



# TWENTY-FIFTH ANNUAL PROGRESS REPORT

For the Period Through August 31, 2012

**Supporting research and extension  
projects based on industry needs and  
designed to directly impact commercial  
aquaculture development.**



United States  
Department of  
Agriculture

National Institute  
of Food and  
Agriculture

December, 2012



## **TWENTY-FIFTH ANNUAL PROGRESS REPORT**

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## **PREFACE**

In 1980, Congress recognized the opportunity for making significant progress in domestic aquaculture development by passing the National Aquaculture Act (P.L. 96-362). The Act established USDA as the lead agency for aquaculture coordination and called for development of a National Aquaculture Plan. The next year, Congress amended the National Agricultural Research, Extension, and Teaching Policy Act of 1977 (P.L. 95-113) by granting, in Title XIV, Subtitle L, Sec. 1475(d) of the Agriculture and Food Act of 1981 (P.L. 97-98), authority to establish aquaculture research, development, and demonstration centers in the United States.

Congress envisioned the Centers as focal points in a national program of cooperative research, extension, and development activities that would be developed in association with colleges and universities, state Departments of Agriculture, federal facilities, and non-profit private research institutions with demonstrated excellence in aquaculture research and extension. Eventually, five such Centers were established—one in each of the northeastern, north central, southern, western, and tropical Pacific regions of the country. Funding for the Centers was reauthorized in subsequent Farm Bills (the Food, Agriculture, Conservation, and Trade Act of 1990 [P.L. 101-624]; the Agriculture Improvement and Reform Act of 1996 [P.L. 104-127]; the Farm Security and Rural Investment Act of 2002 [P.L. 107-171]; and the Food, Conservation, and Energy Act of 2008 [P.L. 110-246]).

Projects that are developed and funded by the Regional Centers are based on industry needs and are designed to directly impact commercial aquaculture development in all states and territories. The Centers are organized to take advantage of the best aquaculture science expertise, education skills, and facilities in the United States. Center programs insure effective coordination and a region-wide, team approach to projects jointly conducted by research, extension, government, and industry personnel. Inter-agency collaboration and shared funding are strongly encouraged.

## **ACKNOWLEDGMENTS**

The Southern Regional Aquaculture Center (SRAC) acknowledges the contributions of the Project Leaders and Participating Scientists involved in the projects reported in this Twenty-fifth Annual Progress Report. Members of the SRAC Board of Directors, Industry Advisory Council, and Technical Committee have provided valuable inputs to the successful operation of SRAC during the past year. We particularly appreciate the assistance of the chairs of our Board, IAC and TC.

We also thank the scientists and aquaculturists from across the country who contributed their expertise and valuable time to review SRAC project proposals and publications. Without their help, it would be impossible to maintain the high quality of this program.



# INTRODUCTION

## **The Need for Aquaculture in the United States**

Population growth, rising per capita incomes, and increased appreciation of the role of seafood in human health have caused global demand for seafood to triple since 1990. Over the same period, foodfish output from capture fisheries did not increase because stocks of ocean fish were fully exploited or, in many cases, over-exploited. The difference between the non-expanding supply from capture fisheries and rapidly expanding seafood demand was derived from aquaculture—the farming of aquatic plants and animals in oceans and inland waters.

Global aquaculture has grown at a phenomenal rate over the last 30 years to meet the expanding demand for seafood. Oddly, the United States, which is the third largest consumer of edible fisheries products in the world, lags behind many countries in aquaculture development, accounting for less than 2% of world aquaculture production. Aquaculture nevertheless plays a significant role in United States trade and agriculture, and there is considerable incentive for further development. Important in this regard, the United States is second only to Japan as the world's largest importer of edible fishery products, resulting in a significant international trade deficit. In 2011 the United States imported \$17 billion of edible fish and shellfish products, with a trade deficit of over \$11 billion. This was the largest deficit item for any agricultural commodity.

United States seafood demand continues to increase as a result of population growth and increased emphasis on eating seafood as part of a healthy diet. Although increased seafood demand provides considerable opportunity for growth of domestic aquaculture, production has been level since about 2000. In light of significant economic and food security benefits accruing from producing fishery products rather than importing them, domestic aquaculture production must grow to meet the increasing demand for seafood by consumers.

