Mississippi Water Resources Research Institute
Annual Technical Report
FY 2004

Introduction

The FY 2004 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 five research projects are included in this summary.

Research Program

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:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	Third
Research Category:	None
Focus Category:	Non Point Pollution, Water Quality, Surface Water
Descriptors:	None
Principal Investigators:	Joseph H. Massey

Publication

1. Ampim, P.A., J.H. Massey, B.A. Stewart, M.C. Smith, A.B. Johnson, K.L. Armbrust and A. A.Andrews. 2005. Factors Influencing Pesticide Runoff from Warm Season Turfgrasses. Mississippi Water Resources Conference proceedings.
2. Ampim, P.A., J.H. Massey, B.A. Stewart, M.C. Smith, A.B. Johnson, K.L. Armbrust and A. A.Andrews. 2005. Factors Influencing Pesticide Runoff from Warm Season Turfgrasses. Mississippi Academy of Sciences proceedings.
3. Ampim, P.A., J.H. Massey, B.A. Stewart, M.C. Smith, A.B. Johnson, K.L. Armbrust and A. A.Andrews. 2005. Factors Influencing Pesticide Runoff from Warm Season Turfgrasses. Southern Weed Science Society proceedings.

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:**	$30,027 Total	($20,998) Direct	($9,029) Indirect

(7) Principal Investigator: Joseph H. Massey, Dept. Plant and Soil Sciences, Mississippi State University, 117 Dorman Hall, Mail Stop 9555; Mississippi State, MS 39762; tel. 662-325-4725; fax. 662-325-8742

Co-Principal Investigators: Barry R. Stewart[1], Kevin L. Armbrust[2], Alton B. Johnson[3]

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

[1]Department of Plant & Soil Sciences, Mississippi State University; [2]Mississippi State Chemical Laboratory; Mississippi State University; [3]Mississippi River Research Center, Alcorn State University, Alcorn State, MS,[4]Water Quality Consulting, Colorado Springs,CO;[5]USDA-ARS, Tifton, GA; [6]Agricultural and Biological Engineering, Mississippi State University; [7]Black Belt Experiment Station, Mississippi State University, Brooksville, MS.

(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***). The proposed research has been identified as addressing issues of importance to the Mississippi Department of Environmental Quality and the Mississippi Department of Marine Resources. Please see the attached **letters of support** provided in **Appendix 1**.

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. 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. 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. 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.

Request for 2nd Year Funding

This proposal is a request for the continuation of the support for research began in 2003. A summary of progress made to date is given in the *Methods, Procedures and Facilities* section of this proposal. In addition to $90,000 (3 yr total) received from the U.S. Golf Association, this research project has received an additional $44,500 (actual dollars) in 2003 from the Mississippi Agriculture and Forestry Experiment Station. A Ph.D. student is to begin working full time on this project in January 2004. For the overall success of this project, it is imperative to continue to receive WRRI support in 2004. Longer-term, the infrastructure and techniques developed in the current project will be used to address urban runoff issues important to water quality in Mississippi. In the future, turfgrass will be investigated as a "sink" for metals, oils, and other urban contaminates in addition to being investigated as a "source" of pesticide and nutrient runoff.

(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

any 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 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 1 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 1 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 1 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.

Runoff Plot Establishment and Design

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.

Overview of Progress to Date

Due to excessive rainfall during the spring and early summer, field operations required for this project were slower than anticipated. As a result, plot establishment is behind schedule and actual runoff experiments will not be conducted until late Spring 2004. In spite weather delays, significant progress has been made: The site was laser-leveled to an exact 3% slope with minimal cross-slope. A Hunter-brand irrigation system was installed for grass establishment and maintenance. The irrigation system will permit rainfall intensities of up 1.5 inches per hour to be studied at our facility. Bermudagrass (*Mississippi Pride)* was sprigged at the study site in early August 2003. We are currently in the grow-in phase with approximately 60% surface coverage. The Bermudagrass plots will be used to determine scalability of runoff and, therefore, represent the bulk of the study area. The smaller (12 x 30 ft) Zoysiagrass and Bermuda-overseeded with ryegrass plots will be established in early Spring 2004. Photographs depicting the field progress made to date are provided in **Appendix 2**.

Current Efforts

Runoff collection apparatus: A prototype runoff collection trough and flume are under construction. Runoff collection troughs will be install throughout the winter months, weather permitting.

Laboratory Experiments: Batch soil equilibrium (slurry) experiments using the Brooksville silty clay soil found at the study site are underway using a variety of turf herbicides (e.g., trifluralin; 2,4-D; simazine). The soil-water partition coefficients derived from these experiments are necessary for modeling pesticide runoff from turfgrass.

Contaminant Runoff During Simulated Rainfall Events

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.

Analytical Methods

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).

Anticipated Problem No. 1: 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.

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

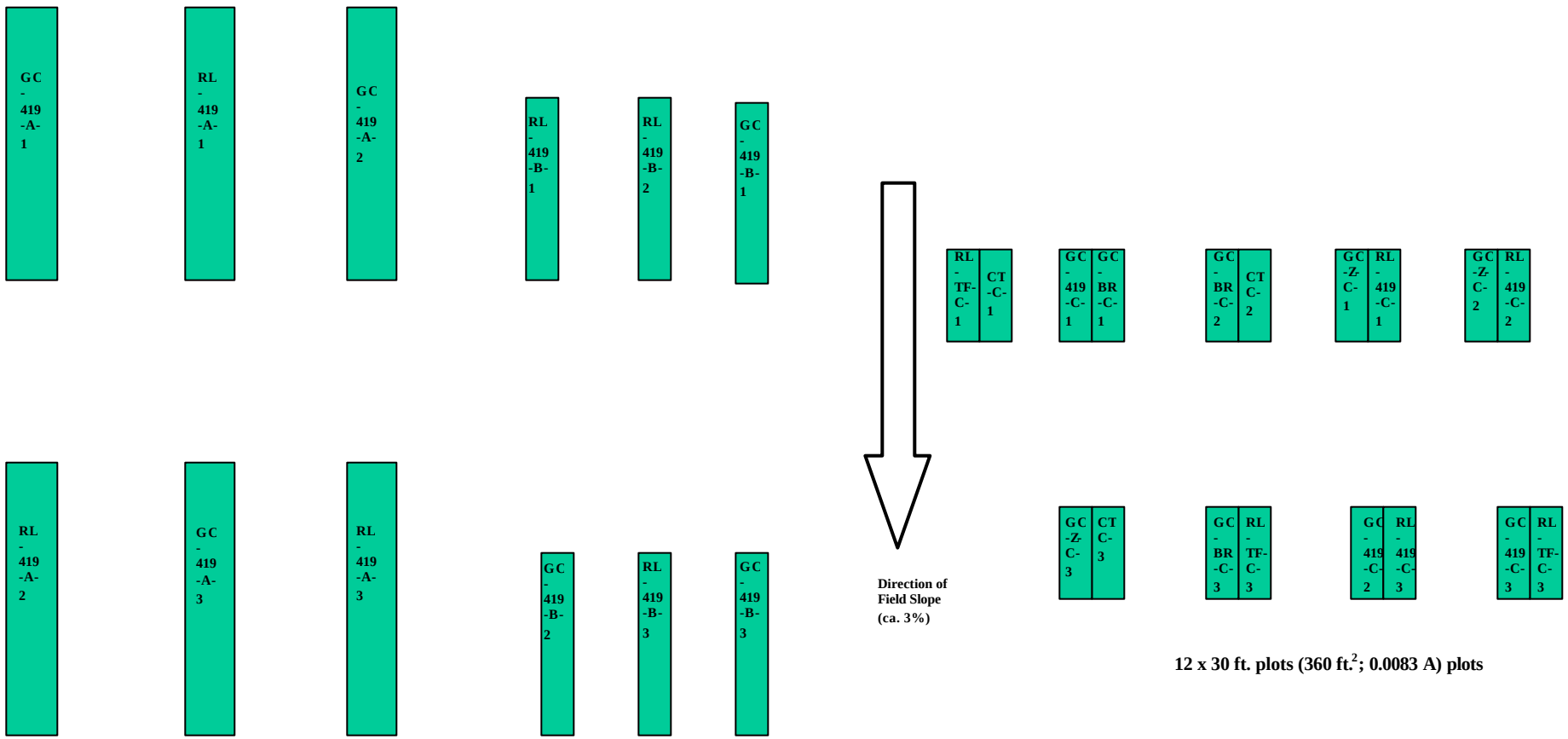
Objective 2: Using results from Objective 1 to refine pesticide (PRZM) and nutrient (RZWQM) runoff model estimates for warm-season turf management regimes.

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.

[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 1st. 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.



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. 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 one to two 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

Letters of Support & Collaboration

APPENDIX 2

Progress Made to Date

Procurement of Matching Funds

The following funds have been secured in 2003 to supplement FY2003 WRRI funding:

Agency	Amount	Use
MAFES[1]-Mississippi State University	$7,500	Laser-leveling of study site
MAFES-Mississippi State University	$5,000	Triplex mower
MAFES-Mississippi State University	$30,000	Sprigging, irrigation system, graduate student support
MAFES-Mississippi State University	$2,000	New roof for runoff equipment shed
U.S. Golf Association	$30,000 ($90,000 over three yrs.)	Measurement of pesticide runoff from MS golf course fairways
Total	**$ 74,500**	

[1]Mississippi Agriculture & Forestry Experiment Station (MAFES)

Figure 1. Laser-Guided Landforming at MS Runoff Field Site (ca. 3 Ac in size).

Figure 2. Elevation Model Showing Three Pads Having 3% Slope Upon Which the Runoff Plots Are Being Installed.

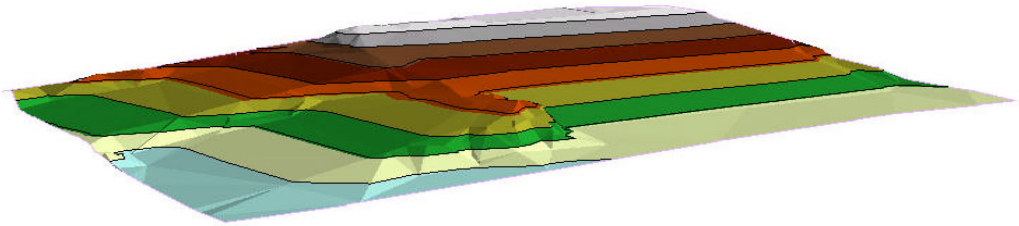


Figure 3. Sprigging of *Mississippi Pride* Bermudagrass at the MS Field Site.

Figure 4. Photograph Depicting Grow-In of Bermudagrass Plots.

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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
Project Number:	2003MS19B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	First
Research Category:	None
Focus Category:	Sediments, Toxic Substances, Water Quality
Descriptors:	None
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

(4) Duration: March 1, 2004 to February 28, 2005

(5)	Federal Funds:	16,540 (Total)	(16,540) Direct	
(6)	Non-Federal Funds:	33,077 (Total)	(19,223) Direct	(13,854) 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 (Table 1). 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.

Table 1. Ecotoxicological 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

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 (Table 2) with two anthropogenic compounds, chlorpyrifos and methylmercury (Table 3), 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.

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

Compound	Formula and Molecular Wt.	Solubility Water (@ 25° 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° 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° 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.

