related agrochemical spending has grown steadily over the past decade and sales are expected to continue growing at 5.5% per year to 6.2 billion dollars by 2006 (Anonymous, 2002). Lawn care and landscape applications generally account for approximately 80% of the total treated turf area and 75% of total turf chemical expenditures (Lin and Graney, 1992). A survey conducted by the Minnesota Department of Agriculture (MDA) indicates that 90% of homeowners apply one or more lawn care products over the course of a growing season (MDA, 1998).

In terms of the *intensity* of use on a mass-per-unit-area basis, pesticide and fertilizer use on residential lawns often exceeds that of agriculture (Gold and Groffman, 1993; Farm Chemicals, 1992). This greater intensity of use is often attributed to the propensity of do-it-yourself applicators to apply, intentionally or unintentionally, more of chemical than recommend by product labels (Landscape Management, 2002). *As a result, the use of pesticides by homeowners may be as high as 5 to 10 lbs. per acre, about ten times more chemicals per acre used by farmers* (Mississippi State University Extension Service, 2001). The intensity of pesticide and nutrient use, coupled with the anticipated continued growth in turf acreage, suggests that concerns over the impacts of turf chemicals on surface water quality will likely increase over time.

Agrochemical Runoff from Turf

While agrochemical use in row crop agriculture has received significant attention as a contributor to surface water contamination, runoff from suburban/urban areas is increasingly recognized as a potential contributor to water quality impairment. Surface waters throughout the nation contain measurable concentrations of pesticides that result from non-agricultural applications (Larson et al., 1995). Wotzka et

[1] Agrochemicals include fertilizers, herbicides, fungicides, insecticides and growth regulators applied to protect turf from pests and/or to improve turf growth, density and appearance.

al. (1994) found runoff in Minneapolis, MN to contain the herbicides 2,4-D, MCPP and MCPA from April through October. The authors attributed early-season, low-level detections to commercial applications to lawns and gardens while the significantly higher herbicides concentrations detected in runoff later in the growing season were attributed to applications by individual homeowners. In contrast to runoff from agricultural fields where peak pesticide concentrations typically occur with the spring flush, detections in suburban/urban runoff have less distinct seasonal patterns and occur over a longer period of time (Larson et al., 1995). The increased duration of urban pesticide detections was attributed to the prolonged time frame during which homeowners apply pesticides to their lawns.

Pesticide and nutrient runoff from residential lawns has been indicated as a source of non-point source contamination in Mississippi (Mississippi Soil and Water Conservation Commission, 1995) that negatively impacts the Gulf of Mexico (Mississippi State University Extension Service, 2001). However, there is a distinct absence of data that determines the turf management system resulting in the greatest runoff losses. Runoff from golf courses has been investigated more than other turf settings due to public concerns over frequent pesticide use and the fact that many golf courses are designed such that runoff flows into ponds and creeks. Researchers measuring the runoff of pesticides from plots simulating greens and fairways have shown that, on average, about 7% of applied chemicals is lost as runoff when rainfall occurs = 48 h after application (Smith and Bridges, 1996; Hong and Smith, 1997; Armbrust and Peeler, 2002). This same trend holds true for NO_3-N losses (Linde and Watschke, 1997). Few studies have compared runoff losses between golf course and residential turf even though residential lawns represent a much higher percentage of land area than golf courses. As compared to professionally maintained golf courses, residential lawns may be especially prone to runoff due to soil compaction and other practices that limit the infiltration rate of rainfall and irrigation water (Harrison, 1993).

Management Differences between Golf Course Fairways and Home Lawns Affect Runoff Potential

A number of cultural differences exist between turf that is professionally managed for golf course fairways and home lawns. Key differences include mowing height and frequency, fertilization rate and frequency, aeration, and irrigation rate and frequency. Of particular interest are those factors that affect turf density. Welterlen et al. (1989) reported that shoot density is affected by soil moisture, N fertility, and mowing height.

Mowing height affects the number of grass plants per unit area (i.e., shoot density). Beard (1997) found that Bermuda grass was over 60% denser when mowed to a height of 0.5-inches as compared to a mowing height of 1.5-inches which had 300 shoots/dm^2. As shoot density increases, the water holding capacity (WHC) of underlying soil increases (Linde et al., 1995). Increased soil WHC reduces runoff losses. Typical mowing heights for residential lawns are 1 to 3 inches and 0.5 1.5 inches for golf course fairways. As a result, one could reasonably expect that differences in mowing height between golf course and home lawns could contribute to significant differences in runoff timing and volume.

A survey conducted in New Jersey indicates that over 80% of the pesticides applied to residential lawns are herbicides as compared to golf courses where 65% of all applications are fungicides (Anonymous, 1992). This indicates another significant difference between golf courses and residential lawns since herbicides are typically more water-soluble than fungicides and, as a general rule, more prone to runoff. Coupled with the propensity of homeowners to over-apply agrochemicals and the proximity of many residential lawns to streets and other impervious surfaces, runoff from treated lawns may quickly find its way to storm drains that are often directly linked to the nearest body of water.[2]

More information is needed to determine if turf maintenance practices contribute to surface water impairment in the south Atlantic-Gulf region, and to devise BMPs for agrochemical use on turf as has been done for agricultural settings. Currently there exists no regulatory exposure assessment tool that can be used by authorities at the Mississippi Department of Environmental Quality or and EPA Region IV to assess the impacts of various turf management scenarios on surface water quality.

Utility of Computer Models for Runoff Assessments

It is impractical to conduct runoff experiments exclusively on a site-by-site basis due to cost and logistical constraints. The only realistic approach is to use carefully calibrated and validated computer models to simulate the movement of nutrients and pesticides under different use scenarios. Model calibration first entails matching model output with the hydrology of the actual runoff event (i.e., timing and extent of water runoff). Next, the rate of transportation of a particular chemical is modeled by entering sorption coefficients, dissipation half-lives and other pertinent parameters so that the computer's results match actual runoff losses observed in the field. Once a computer model has been calibrated for a particular chemical under a given set of environmental conditions, the effect of different environmental conditions (e.g., normal vs. above-normal rainfall) on the runoff of that chemical can be determined. Alternatively, once the hydrology of a particular environment is matched, the model can be used to estimate the transport rate of different chemicals so as to rank their estimated mobility and subsequent concentrations in non-target aquatic systems. For these reasons, runoff models calibrated for various warm-season turf scenarios relevant to the South Atlantic-Gulf region would be valuable tools for regulators and environmental scientists in terms of assessing the fates of turf agrochemicals in Southeastern watersheds.

While there are a number of models used to predict NPS runoff of agrochemicals, the USEPA currently estimates pesticide runoff using a combination of models. The Pesticide Root Zone Model (PRZM) is used to estimate transportation rates and edge of field concentrations. The results of this model serve as inputs to EXAMS (Exposure Analysis Modeling System) that is used to determine the fates and concentrations of agrochemicals in aquatic systems. PRZM estimates daily runoff using the Soil Conservation Service (SCS) curve number technique. Runoff is calculated using a curve number and a continuous functional relation based on soil-water content in the root zone. Currently, regulators only have real world calibration scenarios for agricultural settings. Turf runoff is estimated using agricultural parameters due to deficiencies in turf runoff experiments.

Pesticide and nutrient runoff from agricultural fields can also be estimated using the Root Zone Water Quality Model (RZWQM). Like PRZM-EXAMS, RZWQM relies largely on agricultural settings to estimate runoff from fescue pastures (Ma et al., 1998). However, it is widely recognized that the use of agronomic parameters to estimate turf runoff results in inaccurate runoff estimations and, therefore, inaccurate assessments of potential impacts on water quality and non-target aquatic organisms. To fully determine the impact of turf agrochemical runoff on surface water quality, *real world* turf-specific modeling scenarios must be developed. To do this, critical data gaps must be filled by further experimentation.

[2] Like residential lawns, runoff losses from commercial sod operations are essentially unknown. Because of the need for deep, fertile soils, commercial sod farms in Mississippi are often located on alluvial planes near streams and rivers. Currently, there is no information to assess the impacts of sod farms on surface water quality.

Data Requirements for Improved Turf Runoff Models

The United States Golf Association (USGA) has an interest in ensuring the accurate modeling of pesticide runoff from golf courses. Consequentially, the USGA has recently provided funding to support an effort to establish computer model scenarios for turf runoff that are relevant to the Southeast and Mid-Atlantic regions of the U.S. (*please see Appendix II for USGA letter*). The focus of the USGA project is the improved modeling of pesticide runoff from golf course turf. This issue is also of high interest to manufacturers of turf products (*please see Appendix II for supporting letter*). Runoff model refinements for the USGA study will address data gaps recently identified by the USEPA (Nett, 2002) which include (a) determining the scalability of pesticide runoff processes, and (b) determining the effect of warm-season grass species on pesticide runoff. Dr. Don Wauchope of the USDA-ARS is involved with our project as both a recognized expert in the measurement of field runoff and runoff modeling (*please see Appendix II for supporting letter*). An overview of the USGA-funded project is given below:

Overview of USGA-Funded Turf Pesticide Runoff Modeling Project

This collaborative project represents the first phase of a planned national "turf umbrella" project whose ultimate purpose is to improve understanding of regional differences in agrochemical runoff from turf. This project lays the necessary groundwork for collaboration with researchers at the University of Maryland and specifically seeks to bridge critical information gaps that currently prevent previous runoff research from being fully considered in pesticide risk assessments. The objectives of this project are (1) to develop a standardized field protocol for use in turf runoff experiments based upon input from key stakeholders, (2) to determine plot size effects on runoff (scalability) and (3) to determine warm-season grass species effects on the timing and extent of agrochemical runoff. These deficiencies, recently identified in a meeting held between the USEPA and turf industry representatives, ultimately prevent the accurate estimation of agrochemical runoff from turf since agronomic parameters/conditions are used in the absence of turf-specific information. This project will investigate the runoff of three pesticides having a range of physicochemical properties from replicated plots ranging from 0.01 to 0.1 acre in size. The plots will be planted with either hybrid Bermuda or Zoysia, with the Bermuda plots being over-seeded with ryegrass after dormancy to mimic a common management technique practiced in the Southeast that often increases fungicide applications for disease control. Key factors affecting runoff (e.g., antecedent moisture, soil properties, thatch content, hydraulic conductivity, soil bulk density) will be determined prior to initiating the simulated rainfall experiments.

An important aspect missing from the USGA study that is of significance to water quality issues in the Southern Gulf-Atlantic region is the modeling of nutrient runoff from golf courses and residential turf. Nutrients (nitrogen, phosphorus) are key contributors to surface water impairment in the Southern Gulf-Atlantic region. Moreover, lawns represent a greater portion of turf acreage than do golf courses and, therefore, may ultimately represent a greater source of contamination than golf courses. Unfortunately, few data exist to determine which of these turf management practices poses a more significant threat to surface water quality. Side-by-side comparisons between residential turf and turf maintained to golf course standards would help to establish the impact of turf runoff on water quality. *The funding and resulting infrastructure provided by the USGA represent a tremendous opportunity to develop an extensive regional database for nutrient and pesticide runoff from turf that goes beyond golf-course settings.* The objectives of the current project are given below:

Research Objectives

1. Determine rates of transport for nutrients (nitrogen, phosphorus) and pesticides applied to turf maintained according to USGA superintendent practices for fairways and turf maintained according to MSU Extension recommendations for home lawns. Statistically compare nutrient and pesticide runoff from the various turf management regimes.

2. Using results from Objective 1 to refine pesticide (PRZM) and nutrient (RZWQM) runoff model estimates for warm-season turf management regimes.

3. Compile turf-relevant hydrological parameters and contaminant transport rates for each turf maintenance regime/grass species into database for use by regulatory agencies and environmental engineers/scientists.

(12) Methods, Procedures and Facilities

Objective 1: Determine rates of transport for nutrients (nitrogen, phosphorus) and pesticides applied to turf maintained according to USGA superintendent practices for fairways and turf maintained according to MSU Extension recommendations for home lawns. Statistically compare nutrient and pesticide runoff from the various turf management regimes.