## **Aquaculture in the Southeast**

The farm-gate value of United States aquaculture exceeds \$1.2 billion. The farm-raised catfish industry—centered in the three deep south states of Alabama, Arkansas and Mississippi—is the largest sector of domestic aquaculture, accounting for one-third of U.S. production. The southeast is also home to other large aquaculture sectors, such as farming of crawfish, hybrid striped bass, oysters, clams, and bait and ornamental fish.

Overall, about 70% of the \$1.2 billion domestic aquaculture crop is produced in the southeast, and the regional economic impact goes far beyond the farm gate. Many of the support functions for the industry—such as feed manufacture and equipment fabrication—also take place in the region, and the total economic impact of aquaculture is many times the value of production alone. Further, if the overall economic value of aquaculture is viewed against a generally depressed agricultural economy, it is clear that aquaculture is a critical factor in the economy of the southeastern United States. However, the profitability of catfish farming and other aquaculture activities have declined to historic lows because of competition from imported products, a sluggish economy, and higher production costs.

## **The Role of the Regional Aquaculture Centers**

Technologies that improve production efficiency can help restore profitability to United States aquaculture and provide a reliable domestic source of seafood for domestic consumers. Technology development is, however, costly, and support for research and development in aquaculture differs radically from that for traditional agricultural sectors such as poultry, cotton, and soybeans. Farmers of those commodities rely on a vast infrastructure of private-sector agribusinesses to conduct most of the research needed to sustain industry growth. Aquaculture, on the other hand, receives little private-sector R&D support, relying instead almost entirely on public-sector funds for technology development.

Although government agencies, particularly the United States Department of Agriculture, have provided significant support for aquaculture research and development, much of that funding is earmarked for specific use by specific institutions. The USDA/NIFA Regional Aquaculture Center program is the only funding activity with the flexibility to stay abreast of industry development, identify problems on a region-wide scale, and implement cooperative, interstate projects to solve those problems.

Since its inception in 1987, the Southern Regional Aquaculture Center has become the most important regional aquaculture activity in the southeastern United States. In its 25 years of operation, the Center has disbursed more than \$17 million to fund multi-state research and extension projects. More than 200 scientists from 30 institutions in the southeast have participated in Center projects.

In the past year, SRAC funded 11 research projects totaling more than \$2.5 million. The Center's "Publications" project is in its seventeenth year of funding and is under the editorial direction of faculty and staff at Texas A&M University. This past year, 16 fact sheets and one final project summary were completed with seven fact sheets in progress. To date, the "Publications" project has generated 226 fact sheets and species profiles, seven project summaries and 21 DVDs with contributions from 225 authors from throughout the region.

Productivity from SRAC research projects has been excellent since the Center's inception more than two decades ago. Information derived from SRAC-funded projects has been transferred to producers and other scientists in thousands of scientific papers and presentations. Currently funded projects continue this trend of high productivity.

Beginning with the first projects funded by the Southern Regional Aquaculture Center, interest among aquaculture research and extension scientists in Center activities has been excellent. In fact, funding and project coordination provided by SRAC has become so embedded in the fabric of southeastern aquaculture research and extension that it is difficult to envision what these activities would be like without the program. We are pleased with the participation by our research and extension scientists in the Southern Region in ad hoc Work Group meetings and Steering Committees, and their willingness to serve as Project Leaders and Principal Investigators for the projects. We believe this broad-based representation has resulted in strong, cooperative research that will be of long-lasting benefit to aquaculture producers and consumers, and to the growth of the aquaculture industry in the Southern United States.

This Twenty-fifth Annual Progress Report covers the activities of the Administrative Center during the past year. Progress reports on the ten multi-year research and extension projects supported by SRAC during this reporting period cover the life of the projects from their initiation date through August 31, 2012.

## **ORGANIZATIONAL STRUCTURE**

The Agriculture Acts of 1980 and 1985 authorized establishment of aquaculture research, development and demonstration centers in the United States. With appropriations provided by Congress for the 1987 and 1988 FYs, efforts were undertaken to develop the five Regional Aquaculture Centers now in existence. Organizational activities for SRAC began in 1987, with the first research and extension projects initiated in 1988.

