

Lake Pontchartrain Basin Research Program (PBRP) 2006 Annual Report

Southeastern Louisiana University
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LAKE PONTCHARTRAIN BASIN
RESEARCH PROGRAM

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Lake Pontchartrain Basin Research Program 2005 – 2006 Annual Report

Administration

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Letter from the Director

The year 2006 represented a critical phase in the five year evolution of PBRP. During the spring of the year the program experienced a comprehensive peer review that was conducted by an EPA formulated committee comprised of four environmental scientists. The review team was impressed with the overall program, and, in particular, with the quality of research conducted by the individual investigators and with the caliber and dedication of the participating graduate students. PBRP made a concerted effort in 2006 to establish a technology transfer team whose primary responsibility would be to ensure that the knowledge garnered in its projects will be disseminated widely to technical professionals in the regulatory agencies and to community leaders, as well as to other important stakeholders and decision makers, the general public, and to the news media.

PBRP hosted during the fall a retreat at the Solomon Conference Center in Robert, Louisiana. The participants were the program's Science Advisory Committee (SAC), its principal investigators and staff members. A primary objective of the retreat was to determine whether the mission and target areas of the program should be modified, especially considering the dramatic impact that hurricanes Katrina and Rita had on the Lake Pontchartrain Basin. It was decided that the program should concentrate on the environmental challenge of sustaining cypress ecosystems. The cypress tree is an icon for Louisiana wetlands and a symbol with which the public can readily identify, thereby making it easier to secure needed support among the stakeholder groups.

This report reflects the research activities of 17 projects that have been funded at a total value of approximately two million dollars. The diverse projects include efforts to determine the environmental factors that would enable the restoration of biological diversity in the wetlands of the greater Manchac ecosystem; to utilize a whole system approach for the restoration of the western Lake Pontchartrain Basin; to determine whether polycyclic aromatic hydrocarbons, a point source contaminant in the Lake Pontchartrain Basin, are stressors for lymphocyte development and/or activation; to ascertain whether genetic variation exists between specific species of fish located in the Mississippi River and the Lake Pontchartrain Basin (with regard to anticipated freshwater diversion projects); and to determine whether salinity induces stress on the endocrine system of an economically important species of turtle.

PBRP also supports essential education and outreach programs, as well as projects that focus on aspects of human behavior in the Lake Pontchartrain Basin. In particular, one such study pertains to the subject of contingent valuation and is designed to identify those factors which contribute to a citizen's willingness to pay for the restoration of wetlands associated with Lake Maurepas. Another project chronicles the historical transformation of the Manchac Basin ecosystem, as it pertains to the adverse

influence of man on the ecological degradation of the basin. The study has culminated in the production of a video titled "The Manchac Swamp: Man-made Disaster in Search of Resolution".

A critical component of PBRP is its education and outreach programs, which are designed primarily as hands-on interdisciplinary, educational experiences for both K-12 teachers and their students. The workshop oriented activities introduce participants to the ecology of the basin and attempt to instill in them the important link between the region's ecology and its cultural and economic vitality. Detailed accounts of the projects mentioned in this introduction can be found in the following report.

William N. Norton, Ph.D.
Professor of Biological Sciences
Director of the Lake Pontchartrain Basin
Research Program

General Statement

The EPA supported Lake Pontchartrain Basin Research Program (PBRP) concentrates its resources and collective expertise on investigations of the Lake Pontchartrain Basin, an ecosystem that is recognized nationally for its economic and cultural significance. The Lake Pontchartrain Basin forms one of the largest and most important oligohaline coastal ecosystems in the United States. The mission of PBRP is to determine the ecological stresses, including those associated with human behavior, on the Lake Pontchartrain Basin ecosystem and to provide scientific information to decision makers and stakeholders on methods and policies to stabilize, sustain, and/or enhance its environmental and economic recovery in a manner that is harmonious with the Comprehensive Management Plan for the restoration of the Lake Pontchartrain Basin and the Louisiana Coastal Area. PBRP strongly encourages inter-institutional and interdisciplinary collaborations as mechanisms for an integrative approach to the investigation of the Lake Pontchartrain Basin. In addition to its support of projects designed to acquire information concerning the biotic and abiotic variables of the Pontchartrain Basin, PBRP is also committed to studies that focus on possible links between the social, cultural, political, and economic history of the region and the degradation of its ecosystem.

Science Advisory Committee

PBRP is guided by an external Science Advisory Committee (SAC) that consists of twelve individuals who represent academia, federal and state agencies, the local community, and the private sector. The members of SAC provide council and guidance to the Program Director and through a peer review system, critique submitted proposals for merit and compatibility with the Program Mission. SAC makes funding recommendations to the Program Director who then finalizes the ranking and dispersion of funds to the Principal Investigators (PI's). Semi-annual progress reports are submitted by the PI's to the Director who then provides them to the members of SAC and to the EPA Project Officer for their review. Biennial oral and written progress reports are provided to SAC which meets at approximately 6-month cycles to review the progress of the projects. The members of the SAC, their profession affiliation, and respective area of expertise are listed below.

Members of Science Advisory Committee

Member	Affiliation	Expertise
Mr. Carleton Dufrechou Chairman of SAC	Lake Pontchartrain Basin Foundation	Wetland restoration and community involvement
Dr. Dale Manty	EPA, Washington DC office	Hazardous Substances
Dr. Robert Reimers	Health Sciences Center Tulane University	Bioremediation
Dr. Kenneth Teague	U.S. EPA Region 6	Wetland restoration
Mr. Dan Llewellyn	Louisiana Department of Natural Resources	Wetland ecology
Dr. Len Bahr	Coastal Activities Division Office of Governor of Louisiana	Policy/Environmental regulations
Dr. Fred Kopfler	U.S. EPA Region 4 EPA Gulf of Mexico Program	Coastal restoration
Mr. Gordon Austin	Sewerage & Water Board of New Orleans	Treatment of sewage and waste water
Dr. Mike Livingston	Sobran Environmental Consultants	Environmental toxicology
Dr. Marilyn Kilgen	Head of Biology Department Nicholls State University	Environmental microbiology
Dr. David Constant	Professor and Assistant Director EPA HSRC, LSU	Environmental engineering
Bill Hawkins	Exec. Director GCRL Univ. of Southern Mississippi	Environmental toxicology

Mission Statement

The mission of PBRP is to determine the ecological stresses, including those associated with human behavior, on the Lake Pontchartrain Basin ecosystem and to provide scientific information to decision makers and stakeholders on methods and policies to stabilize, sustain, and/or enhance its environmental and economic recovery in a manner that is harmonious with the Comprehensive Management Plan for the restoration of the Lake Pontchartrain Basin and the Louisiana Coastal Area.

Target Areas

PBRP has established central themes or target areas for investigators to address. Funded projects in Phase V (the most recent phase) were designed to:

1. Identify the various environmental factors, including biotic and abiotic stressors that both positively and negatively impact the Lake Pontchartrain Basin and determine the extent to which they affect that ecosystem.
2. Determine the social, economic and governmental factors that must be considered in order to achieve environmental recovery and sustainability of the Lake Pontchartrain Basin ecosystem.
3. Employ established and effective environmental models to determine the impact of specific variables of the ecosystem. Of particular interest are models that may be used to investigate various parameters associated with proposed fresh water diversion projects, such as the analysis of water quality, the deposition of particles, and flow rates. Principal Investigators who wish to design projects that focus on environmental models should contact the Program Director prior to the drafting of their proposal to be linked to appropriate operational environmental management models.
4. Design projects that will provide pertinent information regarding freshwater diversion programs. Examples of relevant topics include the proliferation of algal blooms, pollutant loading, nutrient loading, the impact of toxins such as benzene, heavy metals, herbicides, and pesticides, an analysis of the socioeconomic impacts, and the effect on threatened and endangered species.
5. Address factors that are specifically associated with Katrina/Rita-induced demographic changes, especially as they pertain to the North shore of Lake Pontchartrain. Examples include environmental stresses induced by significant increases in population, pollutant loading, land use, and wetland loss. Of particular importance are the effects of development and urbanization in the Pontchartrain watershed on the water quality in the estuaries.

Technology Transfer and Outreach Components

All proposals submitted to PBRP must have a Technology Transfer and Outreach section. Funded PI's are expected to address their progress in this effort throughout the project. Technology Transfer is of paramount importance since state and federal agencies are searching for the most scientifically sound methods of managing the complex ecosystems of southeastern Louisiana. This fact is especially critical in the wake of Hurricanes Katrina and Rita. The ultimate recipients of Technology Transfer and Outreach documents will be stakeholders and policy makers who have the authority or resources to promote restoration. The Principal Investigators are consistently reminded that policy makers and stakeholders can not make meaningful and appropriate decisions on the management of our wetlands, including flood protection, unless they are provided meaningful results of research and insightful analysis and recommendations from the individuals who investigate the Pontchartrain ecosystem.

Graduate Student Component

An important goal of PBRP is to train a new generation of young scientists who will continue to address significant environmental problems. Southeastern is a master's level I comprehensive regional university according to the Carnegie classification of universities and colleges. The names of the graduate students who are participating in PBRP funded research projects, their respective Major Professor (Principal Investigator), and the level of their support are presented below.

Phase I Projects

Graduate Student	Major Professor (PI)	Level of Support
Megan Collins	William Font	\$16,000
David Fox	Phil Stouffer	\$16,000
Ellen Geho	Paul Keddy	\$16,000
Brett Henry	Ann Cheek	\$16,000
Demetra Kandalepas	Paul Keddy	\$16,000
Timothy Menzel	Paul Keddy	\$16,000
Sarah Temple	William Font	\$16,000

Phase II/III/IV Projects

Graduate Student	Major Professor (PI)	Level of Support
Lisa Cordes	Kyle Piller	\$16,000
Todd Hymel	Brian Crother	\$16,000
Jessica Klopff	Penny Shockett	\$16,000
Eddie Koch	Gary Shaffer	\$16,000
Leonard McCauley	Gary Shaffer	\$16,000
Erica Perrerr	Penny Shockett	\$16,000
Joe Ramspott	Brian Crother	\$16,000
Roxanne Rudowicz	Colin Jackson	\$16,000
Tiffany Schriever	Brian Crother	\$16,000
Jack Siegrist	Paul Keddy	\$16,000
Spencer Varnado	Gary Shaffer	\$16,000
Jason Zoller	Gary Shaffer	\$16,000

Phase V Projects

<u>Graduate Student</u>	<u>Major Professor (PI)</u>	<u>Level of Support</u>
Colby Morgan	Bill Font	\$16,000
Chris Lundberg	Gary Shaffer	\$16,000

Total Number of Graduate Students in Phases I-V 21
Total Amount of Financial Support for Graduate Students \$338,000

Phase I Projects

Principal Investigators, Their Profession Affiliation, and Area of Expertise

Ann Cheek (SLU), Endocrinology/Physiology
Debbie Dardis (SLU), Science Education
William Font (SLU), Parasitology
Mark Hester (UNO), Plant Physiology
Paul Keddy (SLU), Wetland Ecology
Rob Moreau (SLU), Environmental Studies
Gary Shaffer (SLU), Wetland Ecology
Phil Stouffer (LSU), Ornithology

Research Associates, Their Professional Affiliation, and Area of Expertise

Dan Campbell (SLU), Wetland Ecology
Jonathan Willis (UNO), Environmental Toxicology
Thais Perkins (SLU), Wetland Restoration

Project Titles, Principal Investigators, and Project Budgets

<u>Project Title</u>	<u>Principal Investigator</u>	<u>Budget</u>
Effects of Multiple Stressors on Marshes and Swamps	Paul Keddy, PI	\$95,000
Ecosystem Health and Restoration Needs for Swamps	Gary Shaffer, PI	\$197,701
Constraints on Plant Establishment and Community Composition	Mark Hester, PI	\$187,011
Vegetation and Bird Communities	Phil Stouffer, PI	\$124,519
The Fish Parasite Community	William Font, PI	\$131,960
Effects of Contaminants	Ann Cheek, PI	\$121,425
Teacher Workshops and In-Service Training	Debbie Dardis, PI	\$97,196
Public Outreach and Environmental History	Rob Moreau, PI	\$23,412

Phase II/III Projects

Principal Investigators, Their Profession Affiliation, and Area of Expertise

Brian Crother (SLU), Herpetology
 Debbie Dardis (SLU), Science Education
 Cliff Fontenot (SLU), Herpetology
 Maury Howard (SLU), Environmental Chemistry
 Sam Hyde (SLU), History
 Paul Keddy (SLU), Wetland Ecology
 Colin Jackson (SLU), Microbiology
 Jay Johnson (SLU), Environmental Economics
 Kyle Piller (SLU), Ichthyology
 Rob Moreau (SLU), Environmental Studies
 Gary Shaffer (SLU), Wetland Ecology
 Phil Stouffer (Louisiana State University), Ornithology
 Penny Shockett (SLU), Immunology

Research Associates, Their Professional Affiliation, and Area of Expertise

Tiffany McFalls (SLU), Wetland Ecology
 William Wood (SLU), Wetland Ecology
 Jason Zoller (LSU), Ornithology

Project Titles, Principal Investigators, and Project Budgets

Project Title	Principal Investigator	Budget
Contingent Valuation of the Western Lake Pontchartrain Basin Ecosystem	Jay Johnson	\$30,000
Amphibian and Reptile Monitoring in the Pontchartrain-Maurepas Region	Brian Crother	\$96,000
Restoring Biological Diversity to Wetlands of the Greater Manchac Region	Paul Keddy	\$260,000
The Historical Transformation of the Manchac Basin Ecosystem: Ecological Degradation at the Hands of Man	Sam Hyde	\$65,000
Organic Matter Processing in Western Lake Pontchartrain Basin Wetlands	Colin Jackson	\$221,000
A Whole-System Approach for Restoring the Wetlands of the Western Lake Pontchartrain Basin	Gary Shaffer	\$340,000

Outreach Component for Southeastern Louisiana University Western Lake Pontchartrain Basin Research Program	Rob Moreau	\$54,000
Western Lake Pontchartrain Basin Research Program: Education Outreach Program	Debbie Dardis	\$155,000
Are Polycyclic Aromatic Hydrocarbons Stressors for Lymphocyte Development or Activation in Frog Populations in Bayou Trepagnier?	Penny Shockett	\$100,000√
Genetic Variation Between Lake Maurepas and Mississippi River Basin Fishes	Kyle Piller	\$160,000√
Heavy Metal Contaminants: A Study Investigating the Occurrence, Distribution, and Species of Trace Metal Inputs to Western Lake Pontchartrain	Maury Howard	\$75,000

Phase IV Projects

Principal Investigators, Their Profession Affiliation, and Area of Expertise

Kyle Piller (SLU), Fish Genetics
 Assaf Abdelghani (Tulane),
 Mark Hester (UL Lafayette), Wetland Ecology
 Jonathan Willis (UNO), Wetland Ecology
 Robert Moreau (SLU), Environmental Studies
 Michael Greene (SLU), Biology and Science Education
 Richard Campanella (Tulane), Geography/ GIS
 Volker Stiller (SLU), Plant Physiology
 Roldan Valverde (SLU), Animal Physiology
 Debbie Dardis (SLU), Science Education

Research Associates, Their Professional Affiliation, and Area of Expertise

Rebecca Johansen (SLU), Fish Genetics
 William Wood (SLU), Wetland Ecology
 Jonathan Willis (UNO), Wetland Ecology

Project Titles, Principal Investigators, and Project Budgets

Project Title	Principal Investigators	Budget
Establishment of Baseline Concentrations and Elucidation of Environmental Processes Controlling the Bioavailability and Bioaccumulation of Mercury and Other Toxic Metals in the Lake Maurepas Basin.	Jonathan Willis Kyle Piller, Assaf Abdelghani, Mark Hester,	\$68,000
Development of White Paper, How-To Manual, Outreach Workshops and Website for Mitigation Banking in Manchac Swamp	Robert Moreau, Michael Greene, Richard Campanella	\$77,148
Hydraulic Conductivity and Vulnerability to Xylem Cavitation of Baldcypress (<i>Taxodium distichum</i>) Along a Salinity Gradient as Indicators for Restoration Success.	Volker Stiller	\$77,500
Salinity as a Stressor of the Freshwater Turtle (<i>Trachemys scripta</i>) in the Lake Pontchartrain Basin	Roldan Valverde	\$37,500
Genetic variation between Lake Maurepas and Mississippi River Basin Fishes	Kyle Piller	\$70,000
Western Lake Pontchartrain Basin Research Program: Education Outreach Program	Debbie Dardis	\$15,000

Phase V Projects

Principal Investigators, Their Profession Affiliation, and Area of Expertise

Gary Shaffer (SLU), Wetland Ecology
 Molly McGraw (SLU), Geology
 Hassan Mashriqui (LSU), Ecological Modeling
 William Font (SLU), Parasitology
 Mike Fitzsimmons (LSU)
 Janice Bossart (SLU), Animal Behavior
 Colin Jackson (U of Mississippi), Microbiology
 Assaf Abdelghani (Tulane),
 Philip Voegel (SLU), Chemistry Pinckney,
 Sophia Passey (UT Arlington),
 Robert Moreau (SLU), Environmental Studies
 Thais Perkins (SLU), Science Communication
 Denise Rousseau-Ford (LSU), Science Communication

Research Associates, Their Professional Affiliation, and Area of Expertise

Project Titles, Principal Investigators, and Project Budgets

<i>Project Title</i>	<i>Principal Investigators</i>	<i>Budget</i>
De-energizing Storms with Cypress/Tupelo Buffers: A Plan to Restore the Repressed Swamps of the lake Pontchartrain Basin by Using Point and Non-point Freshwater Sources	Gary Shaffer, Molly McGraw, Hassan Mashriqui	\$136,318
Mitigating the Spread of Zebra Mussels into Wetlands from Mississippi River Diversions	William Font, Mike Fitzsimmons	\$88,492
Development of an Index of Biological Integrity for Lake Pontchartrain Basin Wetlands	Janice Bossart, Colin Jackson, Assaf Abdelghani	\$145,817
Determining the Potential for Algal Bloom in Lake Maurepas: Effects of Changing Nutrient Load from Freshwater Diversion and Changes in Human Population	Philip Voegel, James Pinckney, Sophia Passey	\$87,512
Technology Transfer and Outreach for the Lake Pontchartrain Basin Research Program	Robert Moreau, Thais Perkins, Denise Rousseau-Ford	\$72,179

Phase II-III Final Reports

Contingent Valuation of Western Lake Pontchartrain Basin Final Report

Dr. Jay Johnson, Loyola University New Orleans
Dr. David Bowes, Southeastern Louisiana University

Abstract

A contingent valuation survey was used to estimate an average individual's willingness-to-pay (WTP) for restoration of Maurepas wetlands. A sample of 404 participants produced an estimated WTP of \$21.41. We then calculated an aggregate value of \$35,456,095 (\$985 per acre) by Louisiana residents which substantially offsets the project cost of \$50 million. We also estimated a non-use value between \$5,051,000 and \$24,361,000 (14.3 % to 68.7% of the total). Several significant factors that contribute to these values were identified which can suggest further research strategies and outreach programs one of which is the relationship between home value and storm protection.

Primary Objectives

The primary objective of this study is to produce an estimate of the average individual or household willingness-to-pay (WTP) for restoration efforts of the Southeastern Louisiana wetlands. An average willingness-to-pay estimate can be used as important measure of the value that residents of Louisiana perceive from amenities provided by these wetlands. These values can then contribute to more thorough calculations of benefits that are gained through restoration projects that offset the restoration costs. The estimate of WTP was determined by creating a hypothetical market for a wetland restoration project using a contingent valuation survey.

There are many wetlands areas and restoration projects in Southeastern Louisiana that deliver a variety of benefits and amenities to residents. These benefits accrue to a) private owners of wetland areas, b) users of the public and private wetlands areas for activities such as hunting or fishing and c) nonusers of these areas for amenities such as hurricane and storm surge protection and wildlife habitat. The public goods character of these amenities means that much of their value cannot be observed through normal market processes since market transactions are not made for them, especially the nonuse amenities. The hypothetical market created in a contingent valuation survey can be used to assess the WTP of individuals for projects necessary to preserve or restore these benefits. For the purpose of our study we designed a survey asking about a particular wetlands area in order to create the hypothetical market. We used the river diversion project through the Maurepas Swamp and Wildlife Management Area (WMA) defined as Restoration Project PO-29 under the Coastal Wetlands Planning, Protection and Restoration Act (CWPPRA). (see Figure 1 below). This area is a prime example of wetlands loss due to earlier projects to levee the Mississippi River. It is located in the southwest quadrant of Lake Pontchartrain Basin.

Along with the primary objective of an estimate of the WTP for cost-benefit analysis, we designed the survey to collect a variety of additional information in order to

assess which factors contributed most significantly to the WTP. The length of the survey that we could use was considerably impacted by the nature of the sample population selected and the budget available for the survey. Since we wanted a random sample of the general population around the project site we could not design a survey so long that it would discourage participation in the survey unless some compensation was offered. The budget available for the study also helped determine the size for the survey area and the number of surveys that we hoped to complete. Although a larger sample size could dramatically improve the statistical power of the results, we nevertheless achieved some significant secondary results in identifying critical factors contributing to individual's perception of value from the Maurepas project. Since the non-use value of a public good is the most difficult to assess we wanted to try to calculate this value for our survey results. In order to narrow the range of the estimate more complex survey and set of questions would be required, but a estimated range can be reported from our findings.

Results

The primary objective of the survey is to elicit a response and therefore estimate an average individual's willingness to pay (WTP) for the Maurepas Swamp restoration project which can be used in further studies of wetlands benefits and in cost-benefit analyses of coastal restoration programs. The estimated WTP is \$21.41 from a random sample of 404 participants in a 16 parish area within a 90-mile radius of the center of the Maurepas project.

We used the estimated WTP to conservatively calculate an aggregated value of \$35,456,095 for the Maurepas wetlands. The aggregated value based on WTP does not include any estimate of value derived by private parties directly from the restoration project. The WTP is estimated only for a random sample of the general public of the value of the public good character of the Maurepas Swamp and Wildlife Management Area (WMA). The restoration project is estimated to cost \$50 million, so based on the \$35,456,095 estimate the project would not generate sufficient value to cover its cost. However, the public good estimate would cover a substantial portion of the cost. If private benefits and some other possible issues affecting our value estimate are considered the Maurepas could very well meet the requirement that benefits exceed cost. One key reason that a proper cost-benefit analysis might reconsider a project decision is that a significant impact on the WTP comes from the fact that many still view these projects as research efforts. If larger scale restoration projects are undertaken and prove successful, then the public can be educated about how they were successful and that is very likely to increase their assessment of WTP.

Our aggregate value based estimated WTP can be used to calculate an average value per acre. That calculation results in \$985 per acre for the approximately 36,000 acres affected by the Maurepas river diversion project. The \$985 per acre value falls within the bounds of some of the few other studies to which it may be compared.

We also found that non-use value is an important contributor to the total value of wetlands restoration. The non-use value is attributed to benefits received that are not associated with the actual consumption or use of the wetlands. An important component of non-use for coastal Louisiana wetlands is hurricane and storm protection. Unfortunately, we were unable to narrow the estimate of non-use value. Our estimated non-use value ranges from a lower value of \$5,051,000 or 14.3% to a higher value of \$24,361,000 or

68.7% of the estimated WTP. Our estimate for non-use value can be used as a reference for other restoration projects and for further research as a narrower estimate range would be useful.

One other significant factor determined from the contingent valuation study is the relationship between home value and hurricane and storm protection. For those who view storm protection as an important aspect of wetlands restoration, the higher value of their home meant a greater value to which they would be WTP for restoration. Although this effect is small now efforts to educate the general public as to how wetlands actually provide storm protection could lead to growing value in WTP in the future especially when investments in housing and incomes continue to rise in the southeast Louisiana area.

Discussion

During the period of March 27 to April 11, 2006 the contingent valuation survey was conducted by telephone from the Southeastern Louisiana University (SLU) Polling Center. Students of Dr. Kurt Corbello's Political Science courses at Southeastern were trained and used to conduct the survey for academic credit toward their studies of political polling. Twelve polling sessions were conducted including weeknights, weekend afternoons, and weekday afternoon in order to vary the sample of respondents. Approximately eleven students were available for each session lasting three hours for a total of nearly 400 student-hours. During these sessions the students completed 404 surveys from respondents willing to participate for the period of approximately 10 to 11 minutes necessary to finish the questionnaire.

The survey was conducted as a random sample of the adult population of the sixteen southeastern Louisiana parishes contained within about a ninety mile circumference of the geographic center of the Maurepas Swamp benefited by the river diversion project which is defined as PO-29 by the Louisiana Coastal Wetlands Conservation and Restoration Task Force under CWPPRA. (www.lacoast.gov/reports, accessed 10/04/2006) The Maurepas restoration project is designed to move Mississippi River water back through the swamp bringing nutrients and sediment to rebuild an area of about 36,000 acres. The center of the project was estimated to be 30° 10.3' N and 90° 37.0' W. Jefferson Parish and Orleans Parish were both excluded from the sampling pool for different reasons. Orleans was excluded because in the wake of Hurricane Katrina previous polling experience indicated that a significant portion of the population would still be unreachable by telephone. Jefferson was excluded primarily because given the size of its population the remainder of the pool would be under sampled unless the sample size was significantly increased which was not an option due to budget and time constraints. Table 2 below lists the parishes included in the sampling pool.

The results of the completed surveys were compiled into a database by Dr. Corbello and some of his students with the response for each question reported as a numerical value for a number of possible responses. We then were able to use the response data to create a number of choice variables which could be analyzed for their influence on the key dependent variable which is the respondents' willingness-to-pay for a project to restore a portion of the Maurepas Swamp. The willingness-to-pay was assessed by a set of questions about whether the participant would vote for a proposition to pay an additional amount on their state income tax in support of the 36,000-acre Maurepas Swamp project. The increments of the additional amount were five, ten, fifteen, and twenty dollars. An

additional question for those who responded in the affirmative to twenty dollars was asked about whether they would also vote for a range of twenty to forty dollars, forty to sixty dollars, or more than sixty dollars.

Of the 404 respondents 39 reported that they did not know if they would vote for a proposed income tax payment or refused to answer. However, 365 respondents did report a vote. Of these 365 responses, 57 or 15.6% reported that they would not vote for such a proposal at any payment level. These responses, of course, were coded as a WTP of zero. The remaining 84.4% of the participants reported a positive value for a income tax proposal and the mean WTP for the sampled population was \$21.41. The summary statistics for the WTP variable are reported in Table 1 below. The average value for WTP can be used to estimate an aggregate value of the public good amenities provided by this wetlands restoration project and to analyze the components of the WTP for critical issues that affect its value.

Aggregate Value

Although the sampled population is limited to only sixteen southeastern Louisiana parishes, the value of the Maurepas Swamp project is not necessarily limited to those parishes. Therefore, the aggregated value is calculated for both the entire study area and across the entire state. Some elements of the WTP estimate analysis in the section to follow may provide reasons to limit that valuation in favor of further research on the subject. We may nevertheless use these values as a benchmark for assessing the value of the project.

Based on an average WTP of \$21.41, we calculated an aggregate value for the Maurepas Swamp restoration project in two ways shown in Table 2. We attributed the average WTP to all households in Louisiana numbering 1,656,053 for a total aggregated value of \$35,456,095. This method gives the more conservative estimate of value and a value that is most consistent with the design of the survey, which asked about an individual's willingness to vote for a proposition which would include a payment of various amounts on their next state income tax return. The number on tax returns filed in Louisiana very closely corresponds to the number of households as reported by the US Census Bureau.

The estimated aggregate value is only based on the estimated WTP on the general public and does not include any private value. This value of benefits alone covers a significant amount of the \$50 million estimated costs of the Maurepas project. A complete cost-benefit analysis would also have to include any private benefits that might be derived.

The survey was designed as a household survey, but some participants of the survey are very likely to have responded as an individual, in which case the estimated WTP would have to be attributed to all voting age adults in Louisiana as an individual WTP. Hence, some tax returns would have to reflect two (or perhaps more) payments for each adult member of the household filing under that return. This method is almost certainly an over estimate of the Maurepas value, nevertheless it provides an upper bound for the general public's WTP for the restoration. This method yields of value of \$71,863,350 for the estimated 3,356,532 voting-age adults in Louisiana. A cost-benefit analysis using this measure of benefits would clearly provide a net gain over the \$50 million costs without any consideration of additional private benefits.

There are two key concerns about this method of aggregation that will be discussed more fully in the next section, but should be considered here. One concern is the fact that

our survey study area only includes participants within a 90-mile radius of the project. Our regression analysis includes an estimate of the distance of each participant from the project and that distance measure is not statistically significant which suggests that we can extrapolate the results beyond the limits of the study area. However, if we had been able to survey a much wider area it is reasonable to assume that at some point we would reach point where distance from the project does significantly reduce the WTP and would most likely diminish our estimate of aggregate value for cost-benefit purposes.

A second concern is that we found that the WTP was statistically significantly impacted by a combination of an individual's expressed support for the project as it is and those individuals who are also voicing support for continued research in wetlands restoration. We might conclude from the significance of this factor that WTP is biased upward based on expectations of a successful restoration of the Maurepas swamp. Should this project or other wetlands restoration projects fail to meet the public's expectations for success, individual WTP for wetlands restoration may fall. Thus, it is important to educate the public about what outcomes might be expected from these projects and how success is to be measured, as well as to provide information on progress toward a project's goals. Outreach and education to the public in this respect is likely to increase WTP as long as restorations efforts are working as intended.

Nevertheless, our best estimate of total value, \$35,456,095, yields a result of \$985 per acre of restored wetlands based on the 36,000 acres impacted by the project. Few studies exist to which we can compare our resulting estimate of coastal wetlands, but a recent survey study of Louisiana's Elmer's Island indicated that an appraisal value of \$862 per acre was inappropriate and a lower bound estimate should be approximately \$1700 per acre. Of course, Elmer's Island is an area with a sizeable visitation level and economic impact. Edward Barbier estimated WTP of about \$78 per acre for tropical wetlands amenities more than 10 years ago and his sample set included many areas with far lower income levels and investment than southeastern Louisiana. Therefore, our estimated WTP is within the bounds of at least some other reasonable estimates.

WTP Estimate Analysis

The questionnaire used for the survey included questions about the respondent's prior knowledge of the Maurepas Swamp and Louisiana wetlands restoration. Questions were also related to their attitudes and recreational activities regarding environmental goods and issues. Finally, a number of demographic questions were included, all for the purpose of better understanding which elements most closely correlate with or influence the estimate of WTP for an environmental good in the form of a wetlands restoration. We discovered five significant factors influencing WTP and some clues to other hypotheses that might be important for any future research. The regression analysis that best illustrates the results we discuss here is shown in Table 2.

The most statistically significant factor related to our estimated WTP is household income. Environmental goods are normal goods in that as household income increases the demand for environmental goods and quality increases. The positive coefficient on income is highly significant and is consistent with that common finding. One issue that should be noted regarding income is that raising average income levels are one of the most important factors in improvements in environmental quality. If policies are adopted that allow for continued economic progress and rising income levels in Louisiana, it will be much easier to achieve goals regarding environmental quality, including wetlands restoration.

A second significant factor influencing our WTP estimate is the number visits the respondents reported having made to environmental resources such as beaches, wetlands, and forests for recreational purposes during the previous year. This result suggests that the actual use of these resources is still a significant portion of their value as perceived by Louisianans. It is hardly surprising to observe that fact in a state where a large percentage of the population reports hunting, fishing, and other outdoor activities as a major part of their recreation. In our sample 35.9% of respondents reported having visited an environmental resource more than three times in the previous year. However, 20.7% of the respondents reported not having visited these resources at all and yet the estimated WTP for those respondents was still \$14.71 based on their support for the income tax proposal.

Based on this result we can calculate two estimates for the non-use value of the Maurepas Swamp project. Non-use value is described as the value people place on a good for its mere existence or as a benefit to their environment without directly consuming it. In this case, the Maurepas Swamp is thought to have non-use values related to wildlife habitat, hurricane and storm surge protection, and cultural values as an image that is quintessential Louisiana. The most conservative way to calculate the non-use value for the Maurepas project would be to assume that only those who reported never having visited the Louisiana environmental resources are actually expressing a non-use value in their support of payment. In that case, the estimated WTP of those who had never visited was \$14.71. Since 20.7% of the population had reported never visiting then \$3.05 ($\$14.71 * 0.207$) of our total estimated WTP of \$21.41 would be non-use value. So 14.25% of the total estimated WTP would be attributed to non-use value. For the entire Louisiana population that would result in a non-use of \$5,051,000 ($\$35,456,095 * 14.25\%$).

The previous estimate assumes that those who made a recreational visit have no value of the project from non-use. A more likely interpretation is that some of their value results from use and some from non-use. The maximum estimate of non-use value would then assume that all respondents have the same non-use value and only those who visit these resources are reporting higher values. Based on that assumption, then estimate of non-use value would be \$14.71, the estimate average WTP for non-visitors, applied to all households in Louisiana for a total of \$24,361,000 ($1,656,053 * \14.71). This range of estimates is rather large, but our survey could not include a larger variety of questions needed to narrow the range because of budget constraints. A more intensive survey program would likely be able to further analyze this factor and narrow the range of the estimate of non-use value.

Another factor related to the estimate of the WTP is the level of support for the project and the level of support for research into wetlands restoration. A regression model that includes only a variable measuring a respondent's support for the Maurepas project in particular does not yield a significant result. However, a combined variable measuring both those who support the project and those who support further research combined does suggest a positive and statistically significant affect on WTP. The result suggests that many are still considering large scale restoration projects as an experimental learning process that they do support but are obviously uncertain as to whether the results of these restoration projects will produce the outcomes expected. Nevertheless, the greater the level of support for the project itself or further research, the greater is the estimate of WTP which implies that reports of successful outcomes from restoration projects already undertaken

will likely increase the value of these wetland projects in the future as people realize that wetlands loss can actually be reversed.

Another significant factor in the WTP estimate is the value of the respondent's home. With approximately 80% of the households in the study area owning their own home, home value could have an important impact on the total value of these environmental amenities. The coefficient on the variable for home value is negative and statistically significant meaning that as home value increases their WTP for the Maurepas project is reduced. This result is not unexpected. In general, home value and income are highly correlated. Since we have already controlled for household income, then as home value increases it imposes a budget constraint on the household that is likely to reduce their WTP for other goods as in this case. However, another there is another variable that is positively related to home value and is statistically significant. The participants were asked for what reasons they believed the wetlands in Louisiana were valuable to them. Included among the possible answers were culture and tourism, hurricane and storm surge protection, and wildlife habitat. The variable which measures those who believe storm protection is important is not significant on its own. But, of those who believe that storm protection is important, the higher their home value ("homevalhur" variable in Tables 3 & 4 below) the more they were willing to pay. This result suggests that the more Louisianans become aware of how significant the wetlands are to providing our homes with storm protection and the more we invest in our homes, the more value we will place on the proximity of coastal wetlands for that protection. This result is likely the case for real commercial property and business investments as well, but the small number of respondents reporting real and business property investments in our survey was not enough for a statistically significant result. If so, further outreach and education programs that focus on and illustrate how storm protection is produced by these wetlands will likely lead to greater WTP estimates in the future.

There is another possible hypothesis that could be important for further study related to this issue. It may be that those with higher home values are willing to pay less for hurricane and storm protection through wetlands restoration not because of a budget constraint but because they have other ways to protect their home assets from damage such as better insurance policies and enforcement or investing in more storm-proof options for their own homes such as in the form of raising their homes.

There are a few other variables included in our model to estimate WTP that are not significant but need further comment. The first is distance in miles from center of the Maurepas project. The distance variable was mentioned in the previous section as important to estimate of total value by extrapolating our WTP estimate to others in the state outside of our study area. However, if hurricane and storm protection are a significant part of the wetlands non-use value and use values are also important, it is likely to assume that at some distance from the project that these values would begin to diminish. Unfortunately, the size of our sample area was sufficiently limited to prevent us from observing the distance effect. Any further studies on wetlands valuation will need to work from a design at will allow this important aspect to be addressed.

Another aspect of the evaluation of environmental amenities is what is referred to as a bequest value. One reason for preservation and restoration of the coastal wetlands in Louisiana could be that people want to leave the environment for future generations to enjoy in a way that has been important to Louisianans and their culture for many centuries.

We had thought that the bequest value may impact our estimated WTP through questions about how important cultural factors are and through households with children. Our survey indicated that only a very small percentage of participants attributed value based on cultural effects, so it had no impact. We also observed that the number of children in a household nearly had a statistically significant effect based on our 10% probability interval. However, the effect of children was to lower the estimate of WTP as evidenced by its negative coefficient. (see Table 3) A negative effect from children more than likely indicated a typical result of a budget constraint on the household, so any potential bequest effect to leave their children with approve environmental amenities is masked by the budget constraint. Further research in this area would benefit from a design to better analyze this bequest effect as an investment in a longer term future.

One final variable that should be mentioned is that of an estimate of WTP for other wetland restoration projects in Southeastern Louisiana. Although the limited statistical power of our small survey does not produce a statistically significant result, the coefficient on the WTP of other projects is negative. The negative relationship between the WTP for Maurepas and the WTP of other project indicates that if other projects are undertaken simultaneously to the Maurepas project, to which people might be expected to contribute, then they view multiple projects as substitutes for one another rather than a complementary effect on value by restoring cumulatively more acres across coastal Louisiana. Again, the coefficient on the other WTP variable is not statistically significant, but if the substitute hypothesis were correct, it would reinforce the proximity aspect of the project to the participants and, therefore, suggest that distance from the project would more significantly impact the individual assessment of their value. Clearly, the relationship between multiple restoration projects across coastal Louisiana should be studied further to better understand its effect on value.

Technology Transfer

The overall objective of the contingent valuation study is provide an estimate of valuation of a public good, wetlands, that includes the value of the wetlands perceived from the use of the wetlands asset, but also the non-use value of benefits which cannot be observed in any market activity by individuals. The result of the study is an estimate of WTP which does include that non-use value achieved by the hypothetical market created through the contingent valuation survey method. The estimated WTP and other values derived from it can then be used for better calculations in cost-benefit analyses by agencies working to make decisions on restoration projects and as evidence in support of such projects in legislative inquiries for financial resources. Proper cost-benefit analysis must include all sources of benefit, as well as the cost, and non-use values have not been included in past studies.

Further results from the contingent valuation survey suggest that a number of factors have a particularly important influence on individual's WTP, some of which directly affect individual's non-use value. These results can affect both the direction of future research and the nature of outreach and educational programs to promote wetlands restoration projects. For example, the fact that storm protection and home values combined to produce a statistically significant effect on the WTP estimate suggests that the nature of the process of storm protection should be a key factor in future research studies and that if

what is known about storm protection from wetlands were more widely communicated to the general public, the perceived value of restoration projects may significantly increase.

One final advantage of the results of this particular survey study was the experience provided to students. The process of actually conducting the survey and recording the results was undertaken by students at Southeastern Louisiana University. Their experience in conducting the CV survey is important to developing skills among future researchers in the area of survey and polling studies to the purpose of analyzing in number of other issues important to Louisiana's future.

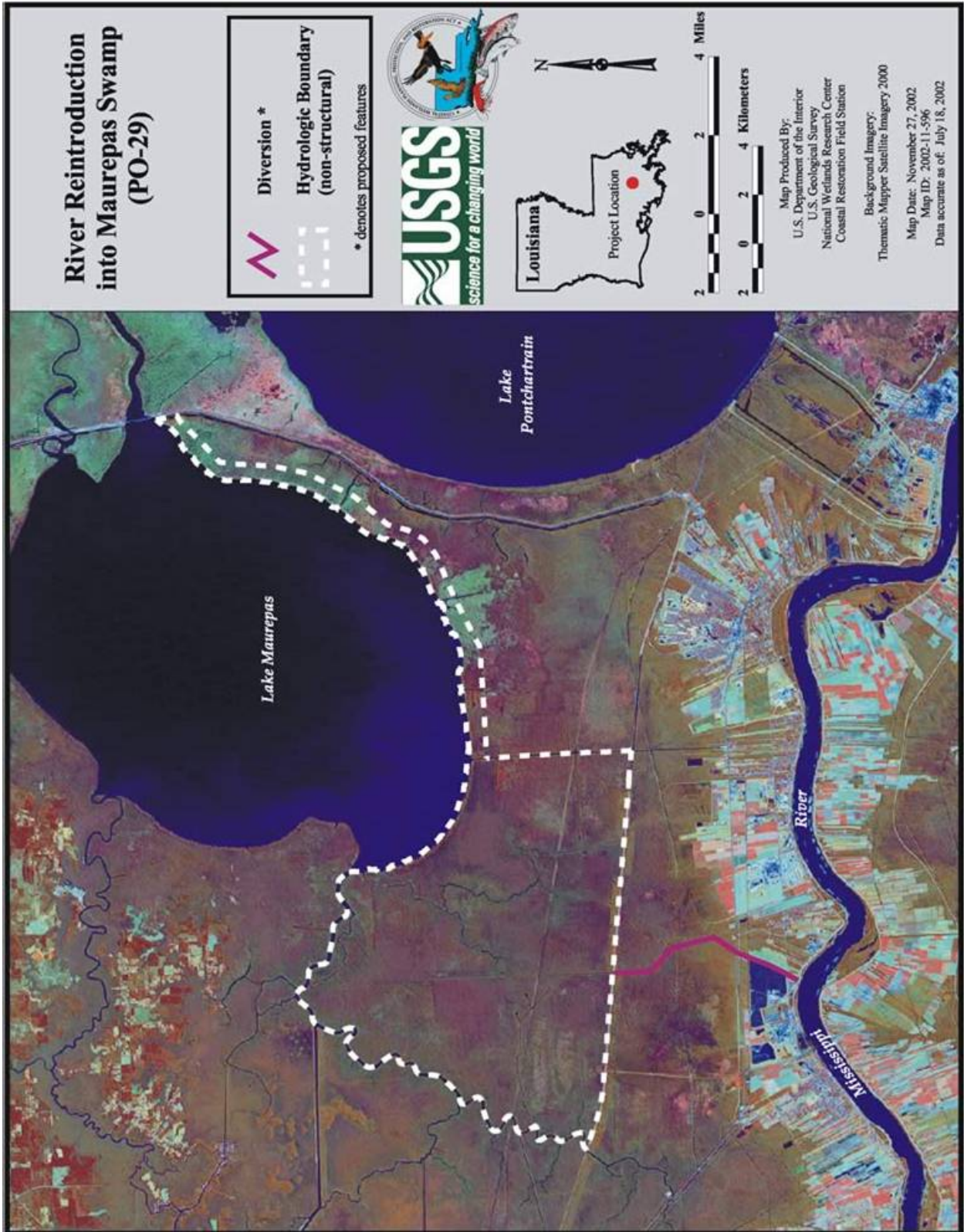


Figure 1

Tabulation and Summary of Responses to WTP

Tabulation of variable WTP			
wtp	Freq.	Percent	Cum.
0	57	15.62	15.62
5	21	5.75	21.37
10	71	19.45	40.82
15	51	13.97	54.79
20	40	10.96	65.75
30	59	16.16	81.92
50	33	9.04	90.96
61	33	9.04	100.00
Total	365	100.00	

Another 39 respondents reported they "did not know" or refused to answer.