Three pesticides having a range of chemical properties (i.e., K_{OC} and water solubility) will be selected and applied simultaneously to allow modeling of a range of pesticide behaviors. Chemical fertilizers will be applied according to standard USGA practices for fairways and MSU recommendations for home lawns, as appropriate. The runoff parameters known to affect runoff that will be collected are given in **Table 1** below:

Soil & Thatch Factors	***Climatic Factors***
Saturated. hydraulic conductivity (k_{sat})	Precipitation at 5-min intervals
Soil texture	Air/soil temp at 5-min intervals
OC content of soil and thatch	Solar radiation
Soil Bulk density	Wind speed at 2-m
WHC at 0, 0.3 and 15 bar	***Pesticide Factors***
Antecedent soil moisture	Soil and thatch sorption coefficients (K_{oc})
Turf density (shoots/dm^2)	Foliar and soil half-life values

Table 1. Field and Chemical Parameters Collected for Runoff Model Refinement.

<u>Runoff Plot Establishment and Design</u>

The turf runoff plots will be established during the spring of 2003 with initial runoff collection beginning in the summer of 2003. The field plot design, as shown in **Figure 1**, consists of three replicated plots for each turf management regime outlined in **Table 2**. Turf maintenance parameters of relevance include mowing height and frequency, irrigation amount and frequency, fertilization and pest control, aeration and thatch management. The turf will be established and managed according to practices recommended by the USGA for warm-season fairways or MSU Extension guidelines, as appropriate. Three untreated control plots, consisting of existing native grass and broadleaf species are incorporated into the design to determine background losses of nutrients. Metal boarders or equivalent will be installed around the plot to delineate the treated areas and to prevent adjacent runoff from entering the plots. Runoff from the plots will be measured using15-cm H-type flumes and ISCO flow meters and auto-samplers.

Plot dimensions will vary depending on the purpose of the study: *Scalability* relationships between treated area and runoff will be determined using three plots ranging in size from 12 x 30-ft to 40 x 125-ft (**Table 2**). All of the scalability plots will be planted in Tifway (419) hybrid Bermuda with three plots of each dimension being managed as either fairway- or residential-type turf. The effect of grass species on runoff will be determined using 12 x 30-ft plots only. Differences in management regimes can be made between plots of similar size.

<u>Contaminant Runoff During Simulated Rainfall Events</u>

Runoff samples will be collected at specific intervals (e.g., 24, 168 and 336 hours after application) using rainfall simulators (Senninger Wobbler™ irrigation nozzles). Simulated rainfall intensity will be ca. 1-inch per hour. Runoff events resulting from natural rainfall will not be sampled but three runoff plots will be instrumented and their runoff measured continuously so that hydrographs can be constructed and used to estimate contaminant losses due to natural rainfall events. This latter arrangement is necessary due to the high cost that would be associated with the sampling and analysis of plots of this size and number.

<u>Analytical Methods</u>

Pesticides: Direct-injection, liquid-liquid partition or solid-phase extraction coupled with High Performance Liquid Chromatography (HPLC) using UV-Vis detection and/or Gas Liquid Chromatography using electron capture detection (ECD) or Mass Selective Detection (MSD) techniques will be used, as appropriate.[3]

Dissolved-phase nutrients (nitrate-nitrogen; ammonium; phosphate) will be determined using ion chromatography using EPA Method 300.1.

Total Kjeldahl nitrogen (TKN) and total phosphate will be determined first by filtering the water through a 1-µm glass fiber filter followed by digestion and detection by auto-analyzer or inductively coupled plasma (ICP) spectroscopy, as appropriate, using methods outlined by Gaudreau et al. (2002).

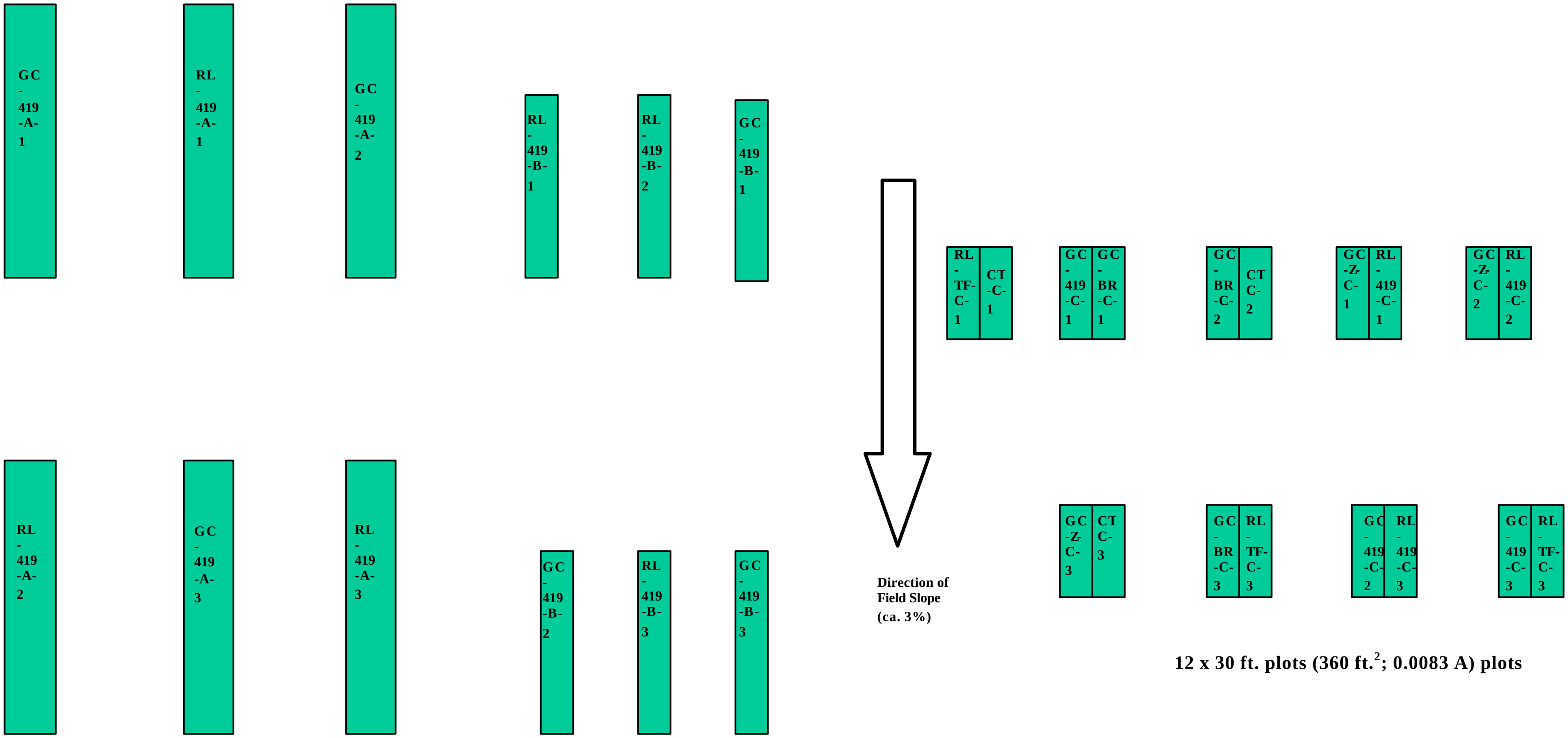
<u>Anticipated Problem No. 1</u>: Reliance upon natural rainfall to generate runoff result in sporadic and erratic results due to lack of control over the timing and intensity of rainfall.

<u>How Problem is Being Addressed</u>: We are using simulated rainfall to generate runoff at prescribed intervals after application and at known intensity.

[3] The actual method(s) used will ultimately depend upon the pesticides that are included in the study during protocol development. Protocol development is underway as part of the larger turf umbrella project and will be completed by early spring of 2003.

Maintenance Regime	*Grass Species*	*Establishment Method*	*Plot Size(s)*	*Mowing Height & Frequency*	*Watering Schedule*	*Fertilization Schedule*
Golf Course Fairway Trt. Codes:* GC-419-A 1-3 GC-419-B 1-3 GC-419-C 1-3	Tifway 419 Bermuda	Sprigging	(A) 40 x 125 ft (B) 20 x 80 ft (C) 12 x 30 ft	0.6 inches 3 to 5 times/wk. By reel mower	1-in./wk.	Early March: 10 lbs of 15-5-10 with 0.92% Oxidiazon (Ronstar)/1000 ft^2 (1.5 lbs of N, 0.5 lbs P, 1.0 lbs of K) Summer: 1.5 lbs N from NH_4NO_3/1000 ft^2 applied May 1, June 1, August 1, Sept 1. Early Fall: 10 lbs/1000 ft^2 of 5-5-20 P and K added per soil tests.
Golf Course Fairway Trt. Code: GC-BR-A 1-3	Tifway 419 Bermuda Over-Seeded With Perennial Rye	Sprigging of Bermuda. Over-Seeding with rye on Oct.1.	(A) 12 x 30 ft	0.6 inches 3 to 5 times/wk. By reel mower	1-in./wk.	Same as above.
Golf Course Fairway Trt. Code: GC-Z-C 1-3	Meyer zoysia	Sod	(C) 12 x 30 ft.	0.6 inches 3 to 5 times/wk. By reel mower	1-in./wk.	Same as above.
Residential Lawn Trt. Codes: RL-419-A 1-3 RL-419-B 1-3 RL-419-C 1-3	Tifway 419 Bermuda	Sod	(A) 40 x 125 ft (B) 20 x 80 ft (C) 12 x 30 ft	2 inches 1 to 2 time/wk By rotary mower.	As needed to maintain vigor	April 15 1.5 lbs/1000 ft^2 13-13-13 (1.5 lbs of N, 1.5 lbs P, 1.5 lbs of K) Summer 1.5 lbs N NH_4NO_3 /1000 ft^2 Applied June 15, August 15 Fall: 10 lbs of 5-5-20 applied Oct 1^{st}. P and K added per soil tests
Residential Lawn Trt. Code: RL-TF-C 1-3	Rebel 3 Tall-Fescue (heat/drought tolerant)	Seeding at 8-10 lbs/1000ft^2	(C) 12 x 30 ft	3-inches 1 time/wk by rotary mower	As needed to maintain vigor	Early Fall: 2 lbs/1000 ft^2 13-13-13 (2 lbs of N, 2 lbs P, 2 lbs of K)
Control Trt. Code: CT-C 1-3	Existing grass and broadleaf species	Not Applicable	(C) 12 x 30 ft	2 inches 1 time/wk By rotary mower.	Same as above	Native fertility, no additional nutrients added. Plots used for background nutrient controls.

Table 2. Proposed Establishment and Maintenance Regimes Used to Determine Scalability, Grass-Species and Management Effects on Nutrient and Pesticide Runoff from Warm-Season Golf Fairways and Residential Turf.



40 x 125 ft. (5,000 ft.^{2}; 0.1 A) plots

20 x 80 ft. plots (1600 ft.^{2}; 0.037 A) plots

Legend:

GC = Golf Course fairway
RL = Residential Lawn

419 = Tifway (419) Hybrid Bermudagrass
BR = Bermuda over-seeded with Perennial Ryegrass
Z = Myer Zoysia
TF = Rebel 3 Tall Fescue

Example:
RL-419-A-1 = Tifway (419) Hybrid Bermuda in plot size A (40 x 125 ft) managed to simulate a Residential Lawn, Replication 1.

Figure 1. Proposed Runoff Plot Layout for Scalability-, Grass Species- and Turf Management-Comparisons.

Anticipated Problem No. 2: Background levels of nutrient runoff may interfere with accurate determination of transport rates of applied fertilizers.

How Problem is Being Addressed: Our field plot design includes three non-treated control plots that will allow the determination of background nutrients with each simulated runoff event.

Objective 2: Using results from Objective 1 to refine pesticide (PRZM) and nutrient (RZWQM) runoff model estimates for warm-season turf management regimes.

The first step in model calibration is to adjust model output to match the actual movement of water across the plots at the different rainfall simulation events. This hydrological calibration involves determining the timing of first runoff event and total runoff volume for the various warm-season turf scenarios under investigation. Once water movement across these plots has been accurately portrayed, chemical concentrations for pesticides and nutrients will be addressed. Dr. Don Wauchope of the USDA-ARS will perform the pesticide calibrations. Dr. Alton Johnson of Alcorn State University will perform the nutrient modeling and assist in characterizing the spatial variability of the hydrological properties of the soil at the field site.

Anticipated Problem No. 3: Soil properties and fertility are heterogeneous by nature. This is especially true for hydrological properties affecting the movement of water. This variability can significantly affect the precision and accuracy by which runoff estimations can be made.

How Problem is Being Addressed: Our study design (**Figure 1**) includes three randomly placed replications per turf management regime that will assist in assessing the variability in runoff processes.