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.

Table 3. Physical-Chemical Properties of Model Compounds

Compound	Formula and Molecular Wt.	Solubility Water (@ 25° 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.

Progress to Date on Original Objectives (Funding Received July 8, 2003 = 4 months)

After receipt of the award letter we have accomplished the following: 1) began characterizing the 82 Mississippi counties to determine locations of field sampling sites, 2) have increased our *Hyallela azteca* population to sustainable levels so we can begin performing single compound and mixture bioassays and 3) graduate student getting trained to use ArcView GIS software and undergraduate getting trained to care for the Hyallela and perform basic bioassays. These results are pertinent to phase III of the original proposal. Our current efforts will focus on phase I and II.

Field Sampling Site Locations

To locate possible field sampling sites state area land use data will be assessed at two levels: county and local area. County level assessments will eliminate large geographic areas not suitable for this study or have a low probability of finding both pesticides and mosquitocides in environmental samples. Local area assessments will define specific locations where there is a high probability that pesticides and mosquitocides will co-occur in the aquatic environment. The two most useful county level characteristics are an active eradication program and data regarding land use devoted to crops. Local area characteristics will be more specific than county level but in general will include the following: 1) detailed information about mosquito control programs including compounds used, amounts applied and application frequencies and locations, 2) agricultural information regarding detailed crop and pesticide data and 3) watershed data, particularly related to streams receiving runoff from agricultural fields. Understanding the spatial and temporal scales related to pesticide and mosquitocide applications are essential to confidently predict locations where these compounds will co-occur and possibly affect the water quality in Mississippi aquatic habitats.

In Mississippi there is no state controlled mosquito eradication effort, cities and counties organize their own programs. Many local government agencies hire pest management services to control mosquitoes. Information regarding which counties or cities currently use a control program will continue to be difficult to obtain until the Department of Health, Division of Epidemiology, compiles data from a recent survey of eradication programs in the state. The database will provide information regarding active control programs and methods being used but will not include specific information such as application frequencies or locations. To date, a few local government agencies and mosquito control services have

been contacted to get information regarding control methods being practiced. Though aerial or truck spraying is in use application of time released larvicides, particularly Altosid™ which contains the active ingredient methoprene, directly into aquatic systems is becoming more common. We have decided to use methoprene as our representative mosquitocide for this study.

Counties can be further characterized as potential field study areas by evaluating agricultural practices. According to the Mississippi Department of Agriculture and Commerce there are approximately 42,000 farms occupying 11 million acres in Mississippi. Commercial forests, which comprise 61% of the state's land area, will not be considered when evaluating counties. We evaluated counties based on their 2002 estimated planted acreage of six crops: corn, cotton, rice, sorghum, soybean and wheat (USDA, 2003). Of the 47 counties growing one or more of the above crops it was evident there is a wide range of acres planted: from 1,100 acres of corn planted in George County to 398,100 acres of all six crops planted in Bolivar County. The county land area devoted to crops ranged between 0 and 80%. Of the counties growing crops approximately 94%, 81%, 66%, 21%, 21%, 19% and 4% grew corn, soybean, cotton, rice, sorghum and/or wheat, respectively. Of the six crops planted approximately 13%, 28%, 26%, 11%, 4% and 19% of the counties grew either 1, 2, 3, 4, 5 or 6 crop types, respectively. Though most counties grew some corn, soybean acreage was greatest at 1,392,400 followed by cotton (1,077,300), corn (524,500), rice (244,600), wheat (204,100) and sorghum (68,700). Counties with large tracts of land devoted to crops and have active eradication programs will receive the greatest consideration when deciding on field study areas. Below is a table listing relevant crop information from selected Mississippi counties with active control programs (Table 4). Approximately 57% of the 82 counties can be eliminated because they do not grow any of the six common crops. Of the counties remaining an additional 41% can be eliminated by only

Tabel 4. Crop data of representative MS counties with mosquito eradication programs.

County	Land Area miles^2	Crops acres	% Land Use: Crops	Number Crops	Largest Single Crop, % Total Planted
Bolivar	876	398,100	71	6	Soybean, 46%
DeSoto	478	45,900	15	3	Soybean, 70%
Hinds	869	27,400	5	3	Corn, 49%
Jackson	727	0	0	---	---
Lee	450	45,500	16	2	Soybean, 86%
Leflore	592	235,500	62	6	Soybean, 38%
Pike	409	0	0	---	---
Rankin	775	9,000	2	2	Cotton, 59%
Washingon	707	326,900	72	6	Soybean, 38%

County land area: US Census, 2003. % land use- crops, was calculated: planted crops (mi^2) /county land area (mi^2) x 100. All other information from USDA, 2003; crop acreage based on 2002 estimates of planted corn, cotton, rice, sorgum, soybean and wheat.

considering those with three or more crop types planted, leaving about 19 counties. These remaining counties can be further reduced to 10 by selecting those with approximately 50% county land use devoted to agriculture. The remaining ten are all located in the Delta area in districts 1 and 4. Of these 10, three are know to have active mosquito control programs and once the Department of Health's eradication program database is complete we will be able to determine if the other 7 have active programs too. As counties are considered for field sampling local agencies or hired control services will be contacted to obtain specific information such as compounds used and application frequencies and locations.

After specific counties have been selected from the above mentioned process local areas within these counties will be evaluated as potential field sampling sites. To obtain the specific information regarding control programs and agricultural practices local governmental agencies and individual farmers will have to be contacted. Detailed data regarding pesticide and mosquitocide application needs to be obtain, e.g. compounds used, amount applied and application frequencies and locations. Mosquitocide information can be obtained from local government agencies or their hired mosquito control services while some crop and pesticide information can be obtained from county land managers individual farmers will need to be contacted regarding specific field conditions. Of the common organophosphate pesticide active ingredients listed by the US EPA's Office of Pesticide Programs only three are used on all six of the crops we have selected: malathion, disulfoton and chlorpyrifos. We have decided to use chlorpyrifos as our representative pesticide for this study. All of the pesticide and mosquitocide data as well as watershed information will be organized and evaluated using GIS technology.

Once co-occurring application areas of chlorpyrifos and methoprene are located they will be further assessed by their relationship with local watershed data. Drainage ditches or small watershed tributaries that receive agricultural runoff are likely aquatic locations where both pesticides and mosquitocides can co-occur. Detailed GIS watershed information can be obtained through state, MARIS (Mississippi Automated Resource Information System) and MDEQ (Mississippi Department of Environmental Quality), and federal, US EPA (Environmental Protection Agency) and US GS (Geological Survey), agencies. It will be helpful to choose local areas that are contained with in a single watershed. Many Mississippi counties have more than one watershed; some are associated with more than one river basin. Below is a table containing watershed information for the same counties selected for the crop data (Table 2).

Much of Mississippi's land area has been eliminated as possible study sites based on county level mosquito management and agricultural practice criterion. Once it has been determined which remaining counties have active mosquito control programs local area characteristics will be assessed. GIS models of chlorpyrifos and methoprene applications, agricultural drainage and tributaries that receive runoff will be used to define a smaller geographic location within counties. Much of the pertinent information can only be obtained from local sources.

Hyallela azteca maintenance and bioassays

The population of Hyallela maintained in the Environmental Toxicology Research Program needed to be increased before bioassays can be performed. The population has recently reached a sustainable level to supply enough individuals for the duration of this study

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Table 5. Watershed data of representative MS counties with mosquito eradication programs.

County	Basin*	Watershed (State)**	US EPA Hydrolic Unit Code (HUC)**
Bolivar	Yazoo River	Lower Mississippi-Helena (AR, MS)	8020100
		Lower Arkansas (AR, MS)	8020401
		Lower Mississippi-Greenville (AR, LA, MS)	8030100
		Big Sunflower (AR, MS)	8030207
		Deer-Steele (AR, LA, MS)	8030209
DeSoto	North Independent	Lower Mississippi-Memphis	8010100
	Streams	(AR, IL, KY, MO, MS, TN)	
	Yazoo River	Horn Lake-Nonconnah	8010211
		Lower Mississippi-Helena (AR, MS)	8020100
		Coldwater (MS)	8030204
Hinds	Black River	Middle Pearl-Strong (MS)	3180002
	Pearl River	Lower Big Black (MS)	8060202
	South Independent	Bayou Pierre (MS)	8060203
	Streams		
Jackson	Coastal Streams	Pascagoula (MS)	3170006
	Pascagoula River	Black (MS)	3170007
	Escatawpa River	Escatawpa (AL, MS)	3170008
		Mississippi Coastal (AL, LA, MS)	3170009
Lee	Tennessee River	Upper Tombigbee (AL, MS)	3160101
	Tombigbee River	Town (MS)	3160102
Leflore	Yazoo River	Tallahatchie (MS)	8030202
		Yalobusha (MS)	8030205
		Upper Yazoo (MS)	8030206
		Big Sunflower (AR, MS)	8030207
Pike	South Independent	Bogue Chitto (LA, MS)	3180005
	Streams	Tangipahoa (LA, MS)	8070205
	Pearl River		
Rankin	Pearl River	Middle Pearl-Strong (MS)	3180002
Washington	Yazoo River	Lower Mississippi-Greenville (AR, LA, MS)	8030100
		Big Sunflower (AR, MS)	8030207
		Deer-Steele (AR, LA, MS)	8030209
		Bayou Macon (AR, LA, MS)	8050002

*Mississippi Department of Environmental Quality, 2003. **US EPA, 2003

Training

The graduate student working on this project has received or is in the process of receiving the following training:

- Mini-course ArcView GIS, August 13-14, 2003
- Taking a graduate level, Geology 500, ArcView GIS
- Enrolled in graduate level Remote Sensing class

ArcView GIS software is going to be loaded onto the graduate student's desktop computer. This software and support is being provided by the University of Mississippi's Geoinformatics Center (UMGC) at no charge to our project (>$10,000 value).

An undergraduate student is being trained to maintain our Hyallela population and on IC_{50} and EC_{50} bioassays using these crustaceans.

(12) Methods, procedures, and facilities: (refer to original proposal for detailed methods and procedures)

Year 2 Goals: Additions and Modifications

Our current year's goals (phase I) were modified after receiving our award notification. We planned to perform a series of single compound bioassays and develop detection/quantification methods appropriate for our analytical equipment. Instead efforts were spent addressing part of our final year's objectives (phase III), particularly assessing likely field sampling locations in the state. This adjustment has proved useful in allowing us enough lead time to obtain the necessary detailed information regarding counties we have been able to designate as having characteristics suitable for field sample sites. We are now satisfied with our progress in assessing field sampling sites to return to our original phase I objectives. So, for the remainder of this year we will focus on completing the necessary single compound bioassays, especially methoprene and chlorpyrifos exposures, and devote more time developing the required analytical methods.

Year two (phase II) will be basically unchanged from the original proposal, crustacean responses to multiple compounds and pre-exposure stress will be evaluated. In addition, if necessary, completion of any phase I objectives will be included into next year's goals. We have determined that our representative compounds will include chlorpyrifos, methoprene and methylmercury. These compounds will be used in binary, three-way mixture and pre-stress studies. By the end of the 2nd year we will be back on the schedule outlined in the original proposal.

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) Related Research:

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.