Summary of WTP					
Variable	Obs	Mean	Std. Dev.	Min	Max
wtp	365	21.40548	18.62381	0	61

Table 1

Estimate of Total Benefits by Aggregation of Households and Adult Individual WTP

Parish Name	WTP	Households	Adult Pop.	Household Value	Individual Value
Ascension		26,691	65,161		
Assumption		8,239	17,188		
East Baton Rouge		156,365	308,974		
East Feliciana		6,699	15,867		
Iberville		10,674	24,451		
Lafourche		32,057	69,042		
Livingston		32,630	80,048		
St. Charles		16,422	36,861		
St. Helena		3,873	7,551		
St. James		6,992	15,418		
St. John		14,283	33,032		
St. Tammany		69,253	163,018		
Tangipahoa		36,558	78,705		
Terrebonne		35,997	78,146		
Washington		16,467	33,333		
West Baton Rouge		7,663	16,031		
Study Area Total	\$21.41	480,863	1,042,827	\$10,295,277	\$22,326,926
Louisiana	\$21.41	1,656,053	3,356,532	\$35,456,095	\$71,863,350

Table 2

Table of Regressions Results for Estimate of WTP

Regression of WTP on income wtp2 homeval real child single contrib visit concern priority hur wl informed swamp research gender homevalhur distance, noconstant

Source	SS	df	MS	Number of obs = 300		
Model	163909.477	18	9106.08206	F(18, 282) =	30.70	
Residual	83632.523	282	296.56923	Prob > F =	0.0000	
-----				R-squared =	0.6621	
-----				Adj R-squared =	0.6406	
Total	247542	300	825.14	Root MSE =	17.221	

wtp	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
income	.0001707	.0000496	3.44	0.001	.0000731	.0002684
wtp2	-.148213	.1680724	-0.88	0.379	-.4790487	.1826226
homeval	-.0000439	.000025	-1.75	0.081	-.0000932	5.40e-06
real	-.7350187	2.923568	-0.25	0.802	-6.489805	5.019768
child	-1.670532	1.034411	-1.61	0.107	-3.706679	.3656154
single	1.325399	2.282501	0.58	0.562	-3.167503	5.818302
contrib	3.550197	2.976618	1.19	0.234	-2.309012	9.409407
visit	1.751202	.8420187	2.08	0.038	.0937621	3.408641
concern	1.731937	2.166919	0.80	0.425	-2.533453	5.997328
priority	3.061469	2.31317	1.32	0.187	-1.491803	7.61474
hur	-4.009654	3.520847	-1.14	0.256	-10.94013	2.920822
wl	.9295225	2.258762	0.41	0.681	-3.516651	5.375696
informed	-2.479807	2.182739	-1.14	0.257	-6.776336	1.816721
swamp	2.283148	2.195538	1.04	0.299	-2.038574	6.60487
research	11.89186	3.442169	3.45	0.001	5.116257	18.66747
gender	-1.970743	2.339828	-0.84	0.400	-6.576489	2.635003
homevalhur	.0000555	.0000276	2.01	0.046	1.12e-06	.0001099
distance	.0039452	.0889116	0.04	0.965	-.1710694	.1789598

Table 3

Table of Description of Variables with number of Observations and Mean Values

Variable	Description of Variable	Obs	Mean
wtp	Willingness-to-pay	365	21.40548
income	Household income	350	48942
wtp2	Willingness-to-pay for other LA projects	404	2.997525
homeval	Value of participant's home	367	114877.4
real	Value of participant's real commercial property	390	.2025641
child	Number of children in the household	400	.625
single	Marital status dummy (married or unmarried)	398	.4547739
contrib	Contribute to other environmental organizations	383	.156658
visit	# of annual visits to environmental resources	401	2.031172
concern	Highly concerned about LA environmental quality	404	.4752475
priority	Higher priority to wetlands restoration projects	404	.6633663
hur	Equal or more important value is storm protection	404	.7450495
wl	Equal or more important value is wildlife habitat	404	.6683168
informed	Considered highly informed about wetland issues	404	.4752475
swamp	Familiar with the Maurepas swamp particularly	404	.5470297
research	Either supports project or further research	404	.8886139
gender	Gender dummy	404	.4925743
homevalhur	Storm protection and home value interaction	367	91934.6
distance	Household Distance in miles from Maurepas project	404	32.52915

Table 4

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Maurepas Swamp Survey Instrument

TELE #: _____
[ON SAMPLE SHEET]

STATION #: [] [] [] []
[ON COMPUTER]

RESP # [] [] [] [] [] []
[ON SAMPLE SHEET]

[GREETING] Hello! May I please speak to _____?

[WHEN RESPONDENT COMES TO THE PHONE...]

Mr./Mrs./Ms. _____, My name is _____, and I'm calling from Southeastern Louisiana University. Since Hurricane Katrina, we are conducting a confidential survey of randomly selected voters in your area concerning loss of natural resources and, if we may, we would like to get your feelings and opinions. The survey should take about ten minutes. Can you participate?

Yes [IF "YES"]---"I really appreciate your help, and please keep in mind that if you wish not to answer a particular question, please say so. First, [GO TO Q1]..."

No [IF "NO"]---"I'm sorry for bothering you. Have a good evening!"

[IF RESPONDENT IS UNAVAILABLE OR CAN'T PARTICIPATE RIGHT NOW, ARRANGE A CALL-BACK]

Q1. ... in an average year, about how often do you visit beaches, rivers, wetlands and forests for recreational purposes? Is it once a year, about two or three times a year, more than three times a year, or never?

- | | | |
|---------------------------------------|------------------|------------------|
| _____ 1. Once a year | _____ 8. DK/Rem. | _____ 9. Refused |
| _____ 3. Two or three times a year | | |
| _____ 5. More than three times a year | | |
| _____ 7. Never | | |

Q2. Now, I'd like for you to picture a numerical scale from 1 to 5, with 1 being very low and five being very high. How would you rank your concern about environmental quality in Louisiana? Or are you not concerned at all? [CIRCLE ONE]

- | | | | | | | | |
|----------|---|---|---|-----------|---------------|------------|---------|
| 1 | 2 | 3 | 4 | 5 | 7 | 8 | 9 |
| Very Low | | | | Very High | Not Concerned | Don't Know | Refused |

Q3. On the same kind of scale, with 1 being the lowest and five being the highest, relative to other priorities that you have for Louisiana, how high of a priority do you think it should be for Louisiana to preserve and restore its' wetlands? Or should it be no priority at all? [CIRCLE ONE]

- | | | | | | | | |
|-----------------|---|---|---|------------|-------------|------------|------------|
| 1 | 2 | 3 | 4 | 5 | 7 | 8 | 9 |
| Lowest | | | | Highest | No Priority | Don't Know | Refused |
| [GO TO Q4.....] | | | | [GO TO Q5] | | [GO TO Q5] | [GO TO Q5] |

Q4. Wetlands might be valuable for a variety of reasons. In your mind, which of the following ranks as the most important reason to place a value on wetlands? Is it culture and tourism, is it hurricane and storm surge protection, is it wildlife habitat, are all equally important reasons, or are none of them important?

- | | | |
|---|---------------------|------------------|
| _____ 1. Culture and tourism | _____ 8. Don't Know | _____ 9. Refused |
| _____ 3. Hurricane and storm surge protection | | |
| _____ 5. Wildlife habitat | | |
| _____ 6. All equally important | | |
| _____ 7. None important | | |

Q5. On a scale of 1 to 5, with 1 being little and 5 being very highly, how informed are you regarding the loss of wetlands in Louisiana? Or are you not informed at all? [CIRCLE ONE]

- | | | | | | | | |
|-----------------|---|---|---|----------------------|------------|------------|---------|
| 1 | 2 | 3 | 4 | 5 | 7 | 8 | 9 |
| Little Informed | | | | Very Highly Informed | Not At All | Don't Know | Refused |

Q6. On a scale of 1 to 5, with 1 being the lowest and 5 being the highest, as far as you know, how effective is fresh water diversion from rivers in rebuilding wetlands in coastal Louisiana? Or is it not effective at all? [CIRCLE ONE]

1 2 3 4 5 7 8 9
Little effective Very effective Not effective at all Don't Know Refused

Q7. Are you familiar with Maurepas Swamp?

_____ 1. Yes _____ 8. Don't Know _____ 9. Refused
_____ 3. No

Q8. Maurepas Swamp is about 36,000 acres of wetlands west of Lake Pontchartrain, and is losing hundreds of acres to subsidence every year. In general, would you support the idea of diverting fresh water from the Mississippi River to restore damaged wetlands and increase wetland acreage in Maurepas Swamp by about 5,400 acres? [PAUSE] Do you feel strongly about this or not?

_____ 1. Support, str. [GO TO Q9] _____ 8. Don't Know _____ 9. Refused
_____ 3. Support [GO TO Q9] [GO TO Q8a] [GO TO Q8a]
_____ 5. No support [GO TO Q8a]
_____ 7. No support, str. [GO TO Q8a]

Q8a. Would you support research into the idea? [PAUSE] Do you feel strongly about this or not?

_____ 1. Support, str. _____ 8. Don't Know _____ 9. Refused
_____ 3. Support
_____ 5. No support
_____ 7. No support, str.

Q9. Now, I'd like for you to consider the following hypothetical proposition. Please try to answer as if this was an actual proposal and your answer is going to result in state policy. Suppose the Louisiana Legislature wants you to vote on a plan to pay for restoring and rebuilding wetlands in Maurepas Swamp. This plan would require you to pay an extra TEN dollars on your state income tax returns for the sole purpose of funding preservation and restoration projects in the Maurepas Swamp area. Would you vote for this proposition?

1. Yes [GO TO Q9a] 8. Don't Know 9. Refused
 3. No [GO TO Q9d] [GO TO Q9d] [GO TO Q9d]

Q9a. Would you still vote for this proposition if the payment were FIFTEEN dollars?

1. Yes [GO TO Q9b] 8. Don't Know 9. Refused
 3. No [GO TO Q9E] [GO TO Q9E] [GO TO Q9E]

Q9b. Would you still vote for this proposition if the payment were TWENTY dollars?

1. Yes [GO TO Q9c] 8. Don't Know 9. Refused
 3. No [GO TO Q9E] [GO TO Q9E] [GO TO Q9E]

Q9c. What is the maximum amount of money you would be willing to pay for a proposition to preserve and restore Maurepas Swamp? Would you pay nothing more? Would you pay from \$21 to \$40, from \$41 to \$60, or more than \$60?

1. Nothing more [GO TO Q9E] 8. Don't Know [GO TO Q10]
 3. \$21 to \$40 [GO TO Q10] 9. Refused [GO TO Q10]
 5. \$41 to \$60 [GO TO Q10]
 7. More than \$60 [GO TO Q10]

Q9d. Would you vote for this proposition if the payment were reduced to FIVE dollars?

1. Yes [GO TO Q9E] 8. Don't Know 9. Refused
 3. No [GO TO Q10] [GO TO Q10] [GO TO Q10]

Q9e. Given the amount you've said you would pay on behalf of Maurepas Swamp, if this proposal included more wetlands across the state, would you pay nothing more, would you pay up to \$5 more, up to \$10 more, up to \$15 more, up to \$20 more, or more than \$20 more?

1. Nothing more [GO TO Q10]
 2. Up to \$5 more [GO TO Q10]
 3. Up to \$10 more [GO TO Q10]
 4. Up to \$15 more [GO TO Q10]
 5. Up to \$20 more [GO TO Q10]
 6. More than \$20 more [GO TO Q10]
 8. Don't Know [GO TO Q10]
 9. Refused [GO TO Q10]

Q10. Now, I'd like to close quickly with some important demographic questions that will help us to analyze our data. First, are you currently a member of any environmental, conservation, hunting or fishing organization?

1. Yes [GO TO Q10a THRU Q10f...]
 3. No [GO TO Q11]
 8. Don't Know [GO TO Q11]
 9. Refused [GO TO Q11]

Q10a. Do currently you belong to "Save Our Lake" (Lake Pontchartrain Basin Foundation)?

1. Yes
 3. No
 8. Don't Know
 9. Refused

Q10b. What about the Sierra Club?

1. Yes
 3. No
 8. Don't Know
 9. Refused

Q10c. Ducks Unlimited?

1. Yes
 3. No
 8. Don't Know
 9. Refused

Q10d. World Wildlife Fund?

1. Yes
 3. No
 8. Don't Know
 9. Refused

Q10e. Greenpeace?

1. Yes
 3. No
 8. Don't Know
 9. Refused

Q10f. Do you currently belong to any other environmental, conservation, or hunting and fishing organization?

1. Yes
 3. No
 8. Don't Know
 9. Refused

Q11. In the past year did you contribute any money, other than any membership fees, to environmental, conservation, hunting or fishing organizations or causes?

1. Yes [GO TO Q11a]
 3. No
 8. Don't Know
 9. Refused

Q11a. About how much did you contribute? Was it \$10 or less, was it from \$11 to \$20, from \$21 to \$50, from \$51 to \$100, or more than \$100?

1. < \$10
 2. \$11 to \$20
 3. \$21 to \$50
 4. \$51 to \$100
 5. > \$100
 8. Don't Know
 9. Refused

Q12. What is your current marital status? Are you married, divorced, separated, widowed, or have you never been married?

1. Married
 3. Divorced
 5. Separated
 6. Widowed
 7. Never married
 9. Refused

Q13. Currently, what is your primary employment status outside of the home? Are you employed full-time, part-time, retired, or unemployed?

1. Full-time
 3. Part-time
 5. Retired
 7. Unemployed
 9. Refused

Q14. How many dependent children do you have, under the age of 18?

- 1. One
- 2. Two
- 3. Three
- 4. Four
- 5. Five
- 6. More than five
- 7. No dependents
- 9. Refused

Q15. Is your annual household income \$20,000 or less, from \$21,000 to \$40,000, from \$41,000 to \$70,000, from \$71,000 to \$100,000, or more than \$100,000?

- 1. \leq \$20,000
- 2. \$21th. to \$40th.
- 3. \$41th. to \$70th.
- 4. \$71th. to \$100th.
- 5. $>$ \$100th.
- 8. Don't Know
- 9. Refused

Q16. Do you own the home in which you live?

- 1. Yes [GO TO Q16a]
- 3. No [GO TO Q17]
- 9. Refused [GO TO Q17]

Q16a. Is the value of your home less than \$50,000, from \$50,000 to \$99,000, from \$100,000 to \$149,000, from \$150,000 to \$249,000, or is it \$250,000 or higher?

- 1. Less than \$50 TH
- 2. \$50 TH to \$99 TH
- 3. \$100 TH to \$149 TH
- 4. \$150 TH to \$249 TH
- 5. \$250 TH or Higher
- 8. Don't know
- 9. Refused

Q17. Do you own other real property such as a farm, commercial building, etc.?

- 1. Yes [GO TO Q17a]
- 3. No
- 9. Refused

Q17a. Is the value of your farm or commercial property less than \$100,000, from \$100,000 to \$249,000, from \$250,000 to \$499,000, from \$500,000 to \$999,000, or is it \$1,000,000 or higher?

- 1. Less than \$100 TH
- 2. \$100 TH to \$249 TH
- 3. \$250 TH to \$499 TH
- 4. \$500 TH to \$999 TH
- 5. \$1 MILLION or Higher
- 8. Don't know
- 9. Refused

This is the end of our survey. Again, I'm from Southeastern Louisiana University and I thank you for your help. Have a great evening (day)!

Amphibian and Reptile Monitoring in the Ponchartrain-Maurepas Region

EPA Annual Report

Brian I. Crother and Clifford L. Fontenot
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Hammond, LA 70402

Abstract

Amphibians and reptiles are good bio-indicators of environmental health because they are in the middle of the food chain, as both predators and prey. In addition, the larval and adult life of amphibians is tied to water, particularly reproduction. Consequently, changes in water quality are often reflected by changes in amphibian populations. This study monitors amphibian and reptile populations in the Lake Ponchartrain-Maurepas Region so that changes in their ecology can be detected. Species lists, relative abundance, and other ecological data are presented, including data on salinity tolerance in Green Treefrogs (*Hyla cinerea*).

Primary Objectives

This study monitors amphibian and reptile populations in the Lake Ponchartrain-Maurepas Region so that changes in their ecology can be detected.

Results

Herpetofauna Monitoring of Ponchartrain-Maurepas Region

A standard transect approach has been undertaken in the Lake Maurepas region (Alligator Island) and in the Pontchartrain region (Manchac swamp) to survey herpetofaunal species diversity and population density estimates. The premise of this work is that amphibians act as the environmental “canary in coal mine” so monitoring their abundances should tell us something about the health of the marsh over the long term. The majority of the survey takes place in the Manchac Wildlife Management Area in St. John the Baptist Parish, Louisiana. The only other project of its kind in this area was in 1989. Transects are in swamp (1 site), levee (2 sites), and marsh (4 sites) habits and monitored monthly by a diurnal and nocturnal sampling period to account for different activity patterns. The monitoring began in October 2002 and has continued with the previous stated methodologies (see previous annual reports and proposals). Amphibian and reptile monitoring surveys are part of a long-term project with no set end date.

In short, the results for species composition leveled off, yet a single new addition of the fauna is reported. *Hyla squirella* was not on the Platt et al. 1989 survey, but was incidentally found calling near one marsh site. In terms of abundances of the most common species founds along the transects, there were no significant changes.

Highway 51 Road Survey

Highway 51 was driven from Ponchatoula to LaPlace and back every third night from January 2004 through June 2005 in search of amphibians and reptiles on the road. Relative abundance showed that *Nerodia fasciata* comprised 61% of the snake assemblage, followed by *Thamnophis proximus* (16%), *Pantherophis obsoletus* (6%), and *N. cyclopion* (4%); other species collected were in relatively low numbers. Details of the data set are pending publication.

The survey produced data (body sizes, location, habitat, etc.) on 296 individuals comprising species of 8 frogs and toads, 12 snakes, 1 turtle and 1 alligator. Figure 1 shows counts of these animals, and whether live or dead. Although most of the animals on the road had been killed by vehicles, dead animals stay on the road longer than live ones and so were more likely to be encountered. Road surveying is a unique method and is biased (as are all methods) by each species' particular behavior, resulting in a different representation than shown by our transect surveys above, where the surveyor walked transects through habitat in search of animals. However, the different methods are complimentary and give a more complete view of the herpetological community.

Effect of Salinity on a Common Frog Species

Salinity tolerance of one of the most abundant frogs in the Manchac swamp area, *Hyla cinerea* (Green Treefrog), is currently being investigated. Saltwater intrusion is among one of the highly detrimental factors contributing to wetland loss in the Lake Pontchartrain area. Studies have documented salinity effects on plant communities in Southeastern Louisiana, but none have dealt with its effect on the anuran community. Anurans are ideal for such a study because of their importance as indicators of environmental health.

This study investigates salinity tolerance by conducting a lethal concentration test of *H. cinerea* larvae as well as look at developmental rate and other effects on ontogeny of the two populations. One population is prone to saltwater intrusion from Lake Pontchartrain (Manchac area) and considered intermediate salinity marshland. The second population is freshwater (West Lake Maurepas) and supports a healthy swamp. Seven salinity levels will be used with three replicates of each for each population.

The lethal concentration test showed divided results between the predominately freshwater site and intermediate salinity site (Fig. 2). Both populations exhibited minimal mortality at 0 and 2ppt, no mortality at 4 and 6ppt. At 10 and 12ppt both populations experienced 100% mortality within 48 to 96 hours. However, at 8ppt a noticeable difference between the populations occurred. For instance, the Manchac population experienced 5% mortality while the West Lake Maurepas population experienced 50% mortality. Developmental rate in the larval stages 17 through 25 (Gosner 1960) was investigated under the previous seven salinity levels. Tadpoles developed to stage 25 in all treatments except in 10 and 12ppt for both populations. Rates were nearly homogeneous for both populations averaging 96 to 120 hours except for 0, 4, and 8ppt for Manchac population, which took 144 hours for complete development.

A similar test investigated salinity tolerance and developmental rate of tadpoles between the stages of 25 and 46 (complete metamorphosis). Salinity levels used are the same except 12ppt was excluded since 100% mortality would have occurred and no information would have been gained from that concentration. One hundred percent mortality occurred at 10ppt in 96 hours for both populations. However, high mortality has occurred at all salinity levels and after 524 hours only a few individuals in 2 and 4ppt for Manchac and 2, 4, and 6ppt for West Lake Maurepas are still alive. A curious observation is no tadpole has progressed past the starting stage of 25 during the 524 hours.

Technology Transfer

- 1. In 5 sentences or less, please describe the overall goal of the project.*

There were three goals to this project. 1) Initiate long-term surveys of reptiles and amphibians in open marsh (Manchac) and in wooded swamp (Alligator Island) to monitor species number and density changes. 2) Initiate long term survey of reptiles and amphibians in long transect between Maurepas and Manchac, 3) Determine salinity tolerance and effects on a frog common in saline marsh and inland freshwater habitats.

- 2. List the restoration or sustainability questions posed by the study*

Can frogs continue to persist in increased saline conditions in Manchac marsh?

- 3. If possible, please list the hypothesized answers to the above questions.*

No.

- 4. What sorts of management recommendations/implications do you hope to make from this study?*

Somehow inhibit saltwater intrusion into the marsh, probably by blocking off ditches that run into the marsh.

- 5. Please list the (a) agencies and geographies (parishes, etc.) and (b) impacted stakeholders (hunters, shrimpers etc.) that will be affected by this study.*

LA Wildlife and Fisheries; St. John the Baptist, Tangipahoa; hunters

In addition to your responses to the questions and statements listed above, which are identical to those that you addressed in your report for last year, please also address the following statement:

6. *With regard to communicating with policy makers and key regulatory agencies, explain how the results of this study may be used to enhance the restoration of Louisiana's wetlands and/or guide those policy makers in their regional planning efforts.*

This is further evidence of the effects of saltwater intrusion on the marsh systems. It is well documented how plants are affected by saltwater intrusion, but now you can add its effect to amphibians.

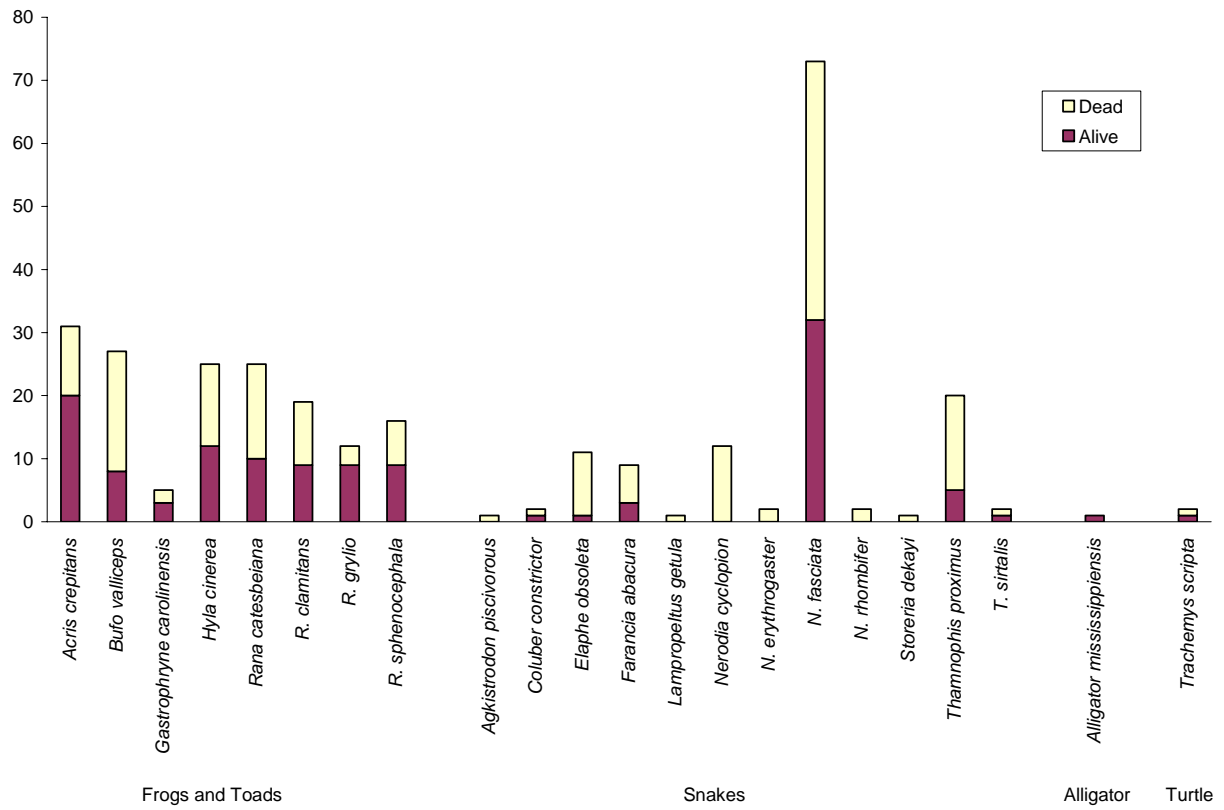


Figure 1. Stacked bar graph of Highway 51 road survey counts comparing alive vs. dead amphibians and reptiles from January 2004 through June 2005.

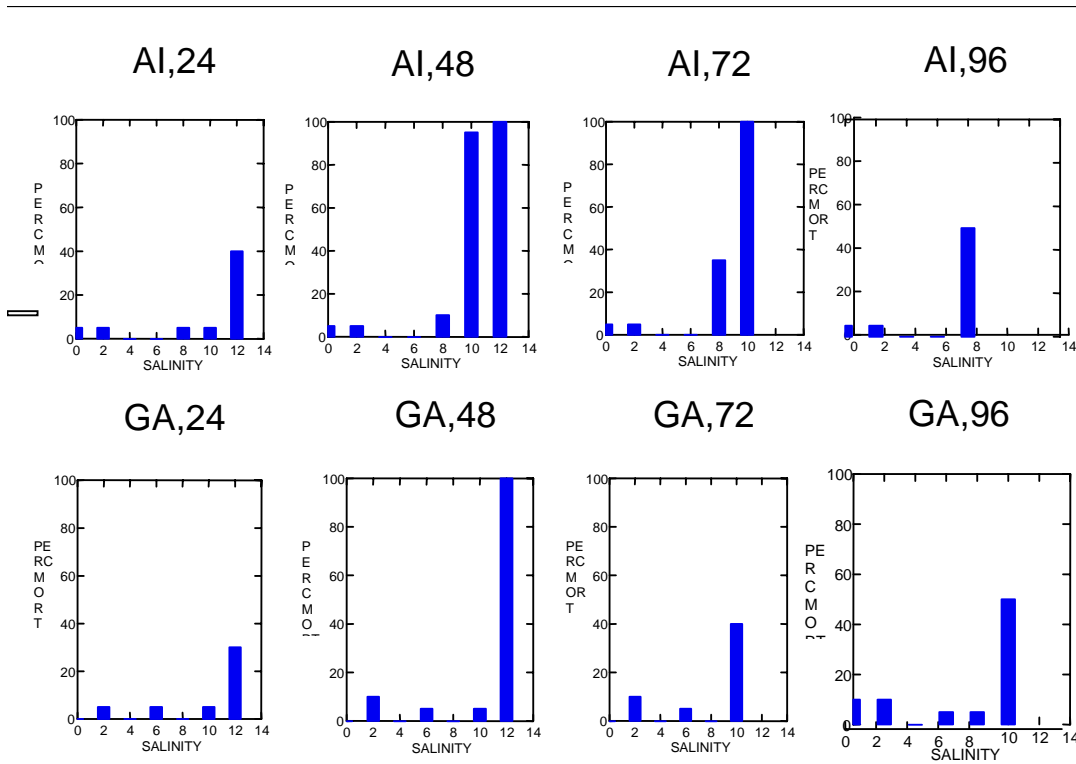
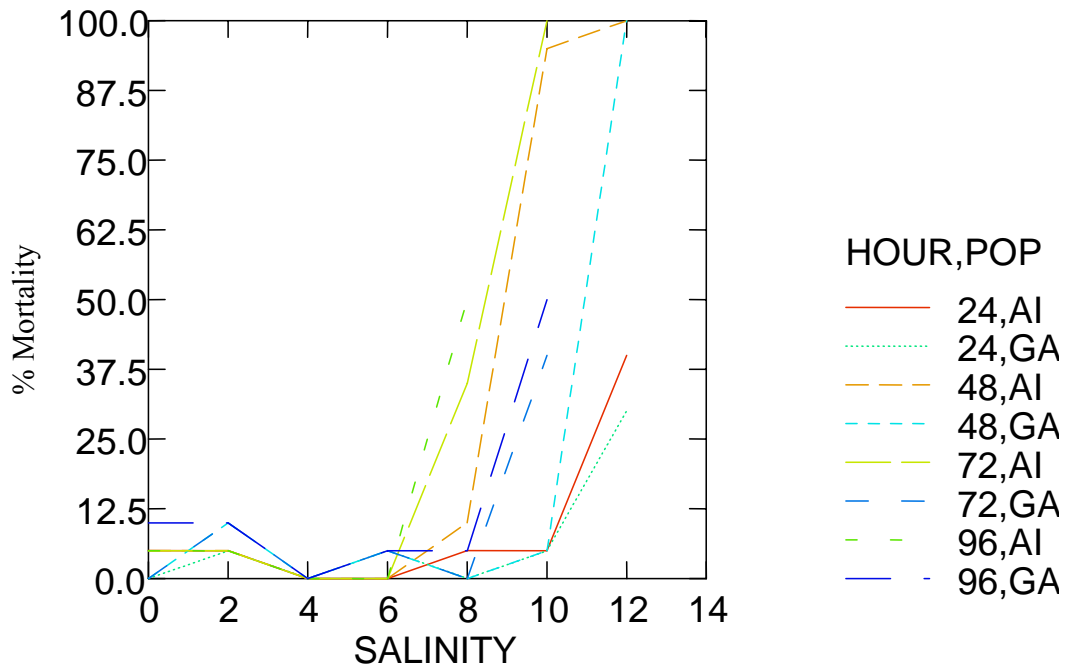


Figure 2. LC50 tests. These tests determine the salinity concentration when the population reaches 50% mortality. The above line graph overlaps all the data. The bar graphs below show the data separately. AI=Alligator Island, GA=Galva. The numbers represent 24 hour periods. The y-axis is % mortality. Note that the freshwater site at 96hr reaches LC50 at 8ppt whereas the brackish site is at 10ppt.

Restoring Biological Diversity to the Wetlands of the Greater Manchac Region

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Abstract

The wetlands of the Lake Pontchartrain basin are one of the largest oligohaline ecosystems along the Gulf coast. Some areas of forest have been converted to marsh by a combination of factors including logging, invasive herbivores and rising sea levels. Our short-term research objective was to explore the ecological factors that are determining biomass and the number of species in the marshes. There were three sets of studies. (1) An initial inventory of 40 randomly located plots documented 107 species of vascular plants. The three main plant communities were marsh, swamp, and shrub-scrub. Marshes of anthropogenic origin differed in species composition from natural marshes. (2) Transplant experiments showed that two biological factors, competition and herbivory, were strongly regulating the species composition of the marshes. (3) Experimental treatments explored the effects of potential restoration tools including fire, herbivore removal, added sediment and added fertilizer. Over a two-year period, increased fertility tended to increase biomass while increased disturbance tended to reduce biomass. There were fewer effects upon species richness. Over a four-year period, many of these effects disappeared. The one exception was in plots that had both a high frequency and high intensity of disturbance – these appear to have been pushed over an ecological threshold.

Primary Objectives

The wetlands of the greater Manchac region (Figure 1) are one of the largest oligohaline ecosystems remaining along the Gulf coast. Over the past two centuries, they have been severely stressed by the construction of levees along the Mississippi River, by saltwater intrusion from navigation canals, the logging of swamps and the spread of non-native species (Shaffer et al. 2005, Keddy et al in press b). Large engineering projects are currently being studied to improve the hydrodynamics of wetlands of the greater Manchac region by diverting sediment-rich waters from the Mississippi River into swamps southwest of Lake Maurepas. The success of these diversions and other measures for wetland restoration will depend upon our understanding of what controls biodiversity and functions of these wetlands and how they are likely to respond to major changes in environmental conditions.

Our short-term research objective was to explore the ecological factors that are determining biomass and the number of species in the marshes. There were two other larger scale objectives. One was to set up a high-profile long-term experiment that would highlight restoration research and attract other researchers to work at the Turtle Cove Environmental Research Station. The other was to determine how biomass and species composition was affected by long-term changes in fertility and disturbance regimes. This report addresses the first of these objectives, which was the focus for this cycle of funding.

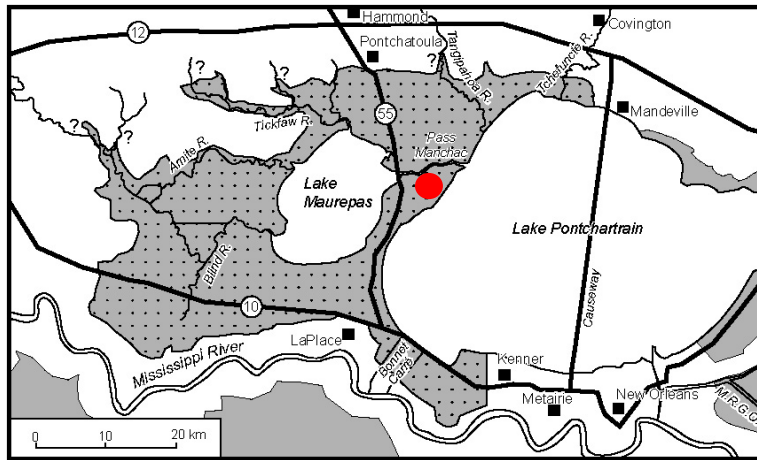


Figure 1: The greater Manchac region. Wetlands are shaded. Study site shown with dot.

Results

Our first objective required research under three topic areas. Each is described separately.

Part 1: Patterns in diversity and composition

To better describe the different vegetation types, species composition were measured in 40 10m x 10m quadrats stratified among four *a priori* vegetation types in the Manchac wetlands during the summer of 2003 (Kandalepas 2004). The sample sites extended from the Tchefuncte River in the east to the Blind and Amite Rivers in the west. After removing infrequent species and floating aquatics, the resultant database consisted of 79 species out of the 107 species originally detected. Table 1 summarizes the plant species observed in marshes and swamps.

Table 1. Species commonly found in the marshes and swamps of the western Lake Pontchartrain and Maurepas area (adapted from Kandalepas 2004).

Marsh Species		Swamp Species	
Species	% Total Cover	Species	% Total Cover
<i>Sagittaria lancifolia</i>	15.8	<i>Nyssa aquatica</i>	31.7
<i>Eleocharis</i> sp.	8.4	<i>Taxodium distichum</i>	12.7
<i>Ipomoea sagittata</i>	6.4	<i>Acer rubrum</i>	8.6
<i>Polygonum punctatum</i>	6.3	<i>Peltandra virginica</i>	7.8
<i>Spartina patens</i>	5.9	<i>Alternanthera philoxeroides</i>	5.2
<i>Lythrum lineare</i>	5.8	<i>Zizaniopsis miliaceae</i>	4.4
<i>Eleocharis cellulosa</i>	5.8	<i>Eleocharis vivipara</i>	3.6
<i>Symphyotrichum subulatum</i>	4.5	<i>Acer rubrum</i> seedling	3.5
<i>Cuscuta indecora</i>	4.0	<i>Cephalanthus occidentalis</i>	2.2
<i>Leersia hexandra</i>	3.3	<i>Morella cerifera</i>	2.2
<i>Paspalum vaginatum</i>	3.0	<i>Sacciolepis striata</i>	2.2
<i>Panicum</i> spp.	2.5	<i>Sparganium eurycarpum</i>	1.5
<i>Cladium mariscus</i> ssp.	2.5	<i>Echinochloa walteri</i>	1.3
<i>Jamaicense</i>			
<i>Vigna luteola</i>	2.3		1.1
		<i>Hibiscus moscheutos</i> ssp.	
		<i>lasiocarpus</i> seedling	
	1.9	<i>Pontederia cordata</i>	0.9
<i>Myrica cerifera</i> (<i>Morella cerifera</i>)			
<i>Eleocharis quadrangulata</i>	1.7	<i>Leersia hexandra</i>	0.8
<i>Echinochloa walteri</i>	1.4	<i>Eleocharis</i> spp.	0.7
<i>Baccharis halimifolia</i>	1.4		
<i>Peltandra virginica</i>	1.2		
<i>Alternanthera philoxeroides</i>	1.2		

Marshes tended to have fewer plant species and less variation in species composition than swamps. TWINSPAN showed ten natural groupings (Figure 2). There was no clear separation between anthropogenic and natural marshes, but rather the differences appear to be attributable to the salinity gradient, with the added occurrence of shrub-scrub community type that may reflect past logging activities. *Morella cerifera* is a common component of this community type. The swamps divided into two major groups: one, with the occurrence of *Sphagnum* moss, the other with saplings of *Taxodium distichum*. This suggests a fertility difference, with *Sphagnum* documenting sites of low fertility. The most speciose plots were those dominated by *Acer rubrum* and *Taxodium distichum*; the mechanism seems to be that many marsh plants can grow in small gaps in the forest, in which case forests contain not only woody plants, but a moderate number of marsh species in addition.

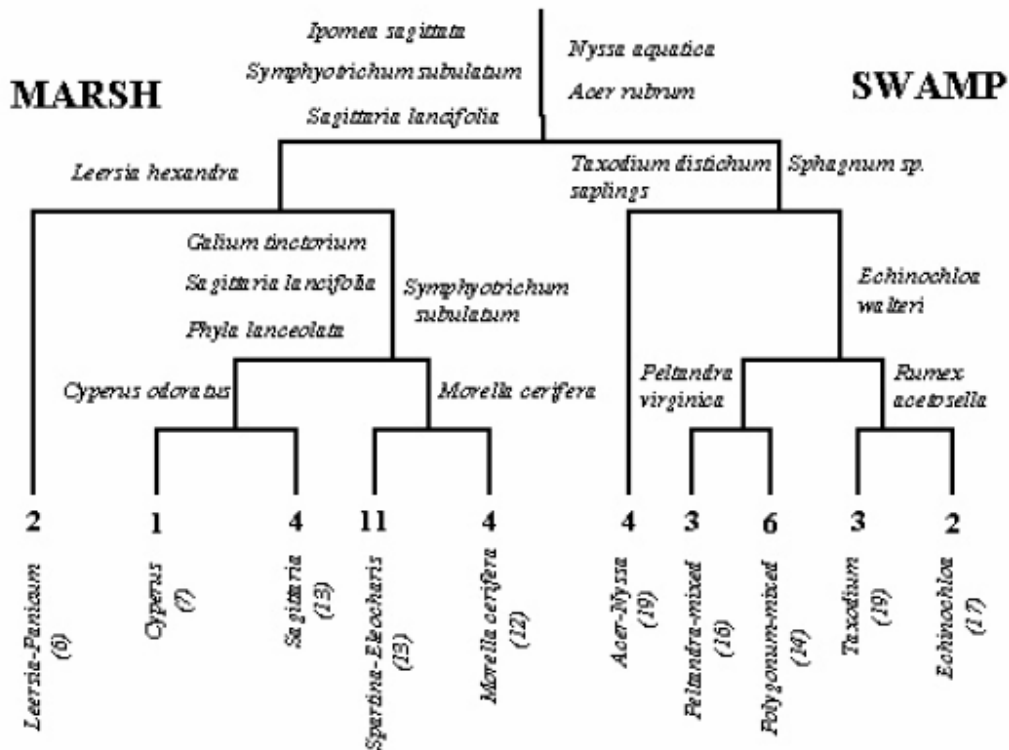


Figure 2. A TWINSpan classification of vegetation types in the northwestern portion of the Lake Pontchartrain Basin (Kandalepas 2004). Numbers directly above vegetation types indicate the number of locations out of 40 assigned to each group. The numbers in parentheses are mean number of plant species found within each group.