Objective 3: Compile turf-relevant hydrological parameters and contaminant transport rates for each turf maintenance regime/grass species into database for use by regulatory agencies and environmental engineers/scientists.

The runoff parameters of interest include transport rates, turf-relevant runoff curve numbers, pesticide-turf extraction coefficients, site-specific fate parameters such as soil and thatch sorption coefficients, soil and thatch degradation rates, and management histories for the various turf scenarios investigated in this study. This information will be compiled in an electronic database (e.g., EXCEL), as batch-files for PRZM-EXAMS, and in peer-reviewed publications and reports. This information will be transferred to the target audience in the manner described in the ***Information Transfer Plan***.

Research Facilities

Field investigations will be conducted on turf plots to be established during the spring of 2003 at the Mississippi State University Black Belt Branch Experiment Station near Brooksville, MS. This site has been used to determine pesticide and sediment runoff from various cotton (Webster and Shaw, 1996; Blanche, 2001) and soybean (Baughman et al, 2001) production systems. The underlying soil is a Brooksville silty clay (fine montmorillonitic, thermic Aquic Chromudert; 3.2% OM, 6.3 pH) with a hydraulic conductivity = 5 mm/h. The runoff plots are equipped with 15-cm H-type flumes. Simulated rainfall (2.5 cm/h) is applied using wobbler irrigation heads (Senninger Irrigation Inc.) mounted to 3-m risers that are spaced 3-m apart. Runoff samples are collected into glass vessels at predetermined runoff volumes using an Isco Model 3700 auto-sampler controlled by an Isco Model 4230 flow meter. A Campbell Scientific weather station measuring rainfall, air temperature, relative humidity, solar irradiance and wind speed is in operation at the Brooksville runoff site.

(13) Related Research

Relation to Completed and On-Going WRRI-Funded Research

Previous WRRI-funded research has supported the measurement of agrochemical runoff from cotton (Baughman et al., 2002) and soybean (Webster and Shaw, 1996) production systems. Through this research, best management practices (BMPs) that meet the specific needs and growing conditions of Mississippi agriculture, such as vegetative buffer strips (Murphy and Shaw, 1997; Blanche, 2001), have been developed. This information was necessary to assess the role of NPS agricultural runoff in contaminating Mississippi's surface waters and to reduce these impacts through BMP development. Similar information is now needed to address non-agricultural runoff of nutrients and pesticides from golf courses and residential lawns.

Literature searches conducted using the *Water Resources Science Information Exchange* (WRSIC) system did not identify any WRRI-funded projects of the nature and scope described in this proposal. To the best of our knowledge, this is the only project of its kind where side-by-side comparisons of nutrient and pesticide runoff from differently managed warm-season grasses are being conducted for the purpose of improved turf runoff modeling and the development of a regional database for use by regulators and environmental scientists in the Southern Atlantic-Gulf region.

Relation to Turf Chemical Runoff Research

The use of small plots for runoff studies is currently favored by researchers. Small plots allow multiple treatments to be examined without utilizing large blocks of land, which makes it relatively easy to develop plots with uniform field conditions (slope, soil type). The use of small plots also avoids the need to apply large volumes of water to generate runoff, and eliminates complications associated with sampling large volumes of runoff. Unfortunately, the use of small plot data to assess the performance of field-scale models to predict turf chemical runoff has produced inconsistent results. Much of the inconsistency appears to be related to the inability of the curve-number method to successfully provide estimates of small plot runoff. Reasonable predictions of the pesticide concentration in turf runoff have required extensive adjustment of the curve number (CN) in small plot modeling efforts (Wauchope, et al., 1990; Durborow et al., 2000).

To accurately simulate runoff from small, dense turf plots in Georgia, Durborow et al. (2000) found it necessary to use a CN recommended for a poor stand of grass grown in much finer textured soil than was actually present at the site. The difference in the CN number specified for the site by the NRCS (Technical Release 55, Urban Hydrology for Small Watershed, CN = 61) and the CN number that was actually used in the calibration of the model (CN= 91) was substantial. To put this into perspective, Haith (2001) reported that changing the CN at a Kentucky site from 58 to 62 increased the predicted runoff at the site by 131% (i.e., from 2.9 to 6.7 mm). In another study conducted at the Georgia site just mentioned Ma et al. (1999) compared actual and predicted runoff using the curve-number method option of the OPUS Model. They found the hydrological component of this model could not adequately predict individual plot runoff. They did however report that there was relatively good agreement between predicted runoff and the mean amount of runoff from all 12 plots at the site. Ma et al. (1999) noted that seemingly uniform small plots can have substantially different hydraulic properties, which the curve number method does not consider. They also stated that one of the reasons why the curve number method works well at the field scale of level of resolution is because spatial differences in hydraulic properties tend to cancel out one another at that level of resolution. Haith and Andre (2000) have proposed a set of

CN for various turf situations. Using runoff data from six different turf sites they were able to demonstrate that use of their CN values explained 78% of the observation variation in runoff. No attempt was made, however, to examine the relationship between the amounts of runoff predicted by their CN approach and size of the plot size being evaluated. This information is needed to examine the scalability of their turf CN approach; "scalability" issues have been found by the USEPA and key turf stakeholders to limit the utility of turf runoff models (Nett, 2002). *Our proposed study is designed to determine the scalability of runoff results from turf managed as either golf course fairways or home lawns.*

Investigations by Pennsylvania State University researchers have shown that grass species can impact the timing and extent of runoff from turf (Linde et al., 1995). Linde et al. (1995) found that runoff from mature perennial ryegrass (*Lolium perenne* L.) plots occurred sooner and in greater volumes than from creeping bentgrass (*Agrostis stolonifera* L. (Huds.)). This was attributed to the stoloniferous nature of bentgrass as compared to ryegrass that has a bunch-type growth habit. The dense mat of stolons is thought to increase hydraulic resistance and water-holding capacity, thereby allowing greater water infiltration in bentgrass, slowing runoff. These differences could be used to refine environmental risk assessments as bentgrass increasingly appears to be the turfgrass of choice for golf courses in the mid-Atlantic region. While differences in root distribution in warm-season turf type have been shown to affect nitrate leaching (Bowman et al., 2002), species effects on pesticide runoff have not been adequately investigated. Determination of differences in agrochemical runoff for different grass species was recently found to limit the utility of turf runoff models by the USEPA and key turf stakeholders (Nett, 2002). *Our study is designed to determine warm-season grass species effects on turf chemical runoff.*

(15) Training Potential

This project will support the education and training of one Ph.D.-level student and two to four undergraduate students. For a Ph.D. student interested in the environmental sciences, this project will allow them to gain valuable hands-on experience in the field and laboratory. The graduate student will play an important role in this project and will interact with regulatory authorities, modelers, environmental chemists, industry representatives and water quality consultants, among others. The student will be engaged in a project that addresses an area that is anticipated to gain considerable importance over time, namely, addressing environmental issues related to turf maintenance and production. This project will involve a blend of field, laboratory and modeling techniques that should prepare the student for successful employment in the environmental sciences arena.

Information Transfer Plan

The problem to be addressed in this work is the improved estimation of nutrient and pesticide runoff from managed turfgrass. Upon successful completion of this project, turf runoff scenarios for golf course fairways and residential lawns grown using warm-season grasses relevant to the South Atlantic-Gulf region will be made available to the target audience of this work (i.e., regulatory and environmental engineers/scientists charged with assessing health risks and environmental impacts associated with non-point source runoff of nutrients and pesticides in surface waters).

The turf runoff model scenarios and other results of this project will be disseminated through referred journals (e.g., the *Journal of Environmental Quality*, *Pesticide Management Science Journal*, *Weed Science*), WRRI annual reports, MAFES experiment station bulletins, oral/poster presentations at regional (Southern Weed Science Society) and national (American Chemical Society; American Agronomy Association, Weed Science Society of America) meetings, water quality conferences, and at quarterly meetings held by the environmental modeling working group (EMWG) hosted by the USEPA.

Once the turf runoff scenarios have been improved, our plans are to host a training workshop on improved turf runoff modeling for interested individuals and regulatory authorities. The training session will be held in conjunction with the EPA's quarterly EMWG meeting held in Washington, DC. Similar training events have occurred in association with these meetings and would provide an excellent avenue for dispersing results of this project since many of the nation's top environmental modelers regularly attend the EMWG meeting. Depending on the response to the workshop, additional workshops might be held as part of the EMWG group or as part of symposium on turf environmental issues hosted by the Agrochemicals Division of the American Chemical Society in 2004/2005.

APPENDIX 1

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Literature Cited

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Chemical Mixtures: Consequences of WNV Eradication on Water Quality

Basic Information

Title:	Chemical Mixtures: Consequences of WNV Eradication on Water Quality
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End Date:	2/28/2005
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Congressional District:	First
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Focus Category:	Sediments, Toxic Substances, Water Quality
Descriptors:	ecosystems, mixtures, pesticides, residues, sediments, toxic substances
Principal Investigators:	Marc Slattery

Publication

RESEARCH PROPOSAL

(1) TITLE: Chemical Mixtures: Consequences of WNV Eradication on Water Quality

(2) Focus Categories: SED, TS, WQL

(3) Keywords: Ecosystems, Mixtures, Pesticides, Residues, Sediments, Toxic Substances, Water Quality

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(5) Federal Funds:	16,540 (Total)	(16,540) Direct		
(6) Non-Federal Funds:	33,081 (Total)	(19,226) Direct	(13,855) Indirect	

(7) Principal Investigator, University and City: Marc Slattery, University of Mississippi, University, MS

(8) Congressional District No: District No.1

(9) Water Problem, Need for Research:

Recent outbreaks of West Nile Virus (WNV) throughout the United States, and particularly in the Mississippi Valley States, have spurred plans to control the vector (= *Culex* mosquito). A probable phase in each plan requires using chemical agents that affect either adult or larval vector life stages. Chemical agents commonly used to control mosquito vectors are non-species specific pesticides that will potentially interact with non-target aquatic organisms. These compounds enter the aquatic environment via direct or indirect routes eventually becoming part of water and sediment matrices. Most of the WNV vector control compounds are hydrophobic. Upon entering the aquatic environment they readily partition from surface waters onto particulate organic matter in the water column or directly onto the sediment. Within aquatic matrices through direct contact, respiration or indirect ingestion non-target organisms are exposed to vector control compounds individually or as mixtures with persistent or transient anthropogenic compounds such as regional crop pesticides and metals. Individually or as mixtures, acting additively or synergistically, these compounds can potentially affect adult and juvenile life stages of non-target organisms. At the present time, there is limited knowledge regarding effects of WNV vector control compounds in mixtures. Evaluating water quality and aquatic habitat are critical to an overall assessment of vector eradication programs.

This proposal directly addresses Mississippi Water Research and South Atlantic-Gulf Region priorities related to water quality, particularly with respect to needs addressing protection of water and sediment from environmental degradation.

(10) Expected Results, Benefits, Information:

Water and sediment quality in aquatic environments are essential indicators of overall success of WNV vector control programs. Aquatic matrices are complex mixtures of natural and anthropogenic compounds. Our proposed research encompasses both individual compound and mixture exposure studies in both water and sediment matrices. We will be able to assess toxicological effects not predicted by individual compound toxicity studies. Our pre-stress exposure experiments will allow us to evaluate model organisms' responses to vector control compounds after pre-exposure to commonly occurring persistent anthropogenic compounds. We will compare critical body residue values determined from controlled laboratory studies to tissue residues from exposed organisms collected from areas during vector control application. By comparing residue levels we can more accurately evaluate risk to aquatic organisms during vector control application periods. During periods of environmental application of vector control compounds we will evaluate water and sediment samples for mixture concentrations of vector control and commonly occurring anthropogenic compounds. By mimicking environmental mixture concentrations in controlled exposure studies we can assess "real-world" chemical mixture toxicological effects in model organisms commonly found in water column and sediment habitats.

In summary, the proposed research utilizes a novel approach to address the issue of chemical mixture toxicity. The model chemicals were selected to assess the influence of WNV vector eradication compound effects in conjunction with two persistent and interacting compounds in the environment that have the potential for occurrence as mixtures. Results of the proposed investigation will contribute to our currently limited understanding of chemical-chemical interactions. Accordingly, this project is directly applicable to Mississippi and the South Atlantic-Gulf because of the importance of accurately assessing ecological risk.