(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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Evaluation of Wetland Floristic Quality Indices as Indicators of Ecological Integrity in North Mississippi Wetlands

Basic Information

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Principal Investigators:	Gary N. Ervin

Publication

1. Bried, Jason T. 2005. Species of adult Odonata from three natural areas in northern Mississippi. Journal of the Mississippi Academy of Sciences (In press)
2. Bried, Jason T.and Gary N. Ervin. Constant abundance but variable sex ratio of dragonflies observed along a wetland buffer gradient. Wetlands (In review)
3. Bried, Jason T. and Gary N. Ervin. 2005. Distribution of adult Odonata among localized wetlands in east-central Mississippi. Southeastern Naturalist (In press)
4. Bried, Jason T. and Steve Krotzer. 2005. New species records for Mississippi: An expected dragonfly and an unexpected damselfly. Journal of the Mississippi Academy of Sciences (In press)
5. Bried, J. T., L. W. Bennett, and G. N. Ervin. 2005. Live mass and length-mass allometry of adult Odonata collected in east-central Mississippi, USA. Odonatologica 34:111-122.
6. Ervin, G. N. 2005. Spatio-temporally variable effects of a dominant macrophyte on vascular plant neighbors. Wetlands 25: 317-325.
7. Ervin, G. N. (In preparation) Experimental evidence for facilitation of plant colonization by a phalanx perennial. For submission to Plant Ecology.
8. Ervin, G. N. and B. D. Herman. 2005. An Approach to Incorporate Invasive Species and Wetland indicator Status into Wetland Floristic Quality Evaluation. Proceedings of the 35th Mississippi Water Resources Conference, April, 2005, D. McBride, ed. Mississippi State University.

9. Ervin, G. N., B. D. Herman, J. T. Bried, and D. C. Holly. (In review) Incorporating information on invasive species and wetland indicator status into wetlands floristic quality assessments. Ecological Applications.
10. Ervin, G. N., C. Anderson, and M. Smothers. (In preparation) Relative importance of wetland type vs. anthropogenic activities in determining site invasibility. For submission to Biological Invasions.
11. Herman, B. D., J. D. Madsen, and G. N. Ervin. (In review) Development of Coefficients of Conservatism for Wetland Vascular Flora of North and Central Mississippi. For submission as a report to the Mississippi Agricultural and Forestry Experiment Station.

ABSTRACT

Project title: Evaluation of Wetland Floristic Quality Indices as indicators of ecological integrity in north Mississippi wetlands

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Focus categories: WL, INV, M&P

Keywords: aquatic plants, bioindicators, biomonitoring, ecological integrity, exotic species, native species, wetlands

Technical Abstract

The proposed research will provide an efficient means by which the overall ecological status of a set of wetlands may be compared with one another and/or with known "reference" wetlands, based on the wetland indicator status of native and exotic herbaceous vascular plants present at each site. The proposed Wetlands Floristic Quality Indices (WLFQI) are based on general Floristic Quality Assessment Indices (FQAI) that have been developed and used extensively in other regions of the United States. The best index, from a set of four proposed methodologies, will be selected by comparison of WLFQI values with hierarchical rankings of study sites, based on immediate and landscape-scale impacts from anthropogenic habitat modification and use (e.g., agricultural use, urbanization, transportation routes, intense recreational use), the principal causes of habitat degradation in any ecosystem worldwide. The proposed indices range from very simple, incorporating only mean wetland indicator status and species richness, to a relatively complex index incorporating the effects of exotic species dilution of native richness.

The resulting index should prove useful in evaluating the success of wetland mitigation, monitoring and assessing restoration efforts, and in determining the impacts of other wetland management practices, such as wildlife management or incorporation of natural wetlands into wastewater treatment programs. The WLFQI is an attractive management and assessment tool because 1) herbaceous plants respond rapidly to both improvement and degradation of wetland health, integrating disturbance at numerous biological scales (from pollutant discharge to urbanization and siltation), 2) numerous regional keys exist for relatively efficient species-level identification of wetland plants (vs. identification of aquatic invertebrates, which is difficult even to the level of Family in some cases), 3) databases of wetland indicator status have been developed for each major region of the US, and 4) an index that incorporates the effects of both native and exotic species will be sensitive to varying degrees of ecological modification in natural and created wetland systems.

(1) Project title: Evaluation of Wetland Floristic Quality Indices as indicators of ecological integrity in north Mississippi wetlands

(2) Focus categories: WL, INV, M&P

(3) Keywords: aquatic plants, bioindicators, biomonitoring, ecological integrity, exotic species, native species, wetlands

(4) Duration: March 1, 2004 to February 28, 2005

(5) FY 2004 Federal funds requested Total: $ 14,851 Direct: $ 14,851

(6) Non-federal funds pledged: Total: $ 29,855
Direct: $ 17,238 Indirect: $ 12,618

(7) Principal investigator: Gary N. Ervin, Mississippi State University, Mississippi State, MS
Mississippi Water Resources Research Institute

(8) Congressional District: 3rd Congressional District

(9) Statement of critical regional water problems to be addressed:

This project will be conducted on public lands in north Mississippi that are subjected to a range of human impacts and are operated under diverse management objectives. The proposed research addresses Mississippi water research priorities by providing a mechanism by which managers and environmental monitoring agencies may directly evaluate wetland ecosystem status (degree of development of hydrophytic vegetation) and ecological integrity (proportion of exotic species within the plant assemblage), and may indirectly evaluate the interactive ecological effects of anthropogenic watershed stressors, such as water quality degradation through the response of wetland plants to point (effluent discharge) or non-point source factors (urbanization, intense agriculture).

By incorporating the multiple ecological scales above, this project also addresses South Atlantic-Gulf Region priorities including water quality and aquatic environmental protection and monitoring. This project also directly incorporates the impacts of one of the nation's most important emerging issues, the effects of exotic species on native ecosystem processes.

(10) Statement of results, benefits, and information to be provided:

This project will enhance the ability of wetland managers to carry out various aspects of wetland management : (1) comprehensive baseline assessment and monitoring, (2) simultaneous quantitative evaluation of the effects of multiple wetland stressors, and (3) consideration of wetland function (support of native hydrophytic vegetation) in comparison with reference wetlands. As such, this project will increase the efficacy with which wetland creation, restoration, or management practices within the state or region, such as mitigation banking sites or lands incorporated into the NRCS Wetlands Reserve Program, may be monitored.

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

The state of Mississippi Department of Environmental Quality and other organizations/agencies have been very involved recently in developing assessment protocols for aquatic and wetland ecosystems. Although much progress has been made nationwide in assessing aquatic systems, primarily streams and rivers, methodology for wetlands evaluation lag considerably. Some of the methods in use for quantifying wetland "health," or integrity, include the HGM Functional Index approach (Smith et al. 1995), various Indices of Biotic Integrity (US EPA 2002), and the Floristic Quality Assessment Index (Andreas and Lichvar 1995).

Floristic Quality Assessment Indices (FQAI) for evaluation of ecological integrity have been developed for a number of states whose flora are well-studied (Illinois: US EPA 2002; Wisconsin: Nichols 1999, US EPA BAWWG 2002; Ohio: Andreas and Lichvar 1995, Lopez and Fennessy 2002; and Michigan: Herman et al. 1997). These indices are attractive management and assessment tools because (Lopez et al. 2002):

1) herbaceous plants respond rapidly to both improvement and degradation of wetland health, integrating disturbance at numerous biological scales (from point-source pollutant discharge to non-point source factors such as urbanization and erosion/ siltation),
2) numerous regional keys exist for relatively efficient species-level identification of wetland plants (vs. identification of aquatic invertebrates, difficult even to the level of Family in some cases),
3) databases of wetland indicator status have been developed for each major region of the US, and
4) an index that incorporates the effects of both native and exotic species will be sensitive to varying degrees of ecological modification in natural and created wetland systems.

Floristic Quality Assessment Indices are calculated as the average per-species *coefficient of conservatism* (*C*), weighted against the square root of species richness, *N*, or

$$\text{FQAI} = \bar{C} \times \sqrt{N} = \frac{\sum C}{N} \times \sqrt{N} = \frac{\sum C}{\sqrt{N}}$$ (Andreas and Lichvar 1995).

Values for *C* are assigned based upon the origin and local or regional distribution of individual species; for example, locally abundant alien species receive very low scores (0), and rare native species receive high scores (10). For most of the U. S., however, there presently exist no comprehensive listings of flora and their distribution that could be used to rapidly develop coefficients of conservatism to be used in the calculation of FQA Indices for use in biological assessment.

Herman et al. (1997) presented an alternative index (termed *Wetness Index*) for use in assessments of wetland vegetation, based upon species' wetland indicator status (Reed et al. 1996), rather than coefficients of conservatism. In this system, each indicator status category is assigned a value from –5 (UPL) to +5 (OBL), termed wetness coefficient, *WC* (Table 1).

Whereas comprehensive records of species coefficients of conservatism are unavailable for most states, regional lists of species' wetland indicator status are available for all of the U.S. from the US Fish and Wildlife Service's Branch of Habitat Assessment (http://www.nwi.fws.gov/bha/). Thus, Wetness Index (WI) could be used similarly to FQAI to indicate the weighted proportion of species present that are adapted to wetland conditions without the need for laborious development of extensive regional lists of conservativeness coefficients.

Table 1. The five major wetland indicator status categories and equivalent wetness indices (Reed et al. 1996; Herman et al. 1997). Signs of wetness coefficients are reversed from Herman et al. (1997) to yield positive numbers for sites with a predominance of wetland species (the desirable condition).

Indicator Status	Probability of occurrence in Wetlands	Wetness Coefficient
Obligate wetland (OBL)	> 99%	+5
Facultative wetland (FACW)	67-99%	+3
Facultative (FAC)	34-66%	0
Facultative upland (FAC)	1-33%	-3
Upland (UPL)	< 1%	-5

Herman et al. (1997) further proposed that the WI should be based on wetness coefficients for native species only because most non-native species in their study area were associated with upland areas. ***However, disregard for non-native species may result in overestimation of ecological integrity, despite accurately indicating the "wetness" of the plant assemblage under investigation.*** In fact, one criticism of using wetland indicator status in the development of indices for ecological integrity of aquatic systems has been the increasing frequency with which non-native wetland-adapted species are encountered in wetlands (US EPA 2002). Exotic species include (but are not limited to) those that are recognized noxious weedy invaders that may degrade wetland ecological integrity through multiple mechanisms, and the presence of even one exotic species may have disastrous consequences for wetland health. Thus, such species should be incorporated into any proposed method of quantifying wetland health.

I am proposing to evaluate the relative effectiveness of floristic indices depicting both "wetness" and "nativeness" of wetland plant assemblages. I refer to these indices as Wetlands Floristic Quality Indices, or *WLFQI* (Note: *WFQI* presently designates the Wisconsin Floristic Quality Index).

(12) Methods, procedures, and facilities

Study Sites

Study areas for this project will include:

1) Yazoo National Wildlife Refuge (a five-refuge complex including Yazoo, Panther Swamp, Hillside, Morgan Brake, and Mathews Brake refuges), located from 4 to 40 miles east of the Mississippi River in west-central Mississippi;
2) Noxubee National Wildlife Refuge, located in east-central Mississippi;
3) Tennessee-Tombigbee waterway, backwater areas in northeast Mississippi; and
4) Tombigbee and Holly Springs National Forests, located across northern Mississippi.