Part 2: Factors controlling plant diversity in marshes

Because these marshes are anthropogenic in origin, they likely originated rapidly (over a few decades, probably from nearby seeds or as seeds dispersed by logging activities). Thus, the few founders of these plant communities may have given rise to the existing marshes. From this perspective, the low diversity of these marshes may be simply a founder effect, maintained by limited dispersal. Further, the few founder species may have rapidly filled the clearings and provided a closed canopy within a few years. A closed canopy would further reduce opportunities for successful establishment by newly arrived propagules. In most natural marshes, large deltaic networks provide vast areas of open mud flats where colonists may establish (Shaffer et al. 1992, White 1993). There is evidence from other vegetation types that the size of the pool of colonists has an important effect on plant species diversity at local scales (Eriksson 1993, Grace and Pugsek 1997). Dispersal limitations in the Manchac area have been hypothesized (Gough et al. 1994). The experimental introduction of plant species can test dispersal as a limiting factor, and explore the relative importance of specified factors as controls on plant distribution. A large transplant experiment (Geho 2004) was conducted to measure the

relative importance of competition, herbivory (mammalian herbivores, primarily nutria), and sedimentation in controlling the number of species in the marsh. Sixteen species were introduced to these marshes (12 herbaceous species; *Acorus calamus*, *Cladium mariscoides*, *Eleocharis* sp., *Juncus effusus*, *Panicum hemitomon*, *Peltandra virginica*, *Pontederia cordata*, *Rhynchospora corniculata*, *Rhynshospora inundata*, *Saururus cernuus*, *Schoenoplectus americanus*, *Typha domingensis* and four woody species *Acer rubrum*, *Cephalanthus occidentalis*, *Nyssa aquatica*, *Taxodium distichum*). Adult plants were transplanted into 3x3 m plots either inside or outside of herbivore exclosures, with or without competition (removed by Rodeo™) from established vegetation, and with or without added sediment (1 cm thick). Each of these four treatments contained one representative of each species, and the four treatments were replicated three times. At the end of one growing season (7 months), above- and below-ground dry biomass was measured. The treatments had significant effects on seven species (Table 2). The exclusion of herbivores resulted in significant biomass increases (2 to 26 times) for *Typha domingensis* and *Taxodium distichum* (Table 2). Removal of competition from neighbors resulted in significant biomass increases (2 to 10 times) for five species: *Acorus calamus*, *Cephalanthus occidentalis*, *Panicum hemitomon*, *Pontederia cordata*, and *Rhynchospora corniculata* (Table 2). In summary, the effects of competition from established plants may be preventing establishment after dispersal. Further, competition is apparently more important than herbivory, and, at least in the short term, added sediment, like that from a freshwater diversion, is unlikely to influence the number of species found. Despite studies supporting the prevalence of flooding and/or salinity at reducing germination, recruitment and survival (McKee and Mendelssohn 1989, Baldwin et al. 1996), the increase in elevation created by the sediment addition, which would ameliorate flooding and salinity pressures, did not increase plant diversity over the short term (Geho 2004). Longer-term experiments are needed to properly assess this hypothesis.

Table 2. Effects of herbivory, competition, and sediment addition upon eight species transplanted into the anthropogenic marsh. Significant *P*-values (ANOVA, $p < 0.005$, Bonferroni adjustment of $\alpha = 0.05$ to adjust for multiple comparisons) are bold and marked with an asterisk (*). Empty cells indicate the interaction term was pooled into the error term ($F < 1.70$) (Bancroft and Han 1983). Species that were unaffected by any treatment are not shown. (Adapted from Geho 2004)

Species	Herbivores (Enclosure)	Competition (Herbicide)	Sediment	Herbivores x Competition	Herbivores x Sediment	Competition x Sediment	Herbivores x Competition x Sediment
<i>Acorus calamus</i>	0.1589	0.0003*	0.0377	--	0.1494	0.0593	0.1811
<i>Cephalanthus occidentalis</i>	0.0283	0.0000*	0.1179	0.0044*	0.1824	0.1978	--
<i>Panicum hamitomon</i>	0.8656	0.0000*	0.0053	0.1041	0.0283	--	--
<i>Pontederia cordata</i>	0.2044	0.0000*	0.075	--	0.0472	--	--
<i>Rhynchospora corniculata</i>	0.2848	0.0002*	0.8181	0.0086	--	--	--
<i>Taxodium distichum</i>	0.0024*	0.0931	0.2819	0.0022*	--	--	0.0694
<i>Typha domingensis</i>	0.0000*	0.0111	0.1722	0.0126	0.1383	0.0375	0.1158

Part 3: Effects of Fertility and Disturbance on Biomass and Richness

Turtle Cove Experimental Marsh was constructed on the Manchac land bridge (Figure 3). The vegetation here was dominated by three species: *Schoenoplectus americanus* (39.0%), *Polygonum punctatum* (18.9%), and *Sagittaria lancifolia* (7.4%).

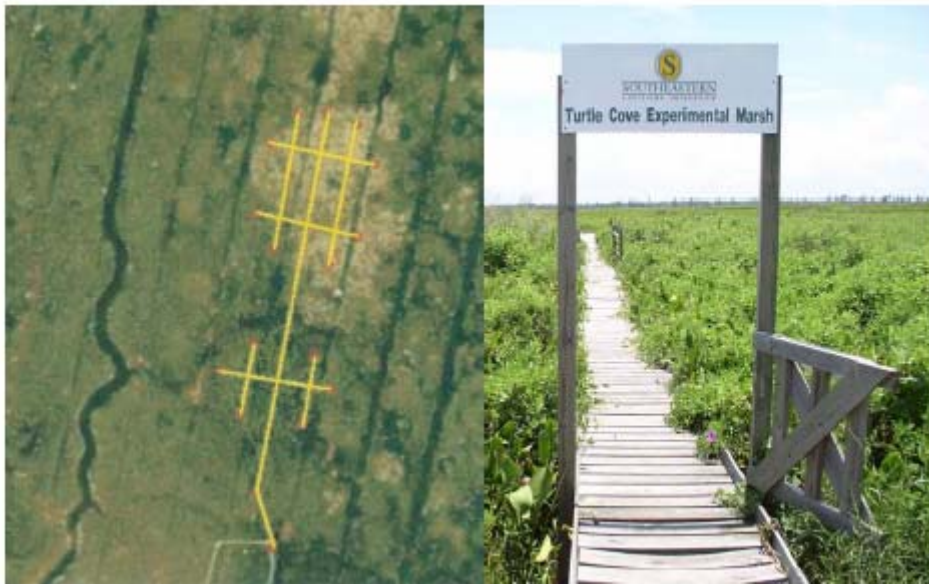


Figure 3. Turtle Cove Experimental Marsh. Left, aerial view with boardwalks and catwalks highlighted. Schlieder's ditch runs to the east of the experiment, and the field station (not shown) is just beyond the bottom of the photograph. Note the parallel ditches left by logging

nearly a century earlier. Right, entrance showing anthropogenic marshes in foreground and a cypress swamp along First Canal in the distance.

The research treatments were designed to provide both multiple disturbance and fertility treatments ranked by intensity and combined in a factorial design (McFalls 2004). Disturbance, defined as any event that destroys plant biomass (Grime 1979), strongly influences species diversity and biomass patterns by creating heterogeneity in ecological communities (e.g., Connell 1979, Grime 1979). Disturbance intensity, measured as the proportion of biomass killed (Grime 1979, Sousa 1984), dictates how much the system is perturbed, while resource availability determines the rate of recovery. In 2002 five disturbance treatments, ranked by order of probable intensity, were applied: no disturbance (control), prescribed fire, herbivory (manipulated with exclosure cages), a single vegetation removal treatment, and recurring double vegetation removal treatments. The last two treatments were intended to simulate two different aspects of disturbance: a single pulse of disturbance in contrast to recurring pulses of disturbance. In more technical terms, the former was a pulse treatment, while the later was a press treatment (Bender et al. 1984). The fertility treatments were designed to include factors that might affect production in Louisiana's rapidly submerging coastal areas, and also were ranked by probable intensity: no fertility enhancement (control), sediment addition, fertilizer addition, and a sediment + fertilizer addition (McFalls 2004). In early 2002, three 40 x 60 m herbivore exclosures were constructed and paired with 3 areas of equal size that were open to herbivory. Exclosures were designed to prevent nutria (*Myocastor coypus*), the principal vertebrate herbivores of the marsh, from entering the plots, but the exclosures may also excluded other less common herbivores such as feral hogs (*Sus scrofa*), marsh rabbits (*Sylvilagus aquaticus*), and muskrats (*Ondatra zibethicus*). The main plots were constructed parallel to one another with a large boardwalk separating them. Access inside of the main plots was provided by 670 m of catwalk. Subplots (3 m x 3 m) for the factorial combinations of the disturbance and fertility treatments were randomly allocated within the main plots. Above ground biomass was measured in July 2003 using destructive sampling techniques near the perimeter of the 9 m² plot perimeter (McFalls 2004). The data were analyzed as 2x4x4 randomized block design split plot factorial analyses of covariance.

Effects after two years

The effects of the first two years of experiments have been fully analyzed for biomass and richness, and are now published (McFalls 2004), in press (Keddy et al. in press a,b) or in review (e.g., McFalls et al. submitted, Keddy et al. submitted). A brief summary follows. The main effects of disturbance and fertility treatments roughly followed our intensity rankings (Figure 4). Linear contrasts showed that the sediment + fertilizer treatment, which simulated Mississippi River flooding events, resulted in increased biomass compared to plots with no fertility enhancement and plots with the sediment only treatment. The sediment treatment was statistically similar to the fertilizer treatment. Linear contrasts demonstrated that fire and the single vegetation removal reduced the amount of biomass relative to the control. Herbivory significantly reduced biomass ($p < 0.0001$). On average, areas protected from nutria herbivory had 1.4 times the biomass of areas open to herbivory. There were complicated two - and three -

way interactions (McFalls 2004) that are not shown here, but will be briefly summarized. Without grazing, biomass increased as fertility increased, whereas outside the exclosures, biomass did not change with fertility. This suggests that nutria consumed a great deal of the increased vegetation that results from enhanced fertility. Further, biomass decreased monotonically with disturbance outside the exclosures while this was much less noticeable inside the exclosures. Apparently, nutria only had an impact upon biomass if another disturbance was present, and they tended to amplify effects of disturbance. The likely mechanism is a preference for newly growing vegetation, a common phenomenon in herbivores (White 1993). Overall, the results show that freshwater diversions that carry sediment and nutrients into the marsh are likely to increase production. These responses are likely to be reduced in the presence of nutria. Although fire occurs in Louisiana marshes, and is a management tool applied to selected marshes (O’Neil 1949, Nyman and Chabreck 1995), the short term results of this experiment indicate that plants regenerating after fire are particularly attractive to nutria and increase impacts of herbivores upon the vegetation. Further, in dry periods fires can consume organic matter in the soil, leading to reduced elevation and increased flooding. This could initiate a positive feedback cycle for further reduction in plant cover and marsh elevation.

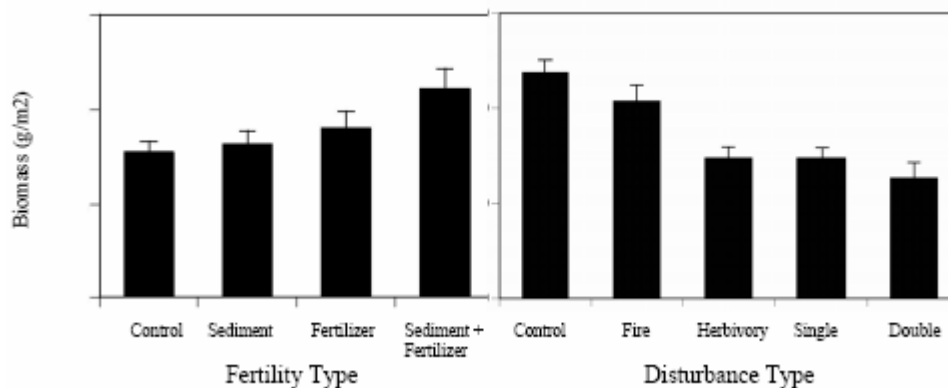


Figure 4. The short term effects upon biomass of increasing fertility (left, including all herbivory and disturbance treatments, mean \pm 1 SE, $n=96$, $F_{3, 70}=10.004$, $p=0.000$) and increasing disturbance (right, including all herbivory and fertility treatments, mean \pm 1 SE, $n=96$, $F_{3, 70}=30.297$, $p=0.000$). Herbivory is included in the latter figure, but was not tested against the other types of disturbance (from McFalls 2004).

Effects after four years

The treatments described above were continued for a further two years. Vegetation data were collected in spring and summer in 2004 and 2005. The most informative results are likely to be those from summer 2005, the longest duration over which analyses were completed. These are therefore the results that this report focuses upon.

Total Cover:

Analysis of variance showed that the effects of disturbance were significant ($p < 0.001$), while the effects of fertility were not ($p > 0.56$). There was a significant ($p = 0.037$) interaction

between disturbance and fertility. There is thus only limited evidence that freshwater diversions will increase biomass (as a main effect) although the nutrients may well interact usefully with other factors in specific cases. For example, a priori contrasts revealed that plots that were burned and fertilized had much higher biomass than control plots ($p = 0.0146$) or plots that were only burned ($p < 0.001$).

The effects of the treatments on spring vegetation were different. In the spring, there was a significant fertility effect ($p < 0.002$) but no disturbance effect ($p = 0.099$). The reason for the reverse pattern in the spring is unclear. It may be that one effect of fertility is to enhance rates of growth, leading to earlier development of cover in the fertilized plots. This is only a hypothesis. Overall, biomass levels in these marshes seem fairly resilient to perturbations by fertility or disturbance, although this resilience is modified by fertility in subtle ways.

A notable exception was the “press” treatments, which received both a high intensity and high frequency of disturbance -- recurring applications of herbicide. This treatment remained consistently lower in biomass ($p < 0.001$). Hence, there is an upper limit of disturbance rates that marsh plants cannot recover from. A single pulse of disturbance has minimal effects, but recurring pulses appear to have cumulative effects. This treatment was designed to create bare plots that were dependent upon dispersal from adjoining plots for recovery. Apparently the process of recolonization of partially bare plots is slow, perhaps because seed recruitment is slowed by recurring flooding and salinity.

Total Richness

Analysis of variance showed that the effects of disturbance were significant ($p < 0.001$), while the effects of fertility were not ($p > 0.099$). There was a significant interaction between disturbance and herbivory ($p = 0.002$). Most of the disturbance effects, however, were the consequence of low richness in the press experiment that had recurring disturbance. As noted above, this treatment was extreme, with both high disturbance rates and intensity, resulting in a richness of only two species per plot. If the double herbicide treatment is removed from consideration, then the single herbicide treatment, fire and herbivory were not significantly different from one another, having 5-6 species per plot.

With the exception of the double herbicide treatment, there were not other effects of the treatments on species richness. All *a priori* contrasts were non-significant. The lack of treatment effects on species richness, however, does not mean that the composition was unaffected, as richness merely describes the total number of species

Total biomass

Analysis of variance showed that the effects of disturbance were significant ($p < 0.001$), while the effects of fertility were not ($p > 0.072$). Again, this disturbance effect was a consequence of the double herbicide treatment; when this is omitted, main plot biomass remains unchanged at ca. 700 gm² across disturbance regimes

Species Composition

The research proposal, and the above analyses, were focused upon changes in biomass and species richness over a two and four year period. Probably the most sensitive indicator of environmental change is changes in species composition. Although these were not part of the initial proposal, our intention was to analyze them to explore the above questions in more detail. Since funding was terminated, we have not been able to carry out the analysis on species composition. The data have been archived and will be analyzed once funding is located.

Summary of experimental results:

Overall, the marsh vegetation is remarkably resilient to the changes we exposed it to. With the exception of the bare (double-herbicide plots), biomass and richness were little affected by the treatments. Thus, there is little conclusive evidence that restoring fresh water diversions, or restoring fire, will have much effect upon biomass or species richness of these wetlands, at least over a four year period. Competition from established plants, and grazing by herbivores, seems to set an upper limit on plant diversity. One might have expected species richness to increase inside the exclosures where effects of herbivores were excluded, but the experiment has to be terminated before these effects were observed. The transplant experiments, however, can be considered as experiments that accelerated rates of dispersal and establishment, and they suggest that in the absence of grazing, woody plants would be able to establish. Cattail invasion might also occur.

Bare spots occur naturally in the marsh. When we experimentally created bare plots with the double herbicide treatment, they turned out to be remarkably limited in recovery over the four years we monitored. Throughout the experiment and data analysis, the double-herbicide (bare) plots stood out as a separate class of plots. Therefore, while the marsh is resilient to relatively small perturbations (e.g. fire, single herbicide event), there is apparently a threshold. Once this threshold is exceeded, the vegetation is unable to recover. A single pulse of disturbance is insufficient to cross this threshold, but four years of disturbances are sufficient to cause major changes in biomass and richness. This suggests that once they form, bare spots will be resilient to recovery. Field observations suggest that these bare spots suffer from lack of accretion, and gradually deepen into pools. If these pools accumulate water which evaporates, leaving higher salinity, it is possible to imagine a positive feed-back loop where saline pools gradually expand and contribute to the loss of marsh. Freshwater diversions would be likely to reverse this process, although our data suggest that rates of recovery will be slow. Clearings may benefit from hand planting of native plants.

Effects of Two Hurricanes

Turtle Cove Experimental Marsh was hit by two hurricanes in 2005. The access boardwalks have been smashed, upturned, draped over the fences meant to exclude nutria, or simply carried away (Keddy 2006 a, b). We could not reach the plots without wading through an eight foot deep channel and picking our way through trees and other debris. The nutria gained access through holes in the fences, or by using debris as walk-ways. The fences have been temporarily repaired but a visit in the summer of 2006 showed that nutria were still able to enter the cages. A refrigerator even drifted into one of the plots (Keddy 2006a, Figure 5). In spite of these many difficulties, Tiffany McFalls made the heroic effort to collect post hurricane data in

the spring and summer of 2006. We will therefore be able to describe changes in the vegetation resulting from the hurricanes, and more importantly, test whether the different pre-treatments changed the degree of resilience to effects from the hurricanes. Although there is said to be interest in determining how hurricanes affect wetlands, our proposals to analyze the changes between 2004 and 2006 have not yet been funded. Again, the data are archived for future analysis.

A General Model for Future Ecological States

To put these results in a larger context, let us therefore consider the options for large and small-scale restoration of the area. Setting targets for restoration of ecosystems is not an easy task and the goals set by restoration have to consider both the original state of the system as well as the desired state (Cairns 1989). If, for example, we use the map of historical vegetation types in Saucier (1963), one might set a target of five percent of the area as fresh marsh, five percent as intermediate marsh, and ninety percent as cypress/tupelo swamp. The plausibility of recreating these conditions depends upon the relative importance of the ecological forces acting upon these wetlands. Given the relative strength of these forces, one could imagine multiple ecological states for the anthropogenic marshes, in particular, as well as the wetlands as a whole (Figure 5). A range of options exists for restoration (natural marsh, *Spartina* marsh, exotic wetlands, baldcypress forest, water tupelo forest, shrub-scrub and mangrove swamp. We have discussed the factors that would control transition among these types in a review paper (Keddy et al in press). The experimental results described above show, for example, that woody plant expansion is limited by the effects of established plants and herbivores.

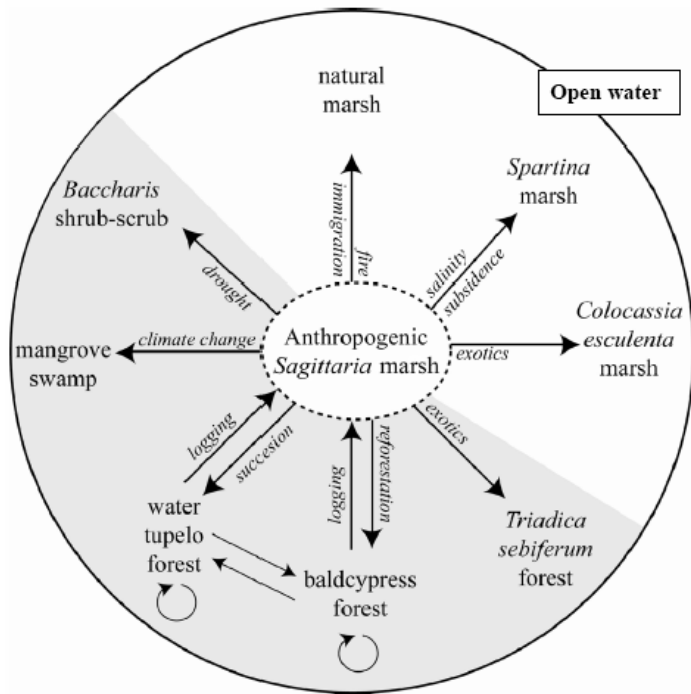


Figure 5. Possible future states for the ecosystem depending upon different combinations of climate change, rising sea levels, fresh water diversions, fire, and invasion by exotic plants. Areas in grey represent forest. Current societal forces acting upon our coastal wetlands are directing wetlands towards the upper right.

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Technology Transfer

Part 1:

1. The plant communities of the greater Manchac area can be divided into swamp, marsh and shrub-scrub. We have documented the main plants in each type for the use of future researchers.
2. The marshes around Turtle Cove are anthropogenic marshes that originated after logging, and they differ in composition from natural marshes.

Part 2:

1. Biological interactions are very important in explaining the composition of marshes. Competition from other plants, and grazing by herbivores, are both strong forces that seem to be limiting plant species diversity.
2. Woody species, and cattails, are particularly susceptible to grazing by nutria. If nutria populations are reduced, we can expect these species to increase in abundance.
3. Computer modeling shows that alligators have the potential to completely eliminate nutria from coastal wetlands – if larger alligators are not trapped and allowed to reach a higher natural density.

Part 3:

After two years (the immediate term)

1. Increased fertility and increased sediment both cause higher plant biomass to accumulate. This will provide more food for wildlife, more protection of the soil, and higher organic inputs for soil accretion.
2. Disturbance from fire and nutria significantly reduces biomass.
3. There are important interactions among these different factors. For example, while biomass increased with fertility (point 1), the presence of nutria eliminated this effect by consuming the added biomass. Similarly, fertilizer actually increased the negative effects of burning, perhaps by attracting nutria to feed upon the newly emerging shoots.
4. Although the treatments had significant effects upon biomass, there were relatively few changes in plant species richness. There is some limited evidence that nutria may enhance species richness, perhaps by creating gaps for regeneration, or perhaps by carrying seeds for dispersal.

After four years (the short term)

1. Overall, the summer data show that the experimental treatments produce measurable effects upon the marsh, while the effects of fertility are minor. The single treatment that continually dominates the treatment effects is the double herbicide, designed to kill adult plants and remove the seed bank. This treatment clearly has a major effect on composition. More minor perturbations, however, do not produce measurable changes in biomass or richness.
2. Fertilizer increases the biomass in plots that were burned. This is the opposite pattern shown after two years of treatments.
3. Neither disturbance nor fertility affected the number of species per plot, with the exception of the double herbicide treatment which provided the treatment of recurring complete plant removal.
4. While the biomass and richness of the vegetation is minimally affected by most perturbations, once bare plots occur, they appear to be resistant to recolonization. Recurring disturbances created plots that were consistently below the other plots in biomass and richness throughout the four years of the experiment.

Long term effects

1. It has taken nearly a century for the effects of natural processes and human activities to create the current wetlands near Turtle Cove (e.g. Keddy et al in press b). Four years of experimentation are insufficient to adequately assess effects of different treatments. The long term effects of fertility, fire and grazing are unknown.

Appendix 2: Communications including Turtle Cove Experimental Marsh

Talks (selected)

Keddy, P. 2006. *Hurricanes, human irrationality, and Gulf Coast ecosystems*. Closing address to symposium Ecological effects of Gulf Coast hurricanes at the Ecological Society of America annual meeting. Memphis, TN. 7 August 2006.

Keddy, P.A. 2006. *Constraints on wooded wetland restoration*. Invited Symposium Session on Forested Wetlands for *Wetlands 2006-Opportunities and Challenges* Association of State Wetland Managers. Traverse City, MI. 30 August 2006.

Keddy, P.A., J. A. Nyman, and J. Siegrist. 2006. *The prospect for biological control of nutria by alligators*. South Central Chapter of Society of Wetland Scientists, U.S. Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, MS. 6 October 2006.

Keddy, P.A. *Maintaining biological diversity in wetlands: fires, flooding, alligators and other unpopular essentials*. Invited address, Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA. April 19, 2006.

Keddy, P. *Twelve priorities for improving the state of the state: a vision for the future*. ESOS. University of New Orleans. 22 May 2005.

Keddy, P.A. *Wetland Restoration: The Good, the Bad and the Ugly*. South Florida Water Management District, West Palm Beach, FL. 30 March 2005.

Keddy, P.A. *Green and Wild: Six Pragmatic Models for Understanding, Conserving and Restoring Plant Diversity*. Department of Ecology and Evolutionary Biology, University of Connecticut (Storrs). 3 February 2005.

Keddy, P.A. *Restoring the Lake Pontchartrain watershed: seven tough questions*. Basics of the Basin, University of New Orleans. May 21, 2004.

Campbell, D. and P.A. Keddy. 2004. Seed banks of an oligohaline marsh along Pass Manchac. Basics of the Basin Research Symposium, Lindy C. Boggs International Conference Center, University of New Orleans Research and Technology Park New Orleans, LA New Orleans, LA. 21 May 2004.

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Keddy, P.A. *Wet and wild: conserving and restoring plant diversity in wetlands*. Distinguished Lecture Series, Joseph W. Jones Ecological Research Center, Ichuaway, Newton, GA. 18 March 2004.

Posters

Geho, E., D. Campbell, and P. Keddy. *Filters influencing species richness in an oligohaline marsh*. Southeastern Ecology and Evolution Conference. Georgia Institute of Technology, Atlanta, GA. March 5-7, 2004.

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Organic Matter Processing in Western Lake Pontchartrain Basin Wetlands

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Final Report

Abstract

Decomposition is a microbially-driven ecosystem process that can determine if sediment accumulates or erodes. Without understanding decomposition it's impossible to predict the full effects of restoration efforts on coastal wetlands. This project studied decomposition in Lake Pontchartrain Basin wetlands, in combination with measurements of microbial activity and community structure. Decomposition in these wetlands is slow, and there are limiting factors on microbial activity. This suggests that changes in water flow and nutrient concentrations associated with restoration efforts will have major impacts on microbial processes. Surveys of bacterial communities indicate that these wetlands also contain many different species of microorganisms, some of which are likely new to science.

Primary Objectives

The research program had three specific objectives. Accomplishing these objectives will provide vital information on the sustainability and functioning of Western Lake Pontchartrain basin wetlands, and how these wetlands will respond at the ecosystem level to restoration efforts.

Objective 1: To determine decomposition rates for organic matter produced in the Manchac area wetlands

Objective 2: To link decomposition rates to microbial activity and community structure, and to generate models from which to predict in situ organic matter processing rates

Objective 3: To determine in situ organic matter processing rates in the western Lake Pontchartrain Basin ecosystem based upon enzymatic decomposition models, and to predict how changes might affect this process

Results

This project contained a number of sub-projects that mesh together to provide an overall picture of microbial organic matter processing in Lake Pontchartrain Basin wetlands. Because these projects also stand alone, results for each are described separately.

(1) Decomposition of fine particulate organic matter in Joyce Wildlife Management Area
Joyce WMA is one of the largest uninhabited swamps in Louisiana and consists largely of secondary baldcypress (*Taxodium distichum*) that has regrown in the area following extensive cypress logging until the early 1900's, as well as various pockets of emergent marsh. The first site was located near the "Swamp Walk" a primitive boardwalk that extends 0.5 km into the swamp roughly 8 miles north of the Lake Pontchartrain estuary. The second site was located 2.5 miles further south near "Kleibert's Ditch", an old hand-dug canoe trail that extends into the swamp. Water depth at both sites varies from essentially no overlying water to over 1 m depth, depending upon long-term (e.g. drought) and short-term (e.g. major storm events) fluctuations. Salinity is only slightly greater than freshwater, but the ecosystem is hydrologically connected to Lake Pontchartrain and minor salinity fluctuations can occur during hurricanes and tropical storms. Sediment at both sites is primarily composed of fine particulate organic matter (FPOM; particles less than 1 mm diameter).

Sediment material was collected from each site in March 2005 and sieved into two size ranges of FPOM (0.25-1 mm "primary particles" and 0.063-0.25 mm "secondary particles"). Sediment was air-dried, weighed, and placed into mesh bags. Sets of litterbags were staked to the sediment surface at each site in April 2005, and samples collected for 1 year to determine organic matter decomposition rates.

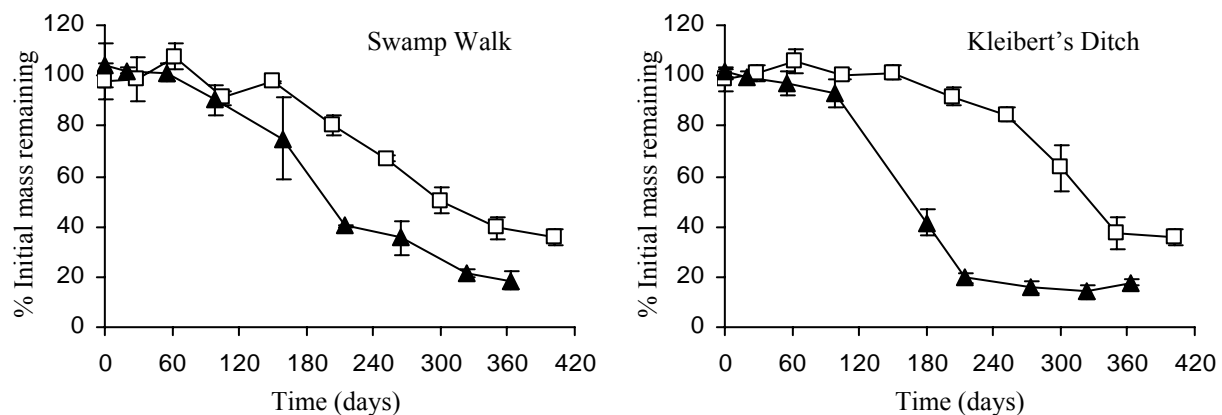


Fig. 1. Decomposition of different size ranges of FPOM at two sites in Joyce WMA. Lines indicate % of initial organic matter remaining over time. Larger, primary particles (open squares) decomposed more slowly than finer, secondarily formed particles (closed triangles) at each site. Values are mean (\pm standard deviation) of three replicates.

Finer (0.063-0.25 mm) particles were initially resistant to decomposition, but after two to three months showed relatively rapid mass loss rates (0.30% per day) at each site. Coarser (0.25-1 mm) particles decomposed more slowly over the course of the study, with mass loss rates

averaging 0.16% per day. Each sample collected for the decomposition study was also assayed for the activity of microbial enzymes involved in lignocellulose degradation (β -glucosidase, NAGase, β -xylosidase, cellobiohydrolase, phenol oxidase, and lignin peroxidase) to determine how the microbial degradative process changed during FPOM decomposition, and ultimately to link decomposition rates to microbial activity (see below).

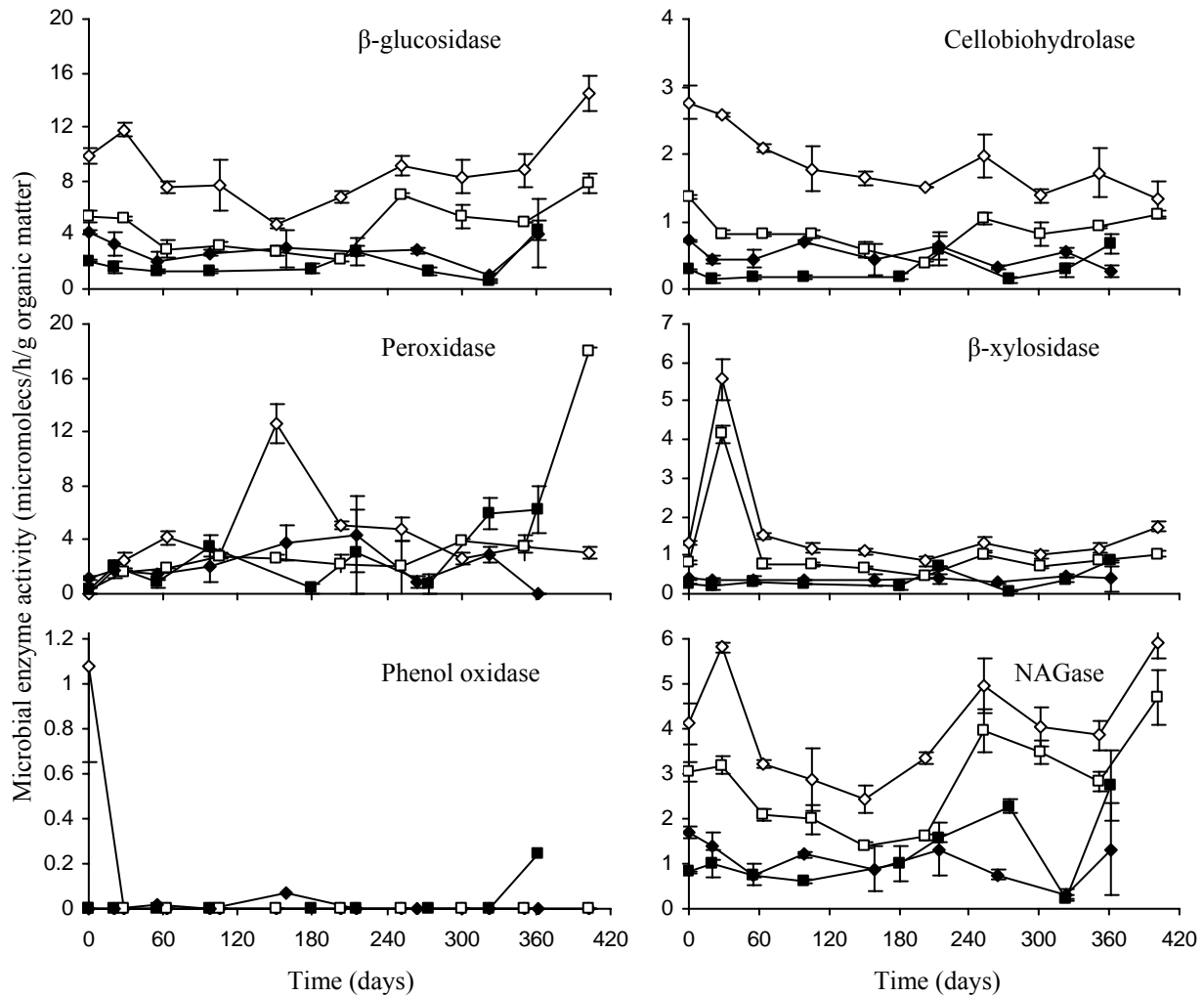


Fig. 2. Patterns in microbial enzyme activity associated with different sizes of decomposing FPOM at two sites in the Joyce WMA. Sites and particle sizes are indicated by different symbols (open diamonds = Swamp Walk 0.25-1mm, open squares = Kleibert's Ditch 0.25-1mm, closed diamonds = Swamp Walk 0.063-0.25 mm, closed squares = Kleibert's Ditch 0.063-0.25 mm). Values are mean (\pm standard deviation) of three replicates.

Activities of the hydrolytic enzymes (β -glucosidase, NAGase, β -xylosidase, and cellobiohydrolase) were generally higher on coarser particles (Fig. 2), and typically higher at the

Swamp Walk site compared to Kleibert's Ditch. Both sites generally showed the same temporal patterns in enzyme activity. Activities of the cellulose-degrading enzymes (β -glucosidase and cellobiohydrolase) were correlated with each other, and with NAGase ($R > 0.8$), an enzyme involved in the degradation of chitin. Activities of lignin-degrading enzymes (peroxidase, phenol oxidase) were less predictable – phenol oxidase activity was essentially non-existent and only detectable a few times over the course of the study.

(2) Linking decomposition rates to microbial activity in wetland sediments

One of the major objectives of this research was “To link decomposition rates to microbial activity and community structure, and to generate models from which to predict in situ organic matter processing rates”. The decomposition and enzyme data described above have been used to generate models that predict instantaneous decomposition rates from a simple measurement of microbial enzyme activity. Linear regressions of % FPOM remaining vs. cumulative enzyme activity were calculated for primary and secondary FPOM particles at each site and each individual enzyme, with the exception of phenol oxidase (which showed only trace activity throughout the study). All regressions were significant although the variability in OM remaining that could be accounted for by the enzyme activity varied in each case. The slope of this regression can be used as a measure of the relative efficiency of that enzyme in degrading that particular class of OM at that site. Clear patterns in the efficiencies of the different enzymes are apparent; with cellobiohydrolase and β -xylosidase being the most efficient enzymes in degrading the two sizes of FPOM at both sites (largely because the activity of these enzymes tends to be lower than others, Fig. 2). There were also substantial differences in the efficiencies of each enzyme in degrading the two different sizes of FPOM. At both sites, all enzymes appeared to be more efficient (3-8 x) at degrading the finer, secondary particles than primary FPOM. There were no consistent patterns between the two sites, although the highest efficiencies tended to be seen at Kleibert's Ditch.

Because all enzymes (save phenol oxidase) showed significant relationships between cumulative activity and FPOM decomposition, the five enzymes were combined into an integrated index of microbial enzyme activity which was related to FPOM mass loss rates. Enzyme data were standardized to a 0-1 scale by dividing the each activity measurement by the highest activity recorded for that enzyme over the study period. Mean standardized microbial enzyme activity was then determined for each date and subsequently integrated over time as for the non-standardized individual enzyme data. Linear regressions of this cumulative standardized microbial activity against % FPOM remaining yielded global enzyme efficiencies for each size fraction of particles at each site. All regressions were significant ($p < 0.001$), and as with the individual enzyme-OM loss regressions, global apparent enzyme efficiencies were greater for the decomposition of secondary FPOM compared to coarser, primary particles. Slopes of regressions (the relative efficiency of the combined microbial enzymes in decomposing FPOM) were -0.4129 ($r^2 = 0.900$) and -0.7468 ($r^2 = 0.857$) for the primary particles at the Swamp Walk and Kleibert's Ditch sites, respectively. Slopes for finer, secondary particles were -1.8550 ($r^2 = 0.961$) and -3.1205 ($r^2 = 0.877$) for the Swamp Walk and Kleibert's Ditch sites, respectively. These global efficiencies can be used to estimate instantaneous mass loss rates for primary and secondary FPOM decomposing in unconfined sediments at these and at other sites in the Lake Pontchartrain wetlands. Essentially they provide a conversion factor from which to predict actual decomposition rates from a measurement of enzyme activity.

(3) Impacts of salinity and changing nutrient status on microbial activity in wetland sediments

Development of the enzyme-decomposition models shows that microbial enzyme activity is directly linked to rates of organic matter decomposition in Lake Pontchartrain Basin wetlands. Therefore environmental changes that affect microbial enzyme activity will also affect how these wetlands function. As part of this study, a microcosm experiment was conducted to examine how changes in salinity (such as associated with saltwater intrusion events during major storms) and

nutrient input (such as associated with potential diversion or wastewater treatment efforts) affect microbial processes in sediments.

Surface sediment was collected from Joyce WMA and 400 ml aliquots added to 54 beaker microcosms. Microcosms were treated with two different salinity levels (ambient, + 3.5 ppt – approximately the salinity that this area was exposed to during Hurricanes Katrina and Rita), three different levels of nitrogen amendment (ambient, + 5 mg/l, + 50 mg/l), and three different levels of phosphorus (ambient, + 0.5 mg/l, + 5 mg/l) in a 2x3x3 factorial design (3 replicate microcosms per treatment). Sediments were sampled after 55 days of exposure to the treatments and assayed for the activity of β -glucosidase (a cellulose-degrading enzyme that can be used to predict rates of decomposition, see above), NAGase (an enzyme that can be used to predicts both rates of decomposition and organic nitrogen mineralization), and phosphatase (an enzyme that yields inorganic phosphate from organophosphates, and an indicator of rates of organic phosphate mineralization).