(11) Nature, scope, and objectives of the research:

The rapid spread of WNV throughout the United States in 2002 resulted in 3231 laboratory-verified infections and 176 deaths (as of October 21st 2002); cases in Mississippi rank within the top 5 nationwide with 178 infections and 9 deaths. Public outcry resulted in hasty plans for eradication of the *Culex* spp. mosquito vectors via insecticide spraying; *these plans often were developed locally and without much consideration to environmental and/or economic consequences.* This proposal directly addresses Mississippi Water Research and South Atlantic-Gulf Region priorities related to water quality, particularly with respect to needs addressing protection of water and sediment from environmental degradation. The following is our three–year approach for assessing impacts of WNV vector control compounds on the aquatic environment.

Phase I - Single Chemical Exposures/Insecticide, Analytical Method Development. *H. azteca*, and *D. magna*, will be exposed to single chemicals to determine concentration threshold values at which adverse toxicological effects occur. In particular, we will focus on those compounds for which this information is not reported in the literature (see Table 1 and 2). Long-term exposures will be conducted to evaluate the effects of individual chemicals on survival, growth and reproduction. Estimates of no observed effect concentrations (NOECs) and EC_{50}'s for individual compounds will be calculated. Whole body residue concentrations and toxicological effect levels will be used to calculate bioconcentration factors and critical body residues for each compound in both *H. azteca* and *D. Magna*. Targeted WNV vector eradication compounds will be spiked into water and sediment for liquid:liquid or liquid:solid extractions/recovery experiments. The extracts will be separated and quantified using LC-MS analysis, and the methods refined for future use in field matrices.

Phase II - Multiple Chemical Exposures, Pre-exposure Stress Responses. Mixture toxicity experiments evaluating binary and three ways chemical-chemical interactions of select vector control compounds (see Table 2) with two anthropogenic compounds, chlorpyrifos and methylmercury (see Table 1), will be conducted during the second year of our investigation. Each vector control/anthropogenic compound mixture study will consist of three binary and one three ways combination at selected concentrations and ratios. Additionally each mixture study will include single chemical concentrations and a control group. Fifteen replicates of each exposure level will be necessary to adequately meet the requirements of the statistical model. Juvenile *H. azteca* and adult *D. magna* will be exposed ten days and seven days, respectively, with survival, growth and reproduction as toxicological endpoints. We will also conduct these experiments in the manner of pre-exposure to a binary combination of chlorpyrifos and methylmercury, followed by addition of a vector control compound to assess the effects of pre-exposure stress on our model organisms' survival, growth, and reproduction.

Phase III - Assessment of Bioaccumulation/Field concentrations. During the third year of investigation, concentrations of the WNV vector eradication compounds, chlorpyrifos and methylmercury in water and sediment from natural waterways throughout Mississippi will be assessed using our LC-MS methodology. Also, whole body residues of the compounds mentioned above will be assessed in field collected *H. azteca* and *D. magna* and respective bioconcentration factors calculated. Additional ten-day and seven-day experiments will be conducted using spiked formulated sediment or water at environmentally relevant concentrations. Bioconcentration of the chemical mixtures will be determined from body residue analysis and chemical concentrations in the water and sediment. Environmentally relevant critical body residues will be derived through correlation of toxicity data (if any) and bioconcentration data.

(12)<u>Methods, procedures, and facilities</u>:

General Methods

Model Compounds. Two model compounds (chlorpyrifos and methylmercury, Table 1) representing environmentally relevant chemical contaminants will be used to assess chemical mixture interactions with the WNV vector eradication compounds (Tables 2). These chemicals were selected due to their persistence, mode of action, and occurrence at concentrations capable of producing adverse toxicological effects. In addition, during a previous study of chemical mixtures (Benson, Block, Steevens, Allgood & Slattery, 2000, Figure 2) we noted that these two compounds provided the most important additive effects (see below: Progress on Work to Date).

<u>Chlorpyrifos</u>. Chlorpyrifos, a model organophosphate, is widely used in the United States with more than 14.4 million pounds applied to cropland each year (USGS, 1997). Chlorpyrifos can enter the environment by volatilization and run-off after application. Following a rainfall event, streams near agricultural fields in northern Mississippi have been shown to receive concentrations of greater than 2.0 ppb chlorpyrifos in runoff 160 days after pesticide application (Smith *et al.*, 1994). Due to the low solubility (1.4 mg/L) and hydrophobic nature (Log Kow 3.31-5.27), chlorpyrifos rapidly partitions from the water and adsorbs to sediment particles (Montgomery, 1993). In the sediment, chlorpyrifos has a long half-life (60-100 days)

making exposure to aquatic benthic organisms possible (Tomlin, 1994). Chlorpyrifos exerts its toxicity by inhibiting acetylcholinesterase, an important enzyme that modulates the concentration of the neurotransmitter acetylcholine.

Methylmercury. Approximately 4,500 metric tons of mercury is released into the environment each year by human activities such as combustion of fossil fuels and other industrial releases (Lindquist *et al.*, 1991). Anthropogenic sources account for nearly 30-60% of the total annual influx of mercury to the atmosphere (Benoit *et al.*, 1994). Global mercury loading trends indicate that atmospheric concentrations are increasing annually by greater than 1 percent (Slemr and Langer, 1992). In Mississippi, concern over mercury in the environment has increased as a result of increased mercury concentrations in fish tissue samples from the Sunflower and Yazoo River Basins and Enid Lake drainage (Bass, personal communication). Methylmercury is persistent in sediments and has been shown to bioaccumulate and biomagnify in fish and invertebrates as reviewed by Suedel *et al.* (1994). Methyl mercury can accumulate by way of an L amino acid transporter and exerts its toxicity by depleting cellular stores of the antioxidant glutathione or by inducing oxidative stress (Lund *et al.*, 1991; Sorenson, 1991; Mokrzan *et al.*, 1995).

Table 1. Physical-Chemical Properties of Model Compounds

Compound	Formula and Molecular Wt.	Solubility Water (@ 25^o C)	log K_{ow}	log K_{oc}	Mode of Action	Stability in Water Soil
Model Cmpds						
Chlorpyrifos [a]	$C_9H_{11}Cl_3NO_3PS$ 350.6	1.4 mg/L	4.70	3.78	Non-systemic Cholinesterase Inhibitor	Low-Mod Persistence Moderate Persistence
Methylmercury [b]	CH_3Hg 215.6				Many physiological systems effected	High Persistence

Sources: EXTOXNET, 1996. Kow = octanol/water partitioning coefficient. Koc = organic carbon partitioning coefficient.

Methoprene. As part of WNV control plans in 2000, officials in New York state planned to use 135,000 pounds of methoprene containing briquets, while Westchester County planned to use as much as 80,000 pounds (Dee, 2000). When applied to larval stages methoprene mimics insect growth regulation hormone preventing metamorphosis to adults (ETOXNET, 1995). Methods of application include ground and aerial spraying, granular, mineral block/pellet and slow release briquette. Due to its low water solubility, 1.4 mg/L, and lipophilic nature, log K_{ow}, 5.21 (Table 2), methoprene readily partitions from surface water into sediments and particulate organic matter in the water column. Methoprene is acutely toxic to some freshwater and estuarine invertebrates and can bioaccumulate in fish and crustaceans (bioconcentration factor: bluegill sunfish is 457 and in crayfish 75, which is 66 times ambient water concentrations. U.S. EPA, 1982). In fresh and salt water methoprene has a half-live of between 10 and 35 days. In soil the reported half-life is 10 days (ETOXNET, 1995).

Table 2. Physical-Chemical Properties of Mosquitocides Targeted for WNV Vector Eradication

Compound	Formula and Molecular Wt.	Solubility Water (@ 25^o C)	log K_{ow}	log K_{oc}	Mode of Action	Stability in Water Soil
Larvicides						
Temephos	$C_{16}H_{20}O_6P_2S_3$ 466.5	0.03 mg/L	4.91	5.0 (est.)	Cholinesterase Inhibitor	Low Persistence Low-Mod Persistence
Methoprene	$C_{19}H_{34}O_3$ 310.5	1.4 mg/L	5.21		Mimics Insect Growth Regulator	Degrades Rapidly Low Persistence
Diflubenzuron	$C_{14}H_9ClF_2N_2O_2$ 310.7	0.08 mg/L (pH 5.5, 20^o C)	3.89 (log P)	4.00	Chitin Synthesis Inhibitor	Low-Mod Persistence Low Persistence
Adulticide						
Malathion	$C_{10}H_{19}O_6PS_2$ 330.3	145 mg/L	2.75	3.26	Non-systemic Cholinesterase Inhibitor	Low-Mod Persistence Low Persistence
Naled	$C_4H_7Br_2Cl_2O_4P$ 380.8	practically insoluble		2.26	Non-systemic Cholinesterase Inhibitor	Rapidly hydrolyzed Rapidly Degrades
Permethrin	$C_{21}H_{20}Cl_2O_3$ 391.3	0.2 mg/L (20^o C)	6.10 (log P)	5.00	Non-systemic Insecticide	Low Persistence Low-Mod Persistence
Resmethrin	$C_{22}H_{26}O_3$ 338.4	37.9 ug/L	5.43	5.00	Non-systemic Insecticide	Low-Mod Persistence Low-Mod Persistence

Sources: Crop Protection Publications, 1994, and EXTOXNET (http://ace.ace.orst.edu/info/extoxnet/, 10/23/02). K_{ow} = octanol/water partitioning coefficient. K_{oc} = organic carbon partitioning coefficient.

Model Organisms. *Hyalella azteca* (Class Crustacea, Order Amphipoda) is a benthic amphipod found in fresh and estuarine waters of North and South America. *H. azteca* is exposed to environmental xenobiotics because it primarily feeds and lives in the upper layers of sediment where the concentration of contaminants is often the greatest. Physiologically, amphipods are similar to crustaceans such as crabs, crawfish, and shrimp (Gardiner, 1972; Pennak, 1989). *H. azteca* is a sentinel testing species for benthic aquatic invertebrates, which are a major food source for commercially important fishes. *H. azteca* has been used to assess bioaccumulation of metals and toxicity of sediments (Borgmann et al., 1991; Ingersoll *et al.*, 1994; Canfield *et al.*, 1994). Furthermore, *H. azteca* has been endorsed as an aquatic invertebrate testing organism for evaluating toxicity as demonstrated by the U.S. EPA standard guidelines for sediment testing (U.S. EPA, 1994b). *H. azteca* obtained from the U.S. Geological Service, National Biological Service are presently cultured in a flow-through aquarium system in the aquatic toxicology research facility of the School of Pharmacy at the University of Mississippi.

Daphnia magna (Class Crustacea, Order Anomopoda) is a cladoceran found in the water column of fresh waters of North America. *D. magna* is exposed to environmental xenobiotics within the water column and from bioaccumulated toxins within its phytoplankton prey; it is an important trophic link between primary producers and macro-predators. Physiologically, cladocerans are similar to crustaceans such as crabs, crawfish, and shrimp (Gardiner, 1972; Pennak, 1989). *D. magna* is a sentinel testing species for aquatic invertebrates, which are a major food source for commercially important fishes. Furthermore, *D. magna* has been endorsed as an aquatic invertebrate testing organism for evaluating toxicity as demonstrated by the American Society for Testing and Materials standard guidelines for acute testing (ASTM 1980: E729-80). *D.*

magna is commercially-available, and have been cultured in a flow-through aquarium system in the aquatic toxicology research facility of the School of Pharmacy at the University of Mississippi.

Toxicological Evaluation. Individual compound and binary and three ways mixtures will be exposed to H. azteca and D. magna. Endpoints evaluated will be survival, growth, reproduction and bioaccumulation (tissue residues). From tissue residues obtained from bioaccumulation experiments and toxicological data from individual compound and mixture studies critical body residues will be calculated.