Research and extension problem areas for the southern region are identified each year by the Industry Advisory Council (IAC), which consists of fish farmers and allied industry representatives from across the region. The Technical Committee (TC), consisting of research and extension scientists from states within the region, works with the IAC to prioritize problem areas. The two groups then work together to develop “Problem Statements” describing objectives of work to solve problems with the highest priority. Using inputs from industry representatives, regional Work Groups of the most qualified research and extension scientists are formed. The Work Groups then plan and conduct the work. Regional aquaculture funds are allocated to participants in SRAC projects approved by the Board and NIFA. Reviews of project proposals, progress reports, and recommendations for continuation, revision, or termination of projects are made jointly by the TC and IAC and approved by the Board.

The thirteen states and two territories represented by SRAC are Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, Puerto Rico, South Carolina, Tennessee, Texas, U.S. Virgin Islands, and Virginia.

### **ADMINISTRATIVE CENTER**

The Administrative Center is located at the Delta Research and Extension Center, Stoneville, Mississippi. Mississippi State University serves as the Host Institution. All necessary support services for the Board, IAC, TC, Steering Committees and project Work Groups are provided by the Administrative Center. This includes monitoring status and progress of projects, preparing and executing Letters of Agreement, tracking administrative and project expenditures, reviewing progress reports, and assisting Project Leaders and participating institutional Grants Offices as needed.

Operation and funding are approved by the Board for inclusion in the Grant Application submitted annually to USDA/NIFA. The Center staff also prepares and submits to USDA/NIFA an Annual Plan of Work covering Center activities and projects to be funded. Following final approval, Letters of Agreement are prepared and executed with all participating institutions. The Center acts as fiscal agent to disburse and track all funds in accordance with the provisions of the grants. Additional Administrative Center responsibilities are detailed in the “Administrative Activities” section of this report.

## BOARD OF DIRECTORS

The Board is the policy-making body for SRAC. Membership provides an appropriate balance among representatives from State Agricultural Experiment Stations, Cooperative Extension Services, 1890 Institutions, and the Administrative Heads Section (AHS) of the Board on Agriculture Assembly (BAA) of the National Association of State Universities and Land Grant Colleges (NASULGC).

The structure of the Board is as follows:

Three members of the 1862 Southern Extension Service Directors Association  
Three members of the 1862 Southern Experiment Station Directors Association  
One member of the 1890 Association of Research Administrators  
One member of the 1890 Association of Extension Administrators  
One AHS administrator from the host institution

Members of the Board are:

Gregory Bohach, Mississippi State University  
Dwight Landreneau, Louisiana State University  
John Liu, Auburn University  
Wondi Mersi, Virginia State University  
Phil Elzer, Louisiana State University  
Joe Street, Mississippi State University Extension Service  
Tony Windham, University of Arkansas Cooperative Extension Service  
Wes Burger, Mississippi State University

Ex-officio Board members are:

Co-chair, Industry Advisory Council  
Co-chair, Industry Advisory Council  
Co-chair for Extension, Technical Committee  
Co-chair for Research, Technical Committee  
Director, SRAC

The Board is responsible for 1) overall administration and management of the regional center program; 2) establishment of overall regional aquaculture research and extension goals and allocations of fiscal resources to ensure that the center develops strong programs in both research and extension; 3) establishment of priorities for regional aquaculture research and extension education activities based on inputs from the TC and IAC and guidance from the National Aquaculture Development Plan; 4) review and approval of annual plans of work and accomplishment reports; and 5) final selection of proposals for funding by SRAC.

## INDUSTRY ADVISORY COUNCIL

The IAC, which meets at least annually, is composed of representatives of state and regional aquaculture associations, federal, territorial and state agencies, aquaculture producers, aquaculture marketing and processing firms, financial institutions, and other interests or organizations as deemed appropriate by the Board of Directors.

The IAC provides an open forum wherein maximum input from private and public sectors can be gained and incorporated into annual and ongoing plans for SRAC. The chairman serves for two years and is elected by IAC members.

Members of the IAC are:

J. Neal Anderson, AR	Lynn Blackwood, VA
Bill Cheek, LA	Sandy Miller, GA
Robert Mayer, KY	Jane Corbin, TN
Ben Pentecost, MS	Jim Ekstrom, TX
Martha Campbell, FL	Shorty Jones, MS
Rob Ellis, NC	Chase Holub, TX
Marty Tanner, FL	Bill Livingston, SC
Butch Wilson, AL	Joey Lowery, AR
Townsend Kyser, AL	Jenny Davis Fagan, TN

IAC members serve up to four-year appointments having staggered terms with options for reappointment. The IAC 1) identifies research and extension needs; 2) works with the TC to prioritize research and extension needs; 3) works with the TC to develop problem statements and recommend funding levels for projects addressing priority research and extension needs; 4) reviews project proposals, progress reports, and termination reports; and 5) recommends to the Board, jointly with the TC, actions regarding new and continuing proposals, proposal modifications and terminations.