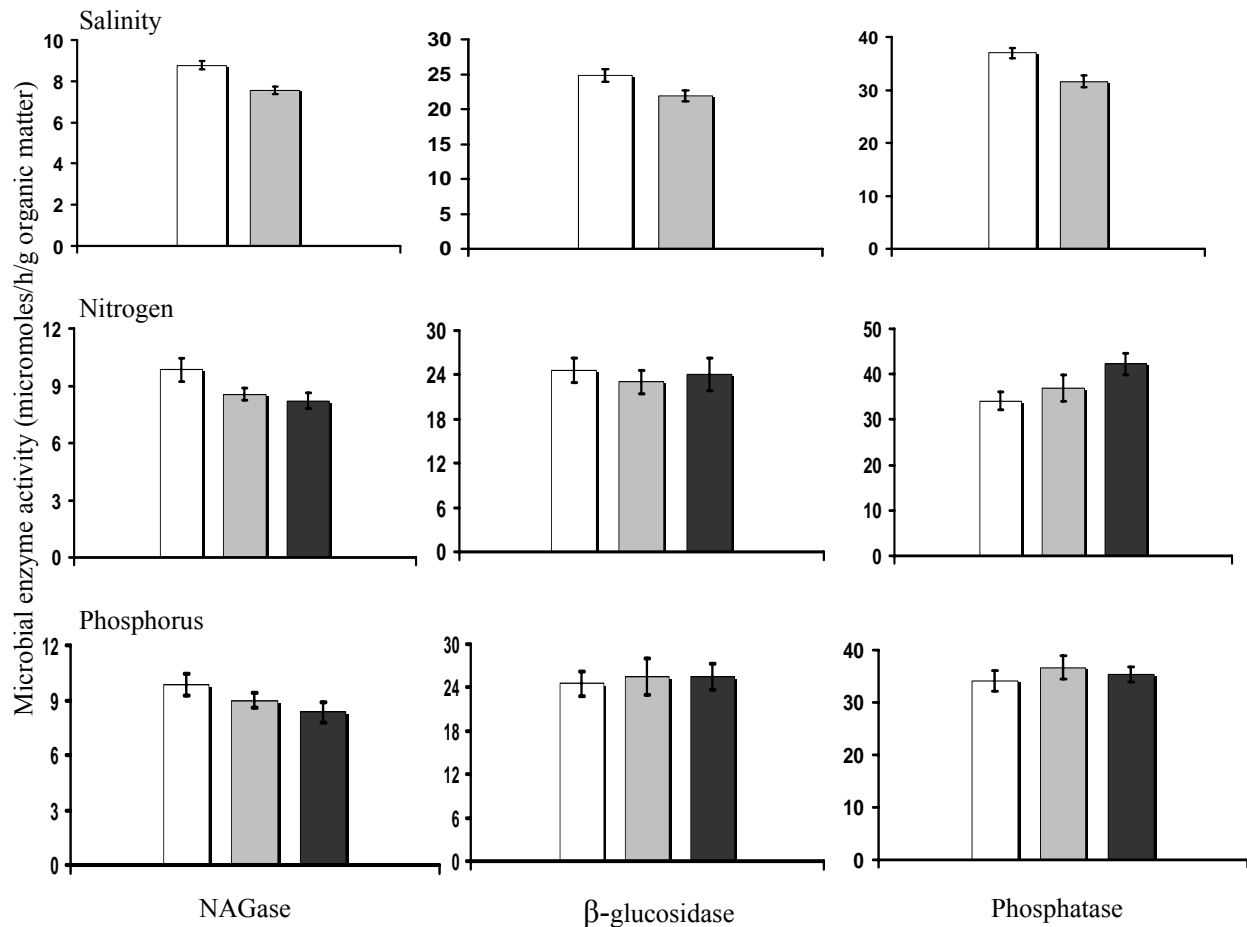


Fig. 3. Effects of increased salinity levels and nutrient amendment on microbial enzyme activity in wetland sediments. Ambient conditions are open bars, treatments are shaded and represent salinity (+ 3.5 ppt), nitrogen (+ 5 mg/l, + 50 mg/l), and phosphorus (+ 0.5 mg/l, + 5 mg/l) effects, respectively. Values are mean +/- standard deviation of 3 replicates per treatment.

Salinity significantly reduced the activity of all three microbial enzymes examined, even at the relatively low level used in this study (Fig. 3). This suggests that modest saltwater intrusion events that occur during tropical storms and hurricanes can substantially alter the rates of microbial processes that occur in sediments in Pontchartrain-Maurepas wetlands. The effects of nutrient additions varied depending upon the particular enzyme examined. Nitrogen additions decreased NAGase activity (an enzyme involved in organic N mineralization), increased phosphatase activity (an enzyme involved in organic P mineralization), and had no significant effect on β -glucosidase (Fig. 3). Inorganic phosphate additions also depressed NAGase activity, but had no effect on β -glucosidase or phosphatase (Fig. 3). Taken together, these results suggest that microbial processes in these wetlands are nitrogen limited. Addition of inorganic nitrogen reduced rates of organic N mineralization by reducing the demand for nitrogen, however, this also increased the demand for phosphate (essentially switching the system from being N-limited to P-limited). Phosphate addition had no effect on rates of P mineralization, confirming that P is not currently limiting to microbial processes in these sediments. Thus, salinity and nitrogen appear to be the two most important drivers for microbial activity in these wetlands.

In addition to examining the effects of nutrient addition and salinity on microbial activity in microcosms, samples were also taken to assay how these treatments may have changed bacterial community structure. Whole community DNA was extracted from sediment in each treatment, portions of the bacterial 16S rRNA gene amplified, and the community examined using denaturing gradient gel electrophoresis (DGGE). DGGE essentially provides a fingerprint or barcode of that community, with each band on the gel representing a different bacterial population. Samples producing similar banding patterns harbor similar bacterial communities. Even though sediments were exposed to treatments for 60 days and showed significant changes in microbial activity over this time, similar banding patterns across treatments suggest that bacterial community structure was not affected (Fig. 4).

Control			Salinity			Nitrogen		
1	2	3	1	2	3	1	2	3

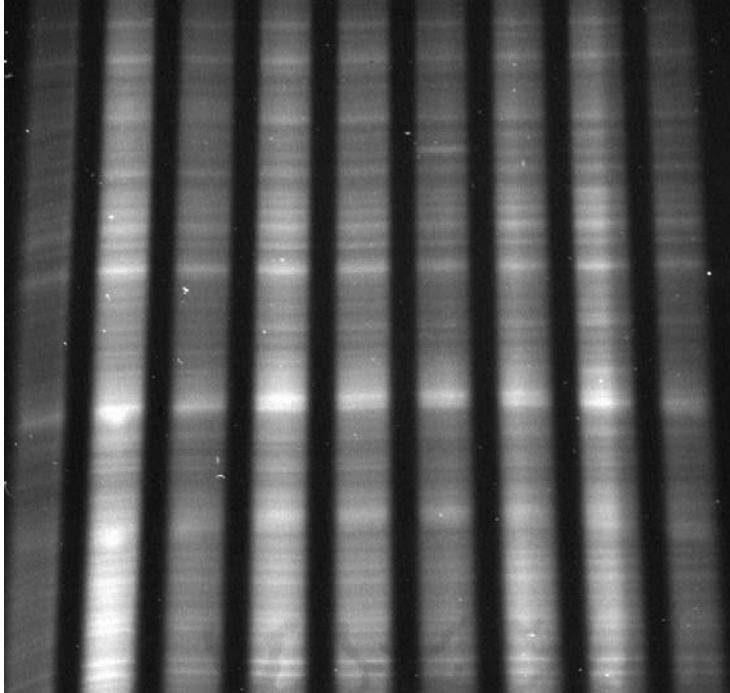


Fig. 4. DGGE analysis of bacterial community structure in wetland sediments exposed to elevated levels of salinity (+3.5 ppt) or nitrogen (+ 50 mg/l), along with control treatments. Numbers designated replicate treatments. Each band represents a different bacterial population. Treatments did not appear to change the bacterial populations present in sediments.

(4) Identification of dominant bacterial populations associated with organic matter in wetland sediments through 16S rDNA sequencing

While the other projects carried out as part of this research yield important information on rates of organic matter processing and other microbial processes occurring in western Lake Pontchartrain Basin wetlands, and how environmental conditions can alter those rates, they provide no information on the composition of the bacterial community in this system. Samples of sediment material were collected from the “Swamp Walk” area of Joyce WMA in early 2005 and the dominant bacterial populations characterized using molecular techniques. DNA was extracted directly from sediment and the 16S rRNA gene amplified and sequenced. DNA sequencing suggests that there are at least 500 distinct species of bacteria in even a few grams of wetland sediment. Many of these bacteria are not closely related to known species, and they represent a number of different groups of bacteria, some of which are very poorly described (Fig. 5).

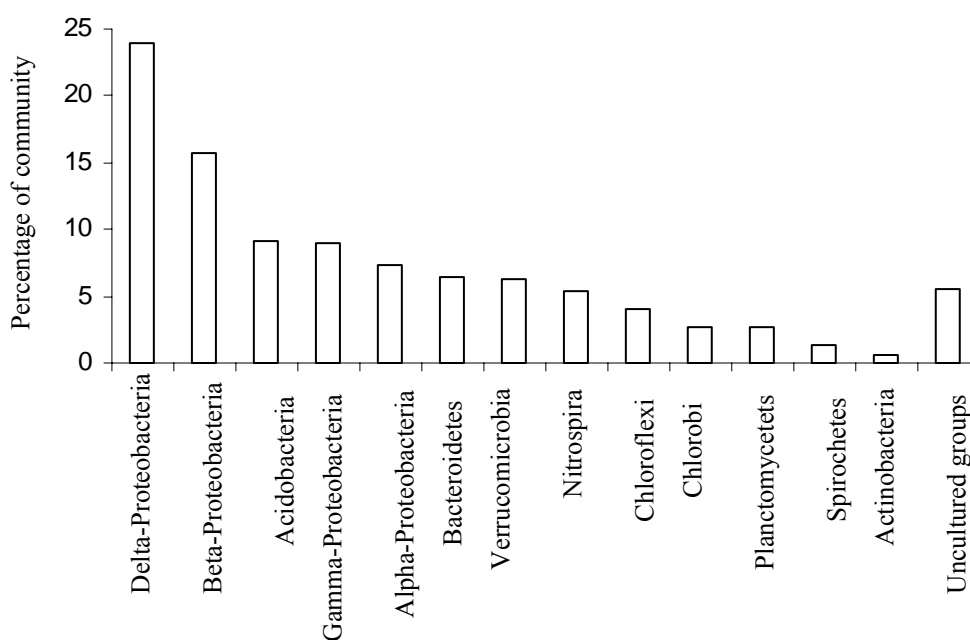


Fig. 5. Major bacterial lineages present in wetland sediments as identified through 16S rRNA gene sequencing. The percentage of the bacterial community occupied by populations in that taxa was determined by sequencing a total of 160 clones.

Delta-Proteobacteria make up almost 25% of the community, including representatives of the sulfate-reducing bacteria and Myxobacteria, a group of bacteria that are involved in organic matter processing (Fig. 5). Other dominant groups include the Alpha-, Beta-, and Gamma-lineages of Proteobacteria, as well as the Acidobacteria and Verrucomicrobia. These latter two groups are poorly characterized, but representatives are typically found in freshwater and soil environments. There are also clear differences between the microbial communities present on different sizes/types of decomposing organic matter. Together, these results suggest that the Western Lake Pontchartrain basin wetlands harbor very diverse and complex bacterial communities. The implications of this are difficult to judge but it suggests that these wetlands are

very resilient to perturbations, and that even if some bacterial populations go locally extinct there are others to take their place.

Discussion

Decomposition of organic material in Lake Pontchartrain wetlands is clearly linked to microbial enzyme activity, although different types of organic matter (different particle sizes) are degraded at different levels of efficiency. Linking microbial activity to decomposition is a major advance as it will allow future studies to simply measure microbial activity at one time point and almost instantaneously determine organic matter decomposition rates.

This was demonstrated with the microcosm studies where the effects of salinity and increased nutrient status could be monitored by an assessment of enzyme activity. While treatments did not measurably alter bacterial community structure (in part because, as revealed by the molecular data, the bacterial communities in this system are very diverse) they do alter microbial enzyme activity. Increased salinity reduced β -glucosidase activity and activity of this enzyme is directly linked to decomposition rate. Thus, moderate increases in salinity (such as occur during major storm events in the Gulf of Mexico) likely result in decreases in decomposition rate. This is of particular interest given that some studies show that in the aftermath of Hurricane Katrina, some Louisiana wetlands actually showed increased sediment accumulation (i.e. while hurricanes may erode some sites, they also result in sediment deposition at others). A concomitant reduction in decomposition rate due to saltwater intrusion essentially means those even though there might be an increase in organic sediment deposition, this material may not quickly decompose and may remain on site.

The microcosm study also showed that the Lake Pontchartrain wetlands are nitrogen limited, and that additions of inorganic nitrogen (as might occur from wastewater addition to these wetlands, or through freshwater diversions) change microbial activity. Of particular note was the increase in phosphatase activity, likely because of an increased microbial demand for inorganic phosphorus. However, neither N nor P additions changed the activity of β -glucosidase, which suggests that while nutrient additions might increase plant productivity, they may not drastically alter decomposition rates.

Molecular analyses of bacterial community structure reveals that these sediments harbor diverse bacterial communities, including species and lineages that have not adequately been described. These diverse communities likely provide a buffer against environmental perturbations and, as shown from the microcosm treatments, bacterial diversity is great enough that even substantial changes in salinity or nutrient status do not appear to cause major shifts in bacterial community structure. This resiliency in sediment microbial communities essentially means that many microbial processes are relatively stable, and while they do change with environmental conditions, the underlying community is still capable of carrying out the fundamental processes of carbon and nutrient mineralization that are required for ecosystem function.

Technology Transfer

Eight presentations based on research conducted as part of this project were given at national scientific meetings:

- 1) C. R. Jackson and S. C. Vallaire. 2007. Enzymatic modeling of fine particulate organic matter decomposition in a Louisiana cypress swamp. Annual Meeting of the North American Benthological Society, Columbia (to be presented in June)
- 2) S. C. Vallaire and C. R. Jackson. 2006. Changes in microbial enzyme activity in response to nutrient addition and elevated salinity levels in wetland sediments. Annual Meeting of the North American Benthological Society, Anchorage. Abstract in Bulletin of the North American Benthological Society 23:394
- 3) R. Rudowicz and C. R. Jackson. 2006. Fine scale spatial heterogeneity in ecosystem function: patterns in microbial enzyme activities in sediments. Annual Meeting of the North American Benthological Society, Anchorage. Abstract in Bulletin of the North American Benthological Society 23:170
- 4) A. V. Camus, S. C. Vallaire, and C. R. Jackson. 2006. Decomposition of fine particulate organic matter in a cypress swamp ecosystem. Annual Meeting of the North American Benthological Society, Anchorage. Abstract in Bulletin of the North American Benthological Society 23:363
- 5) H. L. Echlin, S. C. Vallaire, and C. R. Jackson. 2006. Effects of nutrient addition and salinity on benthic bacterial community structure in wetland sediments. Annual Meeting of the North American Benthological Society, Anchorage. Abstract in Bulletin of the North American Benthological Society 23:361
- 6) Jackson, C.R., and Harrison, K.G. 2005. The diverse bacterial community associated with fine particulate organic matter in wetland sediments. Annual meeting of the North American Benthological Society, New Orleans. Abstract in Bulletin of the North American Benthological Society 22:326
- 7) Vallaire, S., and Jackson, C.R. 2005. Changes in microbial extracellular enzyme activity associated with decreasing organic matter particle size in a Louisiana cypress swamp. Annual meeting of the North American Benthological Society, New Orleans. Abstract in Bulletin of the North American Benthological Society 22:363.
- 8) Harrison, K.G., Dugas, S.L., and Jackson, C.R. 2005. Molecular characterization of the bacterial community associated with fine wetland sediments. 105th General Meeting of the American Society for Microbiology, Atlanta.

Additional presentations were also given by graduate and undergraduate students at a local regional meeting (Southeastern Ecology and Evolution Conference). Manuscripts based on these findings are currently in review at scientific journals or are in preparation, likely resulting in 4-6 journal articles published from this research over the next two years. Before recommendations are made to policy makers we should be absolutely sure that our findings are beyond reproach and peer-review by other scientists at meetings or during the publication process is the best system we have to do that.

Upon publication, efforts will be made to make environmental stakeholders and policy makers aware of research findings. This will involve working closely with the Director and Assistant Director of the EPA/PBRP program so that findings can be integrated with those of other EPA/PBRP investigators, and also because those individuals have greater expertise in this area.

- How rapidly does organic matter decomposition occur in these systems?
- What limits the rate of decomposition and how likely will this change with future perturbations?
- How variable is decomposition spatially? Are there areas with faster decomposition rates than others and does this correlate with areas of land loss?
- Can we use enzyme models to quickly and efficiently monitor decomposition rates and sediment accrual? Are these applicable to other systems as management tools?
- What microbial communities are responsible for decomposition? How resilient are these communities to environmental impacts whether intentional or natural.

As stated previously, because decomposition is a major ecosystem-level process, these are fundamental questions on how these wetlands function. Decomposition rates directly influence whether organic matter builds up in sediments or is quickly recycled. Sediment build up mitigates land loss and is essential in wetland restoration efforts. Knowledge of how ecosystem restoration efforts such as freshwater diversions will alter decomposition dynamics is essential in order to predict the ecosystem-level impacts of those efforts. For example, freshwater diversion efforts might bring in nutrients and oxygenated water that, by all predictions, would likely stimulate the plant community. It's assumed that greater plant growth would result in more organic matter accrual in sediments and reduce wetland loss rates. But what if the microbial decomposer community is also stimulated, so that this additional input of organic matter is decomposed rather than accumulates? The microcosm studies show that salinity depresses microbial enzyme activity, and the decomposition study shows that this enzyme activity directly correlates with decomposition rate. Reduced salinity levels may have the opposite effect – stimulating microbial activity, which in turn would accelerate the decomposition process. If decomposition rates increase, it's very possible that nutrient additions might exacerbate sediment loss rather than reduce it. Obviously such information is vital to policy makers.

The development of the enzyme models that relate decomposition to enzyme activity is probably the most profound impact of this project. Because such models allow the rapid monitoring of decomposition, the approach would have broader management implications. A project that is about to be initiated as part of the Phase V program will monitor microbial enzyme activities in sediments around Lake Maurepas, with a particular focus on sites that are likely to be impacted

by Mississippi River diversion efforts. In collaboration with Dr. Janice Bossart (Southeastern), the enzyme activities will be combined with studies of aquatic invertebrate community structure and diversity to develop holistic biotic indices for environmental quality in this system. Such indices have great value in evaluating both the short and long-term impacts of environmental perturbations.

A Whole-System Approach for Restoring the Wetlands of the Western Lake Pontchartrain Basin

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¹Southeastern Louisiana University, ²Louisiana State University,
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Abstract

In this Final Report, we take a system-wide approach that considers restoration measures that will enhance conditions in the wetlands north and northwest of Blind River, located in the northwestern portion of the Lake Pontchartrain Basin. Specifically, we conducted a feasibility analysis on gapping the Amite River Diversion Canal to increase hydrologic exchange with the adjacent swamp. The area is impounded with approximately 2.2' of stagnant water. We estimate that 9,000 acres of swamp will be restored if this project is constructed. We demonstrate herein that two of our original proposed gaps are not optimally located and need to be relocated closer to Blind River. Project costs could be reduced by gapping the degraded spoil banks of the Petite Amite River and reducing the number of gaps on the Amite River Diversion Canal.

Our project includes three other components that further our understanding of current conditions of Lake Maurepas and its contiguous swamp and enable quantitative assessment of the benefits of future restoration efforts. These include (1) a study of Neotropical migratory birds using the Maurepas wetlands during migration; radar was used to demonstrate that Neotropical birds largely avoid anthropogenic marsh and concentrate in swamp. (2) A Geographic Information System was built to demonstrate that greater than 70% of the Maurepas swamp will not regenerate if logged. (3) A baseline monitoring program was conducted to characterize the benthic invertebrates of Lake Maurepas. These studies have been disseminated through annual presentations at regional, national, and international conferences, local town meetings, and manuscripts are in preparation and review, including a book chapter. In addition, the authors regularly attend meetings of state and federal agencies, serving as consultants on management issues in the Basin. Finally, we are working with our Technology Transfer personnel to produce "Briefs" and other materials to broaden the dissemination of this effort.

Primary Objectives

We had four primary objectives in this project:

- (1) To conduct a feasibility study on gapping the Amite River Diversion Canal to increase hydrologic exchange with the adjacent impounded swamp.
- (2) To study the distribution and abundance of Neotropical migratory birds using the Maurepas wetlands during migration; in particular, do Neotropical birds avoid anthropogenic marsh and concentrate in swamp?
- (3) To build a Geographic Information System to quantify the extent of non-sustainable swamp in the Maurepas sub-basin.
- (4) To conduct a baseline monitoring program to characterize the distribution and abundance of benthic invertebrates, with emphasis on *Rangia cuneata* clams.

Results

Feasibility Study: Four sites with replicate 625 m² permanent stations were established along the Amite River Diversion Canal (eight stations in all). Five litterfall traps, two 16 m² herbaceous plots, and two interstitial salinity wells were installed at each station. Diameter growth of all trees in each of the 625 m² permanent plots (n=460) was monitored annually and litterfall was regularly collected. Species-specific cover values were estimated and biomass was collected twice per year in each of the 16 herbaceous plots.

Working with CWPPRA personnel, we have estimated the optimal number and size of the proposed gaps along the Amite River Diversion Canal, and estimated the expected aerial coverage of benefited swamp. To decrease operation and maintenance, and increase exchange, the originally proposed culverted gaps should be replaced with 40' bridges. Our production data demonstrate that two areas that we thought were highly degraded are relatively healthy (Attachment 1), because several small bayous, not visible aerially, throughput freshwater to these areas. Hydrograph data (EPA/DNR, unpubl., Attachment 2) indicate that the swamp north of the Diversion Canal is definitely impounded and that water from the Diversion Canal would penetrate into the degraded swamp, and allow the swamp to drain several times each year (Attachment 2). We are suggesting that two of the proposed gaps be relocated to a severely impounded area closer to Blind River to achieve maximal swamp production benefits. Rather than gapping the Diversion Canal near Petite Amite we suggest that it would be less expensive, and equally effective, to gap the natural degraded spoil bank of the Petite Amite in several locations. This would cause currently stagnant water to drain from west to east and greatly improve primary production.

Satellite Imagery and Habitat Classification: We obtained a Landsat 7 Thematic Mapper (TM) image of the upper Lake Pontchartrain Basin from May 21, 2002. The image was imported into ERDAS Imagine 8.7 (Leica-Geosystems 2004) for analysis. In a supervised classification, we used the coordinates and known habitat types from 30 of our 40 field plots as training sites to identify the signature profiles of five *a priori* forest types: potentially sustainable swamp (bright green), non-sustainable swamp (yellow), swamp that has converted to anthropogenic marsh (red), natural marsh (purple), and bottomland hardwood forest (dark green) (Figure 1). Ten of our field sites contained attributes of greater than one habitat type (i.e., they were transitional) and these were not used in the training process. Unsupervised classification was then used to classify all of the remaining pixels. The classification results were then compared to higher resolution digital orthophoto quarter quadrangles (DOQQs) downloaded from Louisiana State University's Atlas server (<http://atlas.lsu.edu/>) to verify major landscape features. The final classification was then verified by conducting field trips to a wide variety of locations within each habitat type. Furthermore, the map was shown to several local authorities familiar with the territory to identify potential misclassifications.

Swamp Use by Neotropical Birds: We used radar to sample birds because it allowed us to monitor a much larger area than we could by working on the ground. The nocturnal migration of most songbirds makes them particularly amenable to sampling with weather radar (Gauthreaux and Belser 2003, Larkin 2004). At dusk, birds that will resume migration fly up from where they spent the preceding day. As they enter the radar beam, they are detected, and their density is reflected by the amplitude of the signal returning to the radar (just like rain on the familiar radar

images used to describe weather). We archived radar images from the Slidell airport NEXRAD daily in the spring and fall of 2004 from Weathertap (<http://www.weathertap.com/>). We archived both the base reflectivity (the typical radar map displayed to describe weather) and the radial velocity, which measures the speed of signals. For each night that we analyzed, we used a single image from within 30 minutes after sunset. Using the image just after dusk allows us to assume that birds are detected directly above where they spent the day. We only choose images for which the outline of the lake was still clearly visible (Figure 2).

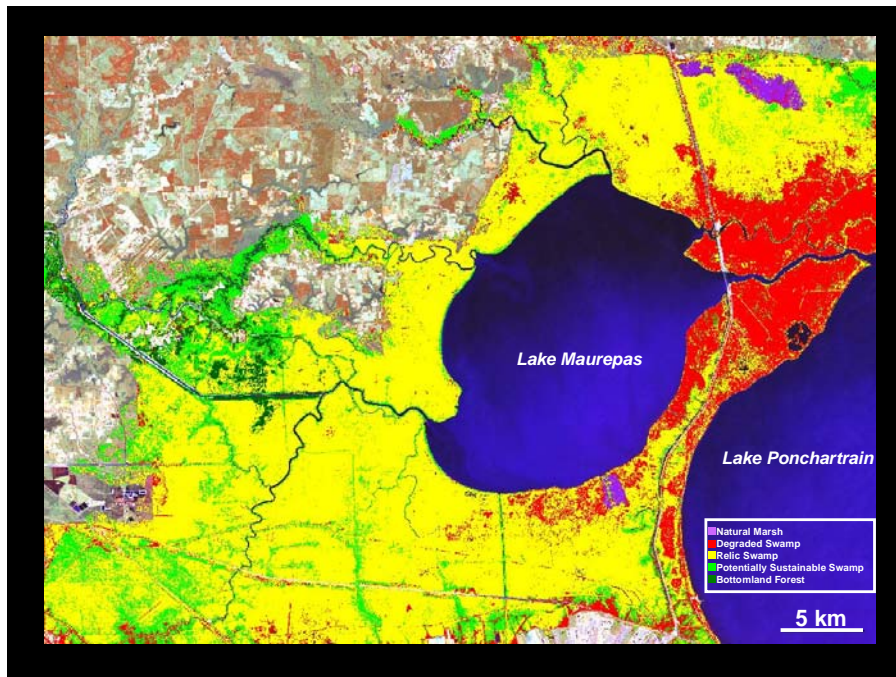


Figure 1. Classification of wetland types in the Lake Maurepas swamp.

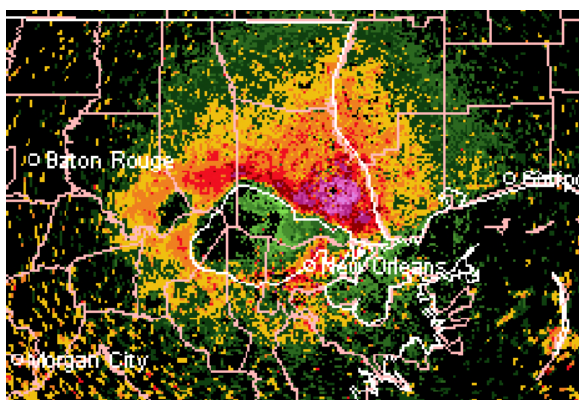


Figure 2. Sample radar reflectivity image from spring 2004 on a night of heavy bird migration. Hot colors indicate higher dBZ. Notice that the effect of Lake Maurepas remains visible; birds have not yet moved far enough from their stopover locations to fill the airspace over the lake.

Our dataset included 13 nights in spring 2004 between 9 April and 19 May and 12 nights in fall 2004 between 14 August and 15 October. We combined these images to form single composite image for each season, with each 1 km² pixel representing the mean dBZ scores of all the nights in the sample.

We added the composite radar image for each season to our landcover GIS (Figure 1). To analyze the combined dataset we overlaid a grid of hexagons 2.48 km on a side (~6 km²). Within each hexagon, we summed the pixels for both landcover and birds to get a total radar score and the proportional landcover of each category (Figure 3).

We began with a qualitative description of bird distribution in the Maurepas wetlands. To test for overall abundance differences between spring and fall, we used a Wilcoxon signed-rank test for paired data. We evaluated the difference in bird abundance around the lake by first assigning a location to all hexagons with (% urban + % water) < 50% (Figure 3). Location was then used as a categorical variable in a repeated-measures analysis of variance with dBZ from spring and fall as the repeated measures.

We used an information-theoretic approach to identify the landcover variables that best explain the variation in bird abundance. This analysis compares models based on their information content, which is a balance of the fit weighted against the number of parameters in the model. For this analysis, we used all hexagons with urban < 50% and water < 90%. This slightly broader number of acceptable hexagons increased the variance in landcover variables among samples. We also wanted to evaluate the effect of distance from the radar, so we included a variable corresponding to the column of hexagons, with 1 on the east side, closest to the radar, and 8 on the west side. Finally, we returned to the reduced dataset used in the analysis of location for a second information-theoretic analysis to consider whether location around the lake, landcover, or a combination of the two best explained bird abundance.

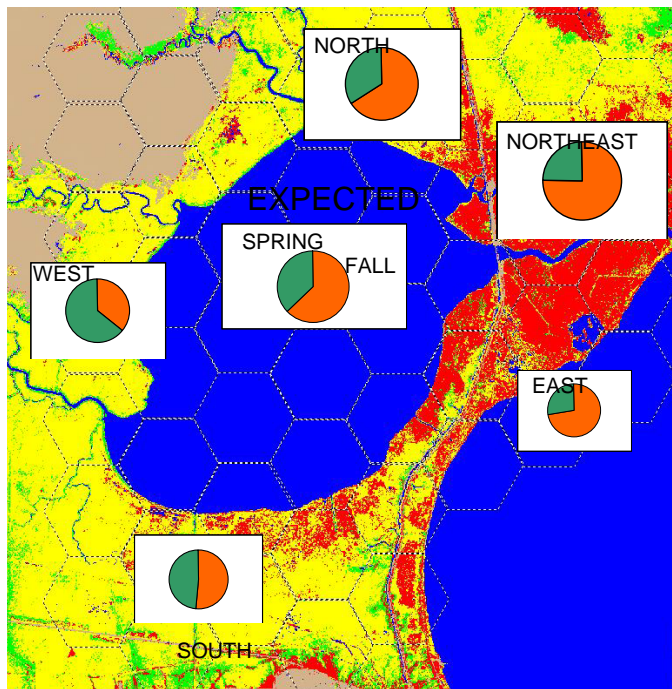


Figure 3. Observed and expected seasonal use of five locations around Lake Maurepas by migrating birds. The expected values come from the overall difference in birds detected between spring and fall. This figure shows only the proportional difference in use at each site, not the differences in bird abundance among sites. The base map includes the main landcover types: marsh (red), relic swamp forest (yellow), potentially-sustainable swamp forest

(green), water (blue), and unclassified + urban (both brown). A few small patches of bottomland hardwood (dark green) appear within sustainable swamp forest.

We detected more birds in fall than in spring (paired T-test, $T=2.264$, $P=0.030$). In addition to the overall difference in bird abundance, we also recorded different patterns of use around Lake Maurepas between the two seasons. South and west of the lake, bird abundance was higher in the spring than in the fall. East and northeast of the lake, more birds were detected in fall than in spring. To the north of the lake, birds exhibited little preference.

Repeated-measures ANOVA revealed significant season, location, and season x location effects (season $F_{1,24} = 28.34$, $P<0.001$; location $F_{4,24} = 8.68$, $P<0.001$; season x location $F_{4,24} = 16.43$, $P<0.001$). These results show that birds avoided the north and northeast corners of the lake in the spring, but strongly favored the northeast in the fall. All locations differed between seasons except the south (Figure 4).

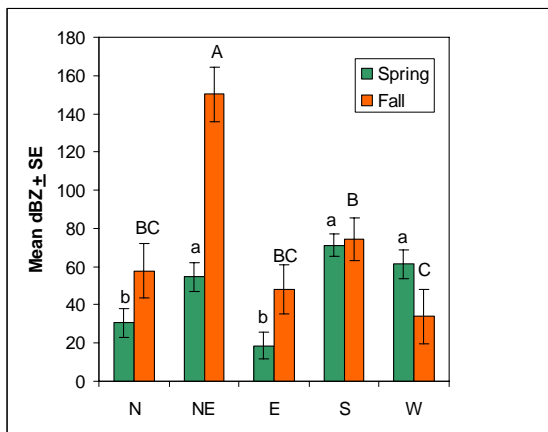


Figure 4. Mean dBZ values by location and season. Locations with the same lowercase letter do not differ in the spring; locations with the same uppercase letter do not differ in the fall (least-square means, $P>0.05$). All locations except south differed between spring and fall.

Bird association with landcover differed between spring and fall, as might be expected from the strong differences within locations between seasons. In spring, the strongest effect was avoidance

of marsh ($\Sigma w_i = 0.78$, Figure 5). The positive relationship between forest (sustainable + relic) and dBZ was less well supported ($\Sigma w_i = 0.41$, Figure 6), but would have been improved with exclusion of three outliers with low proportion of forest but high dBZ scores. These three points were from cells along the southeast corner of the lake.

In fall, distance from the radar was the most important variable ($\Sigma w_i = 1.0$), with more birds detected closer to the radar (Figure 7). Forest cover also was highly weighted ($\Sigma w_i = 0.91$, Figure 8), although this relationship included some low dBZ scores for hexagons with 50-60% forest cover along the northwest corner of the lake. No other variables had $\Sigma w_i > 0.44$. These analyses all excluded the outlier in the northeast corner.

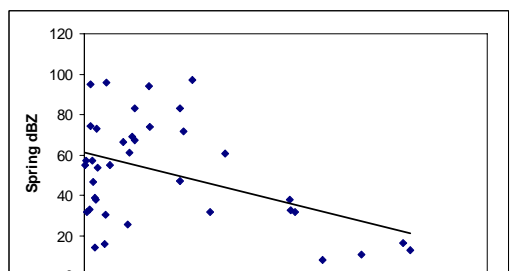


Figure 5. Hexagons with greater proportion marsh had lower dBZ scores in spring. The line is a linear regression ($R^2 = 0.20$).

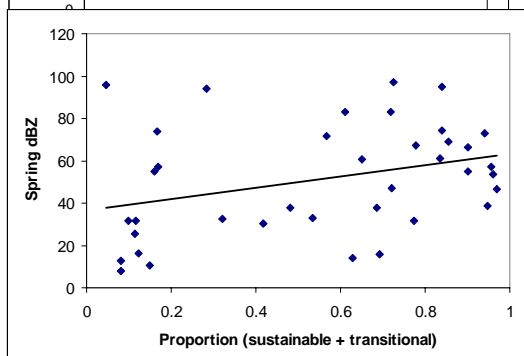
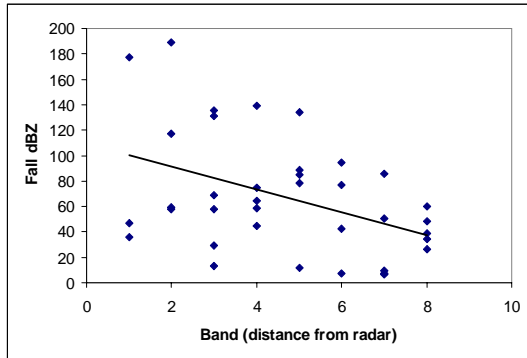


Figure 6. Hexagons with greater proportion of sustainable and transitional forest had higher dBZ scores in spring. The three

points with high dBZ scores at 5-30% forest were all from the southeast rim of the lake. The line is a linear regression ($R^2 = 0.11$).

Figure 7. Fall dBZ scores decreased with increasing distance from the radar ($R^2 = 0.18$).



Fall dBZ scores increased with increasing proportion of sustainable and relic forest. Low dBZ values at about 50-70% forest were all from the northeast rim of the lake. The line is a linear regression ($R^2 = 0.11$).

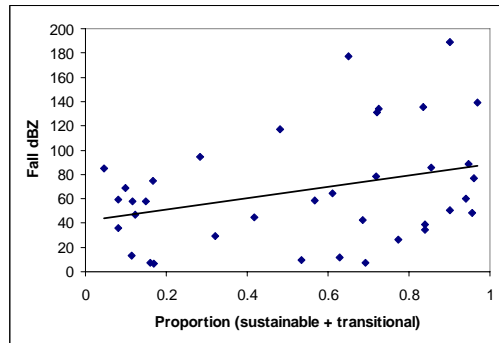


Figure 8.

We also analyzed landcover associations from the reduced dataset that was used for the analysis of location effects (Figure 4). For spring data, we collapsed the location data into the two groups of locations that differed from each other: N+E and NE+S+W. We then modeled dBZ as a function of landcover alone, as a function of location alone, and as a combination of the two (with location as a categorical effect and landcover variables as covariates). In spring, all of the models with location performed better than models with landcover alone, and no model with landcover alone was even moderately supported (all $\Delta AICc > 9.7$). Based only on the models that included location, proportion of water was the most informative variable ($\Sigma w_i = 0.64$). Proportion of forest had approximately the same support as in the analysis of landcover alone ($\Sigma w_i = 0.41$). Proportion of marsh had less effect than in the analysis of landcover alone ($\Sigma w_i = 0.33$), possibly because most of the hexagons with high proportion of marsh were concentrated within a single location (E).

For the fall data, we modeled landcover and location three ways. First, we included only landcover. Second, we combined landcover with location, as in the spring analysis. Finally, we combined landcover with distance from the radar. In all of these analyses we excluded the NE hexagons, which had much higher dBZ than any other location. The best-supported models included landcover and distance from the radar; no models that included location or did not include distance from the radar received appreciable support ($\Delta AICc > 6.0$). These results suggest that, for fall, our analysis of landcover and distance from the radar were adequate.

In both spring and fall, the three sample hexagons from the Pearl River basin had higher dBZ scores than nearly all of the hexagons around Lake Maurepas. In spring, the lowest Pearl value (106.9) was equivalent to the highest Lake Maurepas value (106.8), but no other Maurepas

values were >100, compared to 149 and 280 for the other two Pearl sites. In fall, the hexagon in the northeast corner of the Maurepas sample (dBZ = 290) was comparable to the Pearl values (216, 290, and 323). Three other hexagons in the northeast corner approached these values (dBZ>177), but the other fall samples from Lake Maurepas were considerably lower.

Survey of the Lake Maurepas Benthos: The littoral zone of Lake Maurepas was investigated for submerged aquatic vegetation (SAV) on 5/13/05, 6/9/05 and 12/13/05. In addition, the mouths of the Tickfaw, Amite, and Blind River, as well as North Pass, Pass Manchac, and Ruddock Canal were surveyed. Observations at thirty-three sites in the littoral zone of Lake Maurepas yielded no evidence of SAV. However, SAV was present in the rivers, passes, and canals sampled and the following is a list of species present: *Cabomba caroliniana*; *Ceratophyllum demersum*; *Heteranthera dubia*; *Hydrilla verticillata*; *Myriophyllum spicatum*; *Potamogeton pusillus*; *Vallisneria americana*; and *Zannichellia palustris*.

Nine sites on representative transects were sampled on June 2004, January 2005, June 2005, and November 2005 to determine benthic invertebrate species composition and water quality. Size classes of the abundant clam, *Rangia cuneata*, also were determined. Twenty-seven taxa of invertebrates were identified during the course of this study (Attachment 3). In addition, five epiphytic, colonial species *Cordylophora caspia*, *Moerisia lyonsi*, *Conopeum* sp., *Spongilla alba*, and *Trochospongilla leidii* also were present. These species were not used when determining species diversity in subsequent analyses because individuals are not easily distinguished. The following eight taxa listed in overall order of abundance constituted at least 1% of the total number of individuals over time: *Amphicteis floridus* (25.7%), *Rangia cuneata* (24.5%), *Chironomidae* (13.9%), *Chaoborus* sp. (5.8%), *Streblospio benedicti* (4.3%), *Probythinella protera* (3.6%), *Congeria leucophaeta* (2.0%), and *Oligochaeta* (1.9%).

Shannon-Weiner species diversity values significantly differed by date ($F_{3, 0.05} = 6.670$, $p < 0.001$). Species diversity values averaged between 0.912 ± 0.472 on 6/29/04 and 1.2481 ± 0.357 on 11/11/05. Diversity indices in June 2004 and January 2005 were significantly lower than those of June 2005 and November 2005. Changes in diversity values appeared to be due to changes in the number of individuals among species (evenness) rather than changes in species richness.

Lake Maurepas benthos was dominated by *Rangia cuneata*. Clams were present at all sites sampled. Results indicated that the number of clams varied by date, site, size class and the interaction of the three ($F_{144, 0.05} = 1.736$, $p < 0.001$). Although the interaction was significant, it appeared to be driven by size class. Clams in the size classes 26-30 mm and 31-35 mm were most prevalent, comprising over 80% of the total numbers of clams at each sampling site and up to 9.85 g/m^2 (Attachment 4). No clams were found larger than 40 mm. Low numbers of small clams (< 5 mm to 20 mm) indicate little or no spawning has occurred with the exception of June 2005. A massive spawning event occurred prior to the June 2005 sampling, resulting in up to 100 clams/m^2 in the <5 mm size class (Attachment 5).

Discussion

Feasibility Study: The feasibility study on gapping the Amite River Diversion Canal to increase exchange with the adjacent swamp indicates that the gaps should not be equidistantly located, as originally proposed. The area north of the Diversion Canal near Blind River is severely impounded with approximately 2.2' of nutrient-poor, stagnant water. Swamp is converting to floating marsh in this area and it compares with the most degraded areas in our extensive data base. Conversely, the areas south of the Diversion Canal and north near Petite Amite River approach the healthier sites in the data base, such as those along Hope Canal. A single 40' bridge gap to the southern area and gapping the degraded spoil banks of the Petite Amite in the northwestern area would further enhance primary and secondary production. A

series of 40' bridge gaps would provide extensive exchange to the degraded swamps contiguous with the Diversion Canal and would require far less operation and maintenance efforts.