These areas contain wetlands that are exposed to a variety of disturbances, including logging and recreation (area 4), impoundment, water-level manipulation, and recreational boating (areas 1 & 2), and impoundment, dredging, recreational and commercial boating, and periodic, intensive aquatic weed management (area 3), as well as some wetlands that are relatively isolated from human impacts (areas 1, 2, and 4). No fewer than 3 wetlands will be surveyed within each subunit of each site listed above (a total of 13 units, or 39 individual wetlands).

Using methodology similar to that of Lopez and Fennessy (2002, US EPA 2002; and see *Preliminary WLFQI Evaluation*, below), we will rank the wetlands surveyed based upon intensity of human impact within the immediately surrounding landscape (which has been linked to plant response at the level of species and functional guilds, Table 2; Lopez and Fennessy 2002, Lopez et al. 2002). The results of each *WLFQI* calculation will then be tested against the relative rankings of our study sites to determine the efficacy of each index at indicating impinging anthropogenic disturbance within the region, and thus, indirectly, the potential ecological integrity of wetlands. A preliminary assessment of this methodology is described in the *Preliminary WLFQI Evaluation*.

Inventories will be conducted between May 15 and August 15, 2004 by surveying a stratified series of at least four transects, oriented parallel *and* perpendicular to the primary topographic gradient in each wetland. In each transect, at least 20 quadrats (0.50 m^2 each) will be placed at 10 to 20-m intervals such that the vegetated area of each wetland is uniformly examined (Lopez and Fennessy 2002, Lopez et al. 2002). Each species encountered along these transects will be collected and identified to the lowest possible taxon (repeat visits will be made as allowable to ensure the highest percent of identifications to species). Species accumulation curves will be used to estimate the completeness of each site's inventory, and where this analysis indicates incomplete coverage, additional transects will be surveyed to ensure the fullest possible inventory of each site.

Collections will be preserved for storage in the Mississippi State University herbarium, and results of this study will be made available to each of the agencies managing our study areas (USFWS, USFS, and US Army COE) and the US EPA Biological Assessment of Wetlands Workgroup (BAWWG), in addition to reporting as required under the WRRI grants program.

The methods of *WLFQI* calculation to be tested are:

1. $WLFQI1 = \frac{\sum WC}{\sqrt{N}}$

2. $WLFQI2 = \frac{\sum WC^{*}}{\sqrt{n}}$

3. $WLFQI3 = \frac{\sum WC}{\sqrt{N}} \times \frac{n}{N}$

4. $WLFQI4 = \frac{\sum WC}{\sqrt{N}} \times \frac{\sum f}{\sum F}$,

where *WC** is the Wetness Coefficient (WC, Table 1) for native species only, *n* is the number of native species, *f* is the frequency of native species among all quadrats, and *F* is the total number of all species occurrences among all quadrats. These formulas combine attributes of the Wetness Index described by Herman et al. (1997) and the formula for *FQAI* (Andreas and Lichvar 1995). Formula 1 simply replaces *C* with *WC*, *WLFQI2* is the native-species-only index suggested by Herman et al. (1997), *WLFQI3* weights *WLFQI1* against the proportional richness of native species, and formula 4 weights *WLFQI1* against the proportional frequency of native species.

Regression analysis will be used to determine the degree to which each WLFQ Index correlates with our *a priori* site rankings, as has been done in previous work (Lopez and Fennessy 2002, US EPA 2002). The index or indices that optimally fit the habitat disturbance rankings of the study sites will be presented to regulatory and other pertinent agencies (e.g., MS Natural Heritage Program) to determine the efficiency with which each may be incorporated into assessment programs. For example, it may be that all four indices fit the disturbance rakings equally, in which case the simplest index (WLFQI1) might be most desirable because it would be most easily implemented.

Preliminary WLFQI Evaluation

Using data collected in wetland fringes of eight created impoundments and one beaver pond (representing a "reference," or least-impacted, natural wetland ecosystem) in central and north Mississippi (Brook Herman, unpublished data), I evaluated the four proposed Wetland Floristic Quality Indices to determine whether any may be appropriate for the

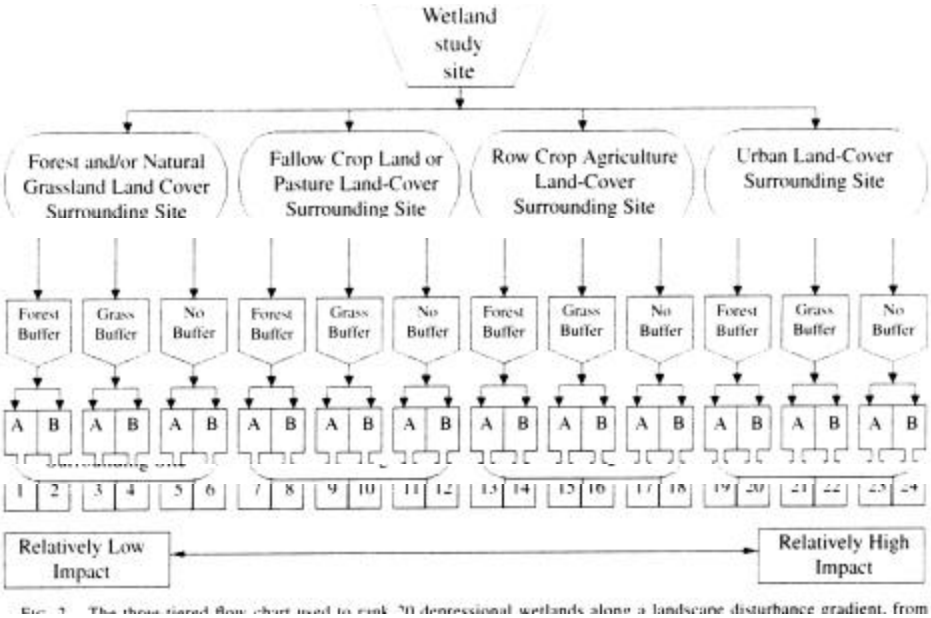

Fig. 1. Flow chart used to rank the plant communities used in the preliminary WLFQI analyses presented in this proposal. Sites were ranked based on (1) surrounding land cover, (2) type of vegetated buffer immediately surrounding the wetland zone, and (3) whether there is (A) no evidence of human hydrologic alteration or (B) clear evidence of altered hydrology. From Lopez and Fennessy (2002).

proposed research and potential application in wetlands management. Before calculation of WLFQ Indices, the sites were ranked according to the methodology of Lopez and Fennessy (2002) and US EPA (2002). This ranking was carried out using the flow chart above (Fig. 1). The resulting disturbance rankings (which ranged from 2 [the beaver wetland] to 16 [three cattle pasture farm ponds]) were then used as independent variables in regression analyses to determine the correspondence of WLFQI values with potential within-watershed human impacts on each of the wetland plant assemblages.

The results of these analyses suggest that all four proposed indices may be effective at representing anthropogenic impacts to freshwater wetlands (Fig. 2). The best Index appeared to be that which included the most information on the importance of invasive species in the habitats surveyed (WLFQI 4). This more complex Index resulted in a correlation coefficient of 73% between disturbance rank and WLFQI Score, with a highly significant statistical P-value (0.003).

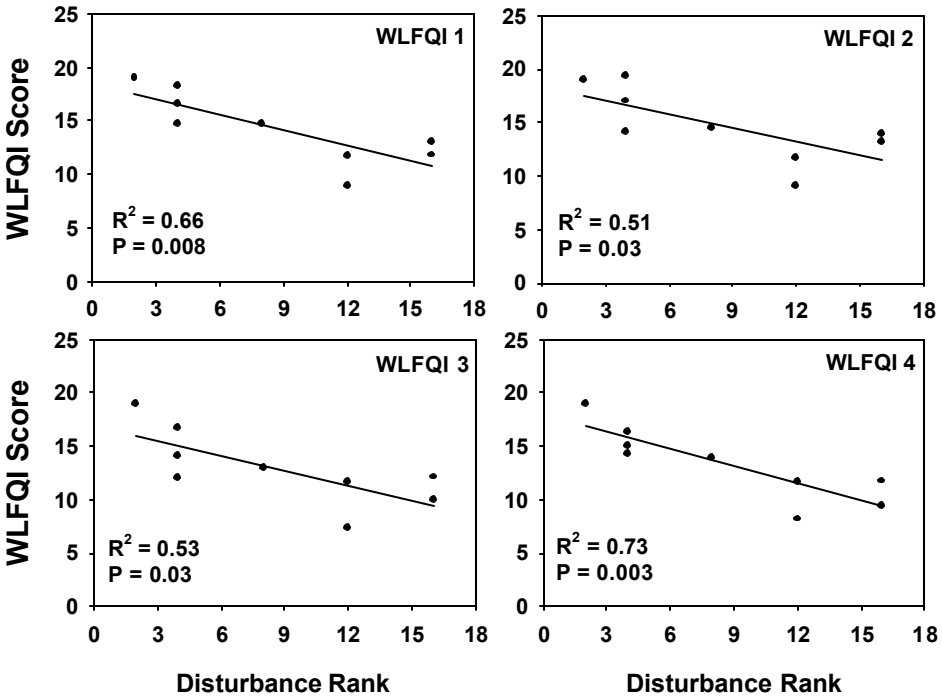

Fig. 2. Results of regression analyses of the four proposed Indices, relative to Disturbance rankings of the nine test sites. Correlation coefficients (R^2) and statistical significance are indicated in the lower-left of each panel.

Methodological concerns

One major concern is the frequently cited correlation of area surveyed with species richness. This phenomenon has been cited as one potential cause of high floristic quality scores in previous assessments (Francis et al. 2000, Matthews 2003). In one study, it was shown that FQAI values increased with area in four of the five wetland types surveyed (classified as floodplain forest, marsh, sedge meadow, and wet meadow) (Matthews 2003). However, the results of that study indicated that in the best fit species-area regression (sedge meadows), area (natural log of hectares) explained only 36% of variance in species richness (R^2 = 0.36) and had a slope of 0.17, indicating that for every additional hectare (2.47 acres) of wetland area, species richness increased by only 0.44 species (back-transformed data). In the other three wetland types, correlation coefficients (R^2) ranged from 0.05 to 0.06, and slopes from 0.05 to 0.12, indicating increases of 0.39 to 0.41 species per ha, with only ~5% correlation of richness with wetland area. These data, although indicating statistically significant relationships between

species and area surveyed, do not provide strong support for a position against using FQAI or similar indices in estimating ecosystem health or quality.

In the preliminary WLFQI evaluations presented above, I found no significant relationship between species richness and disturbance rank ($P = 0.58$) or wetland area ($P = 0.11$), although the smallest site did contain the fewest species, resulting in an apparent but non-significant richness-area trend (however, the largest site *did not* contain the greatest number of species).

Another difficulty in utilizing wetland plants as biological indicators is the slow response rate of woody vegetation to disturbance, such as altered hydrology (Cronk and Fennessy 2000). In fact, responses of trees to altered hydrology may even indicate improved growth conditions because of the alleviated stress from saturated, anoxic soils (Shawn Clark, MS DEQ, personal communication). Forested wetlands also may present another problem, as forest canopy can reduce species richness in the herbaceous understory, and areas adjacent logging operations may exhibit altered understory vegetation resulting from increased light availability, sedimentation, etc (Ron Wieland, MS Natural Heritage program, personal communication).