## TECHNICAL COMMITTEE

The TC consists of representatives from participating research institutions and state extension services, other state or territorial public agencies as appropriate, and private institutions. Membership of the TC includes research and extension scientists representing essentially all states in the region. The TC meets as needed, but at least annually, and has a co-chairman for research and a co-chairman for extension. Co-chairmen serve for two years and are elected by TC members.

Members of the TC for research are:

Brian Bosworth, MS  
Harry Daniels, NC  
Jim Tidwell, KY  
Allen Davis, AL  
Patricia Duncan, GA  
Carole Engle, AR  
Delbert Gatlin, TX  
Chris Green, LA  
Tom Murray, VA  
Cortney Ohs, FL  
Larry Wilson, TN  
Don Bailey, VI  
Bill Shelton, OK

Members of the TC for Extension are:

Jack Whetstone, SC  
Mike Frinsko, NC  
Ron Blair, TN  
Gary Burtle, GA  
Jesse Chappell, AL  
David Heikes, AR  
Michael Masser, TX  
R. P. Romaine, LA  
Michael Schwarz, VA  
Craig Watson, FL  
Forrest Wynne, KY

Technical Committee members serve up to four-year appointments having staggered terms with options for reappointment.

The TC 1) works with the Industry Advisory Council to prioritize research and extension needs; 2) works with the Industry Advisory Council to develop problem statements and recommend funding levels for projects addressing priority research and extension needs; 3) reviews proposals, progress reports, and termination reports; and 4) recommends to the Board, jointly with the IAC, actions regarding new and continuing proposals, proposal modifications and terminations.

## PROJECT CRITERIA

Projects developed within SRAC should meet the following criteria:

- Addresses a problem of fundamental importance to aquaculture in the Southern Region;
- Involves participation by two or more states in the Southern Region;
- Requires more scientific manpower, equipment, and facilities than generally available at one location;
- Approach is adaptable and particularly suitable for inter-institutional cooperation, resulting in efficient use of limited resources and funds;
- Will complement and enhance ongoing extension and research activities by participants, as well as

- offer potential for expanding these programs;
- Is likely to attract additional support for the work which is not likely to occur through other programs and mechanisms;
- Is sufficiently specific to promise significant accomplishments in a reasonable period of time (usually up to 3 years);

## PROJECT DEVELOPMENT PROCEDURES

Research and extension activities supported by SRAC are accomplished by work described in *Project Proposals*. Proposals are developed using either the *Work Group Method* or the *Competitive Proposal Method*. In either case, the IAC initiates the project-development process by identifying critical problems facing aquaculture in the region. The TC and IAC then jointly prioritize problem areas and recommend the most important research and extension needs to the Board of Directors. Once an area of work has been approved, the Executive Committee appoints a *Project Writing Team* to develop the “Request for Pre-Proposals” and recommend to the Board of Directors which project development process appears to be most appropriate. The Board of Directors has ultimate authority to determine which method will be used to develop project proposals.

In the Work Group Method, the Request for Pre-Proposals is distributed to state, territorial or federal institutions and non-profit private institutions within the Southern Region with demonstrated competence in aquaculture research and development. Interested parties respond by submitting a pre-proposal to the SRAC Administrative Office. A *Proposal Review Team* then selects the best pre-proposals to eventually become part of the regional project proposal. The Proposal Review Team consists of three technical and three industry representatives who cannot become funded participants in the project. Once the project participants have been identified, the SRAC Director convenes a meeting of the *Project Work Group*, which consists of individuals selected to participate in the project and members of the Project Writing Team.

The Competitive Proposal Method differs from the Work Group Method in that the Competitive Proposal Method requests that pre-proposals be submitted from multi-state teams of scientists. Each team will submit one proposal addressing all project objectives. Proposals will then be reviewed by a Proposal Review Team, as described above, and one proposal will be selected for funding. After one pre-proposal has been selected for funding, the SRAC Director convenes a meeting of the *Project Work Group*, which consists of individuals collaborating in the selected pre-proposal and members of the Project Writing Team.