Satellite Imagery and Habitat Classification: The vast majority of the Maurepas swamp will not regenerate if logged. Our habitat classification map has been presented to the Governor of Louisiana (Chambers et al. 2005) and has been used in the Comprehensive Habitat Management Plans of The Nature Conservancy and the Lake Pontchartrain Basin Foundation. It also has been used, as part of the Science Working Group's report (Chambers et al. 2005), by EPA to deny a permit request (of Steve Buratt) to log 200 acres of the Maurepas swamp. In addition, we use the image herein to provide evidence that Neotropical birds prefer swamp and largely avoid marsh. Unfortunately, large portions of swamp have converted to marsh over the past 50 years (red areas in Figure 1) and most of the remaining swamp is on this trajectory. A Mississippi River re-introduction at Hope Canal and gapping the Amite River Diversion Canal are two projects that may reverse the trend of swamp loss. Without these restoration projects, most of the Maurepas swamp will be lost within the next several decades.

Swamp Use by Neotropical Birds: Our results show the broad pattern of migration around Lake Maurepas. In spring, birds are more common along the south and west sides of the lake, possibly because they stop their preceding flight before the barrier of the lake. This hypothesis also is supported by the apparent concentration of birds along the southern rim of the lake. We probably underestimated birds south and west of the lake, indicating an even greater difference between the two sides of the lake.

In fall, birds were clearly concentrated along the northeast corner of the lake. Again, this probably indicates that they ended their preceding flight before the barrier of the lake. As expected, we found more birds in the fall, presumably due to the inclusion of young of the year.

The broad patterns of bird movements make it difficult to draw strong inference about landcover associations, as landcover also differs strongly around the lake. Even so, bird abundance was always explained best by models that included landcover variables. *Birds were more common over sustainable and relic forest than over marsh, leading to the fundamental conclusion that degradation of swamp forest removes habitat used by migrating birds.*

At a slightly larger scale, the Maurepas system does not appear to be used as heavily as the more pristine Pearl River basin in spring. In fall, the northeast corner of the Maurepas approached the bird density of the Pearl, although the rest of the Maurepas did not. Qualitatively, we always saw stronger radar signals over the Pearl, even as far north as the central Washington Parish (e.g. Figure 2). The actual hexagons we sampled, however, were only 20-30 km from the radar, close enough that they would be expected to show more detections than the Maurepas sites. Also, birds took off slightly earlier in the east, so more may have been in the air there than over the Maurepas at the time we took our measurements.

We need to mention several additional caveats about these analyses. First, we don't know the species composition of the birds we detected. We assume that the spring birds are mostly Neotropical migrants. In fall, the sample probably includes more short-distance migrants that will remain in south Louisiana for the winter. Second, our sample dates only include nights favorable to migration, without regard to the weather the preceding day(s). Our work does not necessarily identify areas important for migrants in bad weather. Finally, we regret having just one year of radar data to include. Due to changes in the image archives we used, we were unable to apply the same analysis to images from 2005. We suspect that the broad patterns of habitat use over entire seasons are generalizable across years, but we can't yet test this assumption.

Survey of the Lake Maurepas Benthos: The absence of SAV in the littoral zone may be due to a number of factors. A cypress shoreline characterized by an abrupt slope and an unconsolidated, coarse sand substratum creates a high energy environment not conducive to SAV growth. In addition, highly turbid waters in the spring due to riverine discharges may prohibit growth of SAV. Historically, SAV has not been present in similar habitat on the western shoreline of Lake Pontchartrain.

Measures of community structure such as identified taxa of invertebrates and species diversity values were comparable to those reported by Schexnayder (1987), and we also found that *Rangia cuneata* and Chironomidae were the dominant macroinvertebrates. Changes in diversity values in this study appeared to be due to changes in evenness. Although numbers of individuals were dynamic, species composition was relatively stable. Changes in species composition should be closely monitored to determine the effects of a river diversion.

Rangia cuneata dominate the benthos both in Lake Maurepas (Schexnayder 1987) and Lake Pontchartrain (Tarver 1972, Dugas et al. 1974, Abadie 1998). In this study, clams in the size classes 26-30 mm and 31-35 mm comprised over 80% of the total numbers of clams at each sampling site. Schexnayder (1987) and Abadie (1998) also found large numbers of clams of uniform length and no clams larger than 40 mm. Lack of conditions necessary for spawning is the most probable explanation. *Rangia* clams spawn from March to May and from late summer to November but may be continuous (Fairbanks 1963) and a shift in salinity is needed to induce spawning (Cain 1975). Over the course of this study, only one significant spawning event was recorded in June 2005. Clams in the size range of 6 mm – 20 mm were virtually absent from Lake Maurepas with the exception of June 2005, possibly indicating the lack of spawning events, lack of recruitment from Lake Pontchartrain, or high predation rates. The lack of clams larger than 40 mm remains an enigma.

Rangia clams are integral to the holistic integrity of Lake Maurepas. They are a food source by fish, crabs, and waterfowl (Darnell 1958, Perry and Uhler 1988, Ebersole and Kennedy 1995) and an important component of nutrient cycles through excretion, biodeposition of faeces and pseudofaeces, and bioturbation of sediment (Vaughn and Hakenkamp 2001). *Rangia* shell hash provides habitat and refugia for benthic fauna (Vaughn and Hakenkamp 2001) and also stabilizes sediment and modifies/stabilizes shorelines. *Rangia cuneata* should be of the utmost concern when evaluating the success of restoration efforts through river diversions. Furthermore, *Rangia cuneata* improve water quality thru filtration by reducing phytoplankton, reducing turbidity, and reducing the impact of fecal pollution (Spalding et al. in review). Clams may offset negative effects such as algal blooms during river diversions.

Technology Transfer

Our habitat classification map has been presented to the Governor of Louisiana (Chambers et al. 2005) and has been used in the Comprehensive Habitat Management Plans of The Nature Conservancy and the Lake Pontchartrain Basin Foundation. In addition, it was used in a report of NPR (5-26-05), was featured in the Louisiana Levant magazine (Volume 2, 2006), and will be published in the primary literature.

In general, the studies proposed herein will be disseminated through (1) this Final Report (hard bound, CD), annual presentations at regional, national, and international conferences, local town meetings, Master's theses, and manuscripts published in international journals. Dr. Shaffer was appointed as a science advisor to CWPPRA from its inception (1990), worked with agencies

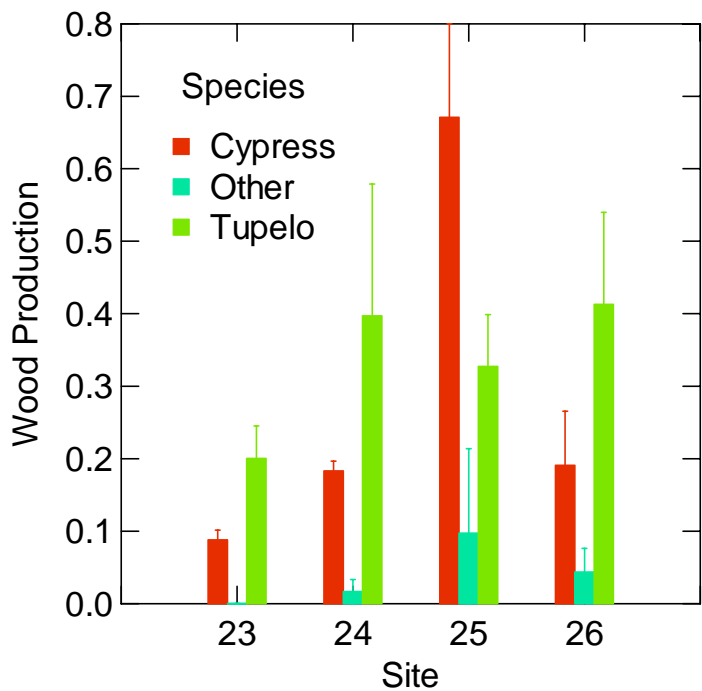
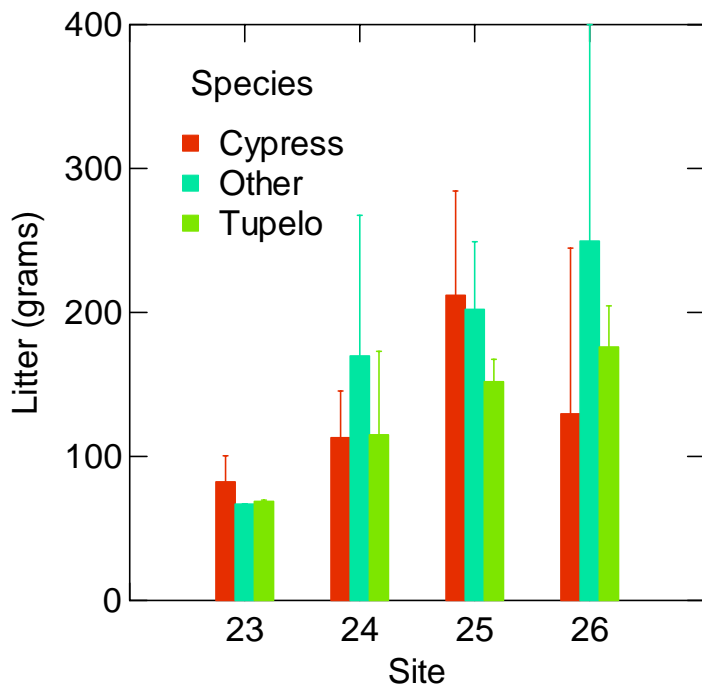
on the Coast 2050 plan, and is actively involved in the LCA effort. All three investigators have worked with state and federal agencies, private enterprise, and environmental groups (especially LPBF and TNC) in attempt to improve the environment of the Lake Pontchartrain Basin. We will continue to attend meetings of state and federal agencies (e.g., USCOE, USEPA, LADNR, LADEQ, LADWF, Governor's Office) serving as consultants on management issues in the Lake Pontchartrain Basin. To date, research out of Dr. Shaffer's lab has been instrumental in locating and sizing the Maurepas diversion, a project sponsored by EPA under CWPPRA and also sponsored by the LCA. Finally, we will be working with our Technology Transfer personnel to produce "Briefs" and other materials to broaden the dissemination of this effort.

Acknowledgments

This research was funded by the Environmental Protection Agency's Pontchartrain Basin Research Program. We thank Dale Manty and SAC members for their guidance and Nick Norton and Thais Perkins for orchestrating funds and meetings. Major contributors to this effort include research associates Bern Wood, Jason Zoller, and Beth Spalding. We would like to thank Glen Martin for his generosity in allowing us access to his land and Jacko Robinson for offering logistical support in the implementation and data gathering aspects of this study. Furthermore, we wish to thank graduate students Eddie Koch, Spencer Varnado, Luke Watkins, Aine Johnson, Susanne Hoepfner, Leonard McCauley, and Demetra Kandalepas, and many undergraduates for their tenacious help in the field.

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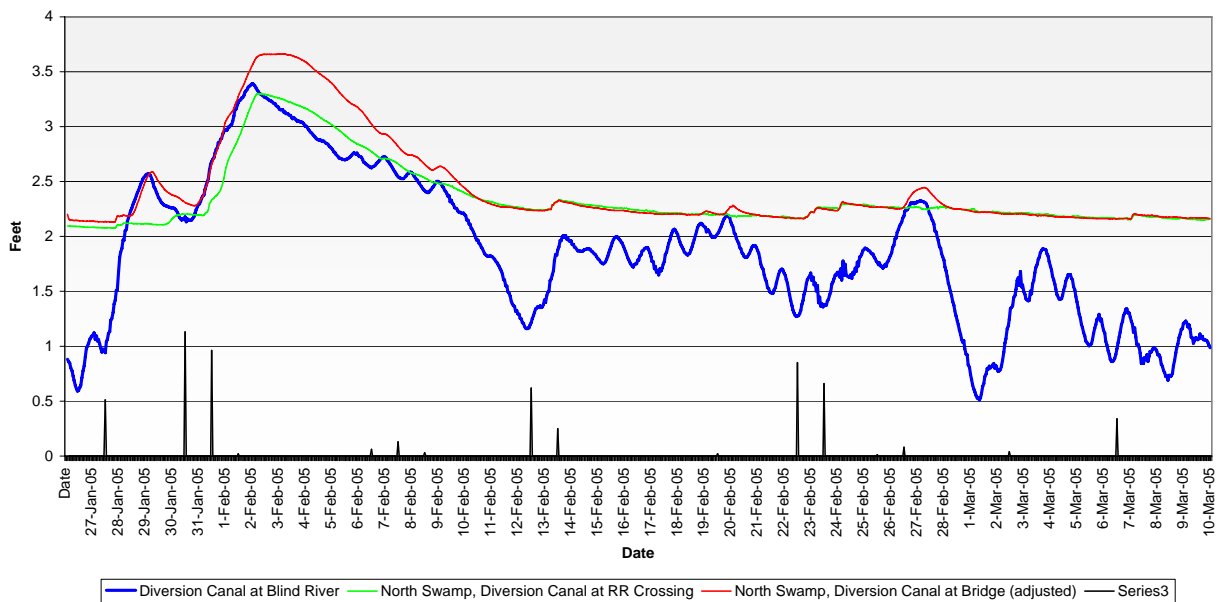
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Attachment 1. Litter (top) and wood production (bottom, measured as the product of diameter increase and basal area), during 2005, for four sites near the Amite River Diversion Canal. Site 23, located north of the Diversion Canal and west of Blind River, is severely impounded and the number of gaps should be increased. Conversely, sites 25 (located south of the Diversion Canal)

and 26 (located north of the Diversion Canal near Petite Amite River) are healthier and require fewer gaps.

Amite Diversion Canal Short Term Stage Data
(Adjusted)



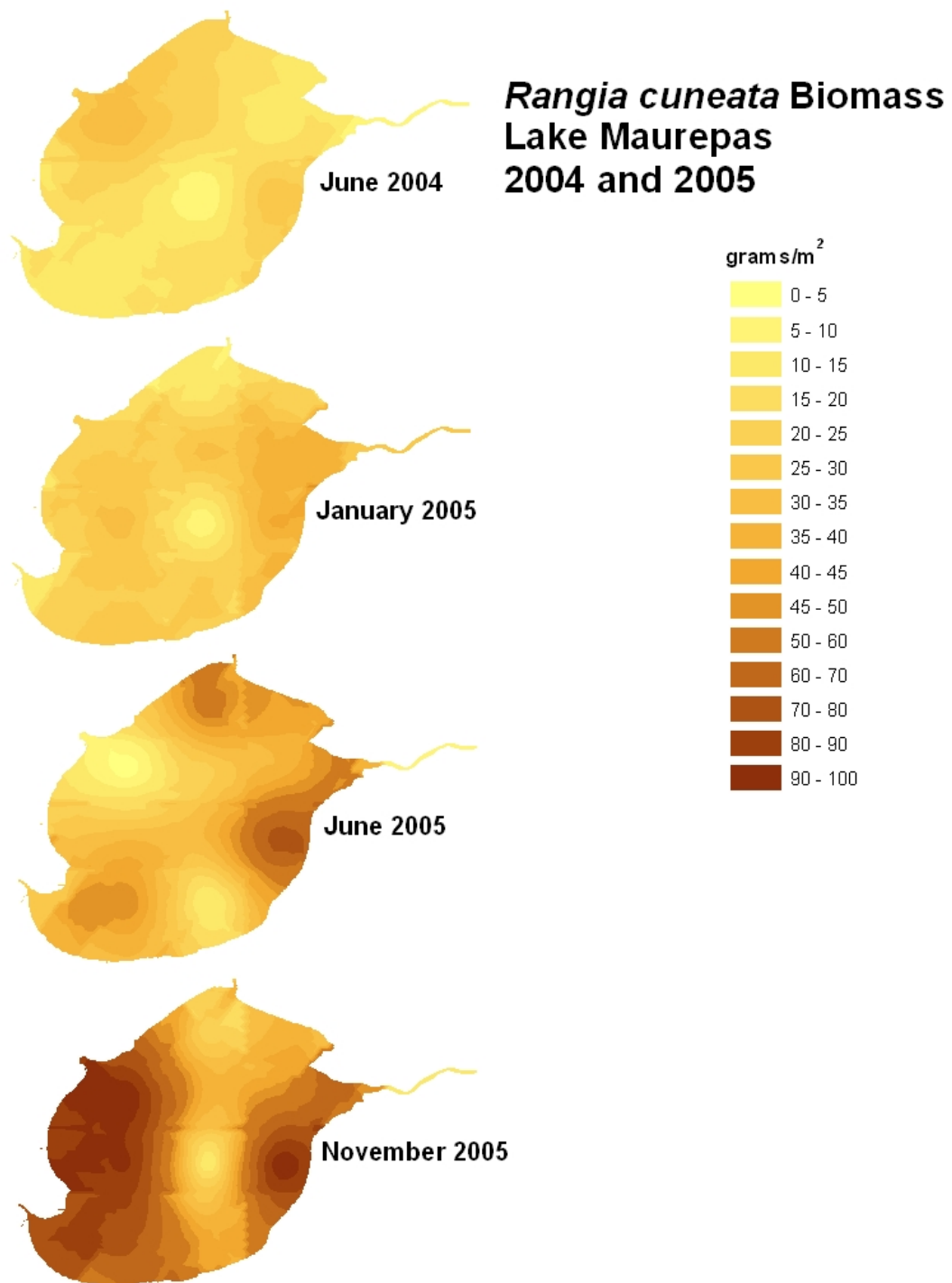
Attachment 2. Hydrograph of gages (of EPA/LADNR CWPPRA team) placed near site 23 (red), site 24 (green), and in the Amite River Diversion Canal where it meets Blind River. Hydrographs of the swamp sites indicate that they are definitely impounded (water never below 2.2'), whereas the hydrograph on the Diversion Canal indicates that gaps made at -1.0' below sea level would allow Diversion Canal water to enter the swamp regularly. The gaps also would allow the swamps to drain whenever the water levels in the Diversion Canal were lower than those of the swamp.

Table 1. Systematic List of Benthic Invertebrates Found in Lake Maurepas*

Phylum	Subphylum	Class	Subclass	Order	Family	Genus & Species	
Annelida		Clitellata	Oligochaeta				
							Polychaeta
				Canalipalpata	Ampharetidae	<i>Sigambra</i> sp.	
						<i>Streblospio benedicti</i>	
Arthropoda	Crustacea	Malacostraca	Eumalacostraca	Amphipoda	Gammaridae	<i>Gammarus</i> sp.	
					Aoridae	<i>Grandidierella bonnieroides</i>	
					Melitidae	<i>Melita</i> sp.	
					Oedicerotidae	<i>Monoculodes</i> sp.	
				Isopoda			
					Mysidacea		
			Maxillopoda	Copepoda			
		Ostracoda					
	Hexapoda	Insecta	Pterygota	Diptera	Ceratopogonidae	<i>Bezzia</i> sp.	
Chaoboridae					<i>Chaoborus</i> sp.		
Cnidaria		Hydrozoa		Hydroida	Clavidae	<i>Coralyphora caspia</i>	
					Moerisiidae	<i>Moerisia lyonzi</i>	
Ectoprocta		Gymnolenemata		Cheilostomata	Membraniporidae	<i>Conopeum</i> sp.	
Mollusca		Bivalvia	Heterodonta	Veneroidea	Dreissenidae	<i>Congeria leucophaeta</i>	
					Mactridae	<i>Rangia cuneata</i>	
		Gastropoda		Neotaenioglossa	Hydrobiidae	<i>Probythinella protera</i>	
						<i>Texadina sphinctostoma</i>	
Nemata							
Nemertea							
Porifera		Demospongiae		Haplosclerida	Spongillidae	<i>Spongilla alba</i> (gemmules)	
						<i>Trochospongilla leidii</i>	

*www.itis.usda.gov – Integrated Taxonomic Information System

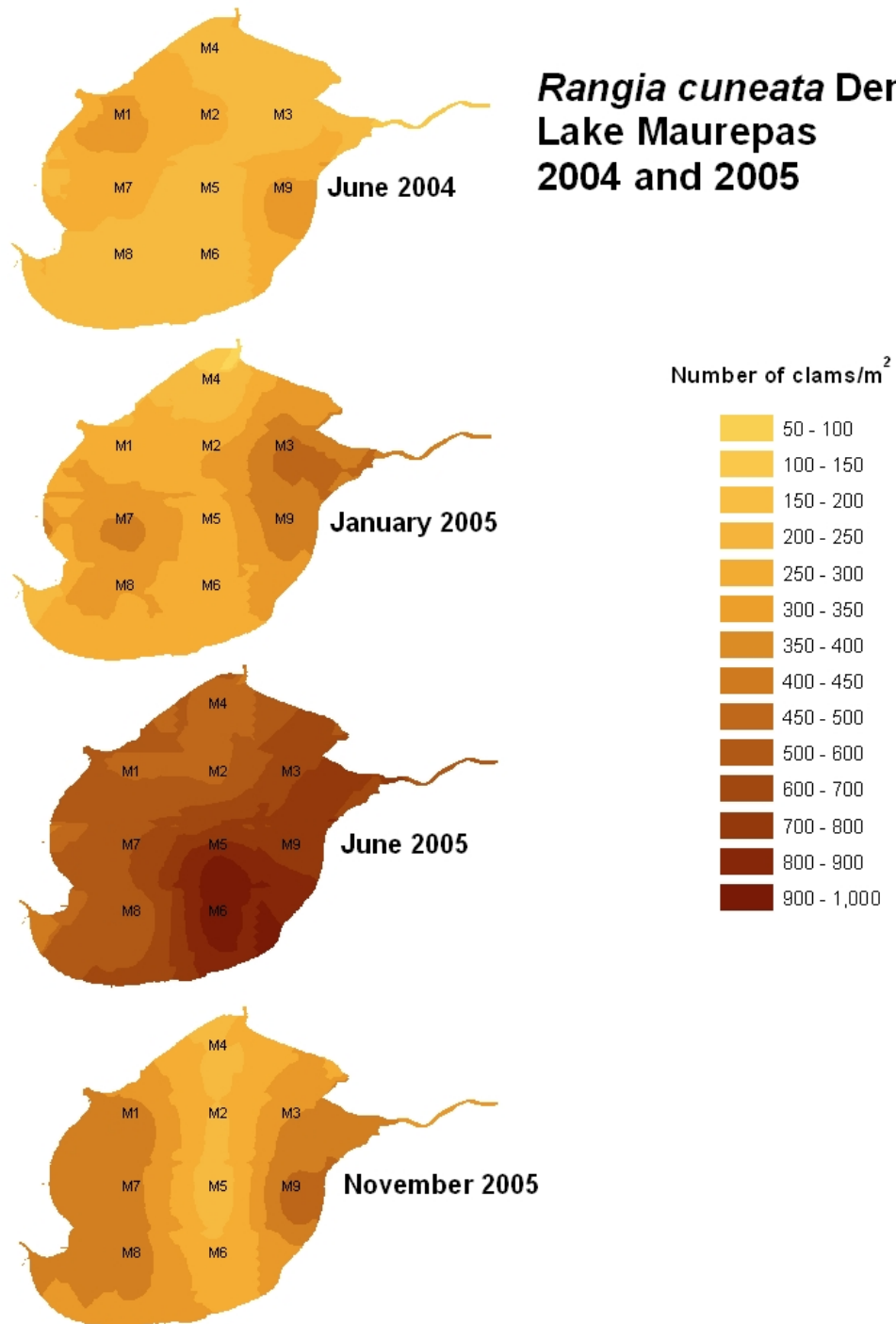
Attachment 3. Twenty-seven taxa of invertebrates were identified during the course of this study.



Maps produced using data collected by the Estuarine Research Lab at the University of New Orleans

Attachment 4. Changes in biomass of *Rangia cuneata* through time.

Rangia cuneata Density Lake Maurepas 2004 and 2005



Maps produced using data collected by the Estuarine Research Lab at the University of New Orleans

Attachment 5. Changes in density of *Rangia cuneata* through time.

**Highlights of Education, Outreach and Technology Transfer Activities at
Turtle Cove Environmental Research Station
Southeastern's Pontchartrain Basin Research Program (PBRP)**

Principle Investigator: Dr. Robert Moreau, Manager of Turtle Cove

Co-PI's:

Dr. Charles Dranguet

Dr. Roman Heleniak

Thais Perkins



Abstract

Southeastern's Turtle Cove Environmental Research Station plays a key role in the education, outreach and technology transfer of information learned by the scientists who use the station as a base of operations. Primary components of this program, funded at \$51,000 over a three-year period, included pontoon boat tours/lectures by university staff and faculty on local wetland waters for university classes, K-12 school groups and other interested organized groups, as well as workshops for K-12 educators. Pre-Katrina use of Turtle Cove (research/outreach/education-related) averaged nearly 3,000 user days of activity. For its part of the grant, Turtle Cove staff produced a website, electronic newsletter and several newspaper and television stories on the program. In addition, a book was produced entitled "Backdoor to the Gulf: an American Paradise Lost: the Pass Manchac Region, 1699-2006," (by Dranguet and Heleniak) which documented the socio-economic and environmental history of the area. 200 copies were sent to educators at all levels, local community leaders, agency professionals, advocacy groups, and political leaders. This book is the authoritative piece on this subject.

Note on Impacts from Hurricanes' Katrina and Rita: Although Hurricanes' Katrina and Rita inflicted severe damage to the research station (main building and Caretaker residence), its infrastructure (nearly 100% damage to boardwalks, bulkheads and wharfs) and its Pontoon Boat—approximately \$2 million worth in total—FEMA and state insurance funding has been committed, and it is hopeful that repairs and upgrades will result in a “better and stronger” facility than ever before. Shortly after the storms of 2005, a new Research Boat House and Office/Education Complex at Galva Canal was completed that will be used as the new site of Turtle Cove field operations until repairs are made at Turtle Cove. At the time of this writing, a new commercial grade pontoon touring boat has been received, and boardwalk repairs are being bid out. Final debris removal and repairs to the EPA experiment sites are set to be completed before the end of the year (December 2006). Therefore it is expected that Turtle Cove education and outreach programs will resume in Spring 2007 in at least a limited mode. Research by PBRP scientists (thru use of Turtle Cove vessels) has continued unabated in the aftermath of the storms.

Primary Objectives

The primary objectives of Turtle Cove's involvement with the PBRP were to communicate information learned from the research efforts through education, outreach and technology transfer activities. Such efforts included use of the following tools:

- Maximum continued use of the Turtle Cove Environmental Research Station resources for PBRP research, education and outreach activities;
- Maintenance of a website that was developed specifically for PBRP;
- Development of two electronic newsletters per year to selected members of the environmental community relating information learned through the PBRP;
- Development of a formal publication (booklet format) highlighting the environmental and socio-economic-cultural history of the Manchac Swamp;
- Use of other media opportunities to communicate information learned through the PBRP (i.e., newspaper, television and radio stories);
- Participation in training workshops developed and managed by other PBRP researchers and educators;
- Identification of key aspects of technology transfer of information learned from the activities undertaken by faculty and staff on the PBRP;
- Development of a Technology Transfer Team to manage all education, outreach and technology transfer issues of PBRP.

Results

Highlights of research, education and outreach-related activities at the Turtle Cove Environmental Research Station relative to the Pontchartrain Basin Research Program (PBRP) included the following areas:

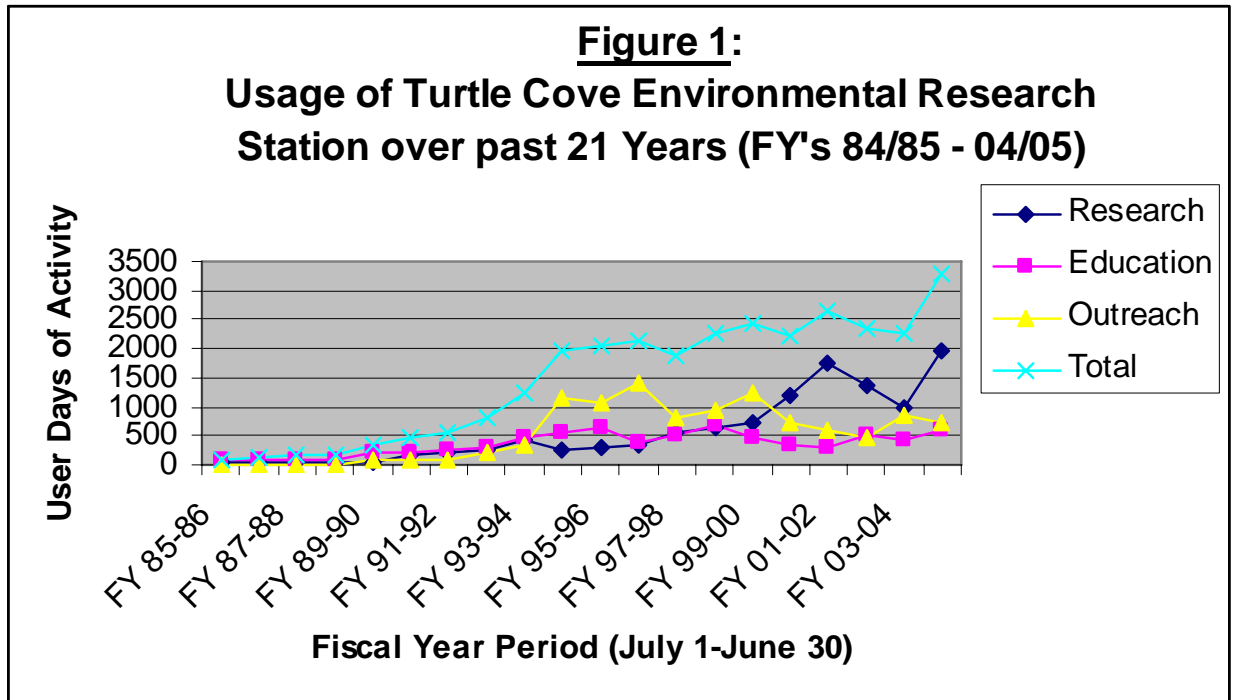
(1) *Continued increasing use of Turtle Cove for research, education and outreach activities related to PBRP:*

Overall use of Turtle Cove facilities, vessels and affiliated faculty/staff/students grew a total of 30.7% in the year just before Hurricane’s Katrina and Rita, with 2,946 total user days of activity. This total includes: an all-time high of 1,629 user days of research, most of which is directly attributable to PBRP (a 65% increase from the previous year), but which also included some new interdisciplinary research from other Southeastern programs and from other institutions; 578 user days of education-related activities such as university courses, field trips and teacher workshops (a 34.1% increase from the previous year), and; 739 user days of outreach-related activity such as K-12 and other group tours to the station and professional meetings and retreats (an 11.6% decrease from the previous year). A breakdown of the past five years of use at the station, which coincides with the start of PBRP, is shown in Table 1 below. **Note: all of the figures mentioned in the above paragraph and in the tables and figures below stop short of FY 05/06 due to extension damage from Hurricanes’ Katrina and Rita, due to the fact that such damage resulted in little or no use of the research station itself (for education and outreach activities) in the first year and a half after the storms.**

Table 1:
Last 5 Years of Use at Turtle Cove Environmental Research Station (FY’s 01/02 – 04/05)
in terms of User Days of Activity

	<u>FY 01/02</u>	<u>FY 02/03</u>	<u>FY 03/04</u>	<u>FY 04/05</u>
Research	1,740	1,383	987	1,629
Education	281	509	431	578
Outreach	<u>615</u>	<u>460</u>	<u>836</u>	<u>739</u>
TOTALS	2,636	2,352	2,254	2,946

Figure 1 below provides a summary of use at Turtle Cove from its inception in FY 84/85 through FY 04/05. Note the dramatic increase overall in user day activity—but especially in research—due primarily to EPA’s SPRP funding.



(2) *Web-Sites for both the Turtle Cove Environmental Research Station and the Pontchartrain Basin Research Program*

The two websites for both Turtle Cove and PBRP were maintained and updated as necessary to coincide with new information learned from researchers involved in the program. The PBRP website is the primary outreach mechanism by which research and education information is provided to the general public, and includes, among other items; information on all of the participating researchers and their work; downloadable reports and scientific papers compiled by the authors on this work; in-depth studies on specific projects by graduate students; and an interactive component that allows readers (i.e., the public) to ask questions and get answers on any of the work being done. The site was developed and is maintained by Thais Perkins, Research Associate at SLU, and was funded by the program. The address of the site is: www.selu.edu/sprp. Additional components of the website includes other technology transfer components such as research briefs. Ms. Perkins also works with other technology transfer and outreach issues associated with Turtle Cove thru other programs funded both internally and externally.

The PBRP website is also listed on the main page of the Turtle Cove website which was produced by Michael Greene, Biologist on Staff and Education/Outreach Coordinator for Turtle Cove. The Turtle Cove site, which was funded internally (and not by this program), includes information on all Turtle Cove programs and activities, staff and outside affiliates, a calendar of events, and even a new on-line boat registration system which now allows researchers and other

users much more ease and flexibility in utilizing the many vessels we have for all programs and projects. The site's address is: www.selu.edu/turtlecove.

Both of the websites are linked to one another and listed in various places on the main university website.

(3) Electronic Newsletter

An electronic newsletter of PBRP was developed in accordance with the PBRP website. Two newsletters were produced each year. The newsletters provided stakeholders with updated information on current findings and events related to PBRP. Each newsletter was designed and maintained by Thais Perkins (who also developed the PBRP website, mentioned above), with the goal of having an updated letter and mailing every six months. Current readers on the list include all researchers and educators associated with the project, the PBRP Advisory Committee, local non-profit and business leaders, public relations personnel at Southeastern, and other administrative leaders at the university. An expansion of the list this during the past year included community organizations, non-profit advocacy organizations, governmental groups, K-12 educators, nearby universities, and additional media outlets around the basin. Copies of the newsletters are available on the website.

(4) Environmental History Research on the Environmental and Socio-Economic-Cultural Aspects of Manchac Swamp

As part of this outreach program housed under Turtle Cove, a research project was undertaken that resulted in a book (soft-bound booklet consisting of 122 pages) entitled *Backdoor to the Gulf: an American paradise lost: the pass manchac region, 1699-2006*. The book was authored by Dr.'s Charles A. Dranguet (Southeastern's History Department.), Roman Heleniak (Southeastern's Social Science Research Center), who collectively have over 40 years of experience in this area. The book helps provide a historical context available to the general public, agency persons, advocacy groups, and decision makers, among others, regarding the history and culture of the Manchac Swamp region. This project, funded by PBRP, is a historical account of how culture, economy and the environment have changed over the past 300 years in the Manchac Swamp. The research consisted of oral interviews of the Reno family (a predominant family in the Manchac region whose first generation were parents to 18 children born in a houseboat on Pass Manchac). The accounts are of a family who has utilized the swamp in a way that has sustained them economically, socially and culturally for over a century. Their jobs in the local area have included trapping; commercial fishing; boat building; wildlife and fisheries law enforcement; employment with Southeastern Louisiana University and the Turtle Cove Environmental Research Station; and seafood resale. The first interviews were conducted with Mr. Hayden Reno, current Caretaker/Facilities Technician at Turtle Cove, in June and July of 2004. Interviews with Dunk Reno (Hayden's father) and Sonny Reno (a cousin who started a boat building business) were conducted in March and April of 2005. Other interviews with other family members who are involved in the businesses mentioned above were completed in early

2006. Some never before seen photographs were also incorporated into the booklet. Dr. Robert Moreau (Southeastern-Turtle Cove E.R.S.) assisted in the project.

Promotion of the book took (and additional technology transfer activity) took place in presentations by Dranguet, Heleniak and Moreau at the Tulane Environmental Conference (April 1, 2006) and the Twenty-Fifth Annual Gulf South History and Humanities Conference, Hilton Garden Inn Pensacola Beach Florida, Friday Oct 6, Program V, Session A. , "Fragile Lands: The Ecology of the Manchac Swamp, Louisiana." Title of paper "Return of Hunting and Gathering in the Manchac Swamp."

(5) Teacher Workshops

Teacher workshops were re-instituted at Turtle Cove in 2002—in part through this program (after a one-year absence). In addition, other various “teaching camps” have been designed and conducted by professors such as Dr. Debbie Dardis. The summer of 2005 produced the last Teacher Workshops conducted (by Dr. Dardis) prior to Hurricane’s Katrina and Rita---three workshops were conducted with 50 teachers from the Jefferson Parish area. Teacher workshops will restart again in the Spring of 2007. To date, a total of 56 workshops have been held at Turtle Cove with a total of 514 teachers participating (from approximately different 18 parishes and 34 different schools). It is estimated that these workshops have impacted over 200,000 students in the Pontchartrain Basin area schools. Many of the teachers have developed their own “in-service” training programs to teach other teachers, so their impact has been even greater. Dr. Dardis will provide more information on the details of the workshops and their success in her reports.

(6) Newspaper/TV Stories, Cypress Planting Outreach Event, and maintenance of On-Line Water Quality Monitoring Stations

Three of our region’s largest newspapers produced major front-page articles on work being done at Turtle Cove by Southeastern and other professionals on cypress and wetland-related issues. These stories are part of the outreach component that Turtle Cove manages relative to work done on SPRP. The stories and their authors are listed below:

The *Times Picayune* (the major New Orleans newspaper) produced a story by reporter Coleman Warner on July 6, 2004 titled: *Swamped in Schoolwork: At Turtle Cove, an SLU research center, teachers learn about incorporating the outdoors into their lesson plans.* The story covered the teacher workshops being conducted by Dr. Debbie Dardis this July. Michael Greene, Turtle Cove Biologist on Staff and Education Coordinator, was also in attendance to discuss other Turtle Cove workshops that are held during the spring and fall semesters and which he administers. Dr. Moreau also accompanied the group to discuss other research, education and outreach initiatives at the station, much of it related to SPRP. The article appeared in the July 11, 2004 edition of the *Times Picayune*, front page of Metro section.

A second story was conducted by the *Baton Rouge Advocate* (the major Baton Rouge newspaper) environmental reporter Bob Anderson, and appeared on the front page of the Sunday edition of the paper on August 15, 2004. The article was titled: *Cypress Calamity: Logging a century ago destroyed Louisiana's majestic cypress swamps, but some people at SLU are working to repair at least some of the damage.* The story covered the history of logging in the southeastern portion of the state, and included many old photographs from the turn of the century on this way of life, and current research and education efforts by university faculty and staff to help address the problem.

A third story was conducted by the *Hammond Daily Star* (major Hammond newspaper) reporter/photographer Roger Zettler on the Marsh Restoration Day conducted by Turtle Cove staff for the Tulane University's Green Club and Alternative Spring Break program. The story was primarily a front page cover story with two pictures and a caption titled *Replanting Swamp.* This annual marsh restoration day provides Tulane's urban-based students with an exercise in marsh restoration—specifically learning “how to” replant cypress trees. The effort involved planting 800 cypress saplings on West Jones Island, and included an overview of coastal wetland loss in Louisiana, and the research currently being conducted at Turtle Cove (specifically thru SPRP) on how to address the issue.

After the storms of the summer of 2005, a story appeared in the *Baton Rouge Advocate* (again by reporter Bob Anderson) titled *Turtle Cove Research Station Survives Storms: SLU Facility will Continue Despite \$2 million in Damage.* The article is a good primer for events that are poised to happen in early 2007 with the repair of the buildings, boardwalks, bulkheads, wharfs and other structures at Turtle Cove.

Turtle Cove also is in a series of partnerships with other universities to construct and maintain water quality monitoring stations in the Lake Pontchartrain Basin. There are currently three such stations, all of which have been enhanced to accommodate both researchers and the general public with information on water quality and weather characteristics of the greater Lake Pontchartrain Basin. The first station is behind Turtle Cove and is a partnership between Southeastern-Turtle Cove and the LSU Agricultural Center. The second is located on the northwestern edge of Lake Pontchartrain and is a partnership between Southeastern-Turtle Cove, Tulane University and LUMCON (Louisiana Universities Marine Consortium). The third is located on the wharf in front of Turtle Cove (on Pass Manchac) is a partnership between Southeastern-Turtle Cove and the USGS. Links to all of these stations can be found on the Turtle Cove website at www.selu.edu/turtlecove.

- (7) *Infrastructure Improvements to Education/Outreach Boardwalks and main Research Experiment Boardwalk at Turtle Cove (not funded thru PBRP or EPA)*

Hurricanes' Katrina and Rita in August and September of 2005 imposed extensive damage (above and beyond damage from Fall 04 Tropical Storm Mathew) to the research station (main building), its infrastructure (nearly 100% damage to boardwalks, bulkheads and wharfs) and the Pontoon Boat, which is the major piece of equipment used in most education and outreach

activities. These damages have been estimated at between \$1.5 and 2.0 million. As of this writing, the following monies have been awarded by FEMA for the damages. The new Pontoon Boat has been received and will be put into use in early December (2006).

Roofing repairs to all buildings:	\$50,000 (done)
Initial Debris Removal and Clean Up	\$42,000 (done)
Pontoon Boat replacement	\$52,700 (done)
EPA Experiment Site (site debris removal and repair)	\$42,000 (underway)
Boardwalk repairs/replacement (2,700 ft)	\$358,000 (1)
Wharf repairs/replacement (6 main areas)	\$48,000 (2)
Bulkhead repairs/replacement	\$640,000 (2)
Turtle Cove main building repair	\$102,000 (3)
Turtle Cove Caretaker Residence repair	\$94,000 (3)

Notes; (1) final contract arrangements are being made with contractor. (2) Jobs will be bid out in public newspaper in December 06 or January 07. (3) State agencies arranging for work to begin.