This research is designed to focus primarily on the herbaceous layer of vegetation because of these difficulties. The herbaceous layer responds rapidly to changes in local environment (Cronk and Fennessy 2000, personal observations), and the presence of local human-induced disturbance (such as logging operations) likely would be accompanied by immigration of exotic and/or invasive species into the herb layer. Thus, the proposed methodology likely would capture the results of any such acute within-watershed disturbances, without being influenced by potentially conflicting signals of the overstory tree canopy. Conversely, analogous natural canopy disturbances, such as the creation of a new beaver impoundment, would be less likely to result in an accompanying introduction of undesirable species, giving those areas a higher Index score than a wetland impacted by human activities.

(13) Related research

Numerous methodologies have been developed for assessing the degree of ecological degradation in human-impacted ecosystems; some of the most successful of these have been the floristic quality indices discussed above. Those indices are relatively easy to implement because of the availability of regional keys for the identification of plant species and the accessibility of regional herbaria and botanists. Indices relying upon aquatic invertebrates as biological indicators have had some success but with noteworthy difficulties in terms of the time and relatively rare expertise required to adequately identify the organisms (not to mention substantial confusion over the acceptability of different taxonomic levels of identification among groups of species; chironomids, for example).

Floristic Quality Indices were first developed in the mid-west, where they were shown to be quite accurate at quantifying expected levels of habitat degradation, using regional coefficients of conservatism for native species. These coefficients typically have been assigned to all species of a region by a panel of well-reputed botanists from a given area. Thus, the development of such a list is fairly time-consuming, initially, but needs little maintenance once established. The difficulty in using the FQI in most parts of the US is that coefficients of conservatism have not

been developed for many areas. However, in most instances where the FQI has been tested as an indicator of ecological health, the method has worked very well.

Native and restored wetlands were examined in North Dakota, using the FQI, compared with species richness and percent exotic species (Mushet et al. 2002). This study indicated that the FQI was much better at distinguishing among sites because values for both species richness and percent exotics overlapped significantly among sites, even when the sites were known to be very different in terms of ecological status (e.g., 2- to 10-year-old restored sites vs. natural wetlands >30 years old). However, another investigation of floristic quality, in Canadian natural areas, suggested that an index of wetness (utilizing the wetness coefficients in Table 1) more accurately reflected a site's hydrology than FQI (Francis et al. 2000).

Floristic Quality Indices were developed originally for use with data on native species only, and this method was justified largely on the basis that invasive species tend to be more adapted to upland conditions (less substantial reasons also have been given for the exclusion of exotic species, e.g., Wilhem and Ladd 1988). The upland nature of exotic species was indicated quantitatively by Wilhem (1992, original not seen; data included in Herman et al. 1997) and supported by Francis et al. (2000), wherein the mean wetness coefficients for adventive species were 1 to 3.5 units "drier" than those of native species across all study sites. Wetter sites displayed a larger discrepancy between native and exotic wetness coefficients than did upland sites (Francis et al. 2000). Thus, the primary argument of Wilhem against including exotics actually would result in those species' appropriately reducing the values of the indices I propose here. Their lower wetness coefficients would decrease the overall value of the WLFQI in which exotic species were included.

Although few studies evaluating the FQI have included non-native species in the index calculation, there exist numerous indications that this practice may result in the loss of much ecologically important information. Not only have exotic species been cited as indicators of ecological stress, but floristic assessment studies in Oregon mitigation wetlands found increased numbers of invasive species (not simply non-natives) in wetlands surrounded by an urban or agricultural landscape than in wetlands of undeveloped areas (Magee et al. 1999). Furthermore, it was found that more than half the species in wetlands of the Portland metropolitan area were non-native. Similarly, an examination of floral diversity in playa wetlands showed more than twice as many exotic species in agricultural (16% of species were exotic) than in grassland landscapes (6% exotic species) (Smith and Haukos 2002).

In assessing the effects of landscape modification on wetland plant assemblages, Lopez et al. (2002) found numerous correlates between land use and the relative contribution of native species. That particular study also found significant effects of land cover on wetland plant indicator status, particularly on the percent of species that were obligate or facultative wetland species (Table 2).

Table 2. Effects of within-watershed land cover on relative contribution of native and wetland-adapted species to wetland plant assemblages (Lopez et al. 2002).

Plant group	Land Cover	Direction of effect	Effect on
All Plants	Grassland	Positive	%FACW spp.
	Open water	Positive	%OBL spp.
	Forest	Positive	%Native spp.
	Agriculture	Negative	%Native spp.
All Woody spp.	Urban	Negative	%OBL
	Agriculture	Negative	%Native
All Herbaceous spp.	Grassland	Positive	%FACW & %Native
	Open water	Positive	%OBL
	Forest	Positive	%Native
	Agriculture	Negative	%OBL & %Native
Emergent Herbaceous spp.	Grassland	Positive	%FACW
	Open water	Positive	%OBL
	Forest	Positive	%Native
	Forest	Negative	%Invasive
	Agriculture	Negative	%OBL & %Native

Numerous other indices have been suggested by the EPA Biological Assessment of Wetlands Workgroup, most of which are similar to the Indices of Biotic Integrity used with data on aquatic invertebrate or fish assemblages. The major drawback of IBI-type floristic indices is that they require in-depth knowledge of individual species traits, such as persistence of plant litter, tolerance to environmental contaminants, and ecological suitability of each site for each species encountered (US EPA 2002). Such information is largely unknown for most wetland plant species, making those indices unlikely candidates for use by most state and federal environmental agency personnel. An index requiring relatively easily collected information and simple calculations, such as that presented here would likely be well-received by managers.

Although the MS DEQ has chosen the HGM Functional Index approach to assessing wetland functional integrity, this method is currently most effective only in certain wetlands of the southern portions of the state because of limited databases concerning indicator plant species for other wetland types and other regions of the state (Shawn Clark, MS DEQ, personal communication). Another drawback of using the HGM approach is the necessity of "reference" systems to use in evaluating a set of wetlands in need of assessment; determination of reference systems is problematic, even when they do exist (Smith et al. 1995). The methods outlined in this proposal could assist in determining, quantitatively, which wetland(s) within a region or landscape represent the "least impacted" state, based on biological integration, by the plant assemblage, of anthropogenic disturbance. Once a least impacted wetland or wetlands are

identified, the HGM approach could be used to evaluate others in the landscape at a finer level of resolution, based on multiple functional parameters.

Thus, the presently proposed project should succeed in providing needed assessment methodology for (Herman et al. 1997, Lopez and Fennessy 2002, Mushet et al. 2002):

1. identification of regional high quality wetlands (reference or least-impacted),
2. determination of the ecological status of a given wetland (relative to others in a region) with a low input of time and effort,
3. among-site comparison during long-term post-manipulation monitoring of created and human-altered systems (e.g., mitigation wetlands), and
4. evaluation of the response of wetland plant assemblages to management efforts.

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(15) Training potential

The project will provide the following training to students directly:

Degree level	Number	Department	Area of study
Master's degree	2	Biology	Wetlands Ecology
Bachelor's degree	1-2	various	Biology, Environmental Sciences

Two graduate students, Jason Bried and Brook Herman, are currently assisting with wetlands research in my lab, in addition to their own thesis research; these students will assist with data collection for this project. Brook Herman has been working with state botanists to develop coefficients of conservatism for a set of created wetlands she is studying for her thesis research. These interactions have generated considerable interest in her research and have indicated a desire among ecosystem managers for development of such methods for evaluating natural ecosystems in the state.

I regularly have one to three undergraduate students working in my lab, assisting with various projects being conducted by myself and the above-named graduate students. These students receive academic credit for Directed Individual Study or Environmental Sciences Practicum credit, for students enrolled in the MSU Environmental Sciences Certification Program. In preparation of required reports, these students often collect data ancillary to a project's primary mission, and most intend to use the experience as preparation for graduate or other continuing studies.

Information transfer plan

Subject matter and problems to be addressed

Two key problems will be addressed in efforts to disseminate the results of this work:

1) the increasing threat posed to natural ecosystems by anthropogenic modification of the landscape, and
2) the negative effects of exotic species on natural ecosystem processes.

This research addresses directly each of these threats and will provide a readily observable quantification of the effects of human habitat alteration and plant invasions.

Target audience, strategies to be employed, and cooperators

The information transfer process will involve multiple audiences. The general public will be targeted through literature disseminated through information outlets at each study site or via each agency (USFWS, USDAFS, USACE). Results of these studies also will be incorporated into my own teaching at the undergraduate and graduate level, through courses such as *Plant Ecology* and *Biology of Aquatic and Wetland Plants.* More technically savvy parties will be targeted through peer-reviewed journal publications and regional or national scientific conferences. Finally, the results will be communicated to regulatory agencies by interacting directly with the MS DNR and DEQ and with the US EPA Biological Assessment of Wetlands Workgroup, who currently tracks such projects in efforts to disseminate wetland management methodologies.

Water Quality Standards: Establishing Nutrient Criteria for Mississippi's Coastal Waters

Basic Information

Title:	Water Quality Standards: Establishing Nutrient Criteria for Mississippi's Coastal Waters
Project Number:	2004MS24B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	Fourth
Research Category:	None
Focus Category:	Water Quality, Nutrients, None
Descriptors:	None
Principal Investigators:	Harriet MacGill Perry

Publication

1) Title: Water Quality Standards: Establishing Nutrient Criteria for Mississippi's Coastal Waters

(2) Focus Categories: WQL, NU

(3) Keywords: Water Quality, Water Quality Standards, Coastal Zone, Estuaries, Nutrients, Eutrophication, Nitrogen, Phosphorus, Suspended Sediments, Algae

(4) Duration: 1 March 2004 - 28 February 2005

(5) Fiscal Year 2004 Federal Funds:	$28,682 Total	($28,682) Direct

(6) Non-Federal Funds Allocated:	($57,364) Total	($21,841) Direct	($25,523) Indirect	($10,000) Cooperator Contribution

(7) Names, University, City/Principal Investigators:
Harriet Perry and Christine Trigg
Gulf Coast Research Laboratory (USM)
P. O. Box 7000
Ocean Springs, MS 39566-7000

Collaborators:
Barbara Viskup
Dept. of Environmental Quality
1140 Bayview Ave.
Biloxi, MS 39530

(8) Congressional District: Mississippi, Fourth

(9) Critical State and/or Regional Problems:

Nutrient overenrichment of estuaries and nearshore coastal waters from human-based causes is now recognized as a national problem on the basis of the Clean Water Act 305b reports from coastal States that list waters whose use or uses are impaired. The National Oceanic and Atmospheric Administration's (NOAA) National Estuarine Eutrophication Assessment indicated that about 60% of the estuaries out of 138 surveyed exhibited moderate to serious overenrichment conditions. The Environmental Protection Agency (EPA) has published recommendations of water quality criteria for nutrients under section 304(a) of the Clean Water Act (66 FR 1671). States must develop water quality standards for nutrients by 2006. The EPA has proposed criteria with the intention that they serve as starting points for states to develop more refined nutrient criteria, as appropriate. States, then, have the option to develop nutrient criteria that fully reflect localized conditions and protect specific designated uses with scientifically defensible approaches as supported by EPA technical guidance manuals. To that end, the MDEQ has incorporated an aggressive monitoring and data gathering initiative into existing programs in order to provide nutrient data to support nutrient criteria development. While much has been accomplished through leveraging resources and funding from existing monitoring programs, there are still many data gaps remaining. Mississippi's estuaries are perhaps the most vulnerable and valuable of the state's waters and it is crucial that we address water quality issues in a timely and thorough manner. Mississippi's coastal ecosystems form a cornerstone of the state's economy by providing a variety of valuable resources and services. Gulf of Mexico fisheries yield more finfish, shrimp, and shellfish than the South and Mid-Atlantic, Chesapeake Bay, and Great Lakes combined. Water quality and wetlands health are vital to the maintenance of fisheries production and to the other water-dependent activities that operate within the coastal zone.