Table 3. Ecotoxicology Information Relevant to Proposed Research

Compound	Percent Active Ingredient	*Daphnia* [b] µg/L		*Hyalella azteca* µg/L	
		LC_{50}	EC_{50}	LC_{50}	EC_{50}
Larvicides					
Temephos (Abate) [a,c]	5 - 43	0.011 - 0.54	----	----	----
Methoprene (Altosid) [a]	----	----	89 [d] - 360 [i]	----	----
Diflubenzuron [e]	25 - 97.6	----	7.1 - 16	----	----
Adulticide					
Malathion [a,f]	57 - 95	----	1 - 2.2	----	----
Naled [g]	58 - 91.6	----	0.3 - 1.55	----	----
Permethrin [i]	----	----	0.60	----	----
Resmethrin [i]	----	----	3.7	----	----
Model Compound					
Chlorpyrifos [a,h]	25.6 - 97.7	0.10 -115	----	0.119 - 0.219 [j]	----
Methylmercury [a]	97.0 [j] (CH_3HgCl)	----	----	3.8 - 23.5 [j]	3.2 - 10 [k]

a: Bioaccumulates or potential to bioaccumulate in aquatic organisms. b: Daphnia species not stated.
c-h: EPA's website, see references. i: Crop Protection Publications, 1994. j: Benson et al, 2000.
k: Borgmann et al., 1993

Water Exposures. Single chemical water-only experiments for both *H. azteca* and *D. magna* will be conducted to characterize the effect-concentration of the model chemicals and the WNV vector eradication insecticides that have not been previously characterized (Benson, Block, Steevens, Allgood & Slattery, 2000, and see Table 3). Acute toxicity, 48-hr, tests will be carried out using a modification of methods outlined by U.S. EPA (1991). Water quality parameters monitored include: dissolved oxygen, pH, ammonia, hardness, alkalinity, and salinity. Exposure chambers, in replicate, consist of a 30 mL beaker containing 25 mL of water/toxicant, nitex substrate, and one adult organism. At termination of the test surviving organisms are counted. *D. magna* reproduction will be evaluated using a 7-day exposure to mixtures of model compounds and vector control compounds following standard methods outlined by U.S. EPA (1989). Static water renewals will occur daily and before transferring adults to the new media one drop of food will be added to exposure chambers. After about the third day *D. magna* should start producing offspring, each day thereafter the number off offspring produced, number of broods and number of live and dead organisms will be recorded. In order to get enough tissue to analyze for bioaccumulation large numbers of *H. azteca* and *D. magna* will be exposed for 96-hours to mixtures of model compounds at concentrations that effects have been observed. Water quality and feeding will be conducted daily. Exposure chambers consist of a 1000 mL glass beaker containing 100 adult organisms, nitex substrate, and 800 mL of test water. At termination of the exposures surviving organisms and offspring will be separated, counted and placed in vials to be stored at -80°C until tissue residues of test compounds can be quantified.

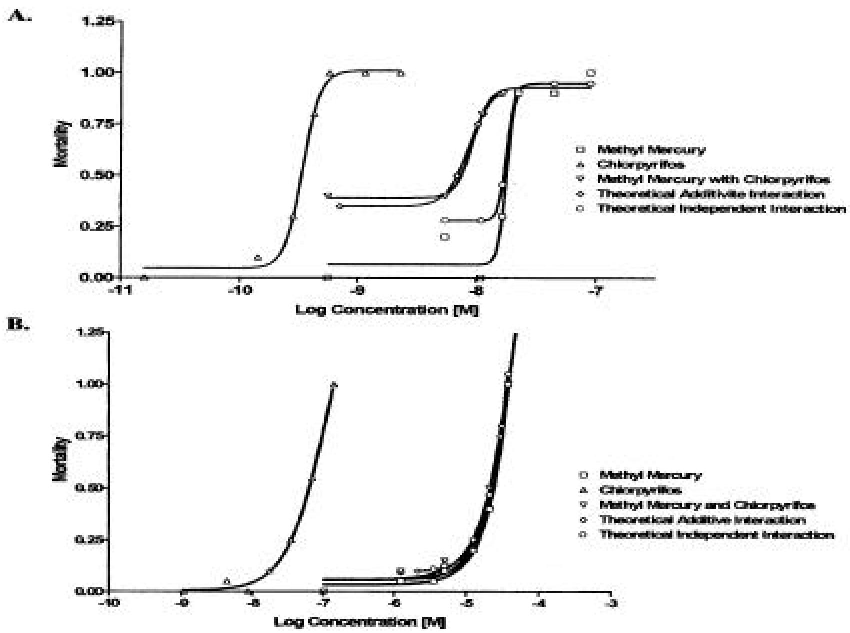

Figure 1. Survival of *Hyalella azteca* following aqueous (A) and sediment (B) exposure to individual and binary chemical mixtures of methylmercury and chlorpyrifos.

Sediment Exposures. Formulated sediments, spiked with model chemicals will be used in ten-day exposures to determine the toxicological effects of chemical mixtures at selected combinations and concentrations determined from acute toxicity tests. Formulated sediments will be utilized to provide a consistent homogenous material representative of freshwater sediment. Sediments will be formulated using Mystic White #18 sand, ASP 400 silt, and ASP 600 and 900 clay, milled humus and dolomite (Suedel and Rodgers, 1994). Components of the sediment will be aged in flowing water for seven days prior to spiking with model chemicals. Guidelines developed by ASTM (1994) will be utilized for sediment spiking. *H. azteca* will exposed to spiked sediment for ten-days according to methods described by Steevens et al. (1998) and outlined by the U.S. EPA (1994b). Sediment chambers will consist of 300 mL lipless glass beakers containing 100 mL of spiked formulated test sediment, 175 mL of overlying water, and ten juvenile test organisms. Overlying water in the sediment chambers will be renewed every 12 hours using a Zumwalt water splitting renewal system (Zumwalt *et al.*, 1994). Organisms will be fed a mixture of 1.8 mg/L yeast, cerophyll, and trout chow daily. Temperature, dissolved oxygen, and pH will be monitored throughout the ten-day exposure period. Water quality parameters including hardness, alkalinity, conductivity, and ammonia are measured at the initiation and completion of the experiment. At termination of the exposure, surviving *H. azteca* will be recovered from the sediment by sieving with a No. 50 U.S. standard sieve and preserved in 8% sugar formalin for length measurements (Tomasovic *et al.*, 1995).

Bioaccumulation and Critical Body Residues. Bioaccumulation is the accumulation in an organism's whole body tissue of a chemical directly from the aquatic environment. The bioconcentration factor is a term describing the bioconcentration of a chemical when an organism is exposed via water. Toxicity data and the bioconcentration factor will be utilized to further characterize the dose-effect relationship for a chemical. The critical body residue is the chemical concentration measured in an organism's whole body that is associated with a measured adverse toxicological effect. It utilizes a one-compartment model to determine the total dose of a chemical that the organism receives from exposure via water, food, or sediment. The critical body residue model accounts for variability in bioavailability of the chemical in the exposure media, metabolism, as well as uptake and depuration kinetics. By measuring tissue residue from field collected organisms and comparing those levels to critical body residues that we calculate from controlled experiments our proposed study will more accurately assess risk to aquatic organism during periods of vector control application.

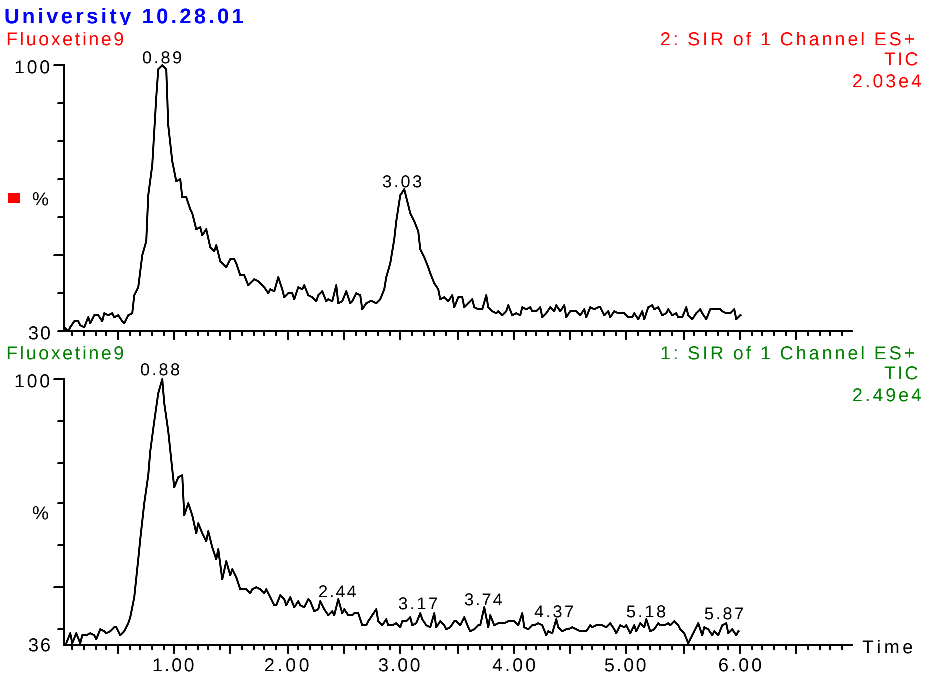

Figure 2. Chromatographs from University, MS wastewater effluent. Top chromatograph is fluoxetine and the bottom is norfluoxetine.

Chemical Analyses. Chlorpyrifos will be analyzed using an enzyme linked immunosorbant assay developed by Ohmicron Rapid Assay® (Strategic Diagnostics). Antibodies raised specifically to chlorpyrifos conjugated to magnetic particles are used to detect analyte concentrations in water and tissue samples. Tissue is prepared and analyzed by methods described by Allgood *et al.* (1997). Analytes are verified by chemical analysis using gas chromatography. Total mercury and methyl mercury is determined by solvent extraction and detected using a Varian Spectra AA-20 atomic absorption spectrophotometer and VGA-76 vapor generation system (Filippelli, 1987). The WNV vector eradication insecticide analysis will be conducted using an LC-MS method that we are currently developing (see Weston, Huggett, Foran, Rimoldi & Slattery 2001 for example, Figure 2).

Experimental Design and Data Analysis. A mixture design will be utilized to characterize the toxic effects of mixtures using survival and growth as toxicological endpoints (Benson, Block, Steevens, Allgood & Slattery 2000). Utilizing preliminary results from single chemical toxicity data, mixture designs are ideal for studying chemical interactions (Svendsgaard and Hertzberg, 1994). The design incorporates five combinations tested at selected concentrations to provide a comprehensive analysis of the potential chemical interactions. Concentrations selected for the mixture design will be based on single chemical toxicity range finding experiments. Chemical mixture interactions based on our experimental design will be plotted using the two-sided isobolographic method. Analysis for interactions deviating from additivity will be determined using three methods including the joint action ratio, combination index, and best-fit regression analysis. The joint action ratio will be used to characterize the binary chemical interactions (Hewlett, 1969). A ratio of the magnitude of a diagonal line drawn from the origin to the observed isobole and predicted additivity describes the type of interaction. Ratios of greater than one describe synergism, and ratios of less than one describe antagonism. The combination index Ic is a mathematical model developed by Berenbaum (1989). The model is the basis for the hazard index that the U.S. EPA presently utilizes to evaluate chemical mixtures (U.S. EPA, 1989). The components of the model (d) is the effect concentration of chemical in the mixture, where (D) is the effect concentration of the chemical alone. Values for the combination index indicate additivity if Ic is equal to one, synergy if Ic is less than one, and antagonism if Ic is greater than one. Best-fit regression analysis will be used to determine the linearity of resulting data as compared to the estimated line of additivity (Gessner, 1995). Data from whole organism responses such as survival and growth as well as biochemical mechanistic data will be evaluated using the described procedure. In the case of single point interaction data, Student's t-test and analysis of variance (ANOVA) will be used. SigmaStat/Plot statistical analysis and graphing software will be utilized (Jandel Scientific).

Facilities

The facilities in the School of Pharmacy's Environmental Toxicology Research Program at The University of Mississippi that are currently available for this investigation can be divided into four major areas: (1) laboratories for basic toxicological research, (2) a Pharmacogenetics Core Facility, (3) an Aquatic Toxicology Laboratory and (4) an Environmental Toxicology Analytical Laboratory.

Basic laboratories are equipped with analytical and microbalances, scintillation counter, centrifuges, refrigerators, water baths, and an ultra-cold freezer. In addition, microscopes (Olympus B-Max 40; Olympus MEIJI), a cryostat (Leica CM1850), a rotary microtome (Olympus HM 315), and paraffin embedding station (Reichert-Jung Histembedder) are available for histological examination of tissues. A digital image analyzer system (Kodak Catseye DKC-5000 with Image Pro Plus version 3.03 software) is available for histological analysis and quantifying the size of adult, larvae, and eggs of aquatic vertebrate and invertebrate species. A TECAN SLT Rainbow UV-VIS scanning microplate spectrophotometer with WinSelect version 2.0 software is utilized for biochemical measurements. Field analysis of water quality is performed with a Hydrolab Quanta water quality monitoring system. There are several desktop and notebook computers available for word processing and data handling and analysis. Recently, the PI equipped these laboratories with an Agilent GC/MS , a Waters LC/MS, and a JOEL SEM to provide greater toxicological identification abilities.