These repairs and upgrades are expected to result in a “better and stronger” facility than ever before. In addition, a new Boat Shed and Office/Education Complex at Galva Canal has been built that will be used as the new site of headquarter operations until repairs are made at Turtle Cove. Therefore it is expected that most Turtle Cove education and outreach programs and projects will resume in Spring 2007 in at least a limited mode. It should be noted that research activities have not been interrupted by the storms (none of the research vessels were damaged).

Discussion

The team that produced all of the deliverables noted in this Final Report plan to continue to write proposals to PBRP for education, outreach and technology transfer initiatives. In fact, Technology Transfer has become a tangible part of the program and the Turtle Cove team is involved in its implementation as of this writing. Technology Transfer issues are discussed in Part V below.

Goals and objectives that will continue despite the ending of this phase of funding include (most probably under the umbrella of the new Technology Transfer Team):

- (1) Continued high use of Turtle Cove resources for PBRP users (research, education and outreach oriented)—although the main building and associated infrastructure will be under intense repair during the coming year from storm damage, new facilities and equipment will allow most PBRP programs and projects associated with Turtle Cove to continue;

- (2) Development of a Technology Transfer group (based out of Turtle Cove offices) that will assist all other researchers in all aspects of education, outreach and technology transfer issues. (see next section below)
- (3) Continued upgrading and maintenance of the PBRP website (should be conducted by technology transfer team);
- (4) Mailing of the electronic newsletter twice per year (during Spring and Fall semesters, thereby communicating the most recent and relevant information from faculty and their graduate students) (should be conducted by technology transfer team);
- (5) Continued use of all monitoring stations for PBRP users and the general public from an outreach and technology transfer standpoint;
- (6) Participation in new technology transfer workshops that will be geared towards local politicians, school board offices, environmental non profits, and other local community groups who are in policy and decision making environments;
- (7) Annual Times Picayune (New Orleans newspaper), Morning Advocate (Baton Rouge newspaper) and Daily Star (Hammond newspaper) cover stories on PBRP programs at Turtle Cove;
- (8) New and recurring stories on Southeastern's cable channel on PBRP programs at Turtle Cove;
- (9) Public Open House at Turtle Cove this fiscal year focusing on PBRP programs and information (once the main building is repaired);
- (10) 4th Annual Marsh Restoration Exercise at Turtle Cove and West Jones Island with Tulane and SLU students (incorporating information learned from PBRP);

Technology Transfer

Technology transfer is an important component of all research projects in PBRP, and is completed in three major ways. First, each researcher in PBRP is required to develop and enact some form of technology transfer for information learned from his or her research project (these Technology Transfer activities are reported separately in each researcher's reports). A second way in which technology transfer is handled in PBRP is through the education and outreach activities of the Turtle Cove Environmental Research Station. Turtle Cove's involvement in PBRP is primarily through use of the station for other researchers, but several of the station's staff and affiliated researchers conduct specific education and outreach programs and activities that directly communicate information learned from PBRP and other sponsored research activities to selected groups of audiences. Therefore, Turtle Cove plays an important role of technology transfer in PBRP.

Finally, in order to better serve the goal of Technology Transfer, the "Technology Transfer Team" based out of the Turtle Cove offices will assist in promoting all aspects of education, outreach and technology transfer issues associated with all PBRP authors and their research. The effective and efficient transfer of research to community and other social groups, teachers, agency representatives, environmental groups, and decision makers (i.e., public

officials), will be communicated in a number of ways by the Technology Transfer Team, including:

- promotion of the PBRP website to a wider audience
- targeting newly developed research highlights and research briefs to more wide-ranging governmental and non-government political groups
- publicizing newspaper, television and radio spots so that a wider audience can be reached
- funding of a technology transfer team to address all of these education, outreach and technology transfer issues each year

The next phase of technology transfer from all researchers involved in PBRP should include all of the above mentioned activities so that the full benefits of all projects within PBRP can be effectively and efficiently communicated to those groups who will be able to then educate (and influence) decision makers.

In summary, the overall goal of this project has been to disseminate research information learned from all PBRP sponsored research to the general public through a variety of channels. These channels include: Teacher Education Workshops; Pontoon Boat tours and lectures in the Manchac Swamp; websites, newsletters and other media information services; and via the completion of a socio-economic and environmental history of the Manchac Swamp and the culture of its human residents. It is now planned that these education and outreach activities will be parlayed into a more detailed and encompassing program of Technology Transfer to other members of the community, including various social and environmental groups, agency personnel and decision makers who serve as the public's representatives.

The questions posed by the various researchers of PBRP—as addressed by their individual research studies, cover a wide array of restoration and sustainability issues. And of course each of these research projects address various scientific hypotheses that challenge the capability of humans to either restore the ecosystem to its pre-human impact state, stabilize it as it is, or predict what further changes we can expect. Recommendations of each of these is part of the Technology Transfer initiative.

In this particular study, groups that are addressed included teachers (primarily from the teacher workshops), and residents, resource users (recreational and commercial), agency representatives, and decision makers from the surrounding parishes of the Manchac Swamp ecosystem---these groups were addressed through the other mechanisms of this project, including the website/newsletters updates on scientific projects, and through the book on Manchac Swamp. Information from all of these media document the changes in both the natural and socio-economic-cultural systems of the area, and it is hoped that information learned will aid these groups in better understanding the state of the region so that both private and public decision-making processes can be made in a more information-rich environment.

Are Polycyclic Aromatic Hydrocarbons Stressors for Lymphocyte Development or Activation in Frog Populations in Bayou Trepagnier?

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Abstract

In the Western Lake Pontchartrain Basin ecosystem, habitat degradation has resulted from multiple stressors including xenobiotics released by the petrochemical industry. Our studies sought to identify stressors influencing immune system integrity particularly in amphibians residing in the region. These studies address whether polycyclic aromatic hydrocarbons (PAH) found in sediments from Bayou Trepagnier impact lymphocyte development or activation in tadpoles developing in the laboratory, in local frog populations, or in mouse lymphocytes. Considered bioindicators of environmental health, frogs have a complex life-cycle and developmental program that are particularly sensitive to environmental influences. Development of the frog immune system occurs in stages tightly linked to the organism's developmental progression. These studies suggest that PAH, such as those detected in sediments from Bayou Trepagnier, reduce RAG-1 mRNA levels in treated bullfrog tadpoles. RAG-1 is a protein required for lymphocyte development. RAG-1 deficiency in frogs residing in contaminated areas of the region would have serious implications for immune system health and survival of these organisms. These studies help lay the groundwork for monitoring immune system health in this region and elsewhere.

Primary Objectives

The major aim of this project is to help identify environmental stressors in the Western Lake Pontchartrain basin region, particularly in Bayou Trepagnier. Our main approach was to identify immune system stressors by looking at effects of known polycyclic aromatic hydrocarbon (PAH) contaminants on levels of RNA corresponding to genes critical for lymphocyte development and activation. This project has helped in the development of protocols for validating the use of frogs as a bioindicator organism for examining immune system effects of environmental contaminants. We plan to continue and extend these studies.

Results

In these studies, we looked for effects of PAH in *Rana catesbiana* (Bullfrog) tadpoles. These animals can be easily manipulated in the lab, and are highly susceptible to contaminant exposure through their skin or by ingestion of contaminated food sources such as insects. Additionally, since bullfrogs are present in the Western Lake Pontchartrain region, the methodology devised to look at gene expression in tadpoles in the lab, can be extended to proposed studies in the field.

While the initial aim of using our devised methods in the field has not yet been completed, we intend to continue these studies with that goal in mind.

Much of the work on this project was carried out by Erica Perrer, MS. and Jessica Klopff, MS. both graduate students at SLU who completed Nonthesis Masters's Degrees.

RNA isolation and Primer Design:

The most significant progress on the project has involved devising protocols for RNA isolation and Real Time Quantitative Reverse Transcription-Dependent Polymerase Chain Reaction (qRT-PCR) amplification of mRNA corresponding to expressed lymphoid genes from tadpole tissues including liver and spleen. We overcame degradation difficulties during tadpole liver RNA isolation by combining two different RNA isolation procedures (Trizol and Qiagen RNeasy columns) (Fig. 1).

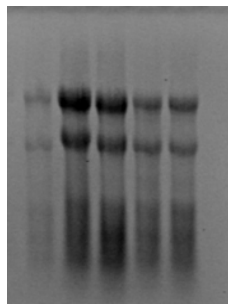


Figure 1: Example of formaldehyde gel demonstrating integrity of input total cell RNA used in these experiments

We designed primers to detect expression of a ubiquitously expressed control gene, EF-1 α . This gene encodes a factor involved in protein synthesis and controls for total RNA input in the reaction and serves as a “housekeeping gene” for normalization of qRT-PCR amplification results. These primers are identical to those used previously to detect expression of Ef-1 α in *Xenopus laevis* (African Clawed Frog) and amplify a fragment of approximately 224 base pairs from Bullfrog mRNA subjected to reverse transcription (RT+), but not the untreated (RT-) control reactions (Fig 2A and 2B).

A.

B. RT + - + - + - + - -

Bullfrog EF1 α

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CCTGAATCACCCAGGCCAGATTGGTGCTGGCTATGCACCTGTGCTGGATTGCCATAC
TGCTCATATTGCTTGCAAGTTTGCAGAGCTGAAGGAGAAAATTGACCGTCGTTCTGG
TAAGAAACTGGAAGAAATATCCCAAGTCCCTGAAATCTGGTGATGCTGCCATCGTTG
ATATGATCCCTGGCAAGCCCATGTGCGTGGAGAGCTTCTCAGACTACCCTC
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EF1 α Xenopus

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CCTGAATCACCCAGGCCAGATTGGTGCTGGATATGCCCTGTGTTGGATTGCCACAC
TGCTCACATTGCTTGCAAGTTTGTGAGCTAAAGGAGAAGATTGATCGCCGTTCTGG
TAAGAAACTGGAAGACAATCCCAAGTTCCTGAAGTCTGGAGATGCTGCCATTGTTGA
CATGATCCAGGAAAGCCAATGTGCGTGGAGAGCTTCTCAGACTACCCTC
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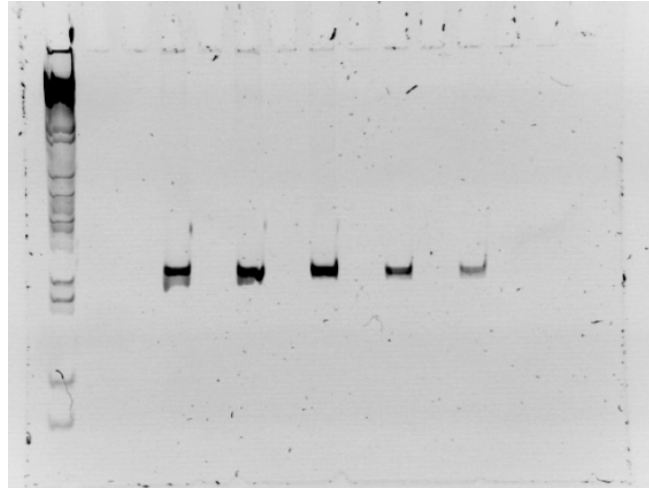


Figure 2: A. Sequence of EF-1a fragment obtained by RT-PCR of Bullfrog liver RNA using *Xenopus*-specific primers. The EF-1a sequence from *Xenopus* is shown for comparison (Greenhalgh et al. 1993). B. Example of gel showing EF1-a amplification in +RT but not –RT liver RNA samples.

Degenerate PCR primers were designed for the Recombination Activating Gene-1 (RAG-1). This gene is critical for V(D)J recombination, a DNA rearrangement process that assembles T cell receptor and immunoglobulin variable region genes during lymphocyte development. Without expression of this gene, T and B lymphocytes cannot express antigen receptors and are blocked in early developmental stages, precluding development of adaptive immunity. The degenerate RAG-1 primers were designed based upon partial consensus with aligned sequences from a variety of vertebrate sources including *Xenopus laevis*. These primers allowed amplification and sequencing of approximately 400 base pairs of RAG-1 mRNA and/or DNA from a variety of frog species including *Rana catesbiana* (Bullfrog), *Hyla cinerea* (Green Tree frog), and *Rana sphenoccephala* (Southern Leopard Frog). The bullfrog sequence obtained was used to design bullfrog-specific primers for use in the qRT-PCR reactions. These primers successfully amplified a RAG-1 mRNA fragment of 161 base pairs from total RNA derived from bullfrog liver subjected to reverse transcription (RT+), but again, not the untreated (RT-) reactions (Fig 3).

RAG-1 seq (predicted)	RAG-1 Product sequence
GCCACTCCATGCTCTCAGAAAT	GCCACTCCATGCTCTCAGAAAT
GCAGAGAAGGCCTTGCTGCCA	GCAGAGAAGGCCTTGCTGCCA
GGGTATCATCCCTTTGAATGGA	GGGTATCATCCCTTTGAATGGA
AGCCTCCTCTGAAGAATGTCTC	AGCCTCCTCTGAAGAATGTCTC
TGCCCCGTACAGATGTTGGCATC	TGCCCCGTACAGATGTTGGCATC
ATGGATGGCCTTTCAGGACTGA	ATGGATGGCCTTTCAGGACTGA
ATCGTTCAGTTGATGAATACCC	ATCGTTCAGTTGATGAATACCC
AGTGGAAG	AGTGGAAG

Figure 3: Predicted and actual sequence of RAG-1 mRNA amplified by RT-PCR from bullfrog liver.

We set out to design primers to amplify Activation Induced Cytidine Deaminase (AID) mRNA. The AID gene encodes a protein that has multiple roles related to B lymphocyte activation, including introducing DNA breaks during somatic hypermutation (SHM) and gene conversion (GC), which add diversity to jawed vertebrate immunoglobulin variable region genes, and immunoglobulin class switch recombination (CSR), which changes the heavy chain constant region gene expressed during the course of an immune response, and subsequent functional capabilities of the immunoglobulin (Fugman, and Schatz. 2002). We tried several different primer sets designed to amplify various regions of the AID gene. Although we had success with one of these primer sets, they were within the noncoding 3' untranslated region obtained from a *Xenopus* genome scaffold and we could not be completely certain that the region was part of the AID gene. Recently, the entire AID cDNA sequence from *Xenopus* was deposited in Genbank. We designed a set of degenerate primers to amplify an upstream coding region fragment spanning Exon 3 and Exon 4 predicted from the human intron/exon structure. This fragment, if amplified, would be splice-specific and thus RNA specific. Unfortunately, these primers failed to amplify a specific fragment that could be cloned, so the amplification of AID mRNA from bullfrog remains an ongoing objective.

We also designed primer pairs for CYP1A1, a gene known to be induced by PAH exposure, but as yet have been unable to amplify mRNA corresponding to this gene.

While our primer set for the RAG-1 gene is "exon internal" rather than "splice-specific", we believe that we have optimized the RNA isolation protocol sufficiently so that little DNA remains in the samples, as evidenced by the absence of (or several orders of magnitude lower signal in) the RT- reactions. The EF-1 α amplification is splice-specific, so only amplifies RNA.

Polycyclic Aromatic Hydrocarbon Exposures:

While working towards designing primers for amplification of Bullfrog genes, we set up a laboratory PAH exposure experiment. We exposed bullfrog tadpoles (C. Sullivan Co, Nashville TN.) to either pyrene, 7, 12-dimethylbenz[a]anthracene, or 2-methylnaphthalene in their water. These compounds were chosen because they had been detected previously at elevated levels in sediments from Bayou Trepagnier as compared to those from Bayou Traverse, a reference site (Mowat. and Bundy 2001). Additionally, of the compounds detected in sediments, these were fairly soluble in aqueous solution. We have collaborated in these studies with Dr. Isiah Warner and Arthur Gates of Louisiana State University's (LSU's) Dept. of Chemistry. They worked towards devising methods for detection of PAH in tissues, and they provided advice for setting up the experiments. Optimization of protocols for analysis of PAH in tadpole tissues is still ongoing. The Warner laboratory utilized EPA Method 8270C for extraction of compounds and analysis of PAH and metabolites by Gas Chromatography/Mass Spectrometry, but have not as yet detected PAH or PAH metabolites from whole animals. We have discussed developing an Enzyme-Linked Immunosorbent Assay (ELISA) to detect PAH in tissue extracts. Future work will also involve attempts to detect PAH and PAH metabolites by fluorescence spectrometry in bile from treated animals.

We used three test concentrations for each compound providing for optimal solubility (pyrene 0.1, 1, and 10 ppb; 7, 12-dimethylbenz[a]anthracene 0.1, 1, 10 ppm; 2-methylnaphthalene 0.1, 1, 10 ppm). The compounds required initial DMSO solubilization, so DMSO control tanks were included. Pyrene tanks contained 0.00005%, 0.0005%, and 0.005% DMSO and 7, 12-

dimethylbenz[a]anthracene and 2-methylnaphthalene tanks contained 0.024%, 0.24% and 2.4% DMSO. In total, 16 tank exposures were performed in 5L of H₂O at pH 7-7.5. Three concentrations of each of the three PAH (9 tanks total), 6 tanks with DMSO, and 1 untreated tank were included. 8 tadpoles were exposed in each tank.

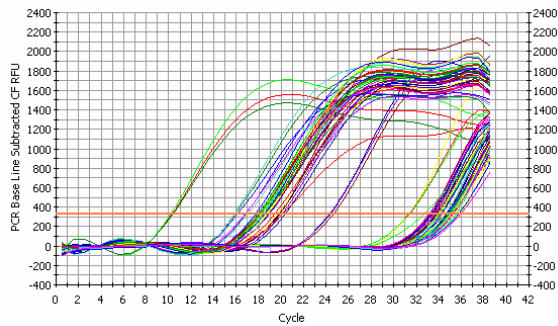
Tadpoles were exposed for a maximum of 14 days. Chemicals were replenished with water changes every three days. Four tadpoles were sampled each at Day 7 and Day 14. Livers and spleens were harvested from three animals and livers weighed (to allow comparison of overall lymphoid cell numbers) before storage for mRNA isolation. Whole animals were also weighed to allow normalizations for liver weights in animals at slightly different stages. One animal from each treatment was harvested undissected for PAH analysis. Livers (from three replicates) were kept individually and spleens pooled from a given treatment and stored for RNA isolation. Additionally, a portion of liver cells containing lymphocytes and their precursors were isolated and stored for RNA isolation.

RNA isolations were achieved with 67% of liver samples. Only one RNA sample of three livers taken at each time point was obtained for several of the treatments. While replicate RNA samples exist for some treatments (63%), thus far we have analyzed only one sample from each treatment. RNA was not obtained from animals exposed to the highest concentration of 7, 12-Dimethylbenz[a]anthracene, because they died early in the experiment.

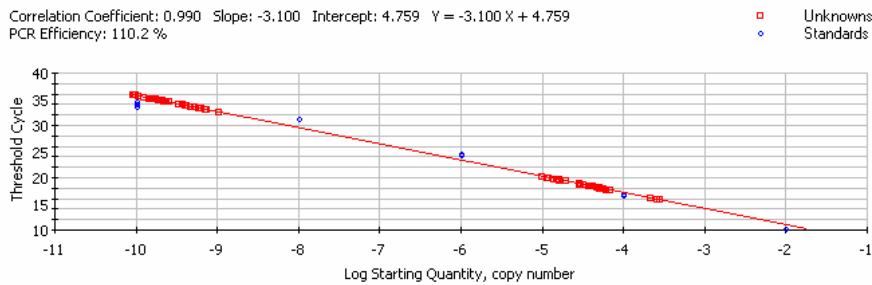
Real Time Quantitative RT-PCR:

For Real Time qRT-PCR, we used a Bio-RAD iQ real time detection system and analysis software. RNA samples were subjected to reverse transcription reactions and subsequently qPCR amplification using iQ-SYBR Green Supermix. Reactions were performed in a volume of 25 ul in triplicate in 96 well plates. For each of the two genes analyzed (Efla and RAG-1), standard curves were derived in each amplification experiment using as input, cloned, quantified, diluted plasmid DNA containing the fragment to be amplified. For quantitation, qPCR is an improvement over standard PCR because it allows precise analysis of PCR products as they are being generated over the course of the reaction rather than simply at the end of the reaction where reagents may be exhausted, detection methods may max out, etc. It also eliminates the need to use multiple dilutions of samples for semi-quantitation. It compares the quantity of product in different reactions in the early, linear portion of their amplification curves at a value called the cycle threshold (CT). In our experiments, this value was determined by the system software and occasionally by the user. To construct standard curves, known varying amounts of input target is plotted vs. signal detected at the CT value. Standard curves were fit with baseline subtraction. Based on the signal detected in the unknown samples at the CT value, the quantity of product is extrapolated from the standard curve. In these experiments, signal obtained with 33 or more cycles of PCR (such as that observed in -RT samples) was excluded as background as determined with a negative control. All -RT signals, possibly the result of minute levels of contaminating genomic DNA, required 33 or more cycles. Specificity of product formed in reactions was confirmed with melt curves (which showed a single major product peak), and in some cases with gel analysis.

A.



B.



C.

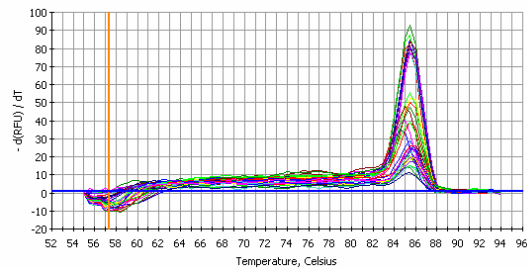


Figure 4: Example of Real Time Quantitative RT-PCR used in these studies. A. Amplification curves detected from a representative qRT-PCR experiment. B. Standard curve constructed in a typical experiment (Blue, Stds., Red, Unknowns). C. Typical melt curve showing a single major product. In these experiments, the efficiency of PCR amplification ranged from 86-122%, the correlation coefficients from 0.98-1.0, and the slope from 3.1-3.7.

PAH Reduce RAG-1 mRNA Levels in Developing Bullfrogs:

qRT-PCR analysis in bullfrog tadpoles treated for 7 days with PAH in their water suggests that pyrene, 2-methylnaphthalene and 7, 12-dimethylbenz[a]anthracene reduce RAG-1 mRNA levels in this system. DMSO appears to increase the level of RAG-1 mRNA (Figs. 5 and 6).

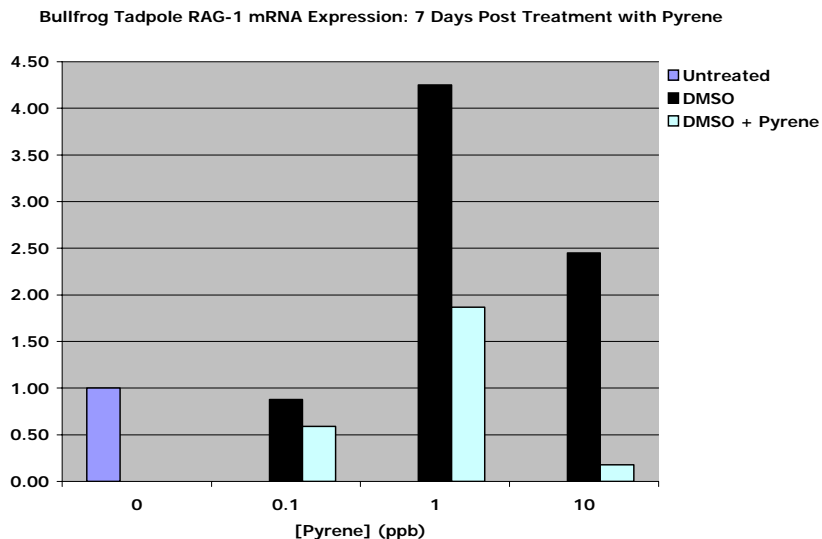


Figure 5. Reduction of RAG-1 mRNA Levels in Bullfrog Tadpoles Treated in their Water for Seven Days with Pyrene. Compared to the untreated control, DMSO, at 0.0005% (as in 1 ppb samples), and 0.005% (as in 10 ppb samples), increases RAG-1 mRNA levels by 4-fold and 2.5-fold, respectively. Despite this stimulation by DMSO, all concentrations of pyrene reduce RAG-1 gene expression in this system. 0.1 ppb, 1 ppb, and 10 ppb pyrene show a 33%, 56% and 93% reduction in RAG-1 mRNA levels, respectively, compared to the respective DMSO controls. Results for RAG-1 mRNA levels have been normalized to those obtained with Ef-1a.

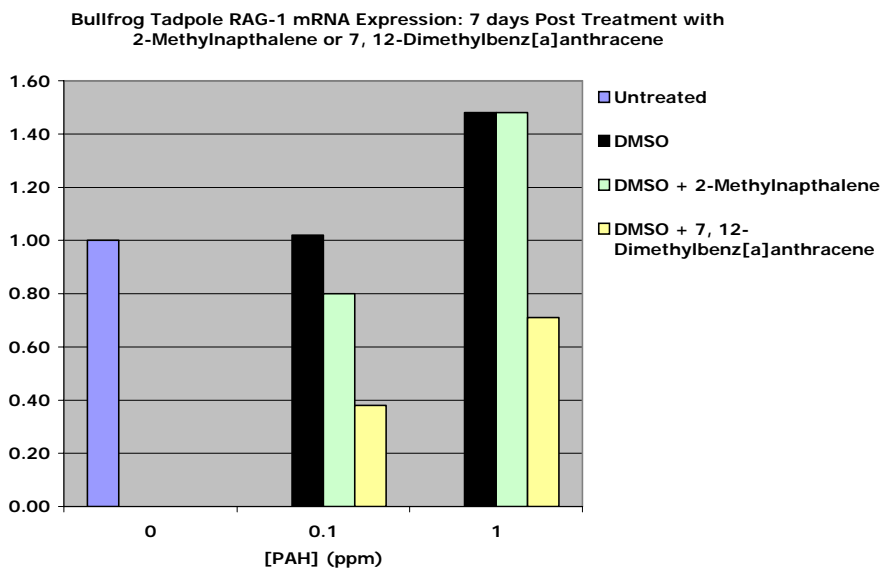


Figure 6. Reduction of RAG-1 mRNA Levels in Bullfrog Tadpoles Treated in their Water for Seven Days with 2-Methylnaphthalene or 7, 12-Dimethylbenz[a]anthracene. Compared to the untreated control, 2.4% DMSO, as used with 2-Methylnaphthalene or 7, 12-Dimethylbenz[a]anthracene (at 1 ppm), increases RAG-1 mRNA levels by 1.5-fold. Despite this stimulation by DMSO, 2-Methylnaphthalene and 7, 12-Dimethylbenz[a]anthracene reduce RAG-1

mRNA levels in this system. 2-Methylnaphthalene (at 0.1 ppm) reduces RAG-1 mRNA levels by 20%. 7, 12-Dimethylbenz[a]anthracene (at 0.1 ppm or 1 ppm) reduces RAG-1 mRNA levels by 63% and 52%, respectively, compared to the respective DMSO controls. Results for RAG-1 mRNA have been normalized to those obtained with Ef-1a.

Data obtained after 14 days of treatment with PAH was inconclusive, as were attempts to assess proliferative capacity of lymphocytes from PAH-treated animals. Additionally, no obvious effect on spleen or liver weights were observed in treated animals. We are continuing to analyze available replicate liver and spleen RNA samples.

Discussion

Our studies seek to determine whether frogs can be used as bioindicators of environmental health in contaminated habitats. The Western Lake Pontchartrain Basin ecosystem has been influenced by a variety of stressors including logging, increased salinity and flooding from coastal erosion, human encroachment, and industrial contaminants. Recently, as a result of the hurricanes, the area (and other nearby areas) were subject to further insults, including the release of hazardous chemicals. These studies seek to develop methods to identify immune system stressors that can help assess environmental health in contaminated areas and assist those carrying out cleanup, conservation and restoration efforts.

We have developed methods for measuring lymphoid-specific mRNA levels in response to PAH exposure in bullfrog, a species that is present in The Western Lake Pontchartrain Basin and nearby areas of Louisiana.

Development of the frog immune system is precisely controlled. Waves of lymphocyte development occur prior to and after metamorphosis so that antigens acquired at metamorphosis are tolerated in the adult (Turner 1994, Du Pasquier et al. 2000). The immunotoxic effects of xenobiotics is believed to be a possible factor in global amphibian population declines, and mass mortalities in frogs have been associated with fungal, bacterial, and viral infections (Carey 2000).

Immunotoxic effects of polycyclic aromatic hydrocarbons (PAHs) and polyhalogenated aryl hydrocarbons (PHAH), such as those known to accumulate in sediments associated with effluents from oil refineries and other industries, are documented in field and laboratory studies. For example, changes in immune function have been observed in fish and fish-eating birds collected from PAH contaminated areas (Luebke et al. 1997). PAHs, at high doses are associated with T and B cell deficiencies, reduced antibody production and macrophage function, and resistance to infectious agents or transplanted tumor cells (Burchiel and Luster 2001). In mice, the intracellular target of PAH, the aryl hydrocarbon receptor (AhR), is a component of a heterodimeric transcription factor that targets and activates expression of cytokine genes in T cells (Jeon and Esser 2000). These studies and others have led to the hypothesis that exposure through the AhR leads to immunodeficiencies in wildlife residing in PAH contaminated environments.

Signaling pathways activated through the AhR in mouse lymphocytes can modulate expression of genes involved in lymphocyte development and activation. In fetal thymic organ cultures, RAG gene expression and immature T cell development was suppressed by an AhR agonist (Lai et al. 1998). An AhR-mediated decrease in Pre/Pro B cell numbers was seen in normal mice and an increase in these cell populations was seen in AhR knockout mice (Thurmond et al. 2000). In

these experiments, AhR mediated effects resulted from TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin) (not PAH) exposure.

Frogs have all of the adaptive components of the immune system found in higher vertebrates including T cells, B cells, and antibodies (Turner 1994, Du Pasquier 2000). We hypothesized that frogs exposed to PAH, like mice, would exhibit immune deficiencies mediated through the AhR. Our studies, thus far, support the hypothesis that PAH, known to be present in sediments from Bayou Trepagnier, negatively impact lymphocyte development in frog larvae. After 7 days of treatment with pyrene, 2-methylnaphthalene, or 7, 12-dimethylbenz[a]anthracene, bullfrog tadpoles show a reduced level of RAG-1 mRNA. RAG-1 is a protein necessary for lymphocyte development and generation of lymphocyte receptor diversity. This protein, in developing lymphocytes, mediates gene rearrangements that encode the Variable Domains of antigen receptors (Bassing et al. 2002). Our studies do not determine at what level suppression of RAG-1 mRNA levels is occurring. Based on results described above in mice, we expect that the reduction that we observe is at the transcriptional (mRNA synthesis) level. However, PAH might also impact mRNA stability. Further experiments will be necessary to distinguish between these possibilities.

Our hope is to extend our experiments to look at additional genes involved in lymphocyte development and activation. These methods may then be applied not only to indicate environmental health in the region dictated by the presence of PAH, but also that dictated by other chemicals, such as pesticides and herbicides and their mixtures. Additionally, these methods may be used to look at environmental health in areas where environmental contamination is expected but uncharacterized.

Future experiments will involve additional Real-Time RT-PCR analysis of RAG gene expression in RNA isolated from liver and spleen of treated and untreated animals, and further in samples taken from animals collected in clean vs. contaminated sites (eg. Bayou Trepagnier, Bayou Traverse, Western Lake Pontchartrain). The initial proliferation assays and others subsequently attempted using a variety of lymphocyte activators and tadpole (or frog) lymphocytes have proven to be difficult. We plan to conduct the experiments using mouse lymphocytes, and the PAH compounds used in the exposure experiment. Additionally, direct effects of these PAH on lymphocyte developmental processes will be assessed using a mouse Pre B cell line in which we can directly measure RAG-1 gene expression and mRNA stability and immunoglobulin gene rearrangements requiring RAG-1.

Technology Transfer

1. The goal of this project is to help identify stressors in the Western lake Pontchartrain Basin by developing a bioindicator system for exposure to environmental contaminants using the frog immune system. The ability to measure changes in expression of genes involved in lymphocyte development and activation in laboratory exposed animals would be transferable to measurements taken in the field in various areas of the Western Lake Pontchartrain Basin or nearby areas. While this proposal focused on individual PAH known to be present in sediments from Bayou Trepagnier, the studies provide a foundation for use of the frog immune system to identify other stressors (such as pesticides, herbicides, or chemical mixtures) that impact environmental health in this region and elsewhere. This study helps lay the groundwork for

future studies addressing environmental stressors in the Western Lake Pontchartrain Basin region and elsewhere in Louisiana.

2. Are PAH (or other contaminants) impacting the immune system of frogs/tadpoles (and possibly other organisms) in the Western lake Pontchartrain Basin? If so, are levels of PAH (or other contaminants) a concern for local ecosystems?
3. Our preliminary studies suggest that PAH previously detected in sediments from Bayou Trepagnier, can impact the immune system of bullfrog tadpoles. RAG-1 mRNA levels (necessary for lymphocyte development) are reduced in animals treated in the laboratory with pyrene, 2-methylnaphthalene, or 7, 12-dimethylbenz[a]anthracene. We have not yet demonstrated effects of these compounds in the field.
4. Contaminant effects on the immune system of developing frogs warrants further work in this area, which should be supported by local programs. Further indications of local contaminant effects on the immune system of developing frogs should necessitate consideration of possible impacts on other organisms and the necessity for development of chemical clean-up scenarios.
5. St. Charles Parish (and potentially others in future studies)
6. This data, in conjunction with additional data supporting an immune suppressive effect of PAH (or other chemicals) in developing frogs (or other organisms) in Southeast Louisiana would alert policy makers of the importance of remediation at contaminated sites.

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The Historical Transformation of the Manchac Basin Ecosystem: Ecological Degradation at the Hands of Man

Dr. Samuel C. Hyde, Jr.

Abstract

The environmental catastrophe currently facing the Pontchartrain Basin relates directly to the actions of man. Despite the irrefutable link between environmental degradation and human habitation, no comprehensive ecological history of the Manchac region exists. The Center for Southeast Louisiana Studies has constructed a comprehensive historical survey of the region, highlighting the environmental stressors that have negatively impacted the northern and western Pontchartrain Basin. Our project provides a historical overview that traces how development in the region from first European exploration to the present contributed to the degradation of this irreplaceable ecosystem by assessing long-term environmental changes wrought by human habitation.

Primary Objectives

Our project consisted of two separate but interconnected projects. First, we aimed to produce a comprehensive ecological history of the region that would highlight the human stressors on the Pontchartrain Basin from prehistory to the present. This phase of the project was designed to influence legislators with what we hoped would be a compelling manuscript, along with numerous pictures to inform policy makers of the project's importance. The Center for Southeast Louisiana Studies' two professionally trained historians, Dr. Samuel C. Hyde, Jr. and Dr. Keith M. Finley, would research and write the manuscript that details the deleterious impact of human habitation in and around the Manchac ecosystem.

Second, we sought to produce a documentary film designed to highlight the material contained in the publication and to rally public support behind the recovery effort. The film provides powerful visual imagery in a dramatic format designed to further heighten awareness of the importance of saving this vital ecosystem. Community outreach represents one of the central missions of the Center for Southeast Louisiana Studies. We turned our efforts during this project toward spreading the word on the need to take immediate action to preserve the fragile Manchac ecosystem.

We designed both projects to appeal directly to the research program's target audience – politicians, policy makers, and the general public. A comprehensive, yet succinct historical analysis coupled with a gripping documentary film we hoped would convey the important link between humans and the degradation of this irreplaceable resource. While researching, writing, and filming our project, we also created an exhibit, independent of the grant, that was designed specifically for school-aged children, or as we like to call them, the policy makers of tomorrow.



Primary Investigator Samuel C. Hyde, Jr. informs area school children of the consequences of human habitation in the Pontchartrain Basin.

Results

Ecological historians, most notably Albert Cowdrey, William Cronon, and Timothy Silver, have gainfully studied the human role in altering the New England and the south Atlantic countryside. Despite the utility of an ecological approach to history, no similar study of southeast Louisiana exists. (Cowdrey, 1983; Cronon, 1983; Silver, 1990) To fill this void, our project explains the human factors that have contributed to the degradation of the ecosystem from the prehistoric era to the present, while also providing a narrative accessible to the literate public that outlines this process. The absence of a chronological record of the transformation of the western Pontchartrain Basin limits the resources available to the scientific community in their efforts to find solutions to the problem. The social, cultural and economic factors that transformed the Basin throughout the region's history are, and should thus be considered, inextricably linked with any future plan to recover the ecosystem. Studies of salinity levels, hydrologic alterations, and the germination of cypress seedlings highlight many of the current problems facing the region, but they tend to offer an opaque analysis so steeped in jargon as to be inaccessible to the general public—the very people whose support is necessary to rally legislators behind the

recovery effort. Our project helps bridge the gap between the scientific and the non-scientific communities.

Our project traces human habitation in the region from the prehistoric era to the present. We explore how the flora and fauna found in the basin changed over time as permanent settlement began. We consider a broad array of subjects, including General Andrew Jackson's decision to close-off Bayou Manchac from the Mississippi River during the 1814 Battle of New Orleans. Although based on strategic considerations, Jackson's decision lessened flooding along the bayou and prompted increased settlement in the region.(Walker, 1856) Soon, rice and eventually cotton production became prevalent, leading first to deforestation, and second to the influx of more inhabitants. (Gray, 1941; Hyde, 1996; Wright, 1978) At the same time, unchecked hunting and trapping of the region's wildlife by farmers, fishermen, and hunters led to the decimation of the Basin's fauna.(Abbott, 2001; Bartram, 1998; Fisher, ed., 1978)

After the American Civil War, northern business interests bought large tracts of land in southeast Louisiana to exploit for timber. Unscrupulous businessmen financed the clear-cutting of the region's virgin forests without a thought given to replanting. From the 1890s to the 1920s, cypress logging in the Manchac ecosystem thus became a profitable enterprise that would continue through the 1950s. (Crown Zellerbach MSS; Louisiana Cypress Lumber Company MSS; Mancil, Ervin MSS) Post-bellum railroad construction encouraged even greater settlement in the region, just as it opened markets for furs, timber, and agricultural products found in the Pontchartrain Basin. (Gautreaux Railroad MSS; National Railway Historical Society MSS; Witbeck Railroad MSS) The trapping of small game that started in the eighteenth century continued through the twentieth until mammals such as the beaver, muskrat, and mink were almost extinct. Inadvertent introduction of nutria and other non-indigenous species into the ecosystem prompted an explosion of non-native plants and animals in the Pontchartrain Basin, leading to the elimination of many native aquatic plants and the marginalization of some of the basin's native mammals. (Nichols MSS)

Later, the introduction of the automobile served as a catalyst for road and highway construction. With southeast Louisianans enjoying greater mobility, the state's population centers of Baton Rouge and New Orleans lost inhabitants as denizens of those cities sought refuge from urban blight in suburbs. The North Shore of Lake Pontchartrain became home for many of these urban refugees. Population pressures magnified the already significant environmental stresses on the area's wetlands. As a result of demographic shifts in the area, industries and towns proliferated along the waterways that empty into the Basin. Today, industrial runoff and human waste released by sewage treatment facilities as far North as central Mississippi continue to find their way into the rivers and creeks that drain into the Manchac ecosystem. (Hebert MSS; Morrison MSS; Regional History - Tangipahoa MSS)

Discussion

The project began with the compiling of resources relevant to the study. We gathered both primary and secondary evidence with an eye on being as comprehensive and as balanced as possible. As we gathered evidence, we began composing the manuscript. Once we had a

working initial draft of the manuscript, we pulled from it the most significant passages for use in the film script. At the same time, we produced a child friendly exhibit that highlighted the main points raised in the manuscript and by extension the documentary film.

Completion of the film titled: “The Manchac Swamp: Man-Made Disaster in Search of Resolution” and the manuscript titled: “‘One of the Prettiest Places I Have Seen’: Politics, Industry and the Destruction of the Manchac Swamp Ecosystem,” occurred roughly in tandem. Both facets of our project were designed to inform the literate public of the crisis facing the Manchac Basin. The docudrama was first shown at the Southeast Louisiana Historical Association meeting, an event attended by the Southeastern Louisiana University campus community, area residents, and local politicians. After that, it made its debut on television. It already has been seen by thousands of people on LPB and the SLU Channel—many of whom are now committed to environmental protection. Thousands more will have the opportunity to view it as it regularly airs on television channels across the state. In addition, the film appeared in the 2006 New York International Film Festival in Hollywood, a fact reflecting the national appeal of the project. The manuscript will also be made available to all state legislators and to area schools so that as many policy makers, both present and future, as possible will be armed with information regarding the causal link between human habitation and environmental degradation.

Technology Transfer

From the beginning, our project sought to inform as many people as possible of the consequences of human habitation in the Pontchartrain Basin as well as to encourage policy makers to actively support and pursue conservation initiatives. Both our manuscript and documentary film reveal the need for Louisianans, and indeed all Americans, to become more effective stewards of the environment. Past inattention to environmental considerations has resulted in the current crisis facing the Pontchartrain Basin. Further neglect will lead to a worsening of conditions and the loss of this vital natural resource. This project was not designed to inform policy makers how environmental recovery can take place—that is the job of the scientists involved in the grant program. This project was designed to inform policy makers that environmental recovery must take place and why the crisis exists in the first place. The constituent parts of our project demonstrate that humans have always altered the environment in which they lived and as technological advances occurred so too did man’s ability to transform his environment. All ecosystems change naturally over time, but mankind possess the ability to alter that landscape faster and more completely than all but the most cataclysmic occurrences, such as hurricanes. Advances in technology enabled man to profoundly alter his environment—we urge that those same technological and scientific advances be turned toward the renewal of the fragile Manchac ecosystem. The story we tell is a sad one, one of shortsightedness and the triumph of profit over principle, but it is also a hopeful one. If Americans in the twenty-first century, devote their energies to preserving the environment with the same vigor that their forebears devoted to destroying it, then the restoration of Louisiana’s wetlands is still possible.