Nutrient overenrichment is a common thread that ties together a diverse suite of coastal problems including harmful algal blooms (red tides), fish kills, marine mammal deaths, shellfish poisonings, loss of seagrass and bottom shellfish habitats, and hypoxia/anoxia. The "dead zone," an area in the west-central Gulf characterized by seasonal anoxic bottom conditions, grows in size each year and is related to nutrient run-off from the Mississippi River. Thorough assessment of coastal waters and the development of clear numerical criteria that will allow discernment of natural nutrient concentrations from heightened anthropogenic concentrations in waters where increased nutrients may cause harm to the quality and

usability are critical to the evaluation and management of Mississippi's estuaries. The proposed research will provide for needed data on diel and tidal variations in nutrient concentrations and other important water quality parameters. Dissolved oxygen (DO) will be carefully monitored because adequate levels are a fundamental requirement for maintenance of populations of benthos, fish, shellfish, and other estuarine biota. Levels of dissolved oxygen are affected by environmental stresses, such as point and nonpoint discharges of nutrients or oxygen-demanding materials. In addition, stresses that occur in conjunction with low DO concentrations may be even more detrimental to biota (e.g., exposure to hydrogen sulfide, decreased resistance to disease and contaminants). Dissolved oxygen levels are highly variable over time, fluctuating widely due to tidal action, wind stress, and biological activity. One of the objectives of this study is to collect data to best represent the DO conditions in the estuaries of the Mississippi Coast. In a pilot study to evaluate the best sampling strategy for DO in Gulf estuaries, continuous meters that measured DO, percent DO saturation, salinity, temperature, water depth, and pH were deployed at eight locations over a 4-month period. Monte Carlo analysis of the eight 4-month records showed that tidal influences during summer months were small and that day-night differences accounted for most of the observed variability with wind stress accounting for most event-oriented phenomena. These analyses revealed that 1, 2, or 3 random instantaneous measures of DO were likely to mis-classify a station with unacceptable DO conditions (*i.e.*, DO <2 ppm for > 20% of time period) as acceptable at a rate of 60-70%. Furthermore, short-term continuous measures of 24, 48, and 72 hours also tended to mis-classify unacceptable sites although not as often as instantaneous DO measures (i.e., 50%). The proposed research will provide information on nutrients and associated water column parameters during high-flow/low-flow periods as well as attempt to characterize peak concentrations associated with the spring runoff. Monitoring will include: total Kjeldahl nitrogen, ammonia nitrogen, nitrite + nitrate, total phosphate, chl *a*, total suspended solids, and field parameters such as dissolved oxygen, temperature, turbidity, transparency, salinity, pH, and depth. Activities will focus primarily on the water column using protocols established by MDEQ in sampling activities supporting USEPA's National Coastal Assessment (NCA) Program. Samples will be analyzed according to an approved QAPP and defined QA/QC procedures. Following field work in Year 1, personnel will work closely with the State's Estuarine Nutrient Taskforce and the MDEQ to evaluate historical data, integrate current data into the database, statistically analyze the data, and propose reference conditions for Mississippi's coastal waters.

(10) Results/Benefits:

Water quality assessment is dependent upon adequate information for characterization of designated use. This research will provide data that will allow for the identification of minimally impacted waters defined as reference sites or least impacted waters and for the determination of impaired waters in coastal estuaries. It will provide numeric data that when combined with existing information, will allow for comparisons and statistical analyses of causal and response variables to help determine effect thresholds and further refine reference conditions. Current condition values will have to be appropriately modified on the basis of examination of the historical record, modeling, and expert judgment. The proposed project will benefit not only Mississippi but also the National Nutrient Strategy Program by providing additional water quality data from coastal waters in EPA Ecoregion XII (MS, FL and AL in the Gulf). This effort has the potential to significantly benefit these coastal states. The GCRL has established a viable collaborative partnership with these states as well as universities, EPA Regional Offices and other federal agencies through the Northern Gulf Nutrient Pilot Study. The findings of this assessment will be transferable to other estuarine/coastal water environments, within the southern states. Most importantly, the findings are of vital importance to the State of Mississippi. Expanded sampling afforded by the proposed project will supplement existing guidance and broaden the base of available information. It uses a scientifically-based sampling approach to define current water quality conditions and provides for evaluation of historic data to establish water quality in coastal waters prior to increased growth and development of the Coastal Zone. This approach will ensure that criteria developed for our estuarine waters are the result of a carefully designed program drawing upon available resources and expertise to benefit the people of the State of Mississippi.

(11) Nature, Scope, Objectives of Research

The work outlined for this project includes water quality monitoring, laboratory analyses, database development and analyses, and development of reference conditions for coastal waters. Water quality data will be collected from the waters in the Mississippi Coastal Region to support Mississippi's nutrient criteria initiative. Specific objectives are to:

1) collect high flow (spring) and low flow (summer) water samples over a 24 hour tidal cycle at nine sites; one shore and two deep water sites in each of the three coastal counties
2) analyze water samples for nitrite-nitrate, ammonia, Kjeldahl nitrogen, total phosphorus, suspended solids, and chlorophyll *a*
3) take hydrographic profiles of the water column at collection sites to include temperature, salinity, dissolved oxygen, and pH (turbidity and Secchi disc readings will also be taken)
4) evaluate historical or legacy data and integrate these data with current data in concert with the Estuarine Nutrient Taskforce and the MDEQ
5) establish numeric nutrient criteria for coastal waters

(12) Methods, Procedures, Facilities:

Field and laboratory work will be carried out by personnel of the Gulf Coast Research Laboratory in accordance with approved EPA/MDEQ methodologies and protocols. A Quality Assurance Project Plan (QAPP) for those field and analytical procedures undertaken during the proposed project is currently in place at the GCRL. Synoptic samples will be taken every 6 hours at nine sites over a 24 hour tidal cycle period during the spring and summer. A shore station and two deepwater stations will be selected in each of the three coastal counties from a list of sites with impaired water quality prepared by the Mississippi Estuarine Nutrient Taskforce. Analytical procedures will be carried out by the Water Quality Laboratory at the GCRL and by the MDEQ laboratory. An overview of field and laboratory procedures follows.

Field Water Quality Data Collection

A global positioning system will be used to locate the sampling sites. The Hydrolab DataSonde 4 water quality probe and the YSI multi-parameter 6920 and 600 XLM datasondes will be used to measure pH, temperature, conductivity, and dissolved oxygen during each sampling event. Detailed standard operating procedures for water column profiling are outlined in the GCRL Quality Assurance Project Plan for Monitoring to Establish Reference Conditions for Nutrients in Estuarine Waterbodies. Site water from target depths will be collected with a horizontal 3-liter Van Dorn sampler. Two liters of water (one liter is preserved with 5 ml concentrated sulfuric acid and one un-preserved) will be iced in the field and returned to the laboratory for nutrient analyses. The remaining liter of water is used for chlorophyll samples and turbidity. Turbidity will be determined with a LaMotte 2020 Turbidimeter.

Samples for water quality analysis will be placed and maintained on wet ice in the field. Dissolved nutrient samples will be maintained at or below 4°C until transported to the GCRL. The samples will be refrigerated upon arrival at the laboratory. Field duplicate and field blank samples will be collected at 10% of the sites to measure any variability associated with sample collection procedures. Sites for duplication will be randomly chosen.

Samples for chlorophyll *a* (chl *a*) will be filtered through Whatman® GF/F glass fiber filters (0.70 ìm nominal pore size) at the same time dissolved nutrients are collected. Sampling for chl *a* will be conducted in the shade. Syringes and forceps will be rinsed with deionized water. Site water will be placed in a container and the syringe rinsed with this water. The syringe will be filled with 60 cc of site water and the sample filtered. This step is repeated 3 more times until 4 filters have been used.(30 cc is filtered through 8 filters when the suspended solid load is too high). The filters are placed in a petri dish. The total water filtered will equal 240 cc. The volume filtered and number of filters used are written on both the petri dish and a protective foil storage bag. The petri dish is placed in the storage bag, sealed, and put in a cooler with dry ice making sure the storage bag is touching the dry ice. Samples for chl *a*

will be maintained at –50°C until analysis at GCRL. Analytical procedures will provide performance equivalent to those of the EPA's EMAP Program and the National Coastal Assessment QAPP, including those for analyses of blanks and standard reference materials. Information will be reported on recovery of spiked blanks, analytical precision with standard reference materials, duplicate analyses and blanks. A database will be maintained to manage sample tracking and laboratory results for the duration of the project.

Sample Handling, Custody Requirements, and Holding Times

Upon arrival at the GCRL, field samples are relinquished to the Water Quality Laboratory where they are logged in by laboratory personnel. The time and date received and the water temperature (temperature check bottle) are recorded on a Chain of Custody Sample Receipt Form. Samples are refrigerated and sample information is recorded on a Sample Login Form for each refrigerator. Station ID, date sampled, and analysis due date are recorded on a master Sample Check List Form. Samples are kept at #4°C, but are not frozen. During sample storage the air temperature of the refrigerator is recorded daily on a Refrigerator Temperature Record Form. Samples are removed from the refrigerator only when aliquots of the sample are taken for analysis. They are placed back in refrigeration as soon as possible in order to minimize temperature change of samples. Samples to be analyzed by the MDEQ Laboratory, Pearl, MS will be transported in coolers on ice at #4°C by GCRL or MDEQ personnel. The samples will be transferred to the MDEQ Laboratory along with the appropriate Chain of Custody and Sample Request Forms, as per GCRL and MDEQ protocols. Samples sent to the MDEQ Laboratory will be transported as soon as possible. Holding times for chemical analyses are listed in Table 1.

Table 1. Chemical methods.