The Pharmacogenetic Core Facility located within ETRP's suite of laboratories has recently been outfitted with state of the art molecular analysis equipment. At the heart of the facility are a Beckman Coulter CEQ 8000 Genetic Analysis System, an Agilent 2100 Bioanalyzer and a BioRad VersaDoc 3000 image analyzer. A technician is on staff to run samples. High quality water is provided by a Millipore Milli-Q system.

The Aquatic Toxicology Laboratory is equipped for specialized research with aquatic invertebrate and vertebrate species. The Laboratory is made up of nine rooms that have individual temperature and lighting controls and Gast Regenair Blowers to provide tank aeration. Ultra pure water is supplied by a Barnstead NANOpure Infinity system. Dechlorinated water is provided by Model 2952 organic bed service exchange carbon for chlorine and chloramine removal (U.S. Filter Systems). Individual Model 2952 systems have been installed in each wet lab. There are numerous exposure systems (30- and 80-L aquaria and Frigid Unit Living Streams). For incubation of eggs, Precision Refrigerated Dual-Program Illuminated Incubators are available.

The Environmental Toxicology Analytical Laboratory occupies approximately 2,000 square feet within a 8,000 sq. ft. facility. Analytical equipment consists of a Hewlett-Packard Model 8452A diode array UV-VIS spectrophotometer with auto-sampler and kinetics software, two Hewlett-Packard Model 5890 Series II gas chromatographs (GCs) with dual electron-capture detectors, a Hewlett-Packard Model 5890 Series II GC with flame photometric and flame ionization detectors, a Hewlett-Packard Model 6890 GC with flame ionization and nitrogen-phosphorous detectors. The GCs are linked with a Hewlett-Packard Vectra 25 GC data station with Hewlett Packard Chemstation software. Also included is a Waters Model 600E HPLC system with Model 484 UV Absorbance Detector, Model 717 autosampler, a fraction collector and Millenium 2010 chromatography software. The laboratory is also equipped with an Ohmicron RPA1 Analyzer for analysis of chemicals using enzyme linked immunosorbent assays. For analysis of metals, a CEM Model MDS-2100 Microwave Digestion System as well as Varian SpectrAA-20 and SpectrAA 400 Zeeman atomic absorption spectrometers are available. A Brucker BioApex 30es High Resolution Fourier Transform Mass Spectrometer is maintained in the School of Pharmacy and is available for use in this project. Through the 1997 National Research Council of Canada/National Oceanic and Atmospheric Administration Intercomparison Studies (NOAA/10) the analytical laboratory has earned a rating of Very Good for accuracy evaluation of sediments and Superior for accuracy evaluation of biological tissues.

(13)<u>Related Research:</u>

Chemical Mixture Toxicity. Chemicals in the environment rarely occur alone, however, most toxicological studies are conducted using single chemical exposures. Therefore, it is necessary to characterize the toxicological hazards and risks associated with multiple chemical exposures (Parrott and Sprague, 1993; Feron *et al*, 1995). Chemicals occurring in complex mixtures have the potential for chemical-to-chemical, toxicokinetic and toxicodynamic, interactions affecting the resulting toxicological response. Chemical mixtures are characterized as having additive, synergistic, or antagonistic interactions and effects on the measured toxicological endpoint

(Calabrese, 1991). Additivity is the summation of toxic responses from multiple chemicals in a mixture. Synergism is the interaction of multiple chemicals in which the toxic response is greater than would be predicted by simple summation. Antagonism is the interaction in which the toxic response is less than would be predicted by summation. The deviation of chemical mixture toxicity from traditional individual toxicological testing makes it necessary to evaluate mixture interactions further so that the hazards and risks associated with multiple chemical exposure may be assessed (Sexton *et al.*, 1995).

To date, aquatic toxicology studies have typically evaluated the interaction of chemicals having similar mechanisms of toxicity. Kraak *et al.* (1994) studied the effects of a mixture of cadmium, copper, and zinc in the Zebra Mussel (*Dreissena polymorpha*) and determined the mixtures to be additive. Similarly, zinc and copper were found to interact additively in the Rainbow Trout (Lloyd, 1961). Spehar and Fiandt (1986) observed mixtures of metals at concentrations acceptable by the individual water quality criteria were not protective of daphnids and fish due to additivity interaction. However, Hoagland et al. (1993) found that atrazine and bifenthrin, having dissimilar mechanisms of toxicity, were additive. Several studies in which chemicals having independent or dissimilar mechanisms of action have demonstrated non-additive interactions, and in some cases found synergistic and antagonistic effects (Marinovich *et al.*, 1996). Classical studies by Triolo and Coon (1966) demonstrated that aldrin antagonized the effects of parathion, paraoxon, as well as several other organophosphates. It is apparent that there have been a variety of conclusions drawn from chemical mixture interaction studies. Chemical interactions are more complex than the assumption of additivity presently utilized to assess the risks associated with multiple chemical contaminants in sediment. Therefore, there is a need to more fully understand the underlying mechanisms of chemical mixtures responsible for deviations from additive interactions.

Bioaccumulation. Contaminated sediments have become an increasingly important issue for human and ecological health. Presently, 15 percent of the nations lakes, 4 percent of the nation's rivers, and 100 percent of the Great Lakes have fish consumption advisories associated with them (U.S. EPA, 1996). Of the fish consumption advisories, greater than 95 percent are due to bioconcentration of chemicals including mercury, PCB's, organochlorine pesticides, and dioxin. Nationally, a reported estimate of at least 29 percent of the benthic community in fresh and marine water is impacted by contaminated sediments (Veith, 1996). Long-term exposure to contaminants in the sediment can result in bioaccumulation of the chemical contaminant reaching concentrations capable of eliciting adverse toxicological effects (Borgmann et al., 1991). Toxicity, bioaccumulation and bioconcentration data can be utilized to further characterize the dose-effect relationship of a chemical. The critical body residue is the whole body concentration in an organism associated with a measured adverse toxicological effect. It accounts for variability in chemical bioavailability in the exposure media, metabolism, and uptake and depuration kinetics. The use of critical body residues in aquatic organisms has been proposed as a method to assess sediment contamination and the potential toxicological effects in aquatic organisms. McCarty and Mackay (1993) suggested the use of critical body residues and corresponding biological responses be studied to validate laboratory and field-based assessments of sediments. Currently, the assessment of sediment contamination is based on measured sediment concentrations of individual chemicals and toxicity to laboratory organisms. Safe sediment concentrations of chemical contaminants in sediment could be determined from the amount of that chemical accumulated and the corresponding measured toxicological effects.

Due to site-specific differences in chemical bioavailability and metabolism, the use of critical body residues may be a better predictor of the degree of ecological risk associated with contaminated sediments than sediment concentrations alone (Landrum *et al.*, 1992; Borgmann *et al.*, 1993).

Electronic databases used to review the literature discussed above include: Environmental Sciences and Pollution Management Abstracts (Cambridge Scientific), Life Sciences Periodical Abstracts (Cambridge Scientific), Biological and Agricultural Index (H.W. Wilson), and Biological Abstracts Inc.

Progress on Work to Date

The current proposal builds upon our previous WRRI work on chemical mixtures (Benson & Slattery: GR-02679-18) and the data we have generated on interactive effects. Specifically we tested three model compounds, chlorpyrifos, dieldrin, and methyl mercury as single- and binary-exposures on *Hyalella azteca*. We evaluated mortality, accumulation, and growth following exposure to the three model compounds, and then assessed a number of biochemical endpoints. Based on this work (and see Benson, Block, Steevens, Allgood, Slattery 2000) we noted that dieldrin acts independently in chemical mixture toxicity assays, while the chlorpyrifos:methyl mercury interaction results in significantly different effects that either compound alone, possibly due to the formation of an unknown complex. Reproductive toxicities and bioaccumulation were also significantly enhanced in the presence of mixtures. Thus, for the purposes of the present study we will focus on the chlorpyrifos:methyl mercury mixture, and examine the effects of this stressor on ability to cope with the relevant WNV vector eradication compounds (Table 2).

We have also initiated work on LC-MS method development for isolation of relevant anthropogenic contaminants in aquatic systems (Figure 1). To date this work, the focus of Mr. Weston's Ph.D., has examined the SSRI's Fluoxetine and Norfluoxetine (Weston, Huggett, Rimoldi, Foran Slattery 2001, and see below). We plan to similarly develop LC-MS techniques for the relevant WNV vector eradication compounds (Table 2), such that we can monitor fate in situ.

(14) Investigator's qualifications:

Marc Slattery is an Associate Professor of Pharmacognosy and a member of the Environmental Toxicology Research Program within the School of Pharmacy at the University of Mississippi. Dr. Slattery attended Loyola Marymount University where he obtained a Bachelor of Science degree in Biology (Marine and Environmental Biology Option) in 1981. He obtained his M.A. in Marine Biology from San Jose State University at the Moss Landing Marine Laboratories in 1987 and then worked for 3 yrs in the San Francisco Bay Area as an environmental consultant of waste-water discharge and marine toxicology. Dr. Slattery obtained his Ph.D. in Chemical Ecology from the University of Alabama at Birmingham in 1994. He has published over 35 scientific publications and has been involved in approximately $1.5 million in grants and contracts dealing with environmental toxicology and chemical ecology. His research activities have focused on assessing the acute and chronic health effects of environmental contaminants including metals, pesticides and endocrine disrupters. He has also examined the direct and indirect effects of natural and anthropogenic stresses on reproduction and disease resistance. See attached resume for additional details.

(15) Training potential:

ESTIMATED STUDENTS RECEIVING TRAINING

Currently a single Ph.D. graduate student (Biology- Environmental Toxicology emphasis) has been targeted for salary support and training under this WRRI program. Jim Weston has been a research scientist in our Environmental Signals & Sensors research program, and he recently decided to return to grad school to complete a Ph.D. This grant will provide the support for him to complete this degree, while performing work closely related to his own interests (the environmental consequences of mixtures of pharmaceuticals in aquatic systems). However our work, and techniques, is multi-disciplinary and it is likely that several other graduate students in my lab and within the ETRP and Biology programs will assist, and be trained, in various aspects of the project. My graduate students include Ph.D. (2 in Pharmacognosy) and M.S. (1 in Biology) candidates, and I currently fund a 4th yr undergraduate as a laboratory technician in environmental toxicology. In addition, environmental toxicology collaborations with Kristie Willett (Pharmacology/ETRP), John Rimoldi (Medicinal Chemistry/ETRP) and Stephen Threlkeld (Biology) suggests that some of their students will also be involved in either field or laboratory-based training associated with this project.

INFORMATION TRANSFER PLAN

A critical issue that has been overlooked in the recent WNV eradication discussions is the impact of spraying on environmental health. While all of the proposed mosquito control agents have been tested utilizing standard EPA protocols, *these have largely focused on single chemical dosing regimes and aquatic systems typically are comprised of chemical mixtures.* These mixtures have the potential to work additively or synergistically, and the stress of exposure to one class of compound may exacerbate the effects of another compound, even if it is applied only transiently. **Thus our goal is to assess the effects of WNV vector eradication agents in two model populations of aquatic invertebrates under conditions of single chemical doses following exposure to a mixture of persistent pesticides.**

This research program targets several important user groups: 1) the health of Mississippi residents who fish our waterways for subsistence or recreation is potentially impacted by bioaccumulation of pesticides and metals, 2) several commercial fishery markets in Mississippi (most notably Crayfish) have the potential to be either directly or indirectly impacted by mosquito adulticides and larvacides, and 3) it goes without saying that the recreation and/or tourism potential of Mississippi aquatic systems might be adversely impacted by changes in environmental health.

Our strategy for dissemination of our data will follow two closely allied approaches. First we intend to provide our results to the scientific community via presentations (budgeted regional & national mtgs) and publications in as timely a manner as possible. We also believe it is important to open a forum for discussion of problem with the lay public and the regional health councils who are developing these eradication plans. We intend to give seminars to regional user groups and develop a link/listserv to the UM ETRP page focused on this issue.

We expect to reach our target audiences via existing collaborations between ETRP and the Field Station Extension Service. And through announcements provided to the WRRI (i.e., LORE newsletter, etc).