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Western Lake Pontchartrain Basin Research Program Education Outreach Component: Phase II and III

Dr. Debbie Dardis

Abstract

The Education Outreach component of Phase II and III provided hands-on, interdisciplinary, educational experiences to both K – 12 teachers and students. Activities introduced participants to the ecology of the basin and southeast Louisiana wetlands, to EPA-funded research on habitat restoration and sustainability in the Manchac area, and to the important connection between the region's ecology and its cultural and economic vitality. During the Summers of 2005 and 2006, four workshops provided professional development for a total of twenty-six teachers, impacting fifteen parishes, twenty-two schools and an estimated nine thousand students in a two-year period.

Primary Goal and Objectives

The goal of this project was to increase awareness of: the deterioration of southeast Louisiana's wetlands, the contribution of human activity to this deterioration, the economic, cultural, and social ramifications of the ecosystem's demise, and the current research performed by local universities on habitat restoration and sustainability.

To reach this goal, four professional development workshops were offered for the teachers of southeast Louisiana. A vital, first step in the process of educating the general public is to educate teachers, who in turn, will impact thousands of students of all ages. Students ultimately become the stakeholders and decision-making citizenry of the future.

Methodology

Introductory Workshops. During the time of Phase II and III, four introductory workshop were offered. Each workshop provided three full days of interdisciplinary instruction and activities at either Turtle Cove Research Station or Tickfaw State Park. Teachers explored the local wetlands by taking canoe trips, nature hikes, and boat rides. Faculty and staff modeled relevant, standards-based activities appropriate for implementation in middle and high school classrooms. Study of the physical, behavioral and physiological adaptations of plant, animal and microbial life served to prepare teachers for the task of creating their own models of wetlands in aquariums or 'in a pan'. Pollution of the lake and surrounding wetlands and the potential negative effects were also studied. Loss of wetlands and the negative impact, food webs and biogeochemical cycles were also studied. A favorite activity of teachers was a scavenger hunt that required digital photography of a variety of wetlands organisms and landmarks. Participants created personal photojournals on Powerpoint (2006) or diversity

maps (2005) using their digital photographs. In addition, teachers learned about several of the environmental research projects being conducted by SLU faculty in the basin.

Teacher Participants. Teachers were recruited from schools in 16 parishes that are in, or adjacent to, the Lake Pontchartrain Basin. These include upper elementary, middle and secondary schools in Washington, St. Tammany, Tangipahoa, St. Helena, Livingston, East Feliciana, East and West Baton Rouge, Ascension, St. James, St. John the Baptist, St. Charles, Jefferson, Orleans, St. Bernard, Plaquemine Parishes.

Results and Discussion

Phases II and III of the Education Outreach program were successful in reference to meeting the stated goal and objectives. Four interdisciplinary workshops were designed and offered to teams of upper elementary, middle and high school teachers during the Summers of 2005 and 2006. In 2005, twenty-three teachers from four parishes (St. Tammany, Jefferson, Tangipahoa, Orleans) and five schools participated. In 2006, twenty-six teachers from eleven parishes and seventeen schools participated. An estimated total student impact potential of 9,000 in the last two years, as teachers continue to impact students yearly.

Phase	Summer	Academic Year	Impact per Year			
			Teachers	Parishes	Schools	*Students
II & III	2005	2004 - 2005	23	4	5	5750
	2006	2005 - 2006	26	11	17	3250

** Students impacted to date: This is calculated by assuming that each teacher has 25 students per class in 5 classes per day. That total is multiplied by the total number of teachers.*

Each workshop provided three full days and evenings of interdisciplinary instruction and activities at Turtle Cove Research Station or Tickfaw State Park. Teachers explored the local wetlands by taking canoe trips, nature hikes, and boat rides. Faculty and staff modeled relevant, standards-based activities appropriate for implementation in middle and high school classrooms.

Study of the physical, behavioral and physiological adaptations of plant, animal and microbial life, prepared teachers for the task of creating their own models of wetlands in aquariums and/or 'in a pan'. Pollution of the lake and surrounding wetlands and the potential negative effects were also studied. Wetland loss and projected ramifications, food chains and food webs, and biogeochemical cycles were also studied. A favorite activity of teachers was a scavenger hunt that required digital photography of a variety of wetlands organisms. Participants processed and used their digital photography to create personal photojournals to be used in their classrooms. In addition, teachers were introduced to the environmental research projects conducted by SLU faculty in the basin.

Evaluation of the summer workshops involved a written survey by teachers. A Lickert Scale rating system of Poor (1) – Fair (2) – Good (3) – Great (4) was used to determine teachers'

attitudes. Teachers gave an average rating of 3.95 for the overall experience of the workshop, a 3.9 for expertise of faculty and staff, and a 3.8 for the wide variety of learning experiences offered. Teachers also appreciated the hands-on and ‘make it, take it’ activities in which they participated. They loved canoeing and boating in the wetlands. For many, this was their first “water experience” in the wetlands.

Budgetary expenditures included salaries for the faculty director and an assistant, funding of a part-time student worker, workshop and office supplies, teaching resources and stipends for teacher participants and presentation and field equipment.

Technology Transfer

All workshops have incorporated discussions of the latest research sponsored by EPA in the Western Lake Pontchartrain Research Project. In the truest sense, this is technology transfer that will reach thousands of our current and future citizens with effective, meaningful learning.

In 5 sentences or less, please state the overall goal of the project.

The goal of this project was to increase public awareness of: the deterioration of southeast Louisiana’s wetlands, the contribution of human activity to this deterioration, the economic, cultural, and social ramifications of the ecosystem’s demise, and the current research performed by local universities on habitat restoration and sustainability.

To reach this goal, four professional development workshops were offered for the teachers of southeast Louisiana. A vital, first step in the process of educating the general public is to educate teachers, who in turn, will impact thousands of students of all ages. Students ultimately become the stakeholders and decision-making citizenry of the future.

List the restoration or sustainability questions posed by the study

None. This is an education outreach project. There are no restoration or sustainability questions posed, only teaching and discussion on such topics.

If possible, please list the hypothesized answers to the above questions.

Not applicable. See above.

What sorts of management recommendations/implications do you hope to make from this study?

In the past, this project has touched hundreds of teachers and thousands of students. During the first two years funding was sufficient to study academic year impact. We saw hundreds of students impacted by what their teachers had learned during summer workshops. This has been previously reported on. I recommend continuing the professional development of teachers as a vital component of technology transfer.

Please list the (a) agencies and geographies (parishes, etc.) and (b) impacted stakeholders (hunters, shrimpers etc.) that will be affected by this study.

As previously mentioned in the report, workshops were designed and offered to teams of upper elementary, middle and high school teachers during the Summers of 2005 and 2006. In 2005, twenty-three teachers from four parishes (St. Tammany, Jefferson, Tangipahoa, Orleans) and five schools participated. In 2006, twenty-six teachers from eleven parishes and seventeen schools participated. An estimated total student impact potential of 9,000 in the last two years, as teachers continue to impact students yearly.

Genetic variation between Mississippi River and Lake Pontchartrain Basin Fishes

FINAL REPORT EPA Phase IV

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Abstract

The objective of this study was to address genetic differentiation between Lake Maurepas and Mississippi River Basin fish populations prior to construction of the Hope Canal. Microsatellite data (five loci) was gathered from 250 blue catfish from the Lake Pontchartrain and Mississippi River basins. Results suggest that there is a low level of genetic differentiation between basins ($F_{ST}= 0.0068$), and within basins (Lake Pontchartrain basin $F_{ST}= 0.011$ and Mississippi River $F_{ST}= 0.0061$), which is consistent with high levels of gene flow related to large population sizes, high fecundity, and dispersal ability.

Overview and Objectives

Historically, the prevailing view in fisheries science was that conspecific populations, stocks, and/or strains could be freely transferred between lakes, rivers, and even drainage basins without detriment to the natives. However, this was most often conducted without examining whether there were distinctive morphological or molecular differences among populations. Recent studies that have incorporated molecular data have shown that species often are composed of genetically distinct stocks that have survived and evolved in isolation from other conspecific stocks and maintain unique features, life-histories, and physiological adaptations. Therefore, from a management perspective, it is critical to identify unique populations prior to stocking or environmental manipulation. Unfortunately, these types of studies have rarely been conducted a priori.

Conceptually, the idea of what constitutes a population seems simple and straightforward, however, empirically this is not the case. Waples and Gaggiotti (2006) summarized commonly used definitions of a “population” under ecological, evolutionary, and statistical paradigms and concluded that none of the working definitions were truly operational. Historically, the criteria that have been used to define a “population” simply have been based on 1). locality such as drainage basin, and/or 2). slight differences in morphology, and/or distinctive aspects of life-history. In the fisheries literature, “stocks” and “strains” have routinely been used in place of “population.” Ihssen et al (1981) defined a stock as an intraspecific group of randomly mating individuals with temporal or spatial integrity. Phillip et al (1993) expanded on this definition and suggested that a stock is defined as a genetically distinct group of fish unique to a particular body of water or basin that has spatial, temporal, and behavioral integrity from other randomly breeding groups of the same species. No matter what definitions are used to identify

unique biological units, it is important to have sound operational methodologies to allow researchers to address and better diagnosis distinct populations, stocks or strains. Molecular techniques offer the most promising aspect to address these types of issues.

Disruption of distinct stocks either through intentional or unintentional avenues can have detrimental effects on the genetic integrity of native fish populations through decreased levels of fitness and lower survivability in offspring derived by hybridized stocks (Allendorf 1991, Epifanio et al 2001, Hindar et al. 1991, Krueger and May 1991, Launey et al 2006). Ecological and genetic effects of fish stocking, in theory, may cause native species to (1) be eliminated (2) have changes in growth and survival, (3) be genetically changed (4) alter community structure, (5) exhibit a combination of the above, or (6) exhibit no detectable changes (Moyle 1986, Krueger and May 1991).

The primary objective of this study is to address genetic differentiation between Lake Maurepas and Mississippi River Basin fish populations prior to construction of the Hope Canal Diversion. The examination of multiple genetic markers from blue catfish (*Ictalurus furcatus*) will assess whether Lake Maurepas and Mississippi River basin blue catfish populations are distinctive from one another prior to mixing of these stocks. Since fishes comprise a major component of this system, collection of a baseline data set on the genetic variability and distinctiveness of these populations is critical to the restoration and long-term sustainability of this resource.

Results

Standard Population Genetic Statistics

Blue catfish (*Ictalurus furcatus*) tissue samples for genetic analysis were by electrofishing or through cooperation with commercial fisherman from five localities in the Mississippi River and Lake Pontchartrain Basins (Table 1). Fifty fin clips were obtained from each locality.

Table 1. Location of tissue samples of blue catfish.

Site	Abbreviation	Basin	N
Pass Manchac	PASS	Maurepas	50
Amite River	AR	Maurepas	50
Blind River	BL	Maurepas	50
Destrehan	DES	Mississippi	50
Atchafalaya	ATC	Mississippi	50

Allelic data was obtained from five microsatellite loci (Ip35, Ip38, Ip189, Ip195, and Ip273) from approximately 250 individual blue catfish using the primers identified in Waldbeiser et al. (2001). Standard population genetic statistics, including allele frequencies, observed and expected heterozygosities, and number of alleles per locus, were generated for each population and locus using GenePop v.3.3 (Raymond and Roussett 1995). Locus Ip35 was the most variable locus with 21 alleles, whereas Ip38 was the least diverse with 11 alleles recovered among 250 individual blue catfish. This level of diversity is comparable to other freshwater fishes, however, it is not possible to make direct comparisons to other blue catfish populations because no comparative studies are available.

Table 2. Observed genetic diversity at five microsatellite loci from five populations of blue catfish from the Mississippi River and Lake Pontchartrain Basins.

Locus	Total # alleles	HE	HO	Allele size range
IpCG0035	21	0.759	0.722	233-337
IpCG0038	10	0.577	0.704	104-160
IpCG0189	14	0.604	0.672	224-281
IpCG0195	11	0.837	0.928	216-255
IpCG0273	19	0.395	0.412	120-186
Mean	11.4	0.634	0.722	

Overall, the average number of alleles averaged across all 5 populations was 40.4 (range=29-56), and observed heterozygosities were 0.629 (range=0.597-0.679) (Table 3). Individually, Pass Manchac was the most diverse population. Fifty-six alleles (11.2 alleles/locus) were recovered from 250 individuals. This high diversity is likely due to mixing of Mississippi River basin blue catfish (through the Bonne Carre spillway) and native blue catfish from Lake Maurepas. The population with the lowest overall diversity was Blind River, which yielded 29 alleles (5.8 alleles/locus).

Table 3. Observed genetic diversity at five microsatellite loci from five populations of blue catfish from the Mississippi River and Lake Pontchartrain Basins including total alleles, average number of alleles (NA), expected (HE) and observed heterozygosities (Ho) for each population.

Site	Total # alleles	NA	HE	HO
PASS	56	11.2	0.679	0.76
AR	39	7.8	0.626	0.744
BL	29	5.8	0.610	0.708
DES	38	7.6	0.597	0.664
ATC	40	8.0	0.635	0.732
<i>I. furcatus</i> mean	40.4	8.08	0.629	0.722

Spatial Genetic Structure

F-statistics are routinely used to assess population subdivision and differentiation (Wright 1951). Fst values are typically inversely related to dispersal abilities. High levels of gene flow typically result in low Fst values, and low levels of gene flow are indicative of population subdivision, fragmentation and/or differentiation and result in high Fs values. In this study, traditional F-statistics were generated using the software program F-STAT (Goudet 1995) and pairwise Fst values were used to test for population differentiation. Pairwise Fst values ranged from 0.0032 (DES vs. BL) to 0.0225 (ATC vs. PASS) (Table 4.). These values are low relative to other studies of freshwater fishes. However, nearly all of these comparisons were statistically significant after Bonferroni correction.

Table 4. Pairwise F_{ST} comparisons among five populations of blue catfish in the Lake Pontchartrain and Mississippi River Basins. Asterisks indicate estimates that are significantly different from zero ($p < 0.05$) after sequential Bonferroni corrections.

	Pass	AR	BL	DES	ATC
PASS	-				
AR	0.0136*	-			
BL	0.0129*	0.0074*	-		
DES	0.0150*	0.0137*	0.0032	-	
ATC	0.0225*	0.0101*	0.0082*	0.0061*	-

Individual organisms are often spatially distributed across a landscape. Within a continuously distributed population, molecular techniques can be used to assess spatial

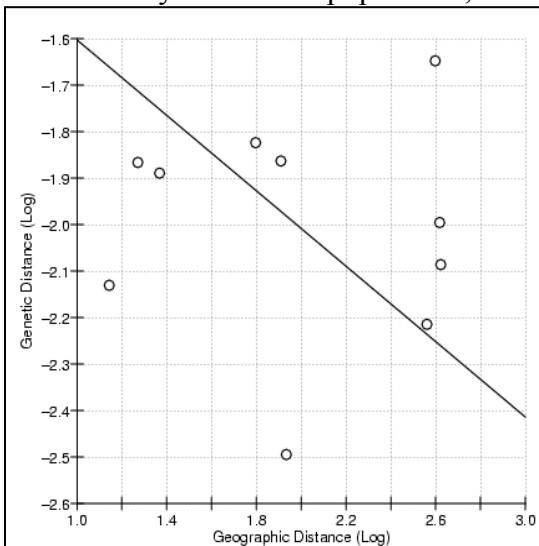


Fig. 1. Isolation by distance graph ($r = -0.0314$, $p = 0.542$)

distribution and its impact on genetic structure. Under the Isolation by Distance Model (IBD), individuals are expected to show increasing genetic distance with decreasing gene flow and increasing geographic distance. A variety of studies have documented this effect. Alternatively, absence of IBD is indicative of high levels of gene flow and this also has been case over both large and small geographic areas. In this study, there was a negative and non-significant relationship ($r = -0.0314$ $p = 0.542$) between genetic and geographic distances. Therefore, blue catfish from Atchafalaya River basin and Pass Manchac, two geographically distant populations, are no more similar to one another than are geographic proximate populations (i.e. Blind and Amite Rivers).

Analysis of molecular variance (AMOVA) is another analytical technique to study molecular variation within a species. More importantly it is a hierarchical method of analysis in which variance can be partitioned as various levels. In this study, most of the molecular variation was intra-populational (98.89%) and there was lesser variation among basins or among populations within basins (Table 5). This analysis further emphasizes the genetic homogeneity within and between blue catfish in both basins.

Table 5. Genetic differentiation of blue catfish based on AMOVA.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among basins	1	3.986	0.00465	0.29
Among populations within basins	3	8.612	0.01297	0.82

Assignment Tests and Distance Analysis

Assignment tests are routinely used to investigate population classification and to measure genetic diversity of natural populations. The software program GeneClass (Cornet et al. 1999) which uses a Bayesian approach to assign individuals to one or more populations based on each individual's multilocus genotype, was used to determine the degree of genetic cohesion among subpopulations within each of the basins. If populations at sampling sites are stable and genetically distinct, then recent migrants to a region or a local site will be detected and the source of these migrants can be identified. In this study, none of the populations could be readily assigned to their own respective populations and the overall average re-assignment success was only 6.4% (Table 6). The highest assignment success was Pass Manchac in which only 28% of the individuals were correctly self-assigned. Other studies have documented a strong correlation between genetic differentiation (i.e. F_{st}) and assignment success. In our study, F_{st} values are low and this likely accounts for the poor assignments.

Table 6..Bayesian assignment test using GeneClass showing self-assignment success.

Population	% Correct Re-assignment	% Mixed Stock
Pass Manchac	28	72
Blind River	2	98
Amite River	0	100
Destrehan	0	100
Atchafalaya	2	98
Mean	6.4	93.6

Finally, to estimate the genetic similarity among populations, a neighbor-joining tree based on shared allelic distances (DSA) (Bowcock et al. 1994) was generated using the Excel Microsatellite Took Kit (Park 2001). If there were distinct genetic structure, one would expect a close relationship between all populations of Mississippi River Basin, and also among all Lake Pontchartrain Basin populations. The results from this analysis (Fig. 2) indicate a lack of genetic structure at both local and basin levels.

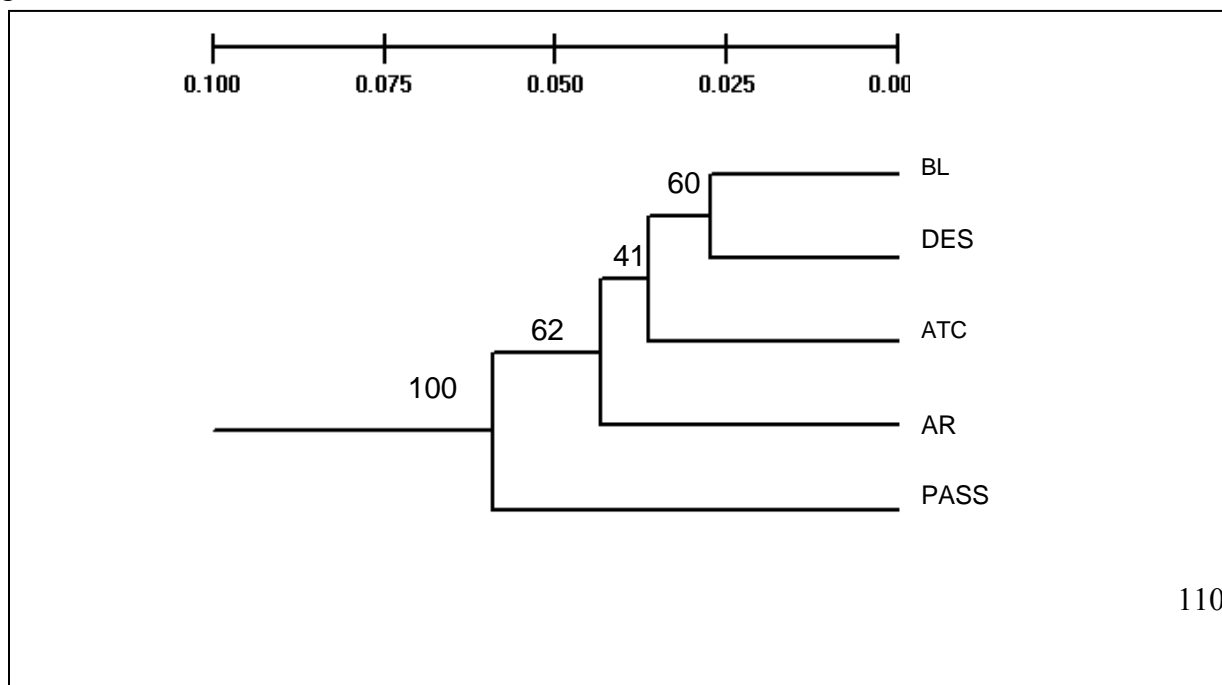


Fig. 2. Neighbor-joining tree based on shared allelic matrix of blue catfish in the Lake Pontchartrain and Mississippi River basins based on five microsatellite loci.

Discussion

In recent years, fisheries science has witnessed an increased usage of molecular methodologies to address issues related to management. Prior to this study, there were no studies of molecular variation of blue catfish in the Lake Pontchartrain and Mississippi River basins, or even elsewhere in the range of this species using high-resolution markers. The five microsatellite loci analyzed in this study were originally designed for the closely related and commercially important channel catfish (*I. punctatus*). The cross-amplification properties of these and presumably other microsatellite loci suggest that future studies may be able to incorporate a larger number of loci, and therefore analyze a larger portion of the genome to get a better sense of variation and molecular diversity in this species. The data generated in this study are important because it represents a baseline by which future genetic studies can be compared. Lack of baseline information is critical if future stocking or restoration efforts need to be implemented in the future.

The overall objective was to assess the population structure of blue catfish in an effort to forecast the potential effects of the impending Hope Canal diversion. Multiple independent data analyses clearly indicate that there is a high level of gene flow within and among populations of blue catfish in the Lake Pontchartrain and Mississippi River basins. Therefore, all populations examined can essentially be treated as one panmictic population. Based on these indirect assessments of population structure it appears as though the impending Hope Canal diversion will not disrupt the genetic composition of blue catfish in these basins. Three reasons likely account for the lack of genetic differentiation between basins. First, temporally, the time for geographic separation between basins has been minimal. Only in the last 100 or so years, have these basins been essentially physically segregated from one another, however the separation is not complete. Occasional opening of the Bonne Carre spillway during flood events still provides an avenue for exchange of individuals between basins, albeit, the exchange is uni-directional (Mississippi River to Lake Pontchartrain). Geographic separation is not required for population subdivision and this has been documented in numerous other studies of fishes.

Second, population size plays a major role in the spatial distribution of genetic variation. Large populations tend to have more alleles and therefore more variation, whereas small populations tend to be isolated and less diverse. Therefore, with a large population, the distribution of genetic variation across a large geographic area appears to be continuous simply because of large populations that have a vast number of alleles within a particular population. Blue catfish are one of the most abundant benthic species in Lake Pontchartrain and Mississippi River basins.

Finally, fecundity and dispersal capabilities also play a critical role in the determination of genetic structure. Production of high numbers of offspring, coupled with the ability to migrate great distances increases the likelihood of a broad distribution, and therefore a more homogenous

genetic structure. Blue catfish have been documented to disperse several hundred kilometers, a fairly large distance relative to other freshwater North American species. From a fecundity standpoint, blue catfish produce between 900 and 1,350 eggs/kg of body weight per year. This is also a large amount relative to other species.

Technology Transfer

Freshwater diversions are routinely being offered as a valuable tool for wetland restoration, however, the potential genetic impacts that this type of restoration project has on native aquatic species has not been thoroughly investigated. In Louisiana, there are multiple on-going or proposed freshwater diversion projects. This present study focused on potential genetic ramifications of this type of restoration technique and was aimed at assessing the potential impacts that freshwater diversion may have on genetic diversity and structure.

Not only has this study generated quantitative baseline genetic data for a commercially and recreationally important species, it is also beneficial for this particular diversion project. More importantly, this data will serve as a model study for any future wetland restoration diversion projects that are aimed at preserving biodiversity and genetic integrity while restoring coastal and wetland habitats. In the study, multiple methodologies failed to identify distinct genetic structure for blue catfish in the region. As stated above, there are several likely explanations for the lack of structure. This study only represents a snapshot of genetic variation of blue catfish in south Louisiana and additional studies that utilize more molecular markers and incorporate a temporal aspect (more generations) are needed to truly address genetic structure in this species. Although the methodologies applied in this study are essentially indirect measures to assess potential impacts, one cannot truly know the consequences without conducting experimental studies assessing fitness and survivability.

This data is critical and will be useful to policy makers including state and federal agencies, including the US Army Corps of Engineers and the Louisiana Department of Wildlife and Fisheries, because these two agencies are the principal players in wetland restoration. The results indicate that the proposed diversion is likely to have minimal influence on the genetic structure of blue catfish in the region. However, to directly assess its impact would require experimentation studies focusing on fitness.

Finally, this study emphasizes the importance of basic research, especially the gathering of baseline data. It is an uncommon occurrence in biology to know that a distinct ecosystem level change is going to occur in the basin (i.e. Hope Canal Diversion). We should utilize this time to strengthen our basic knowledge of the basin, and continue to expand on both basic and applied research. Only in combination can we arrive at a plausible and realistic management plan for the basin.

Phase IV Annual Reports (2006)

Establishment of baseline concentrations and elucidation of environmental processes controlling the bioavailability and bioaccumulation of mercury and other toxic metals in the Lake Maurepas Basin.

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Abstract

Mercury is a well known and highly toxic contaminant affecting many ecosystems worldwide. The capacity of mercury to be introduced into environments through atmospheric deposition as well as its tendency to become methylated in anoxic settings and subsequently become bioaccumulated have resulted in it becoming one of the major contaminants of concern in aquatic systems. Freshwater wetland systems are particularly important for the cycling of mercury and often function as a source of methylated mercury, the most toxic chemical form, to adjacent waters. The ongoing research summarized herein addresses key points of mercury cycling in the tidal fresh water wetland systems of Lake Maurepas that affect mercury bioaccumulation and toxicity.

Primary Objectives

The primary objective of this research is to assess the concentration and bioaccumulation of total and methyl mercury in the wetlands of the Lake Maurepas Basin. To best meet this overarching objective, a series of specific sub-objectives have been generated and studies explicitly designed to address these sub-objectives initiated.

- 1) To determine the current concentrations of total and methyl mercury in the soils, interstitial waters, and vegetation of the wetlands of Lake Maurepas.
- 2) To evaluate the potential transfer of mercury between native sediments, local vegetation, and a common invertebrate.
- 3) To assess interspecific differences demonstrated by tidal freshwater wetland vegetation in the bioaccumulation and translocation of inorganic mercury.
- 4) To determine the roles of major surface water constituents (i.e., nitrate, ammonium, sulfate, and labile carbon) in mercury cycling in freshwater tidal wetlands.

Results to Date

Due to events beyond the control of the granting administration and principal investigators, the grant supporting this research has not been received by the lead research institution and a required upgrade to equipment housed at Southeastern Louisiana University is still pending. However, certain aspects of this research have been undertaken in anticipation of the grant arriving. As proposed, this project is comprised by a field study designed to address sub-objective 1 and a greenhouse study designed to address sub-objective 2. Further, two additional studies have been initiated that address the additional sub-objectives 3 and 4, which were deemed critical to fully understanding mercury cycling in tidal fresh water wetlands such as those surrounding Lake Maurepas. The first of these additional greenhouse studies addresses bioaccumulation of, and tolerance to, high levels of inorganic mercury by local vegetation in a hydroponic setting. The second study evaluates potential shifts in mercury methylation rates resulting from alterations of interstitial nitrate, ammonium, sulfate, and labile carbon in a factorial soil column study.

Regarding the field study, all blocks were established at 4 of the 6 sites for the field study with fall characterization samples collected and preserved for analysis. Blocks at the remaining two sites will be established within the next two weeks. Materials are being gathered to implement the originally proposed greenhouse study, which is anticipated to occur in spring of 2007. Two additional greenhouse studies designed to complement the proposed research have been conducted and relevant sample analysis is currently underway. The hydroponic assessment of local plant species tolerances to elevated inorganic mercury as indicated by photosynthetic characterization (net CO₂ assimilation) are summarized in figures 1 and 2. Strong trends towards interspecific differences in mercury tolerance can be noted, with *Eleocharis microcarpa* net CO₂ assimilation actually appearing to be stimulated by increased mercury concentrations, *Saururus cernuus* and *Juncus effuses* displaying no effect of mercury concentration on net CO₂ assimilation, and *Panicum hemitomon* and *Typha latifolia* demonstrating reduced net CO₂ assimilation with increasing mercury concentrations. Total mercury concentration is currently being determined in biomass partitions (root, shoot, and leaf) to elucidate bioaccumulation potential. Relevance of endogenous plant compounds (phytochelatins, etc) will also be related to mercury bioaccumulation and tolerance.

Future Plans

Sampling and analysis for the field study will continue on schedule for the remainder of the study duration. The originally proposed greenhouse study will be initiated and completed in the spring and summer quarters of 2007. Further, it is anticipated that the analyses for the two additional greenhouse studies will be completed by the end of 2006.

Technology Transfer

Project Goal

The primary objective of this research effort is to determine levels of total mercury (hereafter mercury) and methyl mercury in the Lake Maurepas wetlands and elucidate factors controlling

mercury cycling and bioaccumulation in this environment. As mercury contamination has been shown to be problematic in many wetland systems that have do not have a direct industrial input of this contaminant (e.g., the Everglades) the determination of current levels and seasonal variation of mercury and methyl mercury in the Lake Maurepas wetlands will provide key information for local users of these resources. Further, this data will allow for informed decisions by local managers as many restoration strategies in this area require hydrologic alteration as a component (e.g., spoil bank gapping, river diversions, etc) that could potentially release any currently isolated mercury into Lake Maurepas itself. Finally, through focusing on cycling of mercury in wetland soils and vegetation, bioaccumulation risks and possibilities for sequestration can be addressed, potentially providing tools for not only local managers, but for other managers working in similar environments in the southeastern United States as well.

Sustainability Questions

- 1) What are the current concentrations of methyl and total mercury in the Lake Maurepas wetland soils and interstitial water, and how do they vary seasonally?
- 2) Do the dominant herbaceous plant species of the Lake Maurepas wetlands bioaccumulate mercury, and if so, in what portion of the plant does it tend to be located?
- 3) How do edaphic conditions and the potential alteration thereof affect mercury cycling?
- 4) How is the bioaccumulation of mercury by a common invertebrate affected by mercury concentration, plant species, and edaphic conditions?

Hypotheses

- 1) Although the concentration of either mercury or methyl mercury in the Lake Maurepas wetlands is currently unknown, methyl mercury concentration is anticipated to vary seasonally with the greatest concentrations in the summer due to microbial activity.
- 2) It is anticipated that all of the plant species tested will bioaccumulate mercury to some extent and will tend to store mercury in root material.
- 3) Anoxic soil conditions with moderate levels of available sulfate and labile carbon are expected to lead to maximum methyl mercury production, with increased levels of nitrate tending to ameliorate this production.
- 4) Invertebrate mercury bioaccumulation is expected to be positively correlated with mercury concentrations in both ambient water and plant tissues and to a lesser extent soil mercury concentrations.

Management Recommendations

This research should provide tangible benefit to management officials by estimating the risk of potential mercury release from the Lake Maurepas wetlands into Lake Maurepas by restoration methods that include hydrologic alteration. In the event that significant mercury concentrations are detected, this data should provide insight as to whether restoration methods requiring hydrologic alteration can still be employed in certain seasons where methyl mercury production is minimal. Finally, information on plant species and invertebrate bioaccumulation will allow managers to make informed decisions concerning mercury bioaccumulation risks.

Affected Parties

Agencies

Louisiana Department of Natural Resources
Louisiana Department of Environmental Quality
Environmental Protection Agency

Geographies

St. John the Baptist
Tangipahoa
Livingston

Stakeholders

Hunters
Shrimpers
Fishermen
Recreational Users

Summary Statement

The research conducted for this project is designed to provide key data that will allow for informed managers and stakeholders to safeguard the resources available in the Lake Maurepas Basin. As detailed above this project will generate data on current mercury and methyl mercury concentrations and bioaccumulation risks in the Lake Maurepas wetlands and address potential long-term risks by elucidating factors that control mercury cycling in this area.

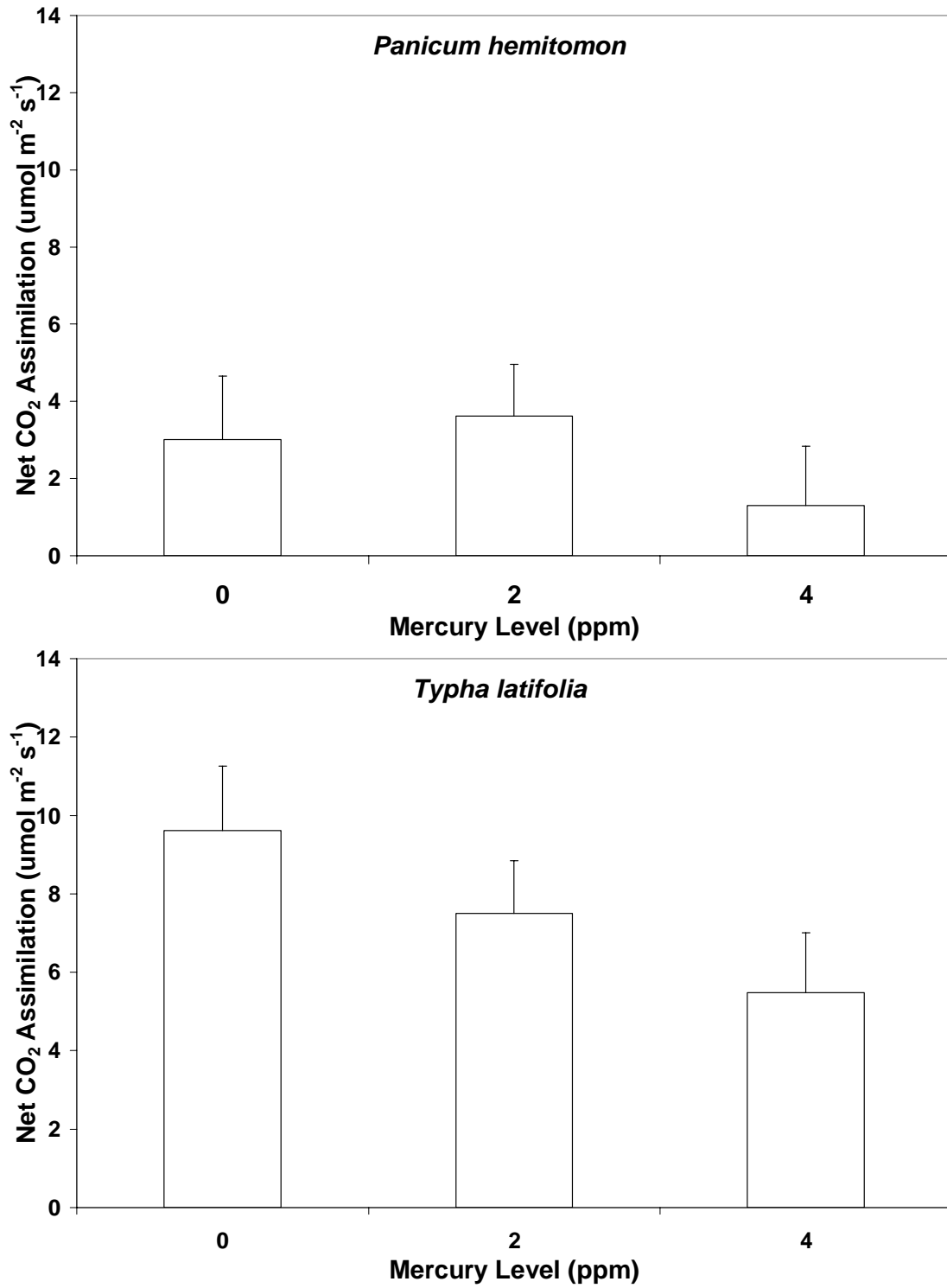


Figure 1. The effect of mercury level on *Panicum hemitomon* and *Typha latifolia* net CO₂ assimilation rate (mean +/- se, n = 4).

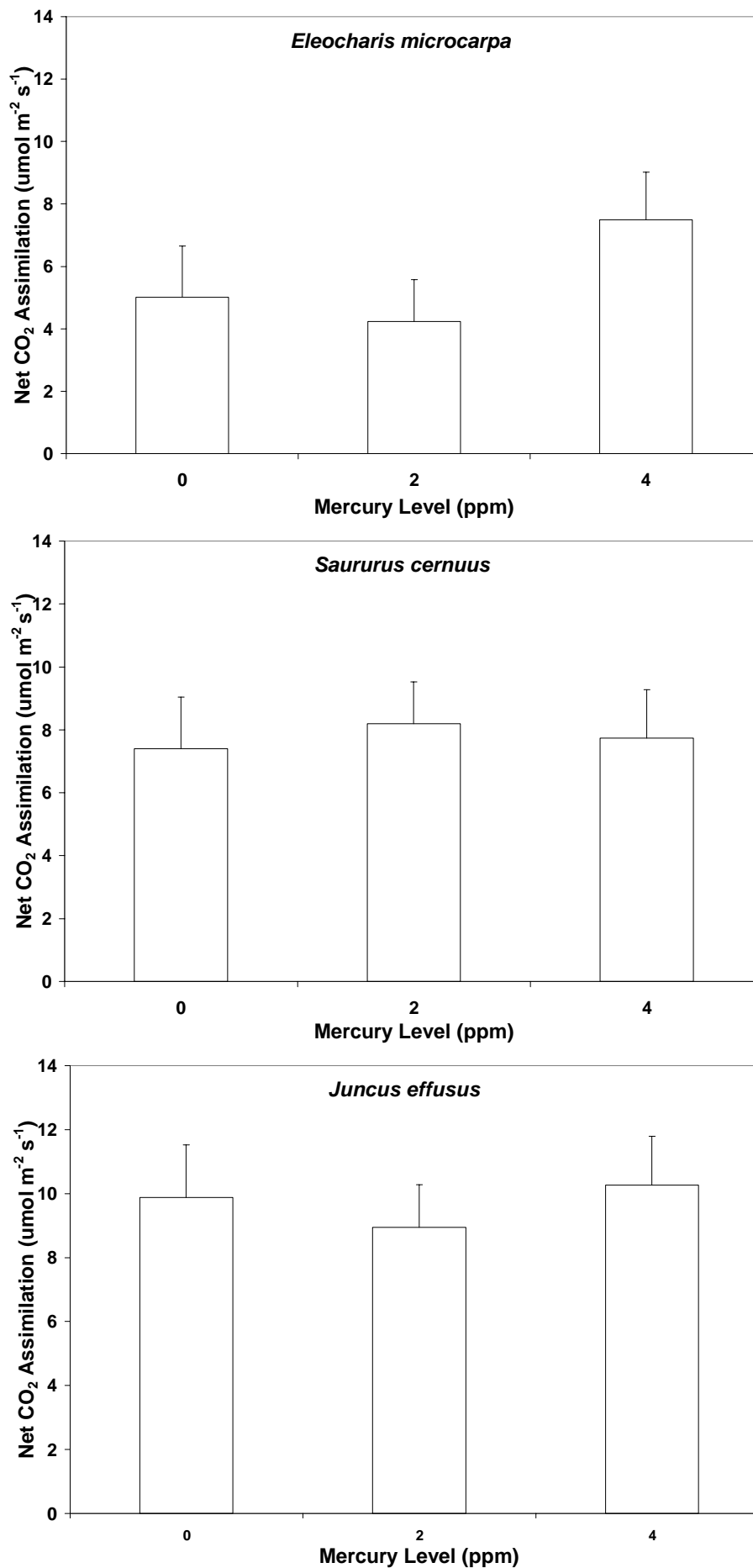


Figure 2. The effect of mercury level on *Eleocharis microcarpa*, *Saururus cernuus* and *Juncus effusus* net CO₂ assimilation rate (mean +/- se, n = 4).

Annual Report on Phase IV of PBRP—Turtle Cove’s Mitigation Project
(Budget #3282, funded at \$77,148):
Year 1 of 2 for Phase IV (Start Date: February 2006)
October 16, 2006

Principle Investigator: Dr. Robert Moreau, Manager of Turtle Cove

Co-PI’s:

James Bartkus, Economics Department (Southeastern)

Richard Campanella, Assistant Director, Environmental Analysis and Remote Sensing/GIS Specialist, Center for BioEnvironmental Research, Tulane University
Michael Greene (Biologist on Staff and Education/Outreach Coordinator for Turtle Cove)

Randy Myers, Biologist Manager of WMA’s, Louisiana Department of Wildlife and Fisheries

Thais Perkins, Assistant Director, PBRP

Fred Stouder, Marsh Restoration Coordinator (Turtle Cove)



Abstract

The purpose of this proposed two-year project is to study the viability of wetland mitigation in the area generally know as the Manchac/Maurepas Swamp. Major deliverables are to develop: (1) a detailed “Whitepaper” on protocols on how to establish, promote, and operate a mitigation bank in the area (geared to state agency representatives); (2) a “How-To” manual designed to communicate this information to the public (geared towards land owners and developers); (3) a workshop in the area to explain the concept to interested members of the

public; and (4) a Web Site devoted to mitigation banking in the study site. Land ownership, suitability maps, and other data sets will be inputted into a GIS model that will aid in selection of viable (and suitable) mitigation sites. This two-year project is funded at of \$77,147.