Analyte	Analysis Methods	Sample Volume	Holding Times	Method Quantitation Limit
Total Suspended Solids (0.1 mg/L)	EPA Method 160.2 Residue, Non-Filterable (Gravimetric, Dried at 103-105EC)	100 L	7 days	4.0 mg/L
Total Ammonia (mg/L)	EPA Method 350.3 Nitrogen, Ammonia (Potentiometric, Ion Selective Electrode)	50 mL	28 days	0.1 mg/L
Total Nitrite + Nitrate (mg/L)	EPA Method353.3 Nitrogen, Nitrate-Nitrite (Cadmium Reduction Method)	25 mL	28 days	0.02 mg/L
Total Kjeldahl Nitrogen (mg/L)	EPA Method 350.3 Nitrogen, Ammonia (Potentiometric, Ion Selective Electrode)	50 mL	28 days	0.1 mg/L
Total Phosphate (mg/L)	EPA Method 365.2 Phosphorus, All Forms (Colorimetric, Ascorbic Acid Method)	50 mL	28 days	0.01 mg/L

General Laboratory Procedures

Water used to prepare standards and reagents is of the highest purity (18Ωohms-cm). Tap water is passed through odor and sediment filters and distilled in a Corning Megapure Distillation Unit. The distilled water is stored in a Corning Collection System. Distilled water is passed through a Barnstead Deionizer and is then polished using a Simplicity Water Purification System. Reagents used in nutrient analyses are analytical grade chemicals meeting ASC specifications. Reagent forms are kept with each analysis. Stock solutions of known concentrations are purchased for use as calibration standards and as reference samples. Glassware is cleaned in a hydrochloric acid solution. Acid-washed glassware is rinsed three times with distilled water.

Instrumentation and Equipment/Data Quality

Instruments and equipment, operation guidelines, and calibration testing procedure for the Genesys 10 Spectrophotometer are followed per manufacturer's guidelines. Instructions for instrument checkout, calibration, and maintenance are filed in the Laboratory. Analytical balances are calibrated annually, and a low range performance procedure is carried out each time a balance is used. Instrument checkout procedures are performed before each analysis, and calibrations are performed on a periodic basis. Record logs of maintenance, calibration, and performance are kept for instruments used in instrumental analysis of nutrients and solids. All data are recorded on data forms kept in a designated file within the Water Quality Laboratory. Data are recorded in Excel format. These data are checked against original data forms by the analyst, analytical QC leader, and the project manager. Data quality for total suspended solids analyses is evaluated through the use of determinations of total suspended solids on quality control samples. These include laboratory blanks (distilled, deionized water samples), field blanks, laboratory duplicate samples (randomly selected by the analyst), field duplicate samples, and reference (QC) samples. Laboratory duplicate samples are evaluated using percent difference between the duplicates and the mean value. Reference samples are evaluated using percent recovery and/or the manufacturers recommended procedures. The overall number of determinations for each quality control sample type is equivalent to a number equal to 10% of the total number of regular field samples taken.

For nutrient samples data quality is evaluated by analyzing concentration of quality control samples. Total ammonia, total phosphate, nitrate-nitrite, and Kjeldahl nitrogen are measured in samples of know concentration. Quality control samples include laboratory blanks (distilled, deionized water samples), laboratory spiked blanks, field blanks, laboratory duplicate and spiked samples (randomly selected by the analyst), field duplicate and spiked samples, and reference (QC) samples. Percent recovery of the analyte is determined on laboratory-spiked blanks, laboratory spiked samples, field spiked samples, and reference samples. Problems and concerns relating to instrument performance and analytical results are brought to the attention of the QA/QC Officer for corrective action. Results of each analysis are reviewed to determine if the analysis meets the performance criteria of the analytical method.

Chemical Analysis/Total Suspended Solids

Total suspended solids (mg/L) are determined on 100 ml of sample using EPA Method 160.2 (Residue, Non-filterable; Gravimetric, Dried at 103-105C). Gelman Type A/E glass fiber filters and Gelman filter assemblies are used in sample filtration. The vacuum assembly consists of a Gast (Model G588DX) vacuum pump and glass manifolds (Houston Glass Co.). Filters and residues are dried in a Precision Economy Oven (Model 51220131). Dried filters are kept in a Sanplatec DryKeeper desiccator or glass desiccator. An OHAUS Voyager Model V1RR80 analytical balance is used to weigh filters and residues to 0.1 mg/L. All data are recorded on a Total Solids Data Form.

Chemical Analysis/Total Ammonia

Total ammonia nitrogen (NH_4-NH_3-N, mg/L) is determined on 50 ml of sample using EPA Method 350.3 (Potentiometric, Ion Selective electrode). An Orion Model EA™ 940 Expandable Ionanalyzer with a Corning Ammonia Electrode (Model 476130) or an Orion Ammonia Electrode (Model 95-12) is used to measure total ammonia concentration of samples. The manufacturer's instruction manuals are followed for instrument check out prior to analysis, for instrument standardization, and for direct measurement of samples. Stock solutions of ammonia are purchased from suppliers and are certified as to concentration. A series of low concentration standards are prepared within the concentration range of 0.00 to 1.00 mg/L. The pH of samples and standards is adjusted immediately prior to analysis by the addition of 1 ml of 10 N sodium hydroxide. A linear regression procedure utilizing five standards is used for instrument calibration. The slope, intercept, and coefficient of determination (r^2) of the regression line are determined. If r^2 is < 0.95, new standard solutions are prepared and the instrument is recalibrated. Measurements are recorded on Ammonia Data Forms.

Chemical Analysis/Total Phosphate

After conversion to orthophosphate by the sulfuric acid-nitric acid digestion procedure (Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998), total phosphate (tPO_4-P, mg/L) is determined colorimetrically using the Ascorbic Acid Method (EPA Method 365.2). Fifty milliliters of the water sampled are digested and 25 ml of the digested sample are used for analysis. A ThermoSpectronic Genesys™ 10 Spectrophotometer with a Sipper System (Model 355982) and 1 cm flow-through cell is used to measure total phosphate concentration. Absorbance is read at 880nm. The manufacturer's instruction manual is followed for instrument check out prior to analysis, for instrument standardization, and for direct measurement of samples. Stock solutions of phosphate are purchased from suppliers and are certified as to concentration. A linear regression procedure (absorbance vs. concentration) utilizing a minimum of five standards is used to standardize the spectrophotometer. The slope, intercept, and coefficient of determination (r^2) of the regression line are determined. If r^2 is < 0.98, new standard solutions are prepared and the instrument is recalibrated. Measurements are recorded on Phosphate Data Forms.

Chemical Analysis/Nitrite-Nitrate

Nitrite-nitrate concentration (NO_2-NO3-N, mg/L) is determined by EPA Method 353.3 (Cadmium Reduction Method). Twenty-five milliliters of the water sampled are passed through a reduction column to convert nitrate to nitrite. Nitrite (NO_2-N, mg/L) is measured using a ThermoSpectronic Genesys™ 10 Spectrophotometer with a Sipper System (Model 355982) and 1 cm flow-through cell. Absorbance of standards is read at 543 nm. A linear regression procedure (absorbance vs. concentration) utilizing a minimum of five standards is used to standardize the spectrophotometer. The slope, intercept, and coefficient of determination (r^2) of the regression line are determined. If r^2 is < 0.98, new standard solutions are prepared, and the instrument is recalibrated. Measurements are recorded on Nitrate-Nitrate Data Forms.

Chemical Analysis/Total Kjeldahl Nitrogen

Total Kjeldahl nitrogen is measured using EPA Method 351.4 (Potentiometric, Ion Selective Electrode). Samples (20 ml) are digested on a RapiDigester block digester with fume extraction system (Econolab, Inc.) according to manufacturer's instruction manual. Previously, the catalyst of choice for digestion has been mercury, however, due to health risks and disposal problems, copper is used as an alternative (Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998). Following digestion, Kjeldahl nitrogen is measured following procedures for total ammonia nitrogen (EPA Method 350.3). Measurements are recorded on Total Kjeldahl Data Forms.

Chemical Analysis/Chlorophyll a

Surface, mid-water and bottom chlorophyll *a* concentrations will be determined for each site from known volumes of water filtered. Measurements will be made by using 90% acetone to extract chlorophyll from GF/F filters collected at each site. Standard Method 445.0 and a Turner Designs fluorometer unit will be used to determine chlorophyll *a* concentrations (this method has been approved by MDEQ and EPA). A spectrophotometer will be used to validate chlorophyll standards. Instrument detection limit (IDL) is ± 0.005 : g/l chl *a*; method detection limit using the present fluorometer is estimated to be ± 0.005 : g/l chl *a*; method detection limit is machine dependent. Chlorophyll will be extracted from the filters without grinding. Only values for chlorophyll *a* will be calculated and reported.

Quality assurance and control measures for each set of samples will include reagent blanks, duplicate reference samples, and calibration standards. Throughout the time frame of the project, all checks of standard reference material, a standard prepared from spinach extract (Sigma Chemical Company), will be within 5% of the calculated value. Duplicate field samples, provided by the GCRL field crew, will have values within 10% of the original duplicate replicate samples. Quality control information will include a QC check sample every 10 samples (90 to 110% recovery) and a calibration curve for the start and end of each sample run (minimum 3 point curve and a regression coefficient).

(13) Related Research:

Related research includes: 1) a nutrient survey of coastal waters conducted quarterly by the GCRL at selected sites in coastal bays and Mississippi Sound, 2) an ongoing study by MDEQ of Bayou Casotte in Jackson County, a heavily industrialized waterbody characterized by periodic elevations of some nutrients, 3) the EPA National Coastal Assessment Study which provides for the collection of water quality samples from stations in Mississippi Sound and adjacent waters during July of each year, 4) the MDEQ ambient monitoring program that is proposed to be reinstated in 2004, and 5) ongoing MDEQ studies in inland lakes and large rivers and historic data from wadeable streams. The proposed low flow/high flow diel sampling, when integrated into the current quarterly nutrient sampling program, will provide data on a critical water quality component and will ensure that criteria developed for Mississippi's estuarine waters are defensible and based on the best available data.

(14) Investigators' Qualifications:

The GCRL has established a viable collaborative partnership with neighbor states as well as universities, EPA Regional offices and other federal agencies through the Northern Gulf Nutrient Pilot Study. Project personnel have received training from MDEQ in field and laboratory protocols and the newly established GCRL water quality laboratory has an approved EPA/MDEQ Quality Assurance document in place for estuarine nutrients. Project Investigators are members of the Estuarine Nutrient Studies Taskforce and were responsible for water quality analyses associated with the Gulf of Mexico Estuarine Inventory and Study conducted in 2001 (Mississippi Tidelands Trust Fund).

(15) Training Potential

One full-time doctoral student in biological sciences and two part-time coastal sciences graduate students/employees at the Master's level will receive training during this project.

(16) Cooperator Letter (see attached)

Information Transfer

Information transfer will occur at several levels. Because states are mandated to develop nutrient criteria, information exchange and data review will take place via workshops and taskforce meetings at the state, regional, and national levels. The data collected during this study will be used by MDEQ, along with other water quality data, in their efforts to establish nutrient criteria for Mississippi's Estuarine and Coastal waters. During the process of developing nutrient criteria, MDEQ will conduct public hearings and comments will be received and considered prior to finalizing the recommendations to be submitted to EPA for final approval. Once approved, the criteria will be published in the Mississippi State Water Quality Standards and implemented. Presentation of data at scientific meetings is also anticipated.

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:	2/29/2004
Funding Source:	104B
Congressional District:	First
Research Category:	None
Focus Category:	Toxic Substances, Sediments, Agriculture
Descriptors:	None
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 58: 489-494.
2. Willett, K. L., S. Ganesan, M. Patel, C. Metzger, S. Quiniou, G. Waldbieser, and B. Scheffler. In vivo and in vitro CYP1B mRNA expression in channel catfish. Marine Environmental Research. Submitted.

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.