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ABSTRACT

Project Title: Chemical Mixtures: Consequences of WNV Eradication on Water Quality

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Focus Categories: SED, TS, WQL

Keywords: Ecosystems, Mixtures, Pesticides, Residues, Sediments, Toxic Substances, Water Quality

Technical Abstract

Recent outbreaks of West Nile Virus (WNV) throughout the United States, and particularly in the Mississippi Valley States, have spurred plans for vector (= *Culex* mosquito) eradication using a variety of control insecticides. Via direct or indirect routes these compounds enter the aquatic environment where they become part of water and sediment matrices. Through direct contact, respiration or indirect ingestion non-target organisms are exposed to persistent and transient anthropogenic compounds and their mixtures. Individually or as mixtures, acting additively or synergistically, these compounds can directly affect adult and juvenile life stages of aquatic organisms. Besides direct effects some anthropogenic compounds found in aquatic matrices are known to bioaccumulate and biomagnify. At the present time, there is limited knowledge regarding effects of WNV vector control compounds in mixtures. Evaluating water quality and aquatic habitat are critical to an overall assessment of vector eradication programs.

The overall goal of the proposed research is to evaluate "real-world" chemical mixtures with toxicological effects not predicted from single chemical toxicity experiments. The amphipod *Hyalella azteca* and the water flea *Daphnia magna* will be exposed to mixtures of three model compounds: methoprene, a mosquitocide, and two regionally persistent anthropogenic compounds, chlorpyrifos and methylmercury. Bioconcentration data along with toxicological indices will be used to determine the critical body residue threshold concentrations at which toxicological effects occur. In the first year (2003-04) toxicological effects of the individual model compounds that have yet to be characterized will be assessed. In addition, we will develop analytical procedures to quantify *in situ* concentrations of model compound in regional water and sediment matrices. The second year (2004-05) will be spent toxicologically assessing mixtures of model compounds. Additional investigations will explore the impacts of pre-exposure stress on the ability of the model organisms to cope with a new toxicant. Finally, in year three (2005-06), bioconcentration data will be used to determine critical body residues. Model compounds in natural water and sediment matrices and residuals in model organisms will be quantified.

Project Coordination of the MS Delta Management Systems Evaluation Areas Project

Basic Information

Title:	Project Coordination of the MS Delta Management Systems Evaluation Areas Project
Project Number:	2001MS14S
Start Date:	4/25/2001
End Date:	8/31/2004
Funding Source:	Supplemental
Congressional District:	Third
Research Category:	Water Quality
Focus Category:	Water Quality, Non Point Pollution, Surface Water
Descriptors:	Best Management Practices, Watersheds, TMDLs
Principal Investigators:	Jonathan Woodrome Pote

Publication

1.

PROBLEM AND RESEARCH OBJECTIVES

The Mississippi Delta Management Systems Evaluation Areas (MDMSEA) Project began in 1995 with two purposes: 1) to assess how agricultural activities affect water quality; and 2) to evaluate Best Management Practices (BMPs) that mitigate agricultural nonpoint source pollution. The project is located in the northwestern portion of Mississippi, an area of intense agriculture referred to as the Mississippi Delta. The project is administered by a Technical Steering Committee comprised of representatives from the U.S. Geological Survey (USGS), the U.S.D.A. Agricultural Research Service (ARS), Mississippi State University (MSU), Mississippi Department of Environmental Quality (MDEQ), U.S.D.A. Natural Resources Conservation Service (NRCS), U.S.D.A. Farm Service Agency, Mississippi Soil and Water Conservation Service, Yazoo-Mississippi Delta Joint Water Management District, and the Pyrethroid Working Group. The primary research agencies in the MDMSEA project are the USGS, ARS, and MSU.

METHODOLOGY

The MDMSEA project focused on oxbow lake watersheds, in which, the lakes served as biological endpoints for improvements made in the watershed. Since 1995, findings from ARS and USGS indicated that sediment was the primary pollutant in runoff and in the lakes: sediment limits visibility in the lake water, which in turn, limits primary productivity necessary for sustaining a healthy aquatic habitat. BMP systems were installed in the oxbow lake watersheds to determine whether a watershed could be improved enough to sustain fish production in the lakes. Edge-of-field BMPs (slotted-board risers and inlet pipes, filter strips, etc.) did not improve lake water quality enough to sustain fish populations. However, conservation tillage systems, with winter cover, were shown to improve lake quality and aquatic habitat to the point of sustaining fish populations.

PRINCIPAL FINDINGS

Within the next decade, agricultural communities like the Mississippi Delta could be faced with greater restrictions on farming to reduce nonpoint source pollution from agricultural fields. Total Maximum Daily Loads (TMDLs) are one such tool that regulatory agencies could use to limit the amount of pollutant loads to streams, rivers, and lakes. MDMSEA data and research findings will play a key role in helping both the Delta agricultural community and Mississippi regulatory agencies adapt protective measures that will meet potential TMDL loading restrictions without excessive impacts to overall profitability and productivity.

The future direction of the MDMSEA project will include two primary objectives. The first objective will be for MDMSEA scientists to expand BMP research and to provide more BMP options for farmers – in other words, to provide a "catalogue" of BMPs that a farmer can tailor to specific landscapes. The second objective will be to provide economic analyses such as construction and maintenance costs, crop yields and profit, and environmental benefits for each BMP studied.

As part of the MDMSEA project extension, the ARS and USGS will be the primary agencies to evaluate the catalogue of BMPs. ARS efforts are more research-oriented and include study of BMPs such as agricultural drainage ditches, slotted-board risers with grass hedges, winter cover crops, constructed wetlands, and conservation tillage practices. ARS will continue to monitor BMP systems in the oxbow lake watersheds but will expand their efforts to include replicated plot studies located at a nearby ARS Field Station. The USGS will also continue monitoring runoff from most of the BMP demonstration fields in the oxbow lake watersheds. However, the USGS proposes to construct new sites in nearby stream watersheds to assess BMPs in watersheds that are more typical of Delta landscapes than were previously studied. The proposed USGS BMP demonstration sites include: 1) a revised slotted-board riser site; 2) an NRCS Wetlands Reserve Program (WRP) landscape; and 3) a conservation tillage site without a planted winter cover. MSU will be the primary agency providing the economic analyses of the BMPs studied. MSU will also provide leadership in generating BMP reports for Delta resource managers.

Functional Assessment of Moist-Soil Management Impact on Wetland Impoundments Created as Part of an Agricultural Lands Reclamation Plan

Basic Information

Title:	Functional Assessment of Moist-Soil Management Impact on Wetland Impoundments Created as Part of an Agricultural Lands Reclamation Plan
Project Number:	2002MS1B
Start Date:	3/1/2002
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	Third
Research Category:	Biological Sciences
Focus Category:	Wetlands, Management and Planning, Water Quality
Descriptors:	wetlands, wetland function, land use, water quality
Principal Investigators:	Gary N. Ervin, Darrel W. Schmitz

Publication

1. Ervin, G.N., J.T. Bried, B.D. Herman, and D.W. Schmitz, 2003, "Assessing functional integrity of moist-soil managed wetlands by comparison with nearby non-managed systems." in the 2003 Proceedings of the 32nd Mississippi Water Resources Conference, Mississippi Water Resources Research - GeoResources Institute, Mississippi State, MS, pgs. 13-25.
2. Krotzer, S. J. Bried, M.J. Krotzer, and R.L. Brown, 2004, Survey of Mississippi Odonata. Argia, (In press).
3. Bried, J., L. Bennett, and R. Brown, 2003, Requesting Mississippi Odonata Information. Argia 15(2):22.
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PROBLEMS AND RESEARCH OBJECTIVES:

The proposed project will be conducted in cooperation with the National Audubon Society to evaluate effects of moist-soil habitat management practices on water quality and wetland function. The study site is part of a 1000-ha farm near Holly Springs, MS, presently undergoing conversion from agricultural land to wildlife habitat under the supervision of Audubon personnel. Mississippi State University was solicited as an institutional partner in examining the success of management practices and effects of habitat manipulations on environmental quality in this headwaters region of the Coldwater River.

This project will address Mississippi water research priorities by monitoring water quality, sedimentation, and biological community development in wetlands managed as habitat for local and migratory wildlife, in comparison to natural wetland ecosystems. The proposed research will evaluate effects of habitat management practices on nutrient and sediment removal, sediment accumulation, and plant community development in three artificial impoundments on lands currently under reclamation from more than 150 years of agricultural use. South Atlantic-Gulf Region priorities to be addressed also include water quality and management during the functional assessment of created and actively managed wetland impoundments. Standard monitoring techniques will be used to evaluate effects of facilities construction and biotic community development in reference to unimpacted natural wetlands located within the same

METHODOLOGY:

Strawberry Plains Audubon Center is a 1000-ha farm located in Holly Springs, MS that entered the care of Audubon in 1998 under its original owners' wishes that it be restored to a more natural landscape, with a focus on birds and other wildlife species. Part of the management plan of Strawberry Plains is the enhancement of riparian areas for bird and other wildlife use. In addition to a number of streams that make up a substantial portion of the Coldwater River headwaters, aquatic resources on the reserve include numerous farm ponds installed to aid in erosion control. Center managers plan to install or enhance water control structures along one major stream and around the two farm ponds in order to increase moist-soil resources for waterfowl and other aquatic animal species, such as amphibians, fish, and mammals.

The aim of moist-soil management is to recreate more-or-less natural hydrologic cycles in managed wetlands to increase the diversity and production of plant and animal species for wildlife food and habitat (Anderson and Smith, 2000). Under moist-soil manipulation, water levels are lowered during the growing season to stimulate seed germination of wetland-adapted plants and to increase the oxygenation of soils to stimulate plant productivity. In autumn, water levels are raised to discourage establishment of non-wetland plant species and increase habitat diversity for invertebrate animals that serve as food for waterfowl and other aquatic wildlife, in addition to seeds that are produced by the moist-soil plant community. These water level manipulations are often accompanied by soil manipulations, such as tilling or disking, that maintain high plant species diversity and high seed production for wildlife (Gray et al., 1999). Moist-soil management practices at Strawberry Plains will include mowing, tilling, and planting in shallow areas of each of three man-made impoundments to enhance early-successional herbaceous plant species for increased seed and invertebrate production.

SIGNIFICANCE:

Strawberry Plains Audubon Center managers installed water control structures in two areas to be managed as moist-soil habitat and repaired the levee on the third managed area. Water control structures were to be donated by Ducks Unlimited. Audubon has installed stage height gages in two of the six sites selected for continued monitoring during 2003-2004.

Monitoring data continue to be collected, at bi-monthly intervals for general field parameters, less frequently for other variables, such as suspended solids, chlorophyll, and nutrient concentrations. In addition to vegetation analyses, further biotic data are being collected on amphibians and macroinvertebrates during the 2003 field season. Audubon is purchasing four automated recorders for collecting data on amphibian use of the sites, and Brook Herman is collecting macroinvertebrate samples as part of her thesis research.

Results to date were presented in a poster at the annual Mississippi Water Resources meeting in April 2003, which also was submitted for publication in the Proceedings. The presentation was entitled, "Assessing functional integrity of moist-soil managed wetlands by comparison with nearby non-managed systems."

A Single Technology for Remediating PNAs, Nitro/Nitrate Residues, PCBs, CAHs, Herbicides and Pesticides from Soils and Sludges with Na/NH3(l)

Basic Information

Title:	A Single Technology for Remediating PNAs, Nitro/Nitrate Residues, PCBs, CAHs, Herbicides and Pesticides from Soils and Sludges with Na/NH3(l)
Project Number:	2002MS6B
Start Date:	3/1/2002
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	Third
Research Category:	Ground-water Flow and Transport
Focus Category:	Toxic Substances, Groundwater, Treatment
Descriptors:	water quality, groundwater treatment, hazardous wastes, toxic substances, chlorinated aliphatic hydrocarbons, polychlorinated biphenyls, declorination, pesticides, herbicides, soil decontamination, reductions, sludge, subsurface drainage, water treatment
Principal Investigators:	Charles U. Pittman, Jr.

Publication

PROBLEMS AND RESEARCH OBJECTIVES:

The key goal of this project is to develop Na/NH_3 solvated electron reductions as a technology to rapidly remediate soils and sludges which have been contaminated with one or more of the following classes of polluting compounds:

(a) polychlorinated biphenyls (PCBs)
(b) chlorinated aliphatic hydrocarbons (CAHs)
(c) nitro/nitrate wastes (military wastes)
(d) pesticides and herbicides (chlorinated/brominated and phorphorous compounds)
(e) polynuclear aromatic hydrocarbons (PNAs)

This single technology has the potential to remove all these classes of toxic compounds from contaminated soils simultaneously. Over the past three years we have thoroughly investigated and demonstrated the rapid destruction of neat PCBs, CAHs and several pesticides as well as soils (with up to 20%wt. water) contaminated with these three classes of pollutants [1-12]. Furthermore, several samples from superfund sites, which were contaminated with PCBs, CAHs, dioxins and nitro compounds, were successfully remediated to very low (or nondetectable) pollutant levels [8,10].