Primary Objectives

Primary objectives of the project are to create a series tools that will aid all stakeholders in the mitigation arena in development of mitigation sites in the Manchac/Maurepas Swamp. Specific objectives include:

- (1) Development of a White Paper that is designed to provide mitigation information to local, state and federal agency representatives involved in mitigation in the area. The white paper will include information and maps on attributes such as: ownership of large tracts of lands (shown as private vs. public); suitability of sites for mitigation (based on LIDAR produced tree canopy data, fresh water inputs, elevation and salinity data, among others); and other geographic and demographic data.
- (2) Development of a How-To Manual that is designed to provide the information noted above to land owners, developers, and the general public at large.
- (3) A workshop at the end of the process, and at a location near the study site, that will promote these and other materials to the various stakeholders addressed in the study (agency representatives, land owners, developers, non profit groups, the general public, etc.).
- (4) Development of a website that will serve as a clearinghouse of information for the above mentioned deliverables.

Results to Date

The project was started in February 2006 (a delayed start due to the storms of 2005), and pertinent information learned thus far has been helpful in fine-tuning the remainder of the study. Some key findings learned to date include:

- (1) The study site was defined as the two-foot contour around the Manchac/Maurepas swamps, and consists of a terrestrial area of approximately 460.5 sq miles (294,735 acres);
- (2) Growth rates along the north shore in St. Tammany parish are nearly five times that of the national average, and habitat developed there is mainly marsh, wetland forest, and scrubbrush wetland (making the Manchac/Maurepas Swamp a natural area for comparable mitigation);
- (3) Growth rates in St. Tammany Parish are even higher since the storms of 2005 (rates of 40% growth);
- (4) Public lands may be more difficult to put into mitigation than private lands for a variety of reasons.
- (5) Impediments to mitigation in the study site include: appropriate land suitability; competing land owner choices (other programs besides mitigation); land owner ignorance about mitigation; complexity of mitigation guidelines; and access to appropriate sites.
- (6) There are currently only three mitigation sites in the Manchac/Maurepas Swamp;

Discussion

Establishment of the two foot contour for the study site is appropriate because it divides the land neatly in to undeveloped marsh and swamp habitat from more developed agricultural sites. The designation is also cadastrally appropriate. The population growth rates (seen as a proxy for development) on the north shore, particularly in St. Tammany Parish, point towards the need to have comparable mitigation sites available---the Manchac/Maurepas Swamp is appropriate from that standpoint (if of course appropriate specific suitable mitigation sites can be defined).

Ownership of land has a significant impact on mitigation potential. In general, public lands may be more difficult to designate as mitigation sites, mainly because such lands are already assigned some sort of management status (and may not be appropriate to “upgrade” into mitigation, since they are already being conserved or preserved in one way or another). One very interesting development however is the capability of public agencies (such as LDWF) to purchase and for public use and have it then be “preserved” as a mitigation site (e.g., the purchased land, if in good shape environmentally, may be used to offset development---so it is purchased as an already completed mitigation project, but is in much better value environmentally than land that has to be mitigated).

Private landowners have many other government-sponsored programs that compete with mitigation. The list includes, among others, the Conservation Reserve Program (CRP); Wildlife Habitat Improvement Program (WHIP); forthcoming Carbon Sequestration Programs; and others. Most of these programs are sponsored through annual Farm Bill Policy programming, and do not allow for “double dipping” (having land placed under more than one designated category). And of course another competing issue to mitigation is land owners availability to use their land on the “open market” for things such as leasing (hunting and fishing or mineral rights), outright sale of land, and other market-based types of activities.

There are currently only two mitigation sites in the Maurepas Swamp part of the study site, and they average about \$10,000 per acre. One is a private bank that entails 1,000 acres of cypress replantings and the other is a WLF site purchased in an already “preserved” (mitigated) state of about 2,500 acres .On the Manchac side of the site, there is one site managed by Southeastern but with very limited success rates for the cypress replantings and limited acres (180) in play.

The lack of more sites suggests that a major impediment to mitigation there is site suitability and the probability for long term success of cypress plantings.

The GIS model for this project will consist of a series of data sets. First is the land ownership issue which has been identified at approximately 31% public vs. 69% private. Land ownership issues are important for a variety of reasons in mitigation, mainly dealing with ease of assignment to a mitigation site (public lands are more difficult in this arena given bureaucratic and political constraints, as well as the current state of preservation/management of such public lands). Environmental parameters used in determination of mitigation suitability will include the following variables: tree canopy height (determined by LIDAR data readings); soil elevation; salinity; and other variables to be determined.

It is hoped that this research will aid all stakeholders in the region in obtaining the latest knowledge and information about the viability of mitigation activities in this area.

Statement on Technology Transfer

This project is, in itself, an exercise in technology transfer. Information learned in the research of mitigation banking potential in Manchac Swamp will be disseminated to a wide group of decision makers, including: natural resource agency personnel; land owners; financial interest entities; community and environmental groups; local politicians; and other interested members of the public. This information will be transferred through a variety of media incorporated in the deliverables of the project, including: journals (for the white paper); a website; workshops; and of course the availability of a hard copy and internet available “how-to manual.”

Statement on Start Date of Project

Hurricanes’ Katrina and Rita in the early Fall of 2005 severely hampered the researchers’ ability to begin the project during October, which was to be the official start date. At a recent SPRP meeting with EPA representative Dale Manty and the Science Advisory Committee (SAC), it was requested that certain projects (including this one) be allowed to push the start date back until at least January 2006. That motion was approved by the SAC. Therefore, this project will start at the opening of Southeastern’s Spring Semester, approximately mid-January 2006.

Hydraulic conductivity and vulnerability to xylem cavitation of Baldcypress (*Taxodium distichum*) along a salinity gradient as indicators for restoration success.

Volker Stiller, Ph.D.

Project Abstract

In the past, considerable efforts have been undertaken to restore Baldcypress trees around Lake Pontchartrain. Vast amounts of cypress trees have been planted with varying rates of success. The project aims at supporting future restoration efforts of Baldcypress by investigating the role of plant hydraulics on restoration success. The specific goals of the study are a) to evaluate the amount of “drought” stress young Baldcypress trees are subjected to, due to increased salinity in their habitat, and b) to evaluate if the xylem of Baldcypress possesses the inherent plasticity to acclimate to increased salinity. The answers to these two, very basic, questions are currently unknown and will provide an invaluable tool in the decision process when and where to plant cypress trees.

Primary Objective

The project will achieve its goals by conducting 4 simple experiments. The first experiment will evaluate the vulnerability of Baldcypress to xylem dysfunction by establishing so-called “vulnerability curves”. These curves show the percent loss of hydraulic conductivity in relation to negative xylem pressure and are a fundamental parameter to understand a species ability to grow in a specific environment. In two field experiments, the in situ the hydraulic conductivity and the amount of xylem cavitation in Baldcypress will be measured along a salinity gradient. In addition to these field studies, a greenhouse study will be conducted, in which the ability of cypress trees to acclimate to increased salinity will be evaluated. Baldcypress will be grown in the SLU greenhouse and the salinity of the irrigation water will be gradually increased from 0ppt to 5ppt. Transpiration and growth measurements throughout the experiment will detect possible acclimation to salinity.

Results for 2006

3.1 Field experiment

Two field sites were selected for field measurements. The control site was located on the Tchefuncte River (Fairview-Riverside State Park, 30°24'42.50"N, 90°08'35.30"W), the elevated salinity site (ES-site) was located on the North Shore of Lake Pontchartrain (Fontainebleau State Park, 30°20'20.50"N, 90°02'55.70"W). In both sites, leaf water potential as well as native xylem embolism was measured on 6-8 plants and stem segments were collected and brought to the lab for vulnerability curve measurements. Salinity at the ES-site was 5.9 ppt, at the control site 2.3 ppt. The relatively high salinity at the control site was due to low river water levels and due to the fact that measurements had to be made at noon shortly

after a high tide. Midday leaf water potentials were significantly different at both sites (-1.62 ± 0.12 MPa at the control site and -2.12 ± 0.34 MPa at the ES-site).

Vulnerability curves measured on plants from both sites were not significantly different (50% loss of hydraulic conductivity at -2.75 MPa and -2.50 MPa for control and ES-site, respectively; Fig 1a,b). In situ xylem embolism was negligible at both sites and agreed well with what the vulnerability curves predicted. In situ hydraulic conductivity of the collected stem segments was not different at both sites.

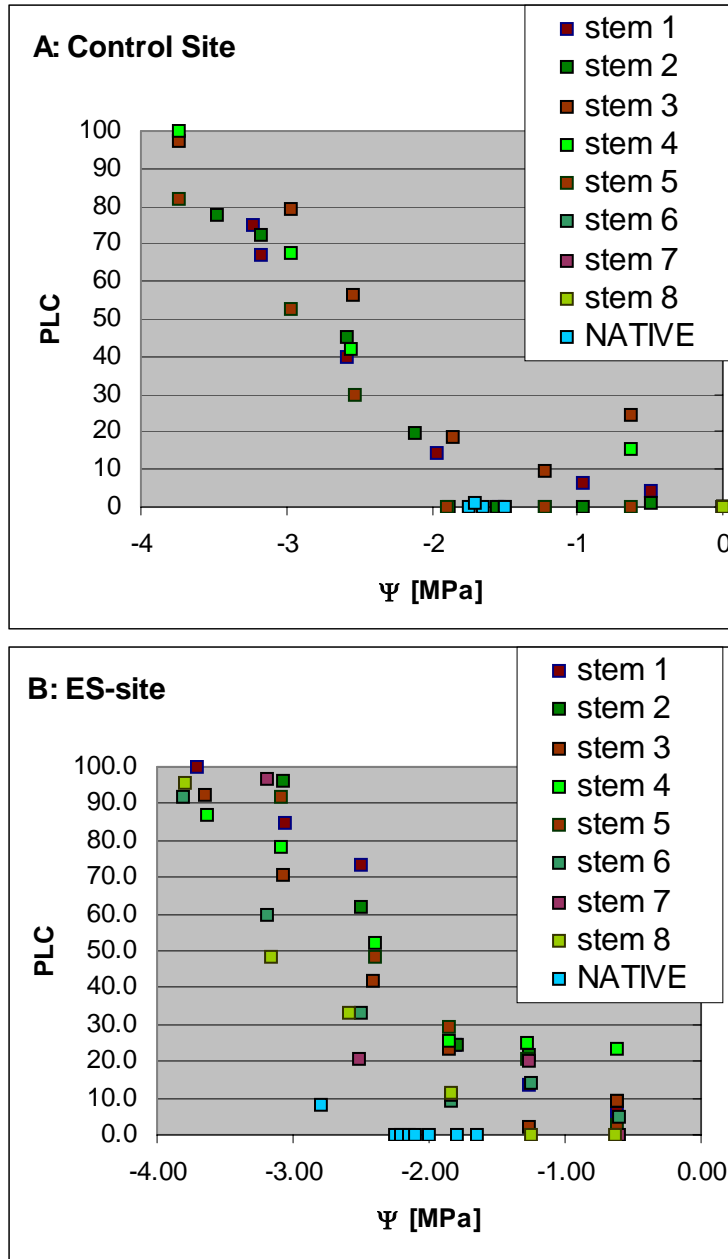


Figure 1 Vulnerability curves, showing the centrifuge-induced percent loss of hydraulic conductivity (PLC) with decreasing xylem pressure in Baldcypress stem segments sampled at

A) the control site (salinity 2.3 ppt) and B) the elevated salinity site (ES-site, salinity 5.9 ppt). In situ embolism (“Native”, blue symbols) was almost always zero.

Greenhouse experiment

Preliminary experiments showed that greenhouse grown Baldcypress seedlings were very vulnerable to xylem cavitation (50% loss of hydraulic conductivity at -1.59 MPa xylem pressure for one-year old stems, Fig. 2) and that new growth was even more vulnerable (50% loss at -1.23 MPa, data not shown).

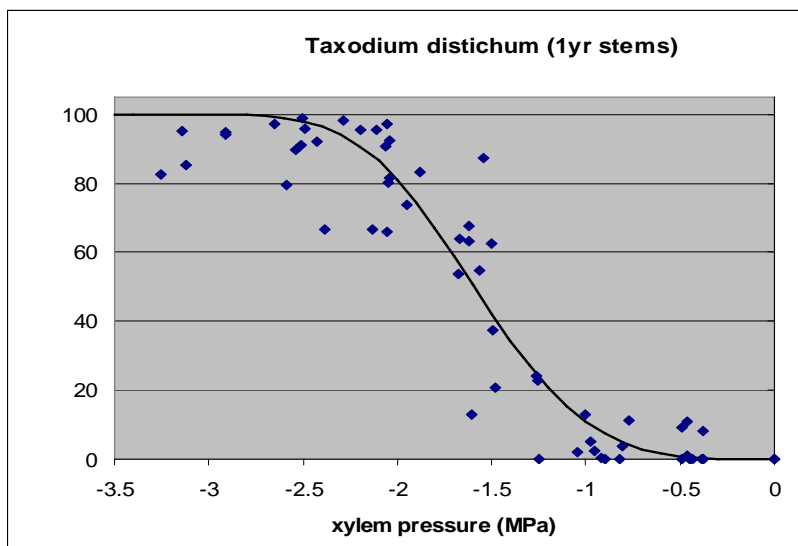


Figure 2 Vulnerability curve, showing the centrifuge-induced percent loss of hydraulic conductivity (PLC) with decreasing xylem pressure in greenhouse-grown Baldcypress seedlings. A Weibull function was fitted to the data set (solid line).

During the summer of 2006, approximately 50 Baldcypress seedlings were grown in the greenhouse of Southeastern Louisiana University. Control plants were irrigated with water twice daily; treatment plants were irrigated twice daily with 5ppt salt water. The salt treatment did not influence diameter growth of stems (Fig. 3) or the relative growth rate (RGR) of both treatments (0.41 ± 0.13 mm vs. 0.39 ± 0.18 mm for treatment and control plants, respectively).

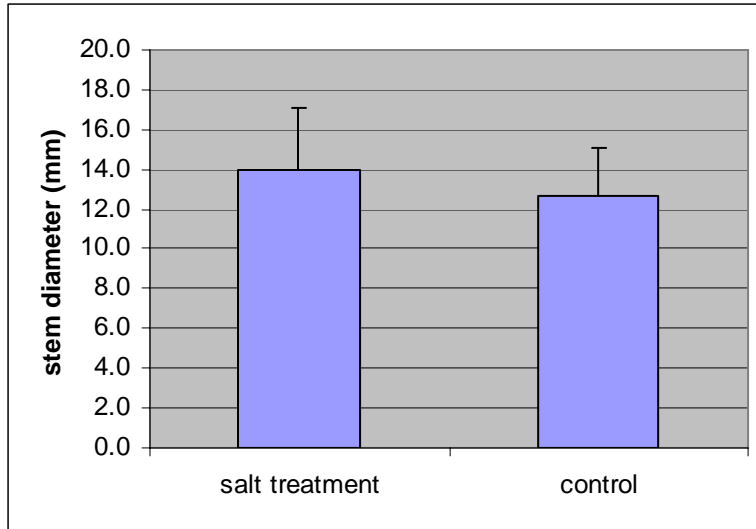


Figure 3 Stem diameters of control and treatment plants

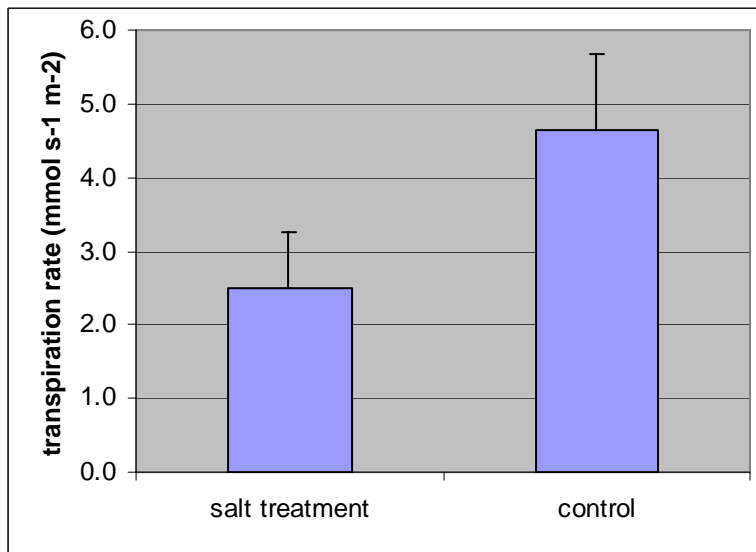


Figure 4 Transpiration rates of control and treatment plants

In contrast, transpiration rates (E, Fig 4) and stomatal conductance (gS, data not shown) of treatment plants were significantly reduced (E: 2.5 ± 0.8 mmol s⁻¹ m⁻² vs. 4.7 ± 1.0 mmol s⁻¹ m⁻² for treatment and control plants, respectively; and gS: 215 ± 64 mmol s⁻¹ m⁻² vs. 436 ± 127 mmol s⁻¹ m⁻² for treatment and control plants, respectively). At the end of the growing season (late October to early November), 6-10 treatment and control plants will be harvested and xylem vulnerability to cavitation will be measured. The remaining seedlings will be planted next season in a saline environment to evaluate survival rates.

Conclusion and Future Plans

Our results suggest that mature Baldcypress trees are well acclimated to the growth conditions at both field sites but also that they are exposed to relatively low midday water potentials. A comparison with the preliminary data discussed above suggests that the water potentials measured in the field could cause more than 50% loss of hydraulic conductivity in 1-yr old seedlings and moreover would almost certainly completely embolize new growth in Baldcypress seedlings. Whether seedlings grown under elevated salinity can acclimate and are less vulnerable to xylem cavitation will be measured at the end of this growing season. The less vulnerable stems of mature trees suggest that Baldcypress does possess the inherent plasticity to acclimate to increased salinity. Whether this acclimation ability translates into greater survival rates will be the focus of next year's field experiment.

Technology Transfer

The overall goal of the present study aims at supporting future restoration efforts of Baldcypress by investigating the role of plant hydraulics on restoration success. The specific goals of the study are a) to evaluate the amount of “drought” stress young Baldcypress trees are subjected to, due to increased salinity in their habitat, and b) to evaluate if the xylem of Baldcypress possesses the inherent plasticity to acclimate to increased salinity. The answers to these two, very basic, questions are currently unknown and will provide an invaluable tool in the decision process when and where to plant cypress trees.

The anticipated results from this study not only provide new insights into the limitations of water transport in Baldcypress, but also lead to a better understanding of the hydraulic constraints on restoration efforts. These results should be of interest for all groups and management agencies that are involved with the restoration of Baldcypress.

Furthermore, results from the greenhouse “acclimation study” can help develop possible preconditioning treatments for cypress trees in nurseries. Such preconditioning treatments could lead to hardier cypress trees that would be better suited to withstand the transplantation shock when planted in brackish or saline habitats and could be a valuable tool for nurseries that provide the plant material for restoration projects.

Salinity as a stressor of the freshwater turtle, *Trachemys scripta* in the Lake Pontchartrain Basin

PI: Dr. Roldán Valverde

EPA Phase IV Annual Report

Abstract

The Lake Pontchartrain System, constituted by Lakes Pontchartrain and Maurepas, are under increasing influence of salt water intrusion from the Gulf of Mexico. This influx of saltwater is thought to place stressful physiological constraints on the biota of the basin, particularly of freshwater species. Research efforts during the first year of the grant have focused on solving logistic aspects of field and lab work, such as developing an effective method to trap turtles and establishing the best set up to expose turtles to various salinities in the lab and testing them for stress. Currently, laboratory trials are underway as well as development of basking traps.

Primary Objective(s)

The primary objective of this study is to demonstrate that the red-eared slider freshwater turtle is a good physiological model to serve as an environmental sentinel with regard to salinity in the Lake Pontchartrain Basin. This objective will be accomplished by pursuing the specific objectives listed below.

1. To test the hypothesis that red-eared slider turtles living at higher salinity exhibit a hyperactive adrenal gland. This hypothesis will be tested using animals captured in the field. A hyperactive adrenal gland would suggest that the turtle lives in a stressful environment.
2. To test the hypothesis that increases in environmental salinity induce an endocrine stress response in the red-eared slider turtle. This hypothesis will be tested in the laboratory using captive-raised animals.
3. To test the hypothesis that the red-eared slider turtle is more abundant in less saline environments within the Lake Pontchartrain Basin. This hypothesis predicts that red-eared slider is more abundant in a Lake Maurepas' tributary where salinity is lowest within the Lake Pontchartrain Basin.

Results

1. To test the hypothesis that red-eared slider turtles living at higher salinity exhibit a hyperactive adrenal gland.

During the past year we have dedicated our efforts to identify specific optimal locations on the Lake Pontchartrain basin to trap turtles (Table 1). The criterion we have used is to find

locations that represent the salinity gradient in the Basin where turtles have been observed. The original locations proposed were Natalbany River, near the area where the Tickfaw River drains into North Lake Maurepas (low salinity), Stinking Bayou on Pass Manchac, and Cane Bayou adjacent to Big Branch National Wildlife Refuge. These locations were selected after consulting with colleagues that have several years of experience working in the basin. In every case we were assured that turtles were present in these sites in meaningful numbers. However, after conducting several sampling trips we noticed that the number of turtles was not as abundant as we were led to believe. In addition, we have noticed a significant decrease in the number of turtles since these animals became active in March-April, particularly in the Tickfaw River and in Cane Bayou areas. Also, we have not seen any turtles in Stinking Bayou since the beginning of the field sampling component in the Spring. Recently we have decided to change our efforts to the West side of Lake Maurepas, close to the Hope Canal Diversion Project site. The site is the Blind River. This area is very promising as we have seen a significant number of red-eared slider turtles here.

Table 1. Location of the various sampling sites in the Lake Pontchartrain Basin.

Site	Abbreviation	Latitude (N)	Longitude (W)
Blind River A	BR A	30° 13.131	90° 38.388
Blind River B	BR B	30° 12.796	90° 37.242
Blind River C	BR C	30° 12.821	90° 38.126
Blind River D	BR D	30° 14.063	90° 38.573
Tickfaw River A	TF A	30° 21.978	90° 29.468
Tickfaw River B	TF B	30° 21.960	90° 29.693
Tickfaw River C	TF C	30° 22.507	90° 29.949
Stinking Bayou 1	SB 1	30° 18.997	90° 18.752
Stinking Bayou 2	SB 2	30° 18.768	90° 19.277

As seen in Table 2, the salinity in the Blind river area is similar to that of the Tickfaw River. This similarity in salinity and the higher number of turtles observed in the Blind River sites suggests that salinity is not reason for the low number of basking turtles observed in the Tickfaw River sites.

Table 2. Mean temperature and salinity (\pm standard deviation) measured at the different sampling sites in the Lake Pontchartrain Basin.

Site	Temperature ($^{\circ}$ C)	Salinity (‰)
TF A	28.10 \pm 0.57	1.35 \pm 0.07
TF C	28.05 \pm 0.64	1.00 \pm 0.00
STB 2	28.57 \pm 0.21	7.13 \pm 0.81
BR A	28.1 \pm 0.42	0.95 \pm 0.21
BR B	29.30	1.1
BR C	27.55 \pm 0.92	1.25 \pm 0.07
BR D	28.7	0.7

To date we have been unable to capture turtles in our hoop nets, even though we have deployed hoop nets in the Blind River sites and followed the advice of experienced colleagues. Thus, we feel that the problem may be the hoop nets themselves, not the availability of turtles.

2. To test the hypothesis that increases in environmental salinity induce an endocrine stress response in the red-eared slider turtle.

Our efforts to address this objective have focused on obtaining blood from small experimental turtles. Although the PI has extensive experience obtaining blood samples from large sea turtles, small turtles present a challenge, particularly in light of the intensive sampling regimes that must be followed with a large number of small animals in the lab. Indeed, a single experiment with a control and three different salinities involves 48 turtles. During an experiment, a large proportion of these animals must be sampled within a minute or two. Thus, every experiment requires several individuals to handle turtles, samples, records, etc. Because the cold months approach and wild turtles will be inactive, we are planning to conduct a series of experiments to address this objective during time. We plan to test the effect of various exposure times (12 days, 1 month, 3 months) on the stress system of the turtles to determine which exposure time will show a physiological response.

3. To test the hypothesis that the red-eared slider turtle is more abundant in less saline environments within the Lake Pontchartrain Basin.

We had originally planned to address this objective by deploying several basking platforms at key locations representing the salinity gradient of the Lake Pontchartrain basin. However, after testing several platforms at the three originally proposed sites we have decided to desist from our efforts. The main reason is that turtles did not use the platforms as actively as we expected. Thus, the platforms appear inadequate to undertake this part of the study. In addition, the weak construction of the platforms (Styrofoam) led the structures to suffer damages, mostly caused by wind. We are now considering to conduct transect censuses along bayous and canals in the areas where traps will be deployed to generate an idea of turtle abundance.

Future Plans

At its onset, the project suffered several drawbacks that caused significant delays. For instance, due to University administrative problems project funds were not available until early in the Fall, just before turtles became inactive due to cold weather. Also, the occurrence of Katrina and related damages prevented us from traveling to the lake. Specifically, damage to the Turtle Cove station and boating system further delayed our field work. By the time we were able to go out cold weather had set in and the turtles were no longer available.

Another significant drawback is the lack of a graduate student that can assist with the work. Early in the project a graduate student and Fulbright fellow was assisting with the work.

However, the student did not qualify to pursue his MSc thesis and later withdrew from the project. It is well known that teaching loads at Southeastern are heavy. This situation severely limits the ability of the PI to address the logistical problems encountered to date, particularly those pertaining to field and laboratory sampling. To address this situation, the PI has recruited

two excellent undergraduates to assist with the experiments. However, they have agreed to participate even though they receive no monetary compensation for their work. Currently, the two undergraduates are being trained to work with the turtles in the laboratory and to assist with field sampling. The undergrads are also assisting the PI to build new basking turtle traps. We feel that these new traps are very promising since they take advantage of the behavior of these turtles to bask on above-surface structures. The new basking traps will be tested before the beginning of upcoming the cold season. We expect to be traveling to the lake to trap turtles in full force by the March, when the turtles become active again.

Technology Transfer

In 5 sentences or less, please describe the overall goal of the project.

The overall goal of this project is to demonstrate that the red-eared slider freshwater is an excellent animal model to study the physiological effects of elevated salinity due to saltwater intrusion into the Lake Pontchartrain basin. The main reasons to support this tenet is that this species is abundant in the basin, is not adapted to saltwater and it is easy to manipulate.

List the restoration or sustainability questions posed by the study.

Does salinity induce a neuroendocrine stress response in freshwater vertebrates?

Does salinity limit the abundance and distribution of freshwater vertebrates in the Basin?

If possible, please list the hypothesized answers to the above questions.

I hypothesize that salinity is an environmental stressor to the freshwater turtle. As such, salinity triggers a neuroendocrine stress response in order to mediate the adaptation of this vertebrate to a challenging environment.

If salinity is stressful to the turtle it is expected to impact the abundance and distribution of the turtle in the Basin.

What sorts of management recommendations/implications do you hope to make from this study?

The hypotheses of this study have predictive and, as such, management value. For instance, the study can help predict the impact of sustained decreases or increases of salinity in the basin on freshwater vertebrates. This is particularly relevant to water diversion projects as they may need short-term biological indicators to evaluate the effect of increasing freshwater input in the basin. Also, it may help environmental biologists assess the damage to the basin by sustained salt water intrusion as exacerbated by land loss driven by subsidence (long term), seasonal storms (short term), etc.

Please list the (a) agencies and geographies (parishes, etc.) and (b) impacted stakeholders (hunters, shrimpers etc.) that will be affected by this study.

a) The scope of this project is expected to be of interest to local, state and federal agencies in charge of protecting the environment such as Louisiana Department of Environmental Quality, the Louisiana Department of Wildlife and Fisheries, the Coastal Restoration and Management Division of the Louisiana Dept. of Natural Resources, the US Geological Survey, and the Environmental Protection Agency. The results of the project are expected to be of relevance to all Parishes found in Southeastern and Coastal Louisiana that are affected by salt water

intrusion. Among these are the Parishes of New Orleans, St. Tammany, Tangipahoa, St. Bernard, Jefferson, etc.

b) Because the project is focused on the effect of salinity on a species of high economic importance to Louisiana, the freshwater red-eared slider turtle, Louisiana turtle farmers are expected to be the most benefited by the results of this project. However, because the project considers the physiological mechanistic impact of salt water intrusion on the turtle the results of the project are expected to have broader implications at the level of the wider Basin as the abundance and distribution of many other freshwater vertebrates may be limited in the Basin by similar mechanisms.

With regard to communicating with policy makers and key regulatory agencies, explain how the results of this study may be used to enhance the restoration of Louisiana's wetlands and/or guide those policy makers in their regional planning efforts.

The value of the results to be derived from this study can be enhanced significantly if conducted also after Mississippi River Diversion projects come into effect. This study must be seen from the stand point of the establishment of a baseline on the status of salt water-sensitive vertebrates in the basin. If this study is conducted again after Diversion projects come into effect then the impact of such Diversion can be realized from the stand point of these salt-sensitive vertebrates. In essence, the hypothesis of this project predicts that Diversion of Mississippi River waters into the Lake Pontchartrain basin will induce a significant, sustained decrease in salinity throughout the basin. This, in turn, will result in the proliferation of salt water-sensitive species, which will no longer see their distribution limited by salt water intrusion. Specifically, the results of this project may allow managers to determine the optimal amount of freshwater that may be diverted into the Lake Maurepas to provide freshwater species with a suitable environment to live.

In specific terms, high salinity is expected to induce an increase in corticosterone, the main stress hormone in turtles. A challenge to this project is to ascertain what the sensitivity of these turtles to salinity stress is.

Western Lake Pontchartrain Basin Research Program Education Outreach Component: Phase IV Annual Report

Dr. Debbie Dardis

Abstract

Phase IV of the Western Lake Pontchartrain Basin Research Program's Educational Outreach Component is a continuation of Phases I - III activities. Phase IV will continue to provide hands-on, interdisciplinary, educational experiences to K-12 teachers. Activities will introduce participants to the ecology of the basin and southeast Louisiana wetlands, to EPA-funded research on habitat restoration and sustainability in the Manchac area, and to the important connection between the region's ecology and its cultural and economic vitality.

The budget was not impacted this past year due to sufficient funding in Phase III to meet the budgetary needs of teacher workshop activities. Phase IV will be implemented during the Spring and Summer of 2007. Planning for one K-12 teacher workshop will begin in February 2007 and continue through May 2007. The workshop is planned for June 2007.

Primary Goal and Objectives

The goal of this project is to increase public awareness of: the deterioration of southeast Louisiana's wetlands, the contribution of human activity to this deterioration, the economic, cultural, and social ramifications of the ecosystem's demise, and the current research performed by local universities on habitat restoration and sustainability.

To reach this goal, we will continue to offer professional development workshops for the teachers of southeast Louisiana. A vital, first step in the process of educating the general public is to educate teachers, who in turn, will impact thousands of students of all ages. Students ultimately become the stakeholders and decision-making citizenry of the future.

Results and Discussion

This phase of the Western Lake Pontchartrain Basin Research Program is a continuation of Phases I - III activities. Phase IV funds were not impacted this past year due to sufficient funds in Phase III to meet the budgetary needs of the two teacher workshops offered during the Summer of 2006.

Future Plans for 2007

Phase IV will be implemented during the Spring and Summer of 2007. Planning for one K-12 teacher workshop will begin in February 2007 and continue through May 2007. The actual workshop is planned for June 2007. Teachers will be recruited from schools in 16 parishes that are in, or adjacent to, the Lake Pontchartrain Basin. These include upper elementary, middle and secondary schools in Washington, St. Tammany, Tangipahoa, St. Helena, Livingston, East Feliciana, East and West Baton Rouge, Ascension, St. James, St. John the Baptist, St. Charles, Jefferson, Orleans, St. Bernard, Plaquemine Parishes.

Technology Transfer

The workshop will include discussions of the latest research sponsored by EPA in the Western Lake Pontchartrain Research Project. In the truest sense, this is technology transfer that will reach thousands of our current and future citizens.

1. In 5 sentences or less, please state the overall goal of the project.

The goal of this project is to increase public awareness of: the deterioration of southeast Louisiana's wetlands, the contribution of human activity to this deterioration, the economic, cultural, and social ramifications of the ecosystem's demise, and the current research performed by local universities on habitat restoration and sustainability.

To reach this goal, we will continue to offer professional development workshops for the teachers of southeast Louisiana. A vital, first step in the process of educating the general public is to educate teachers, who in turn, will impact thousands of students of all ages. Students ultimately become the stakeholders and decision-making citizenry of the future.

2. *List the restoration or sustainability questions posed by the study*

None. This is an education outreach project. There are no restoration or sustainability questions posed, only teaching and discussion on such topics.

3. *If possible, please list the hypothesized answers to the above questions.*

Not applicable. See above.

4. *What sorts of management recommendations/implications do you hope to make from this study?*

In the past, this project has touched hundreds of teachers and thousands of students. During the first two years funding was sufficient to study academic year impact. We saw hundreds of students impacted by what their teachers had learned during summer workshops. This has been previously reported on. I recommend continuing the professional development of teachers as a vital component of technology transfer.

5. *Please list the (a) agencies and geographies (parishes, etc.) and (b) impacted stakeholders (hunters, shrimpers etc.) that will be affected by this study.*

As previously mentioned in the report, The actual workshop is planned for June 2007. Teachers will be recruited from schools in 16 parishes that are in, or adjacent to, the Lake Pontchartrain Basin. These include upper elementary, middle and secondary schools in Washington, St. Tammany, Tangipahoa, St. Helena, Livingston, East Feliciana, East and West Baton Rouge, Ascension, St. James, St. John the Baptist, St. Charles, Jefferson, Orleans, St. Bernard, Plaquemine Parishes. As many as 12 schools may be impacted and hundreds of students.

Genetic variation between Lake Pontchartrain and Mississippi River Basin Fishes: Phase IV

ANNUAL REPORT

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Abstract

The Lake Pontchartrain Basin harbors a distinctive and every changing fish community that will likely change in the near future due to the construction of an interbasin canal that will connect the Mississippi River and Lake Pontchartrain Basins. Although the influx of water is aimed at restoring the wetlands around Lake Maurepas by providing freshwater to the system, it also may negatively impact the ichthyofauna of basin through homogenization of genetically distinctive stocks of fishes. The objective of this project is to examine genetic differentiation between Lake Maurepas and Mississippi River Basin fish populations of bluegill (*Lepomis macrochirus*) using microsatellite markers.

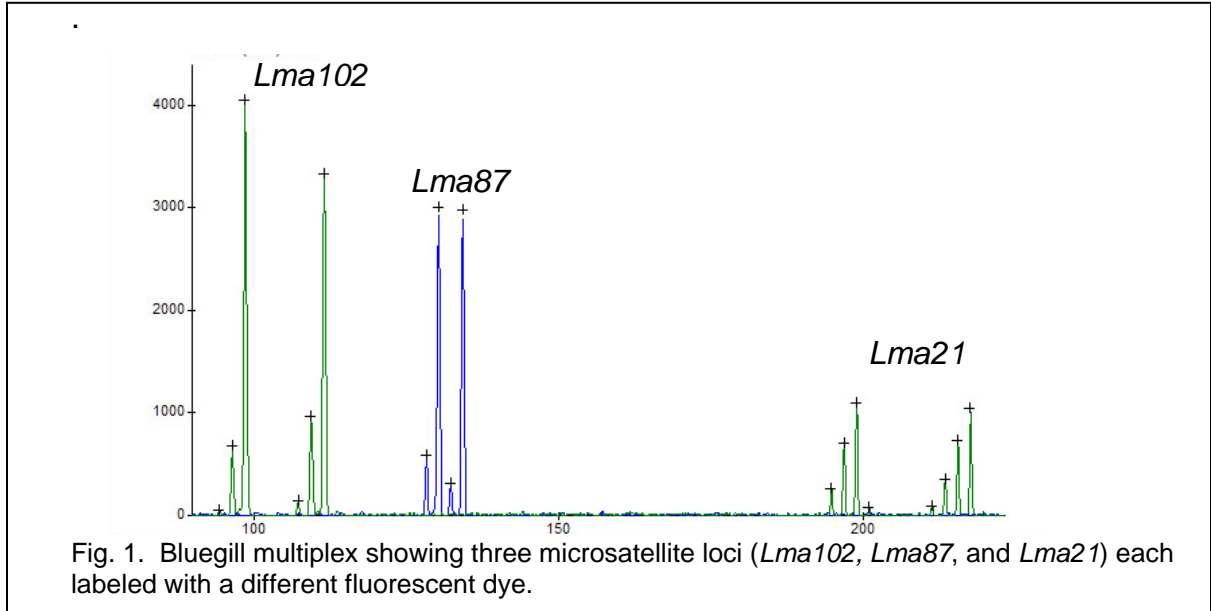
Results

To date, 207 individual bluegills (*Lepomis macrochirus*) have been collected from five locations in the Lake Pontchartrain and Mississippi River basins (Table 1).

Table. 1. Sampling localities, abbreviations, and number of specimens collected.

Site	Abbreviation	GPS	Basin	Species	N
Pass Manchac	PM	30.265N 90.399W	Maurepas	<i>L. macrochirus</i>	40
Carthage Bluff	CB	30.308N 90.588W	Maurepas	<i>L. macrochirus</i>	48
Blind River	BR	30.212N 90.594W	Maurepas	<i>L. macrochirus</i>	31
Devil's Swamp	DS	30.317N 90.423W	Mississippi	<i>L. macrochirus</i>	28
Atchafalaya	AtR	30.513N 91.719W	Mississippi	<i>L. macrochirus</i>	60

DNA from all of these individuals has been extracted and microsatellite data collection has begun. Two multiplex reactions, three loci in each, have been optimized using previously published primer sets (Fig. 1) (Colburne et al. 1996, Neff et al. 1999). Data from these six loci (*Lma20*, *117*, *121*, *102*, *87*, and *21*) has been collected from 207 individuals



The results suggest that the loci chosen in this study are highly variable and do provide informative information regarding gene flow within and among both drainage basins. Across the six loci, the number of alleles per locus ranges from 20-36 (Table 2). Locus *Lma20* is the most variable locus, with 36 alleles whereas *Lma87* is the least variable with 20 alleles recovered from the 207 individuals that have been genotyped.

Table 2. Summary of genetic variation within *Lepomis macrochirus* by locus.

Locus	Total # alleles	H _E	H _O	Allele size range
LMA102	27	0.864	0.817	77-141
LMA87	20	0.823	0.734	108-170
LMA21	35	0.919	0.908	144-236
LMA20	36	0.932	0.938	67-147
LMA117	27	0.874	0.914	127-189
LMA121	21	0.887	0.945	138-200
LMAR10	34	0.938	1	168-344
Mean	28.57	0.891	0.894	

Genotypic data has been gathered from five populations. The Devil’s Swamp population (Mississippi River basin) is the most variable, averaging 21.29 alleles per locus. The Blind River (Lake Pontchartrín Basin) is the least variable, averaging only 15.71 alleles per locus (Table 3). The levels of variability for these microsatellite loci are much higher compared to the levels of variability observed among blue catfish from similar locations.

Table 3. Summary of genetic variation by population.

Site	Total # alleles	N _A	H _E	H _O
PM	124	17.71	0.9082	0.9036
CB	130	18.57	0.9045	0.9077
BR	115	15.71	0.8668	0.8596
DS	110	21.29	0.8777	0.869
AtR	149	16.43	0.8979	0.9286
<i>L. macrochirus</i> mean	125.6	17.94	0.891	0.8937

Future Plans

This project will be completed shortly after the new year (Jan 2007). We are continuing with the data analysis and summary of the results and hope to have a manuscript ready to be sent out for review in early spring 2007.

Technology Transfer

Freshwater diversions are routinely being offered as a valuable tool for wetland restoration, however, the potential genetic impacts that this type of restoration project has on native species has not been thoroughly investigated. This is particularly the case in Louisiana, which has many on-going or proposed freshwater diversion projects. This present study focuses on the evaluation of this restoration technique and is aimed at assessing the potential impacts that it has on genetic diversity and biodiversity. Not only will quantitative baseline genetic data be beneficial for this particular diversion project, but more importantly, this data will serve as a model study for any future wetland restoration diversion projects that are aimed at preserving biodiversity and genetic integrity while restoring coastal and wetland habitats. The data from this EPA project (bluegill), and the PI's previous recently completed EPA project on blue catfish also should provide a comprehensive overview of the impacts of life-history on genetic differentiation for two species with varying fecundities, migratory abilities, and sensitivities to fishing pressure. This data is critical and will be useful to policy makers including state and federal agencies, including the US Army Corps of Engineers and the Louisiana Department of Wildlife and Fisheries, because these two agencies are the principal players in wetland restoration.