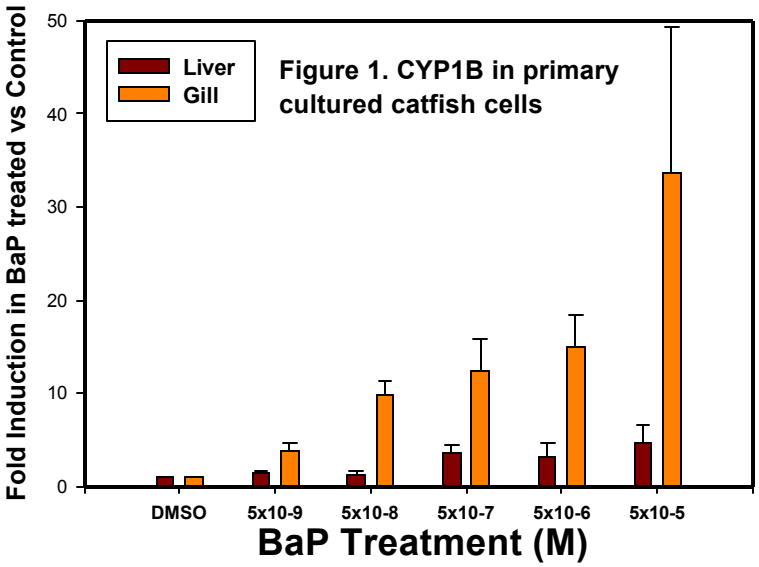
3). Characterize the utility of CYP1B1 as a biomarker of exposure compared to CYP1A and sediment concentrations in fish collected in Mississippi lakes and rivers.

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.

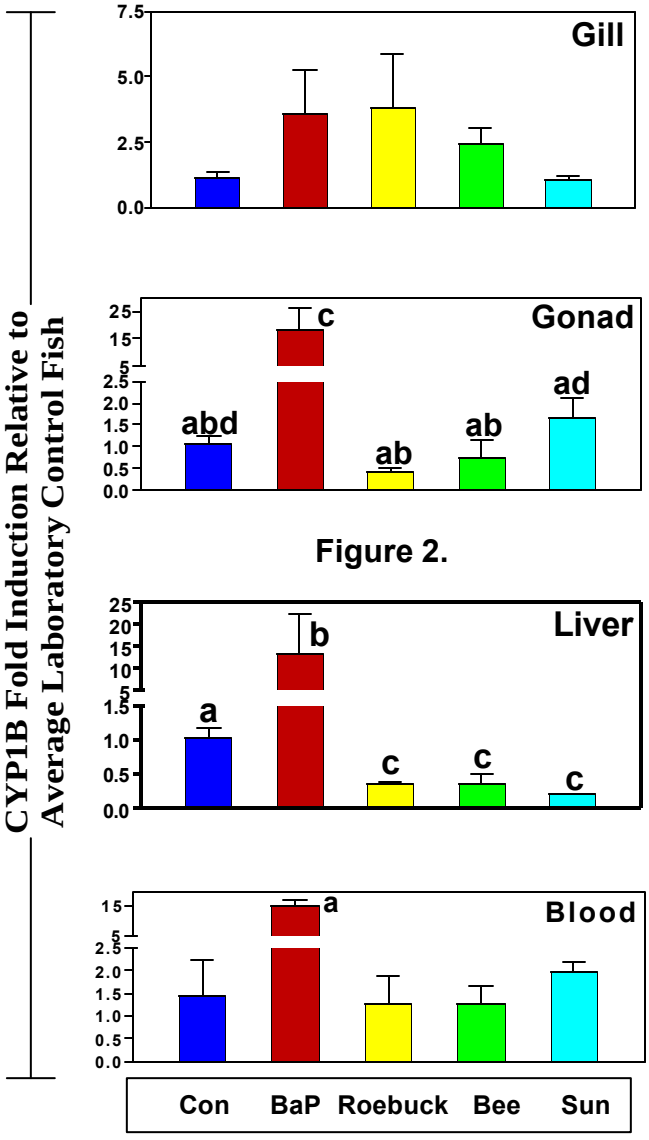


Figure 2.

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.

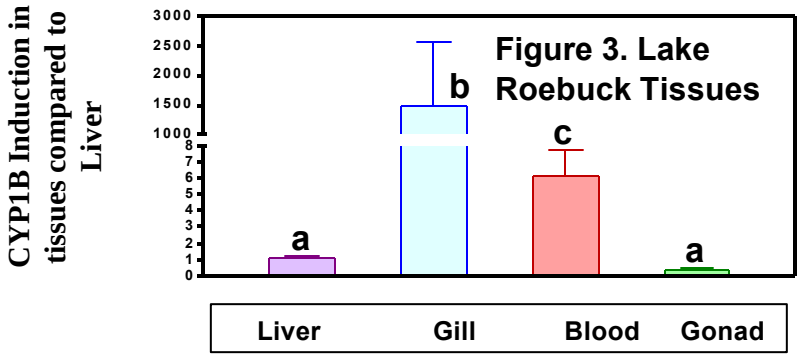


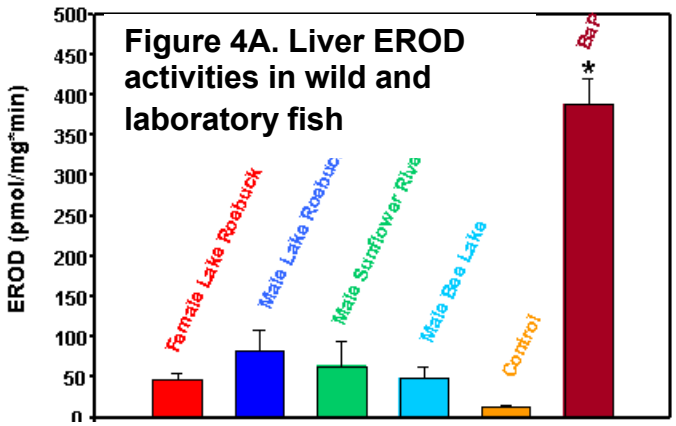
Figure 3. Lake Roebuck Tissues

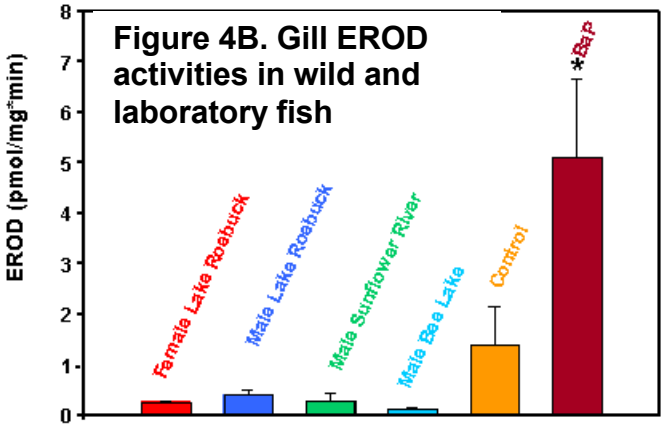
All of the PCR data in the previous figures has been normalized to the laboratory control fish within tissue. From the previous figures one has no insight into relative tissue levels of CYP1B in the various tissues from the same fish. Figure 3, however, shows that when all the tissues from the Lake Roebuck 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.





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.

Information Transfer Program

Information Transfer Program - Conferences

Basic Information

Title:	Information Transfer Program - Conferences
Project Number:	2004MS40B
Start Date:	3/1/2004
End Date:	2/28/2006
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. 1. 2004, Mississippi Water Resources Conference Proceedings, Mississippi Water Resources Research GeoResources Institute, Mississippi State, MS, 222 pgs.
2. 2. 2004, Mississippi Water Resources Conference Program and Abstracts, Mississippi Water Resources Research GeoResources Institute, Mississippi State, MS, 77 pgs.

1. 2004, Mississippi Water Resources Conference Proceedings, Mississippi Water Resources Research – GeoResources Institute, Mississippi State, MS, 222 pgs.
2. 2004, Mississippi Water Resources Conference Program and Abstracts, Mississippi Water Resources Research – GeoResources Institute, Mississippi State, MS, 77 pgs.

Informaiton Transfer Program - Newsletter

Basic Information

Title:	Informaiton Transfer Program - Newsletter
Project Number:	2004MS41B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	Third
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	Newsletter
Principal Investigators:	David R. Shaw

Publication

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

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 - Publications

Basic Information

Title:	Information Transfer Program - Publications
Project Number:	2004MS43B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	Third
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	Publications
Principal Investigators:	David R. Shaw

Publication

1. 2004, Annual Report: 2003-2004, Mississippi Water Resources Research GeoResources Institute, Mississippi State, MS, 34 pgs.

1. 2004, “Annual Report: 2003-2004”, Mississippi Water Resources Research – GeoResources Institute, Mississippi State, MS, 34 pgs.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	7	0	0	0	7
Masters	8	0	0	0	8
Ph.D.	3	0	0	0	3
Post-Doc.	0	0	0	0	0
Total	18	0	0	0	18

Notable Awards and Achievements

Ervin - Two undergraduates also assisted with the project, in conjunction with funding from a National Science Foundation Research Experiences for Undergraduates grant to the Department of Biological Sciences. These individuals were Cori Anderson from Birmingham Southern College and Melissa Smothers from Humboldt State University in CA.

Ervin - Two graduate students mentioned above, Brook Herman and Jason Bried, both completed research for their Masters theses with the assistance of funding from this project. Bried finished his degree in May 2005, and Herman is to defend her thesis in Fall 2005.

Ervin - Jason Bried, with the support from this grant, also was awarded a Society of Wetland Scientists Student Research Grant and was selected for the Best Student Presentation Award for a talk he gave at the Spring 2005 joint meeting of the South Central and South Atlantic Chapters of the SWS.

Massey - Peter Ampim, 2nd place oral competion, 2005 Mississippi Academy of Sciences.

Massey - Carefully designed and conducted studies have been conducted that will be used to improve the estimation of pesticide runoff from turf grasses grown in the Southeastern US.

Willett - The full-length catfish CYP1B cDNA was cloned for the first time (GenBank Accession #DQ088663), and it is 2417 nt to the polyA tail and encodes a putative protein of 536 amino acids. It has 67% amino acid similarity to carp and zebrafish CYP1B and 68% similarity to carp CYP1B2

Willett - To further screen other possible inducers and/or inhibitors of catfish CYP1B mRNA, in vitro studies using primary cultured cells were developed. Induction of CYP1B message by BaP showed a dose-response relationship in gill but not liver cells. When primary cultured gill cells were treated with increasing concentrations of 2,3,7,8-TCDD, and PCBs 77, 126 and 169, CYP1B mRNA was induced more than 10-fold while PCB153 and 4,4DDT did not cause significant CYP1B induction

Willett - These experiments have demonstrated that the CYP1B gene is present in channel catfish, and its transcription is inducible both in vivo (BaP) and in vitro (BaP, TCDD, and coplanar PCBs) by classical AhR ligands.

Perry - Claudia Dreszer is a German student from the University of Duisburg, Essen, Germany. Her honor's thesis research is titled "Monitoring of Hydrology, Nutrients, and Fecal Coliforms During Storm Events in the Back Bay of Biloxi, Mississippi" This study will directly compliment our USGS funded research and was conducted with guidance from the MDEQ.

Publications from Prior Projects