METHODOLOGY:

Based on this highly successful research we began to test the reduction of a large series of polynuclear aromatic hydrocarbons (PNAs) and showed that neat samples were essentially totally reduced, leaving almost no traces of the residual PNAs [8,12]. Thus, the focus of the work turned towards identifying the reduction products produced in these neat reactions and examining the remediation of model PNA-contaminated soils [12,13]. The key findings are summarized as follows without going into the details.

1. With the exception of naphthalene and anthracene, very complex mixtures of reduction products result with more complex PNAs such as phenanthrene, chrysene, benzo-substituted anthracenes, pyrene etc [12,13].
2. Determining the reduction product distribution in treated soils was very difficult or impossible.
3. The product distribution changes with reduction time, soil makeup, water content in the soil and subsequent exposure of the remediated soil to air [13].
4. Environmental samples contaminated with PNAs typically contain complex mixtures of these pollutants. Therefore, after treatment with Na/NH_3, the product distributions of reduction products are extremely complex. Accurate analysis of these distributions in the remediated soils is too complex to be tractable.
5. The amount of sodium consumption required to remediate the contaminated soils was far greater than that required to remediate neat samples to the same low PNA residue concentrations (1ppm for example) [12,13].
6. The high sodium consumptions required during soil treatments (relative to treating pure PNAs) are due to the relatively slow PNA reduction kinetics in Na/NH_3 media. Therefore, water and other impurities in soil are able to complete with the PNA reductions and consume more sodium. Thus, higher amounts of sodium are required.

RESULTS/DISCUSSION:

In our most recent work, we have demonstrated that soils containing PNA mixtures (for example napthalene, anthracene, pyrene, chrysene and benzo["]anthracene) may be successfully remediated. Thus, all of the PNA species in the soils can be reduced to low levels using excess sodium in NH_3. Since, this generates very complex, unanalyzable product mixtures in the soil, there is a requirement that one demonstrate that these product mixtures are safe to leave in the soils. Each particular PNA mixture will give a unique product distribution and this distribution will be different for every different soil composition encountered (even if the PNA contaminate mixture's composition is held constant). Thus, we have started to work with Professor Shiao Wang at the University of Southern Mississippi to develop an evaluation method to see if the PNA-contaminated soils are safe to return to the environment after Na/NH_3 remediation treatments [14]. This project is now combining the recent methods of *biotechnology* with *chemical reduction methods*.

A series of individual PNA-contaminated soils were reduced with Na/NH_3. We have also reduced soils contaminated with PNA mixtures. These samples, along with samples of the reduction products from pure PNA compound reductions, have been sent to Professor Wang at USM. Professor Wang will expose water to these samples individually. This water will then be used as a medium to grow both minnows and/or freshwater mussels. The same species of freshwater mussels and minnows will also be raised, simultaneously, in identical tanks, using pristine water (which never encountered the PNA reduction product or remediated soils with the PNA reduction products). Then, DNA-chip technology will be employed to do scans of a large series of genes to see which genes have been activated (e.g. gene expression turned on). By sampling hundreds or thousands of genes, a general DNA expression array will be obtained for these organisms when living in the pristine water versus that when living in the water exposed to the PNA reduction products. These organisms will serve as environmental sentinels by comparing the up-regulation versus down-regulation of their gene expression as shown by the DNA arrays on a chip. Early indications of environmental stresses placed on the organisms will be revealed by these patterns of gene expression. This will occur long before these species exhibit signs of sickness or die. This should be a far more sensitive technique than looking at organism death rates.

Experiments with minnows are going on at USM now. The DNA-chip technology is in a start-up phase in Professor Wang's lab. His students have about 10 samples from our laboratory waiting to be studied. A chip-reader at the Army Corp of Engineers (WES) in Vicksburg will be employed. We can do nothing at MSU to speed up this progress. We are significantly in front of USM in accomplishing our portion of this work.

While waiting for the USM experiments to begin producing results, we are conducting experiments on PNA model compounds and model mixtures, which are mixed with PCBs, or a model nitroaromatic compound. Then these mixtures are being subjected to dry Na/NH_3 or Na/NH_3 with 2%wt. water added. For example, when the PNA, phenanthrene, and dinitrobenzene were mixed and subjected to Na/NH_3 treatment, these two pollutants were reduced from concentrations of 2000 ppm each to levels below 5 ppm when 2%wt. water was present.

Information Transfer Program

Information Transfer Program - Conferences

Basic Information

Title:	Information Transfer Program - Conferences
Project Number:	2002MS15B
Start Date:	3/1/2001
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	Third
Research Category:	Not Applicable
Focus Category:	Surface Water, Models, Wetlands
Descriptors:	Bioassessment, water policy, surface water, contaminants, models, social impacts, TMDL, coastal wetlands,
Principal Investigators:	David R. Shaw

Publication

1. 2003, Mississippi Water Resources Conference Proceedings, Mississippi Water Resources Research - GeoResources Institute, Mississippi State, MS, 172 pgs.
2. 2003, Mississippi Water Resources Conference Program and Abstracts, Mississippi Water Resources Research - GeoResources Institute, Mississippi State, MS, 49 pgs.

INFORMATION TRANSFER PROGRAM - CONFERENCES

PROBLEM AND RESEARCH OBJECTIVE

Need to provide interactions among water resources researchers and federal, state, and local agencies, policy makers and the interested public. Likewise, there is a need for researchers to present their current and ongoing research to this diverse group.

METHODOLOGY

The Mississippi Water Resources Research Institute is the lead sponsor of an annual Mississippi Water Resources Conference which is co-sponsored by the Mississippi Department of Environmental Quality's Offices of Land and Water Resources and Pollution Control, the Mississippi District Office of the U.S. Geological Survey, and the Mississippi Water Resources Association. The Conference provides a forum for the interaction referenced above.

SIGNIFICANCE

The Conference provides a well-known and respected opportunity for researchers, students, agencies, and the interested public to formally discuss current water resources research and informally critique the applicability of that research to priority water resources research needs in Mississippi, the Southeastern United States, and the Nation. The conference's proceedings, which are distributed to all conference attendees and available for sale to others, are perhaps the preeminent source of current information about the diversity of water and related land resources research in Mississippi.

Information Transfer Program - Newsletter

Basic Information

Title:	Information Transfer Program - Newsletter
Project Number:	2002MS16B
Start Date:	3/1/2001
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	Third
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	Newsletter
Principal Investigators:	David R. Shaw

Publication

1. LORE (Lakes, Oceans, Rivers & Estuaries) Newsletter published quarterly (February, May, August, November), Mississippi Water Resources Research - GeoResources Institute, Mississippi State, MS, 4 pgs.

INFORMATION TRANSFER PROGRAM - NEWSLETTER

PROBLEM AND RESEARCH OBJECTIVE

The need to inform researchers, policy makers, and the interested public about the Institute's activities, the annual conference, and funding opportunities.

METHODOLOGY

The Mississippi Water Resources Research Institute's quarterly newsletters – LORE – include 1) request for proposals for USGS competitive grants, 2) a "call for abstracts" for the annual conference, 3) the conference program and 4) summarizes activities at the conference.

SIGNIFICANCE

The newsletter is one of the Institute's primary mechanisms to inform federal, state, and regional agencies in Mississippi and the interested public about the Institute's activities.

Information Transfer Program - Publications

Basic Information

Title:	Information Transfer Program - Publications
Project Number:	2002MS17B
Start Date:	3/1/2001
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	Third
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	Publications
Principal Investigators:	David R. Shaw

Publication

1. 2003, "Annual Report: 2002-2003", Mississippi Water Resources Research - GeoResources Institute, Mississippi State, MS, 10 pgs.

INFORMATION TRANSFER PROGRAM - PUBLICATIONS

PROBLEM AND RESEARCH OBJECTIVE

It is necessary for Mississippi State University, political and administrative decision-makers, and the interested public to have a mechanism to evaluate the Institute's effectiveness.

METHODOLOGY

These mechanisms take two related but separate forms: 1) a Mississippi State University reporting requirement which encompasses a variety of other multi-disciplinary water and related land management research funded through the Institute and related information about the extent and sources of the Institute's funding; and 2) a discretionary annual report that includes a summary of these assorted projects, their accomplishments, researchers associated with the Institute, and students supported by these projects.

SIGNIFICANCE

The MSU report is necessary for the University to evaluate the effectiveness of the Institute in meeting its mission and goals. The *In Review* publication is discretionary but has historically provided a timely concise overview of the Institute's activities and associated researchers and students.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	9	0	0	1	10
Masters	6	0	0	0	6
Ph.D.	3	0	0	0	3
Post-Doc.	0	0	0	0	0
Total	18	0	0	1	19

Notable Awards and Achievements

Willett - Cloned full lengh RNA sequence of channel catfish CYP1B. The PCR product was 1769 bp coding for a 510 amino acid sequence. Amino acid similarity with humans, plaice and carp are 55, 61 and 71%, respectively.

Willett - Sediments were collected at the five Delta waterways (Lake Roebuck, Bee Lake, Wolf Lake, Sunflower River, and Cassidy Bayou). The sediments were chemically extracted and analyzed in H4IIE rat hepatoma cell bioassay which measures the potential of the sediment extracts to cause CYP1A induction. Bioassay results were compared to analytical chemistry results (quantitated both organics and metals).

Willett - Optimized and published a microsomal estrogen metabolism assay with GC/MS quantitation for seven estrogen metabolites. Tissues were analyzed from control, BaP-exposed, and Mississippi Delta wild catfish.

Willett - Found that 2-hydroxyestradiol and estrone were the predominant metabolites generated by catfish liver microsome preparations.

Willett - BaP exposure caused a statistically significantly higher increase in the 4-hydroxyestradiol compared to the 2-hydroxyestradiol formation. This suggests that environmental contaminants could affect how estrogen is metabolized in vivo in catfish.

Davidson - This project is a two-year investigation that will complete the data gathering phase this summer. Long term measurement of hydraulic head, geochemistry, and isotopic composition in a series of nested piezometers was completed in the Spring of 2004. The final phase of data collection will be a collection of shallow cores around the piezometers to characterize the near surface geology.

Massey - A standardized turf protocol was developed with input from the USEPA, modelers, environmental consultants, industry, and university researchers to ensure study conduct will be comparable between study test sites and will provide data necessary to improve runoff estimations based upon regional differences in turf type and management.

Massey - An HPLC-UV method has been developed that will allow the simultaneous analysis of the three pesticides (2,4-D herbicide, chlopyrifos insecticide, flutolanil fungicide) in runoff that serve as the test compounds in the standardized turf runoff protocol.

Massey - Batch soil slurry adsorption studies involving the three pesticides have been initiated in the laboratory for ultimate use in modeling runoff from these turf runoff experiments.

Slattery - Eliminated 72 of possible 82 Mississippi Counties as potential areas for phase III field testing.

Slattery - Determined most likely agricultural pesticide to co-occur with mosquitocide: chlorpyrifos and methoprene, respectively.

Slattery - In contact with MS Department of Health, Department of Epidemiology, waiting for completed data set on mosquito control programs in Mississippi counties.

Slattery - Confirmed acute toxicity of adult Hyalella azteca to chlorpyrifos, 0.119-0.219 ug/L. Established approximate acute toxicity of adult Hyalella azteca to methoprene, 200-300 ug/L.

Ervin - Project is completed and a final technical report is being developed. Related follow-on funding has been obtained from the National Audubon Society (with Jeanne Jones of Wildlife and Fisheries) for $22,555 (2003-06) for the research work on "Effects of mammalian herbivore exclusion on understory habitat recovery at Strawberry Plains Audubon Center, Holly Springs, MS".

Publications from Prior Projects

1. 2001MS2601B ("Spatial Pattern in Land Use: Its Role in Determining Surface Water Quality") - Water Resources Research Institute Reports - Matlack, Glenn, 2003, Final Completion Report, "Spatial Pattern in Land Use: Its Role in Determining Surface Water Quality", MS Water Resources - GeoResources Institute, Mississippi State University, Mississippi State, MS, 19 pages.