The Project Work Group prepares the project proposal, which is reviewed by the IAC, TC, all project participants and designated peer reviewers from within the region and from outside the region. The SRAC Director submits the project proposal and peer reviews to the Board of Directors for review and approval. Proposals not approved by the Board are returned for revision or eliminated from consideration.

The Director prepares an annual plan of work, including all project proposals approved by the Board, and submits the plan to NIFA for approval. Pending a successful review of the project plan and budget, NIFA notifies SRAC of final approval. Letters of Agreement (subcontracts) between SRAC and participating institutions are then prepared and forwarded for approval and execution by the authorized institutional official. At that point, formal work on the project begins.

## ADMINISTRATIVE ACTIVITIES

A wide variety of support functions for the various SRAC components, including the Board, TC, IAC, Steering Committees and project Work Groups are provided by the SRAC administrative staff:

- Center Director serves as an ex-officio member of the Board, TC, and IAC.
- Monitor research and extension activities sponsored by SRAC.
- Solicit and receive nominations for memberships on the TC and IAC.
- Coordinate submission of written testimony to the U.S. House Agriculture, Rural Development, and Related Agencies Subcommittee on Appropriations regarding RAC support.
- The Director of SRAC serves as a member of the National Coordinating Council for Aquaculture which consists of the Directors of the five Regional Centers and appropriate USDA/NIFA National Program staff.
- Prepare and submit Grant Application to USDA/NIFA entering into funding agreement for each fiscal year, Annual Plan of Work and Amendments.
- Develop and execute appropriate Letters of Agreement with participating institutions in each funded proposal for the purpose of transferring funds and coordinating and implementing projects approved under each of the grants.
- Serve as fiscal agent to review and approve invoices and distribute funds to participating institutions as approved under the grants and as set forth in the Letters of Agreement.
- Prepare budgets for the Administrative Center, track administrative expenditures, and obtain USDA/NIFA approval for project and budget revisions.
- Prepare budget reports for the Board of Directors, tracking expenditures and status of funded projects and the Administrative Center.
- Assist with preparation and revision of proposals for technical and scientific merit, feasibility and applicability to priority problem areas.
- Solicit and coordinate national reviews of project proposals.
- Distribute fact sheets to research and extension contacts throughout the Southern Region, other RACs, and USDA personnel.
- Produce and distribute the “SRAC Annual Progress Report,” which includes editing and proofreading the project reports.
- Produce and maintain the web site for SRAC which provides downloadable copies of all SRAC fact sheets, the Operations Manual and Annual Reports, as well as lists of other research publications and extension contacts in the Southern Region.
- Prepare and distribute Requests for Pre-proposals to research and extension directors and other interested parties throughout the Southern Region.
- Respond to requests from aquaculture producers, the public, and research and extension personnel for copies of fact sheets, research publications and videos produced by SRAC and the other Centers, as well as requests for general aquaculture-related information.

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## PROGRESS REPORTS

The following cumulative reports detail the progress of research and extension work accomplished for the duration of the respective projects through August 31 of the current year. These reports are prepared by the Project Leaders in conjunction with the institutional Principal Investigators.

Publications, Videos and Computer Software .....	10
Improving Reproductive Efficiency of Cultured Finfish .....	15
Using National Retail Databases to Determine Market Trends for Southern Aquaculture Products .....	40
Evaluation of Impacts of Potential “Cap and Trade” Carbon Emission Policies on Catfish, Baitfish, and Crawfish Farming.....	65
Development and Evaluation of Cool Water Crawfish Baits .....	73
Identifying Determinants for Development of Live-Market Grading Standards for Crawfish .....	81
Potential Marketing Structures for the Catfish Industry .....	91
Reproduction and Larval Rearing of Freshwater Ornamental and Marine Baitfish .....	109
Effects of Mosquito Abatement Pesticides on Various Life Stages of Commercially Important Shellfish Aquaculture Species in the South .....	125
Development of Baitfish, Goldfish, and Ornamental Fish Hatchery Methods .....	139
Improving Catfish Broodstock Management by Manipulating Diet, Stocking Densities and Sex Ratios .....	150

## **PUBLICATIONS, VIDEOS AND COMPUTER SOFTWARE**

### **Reporting Period**

March 1, 1995 - August 31, 2012

<b>Funding Level</b>	Year 1 .....	\$ 50,000
	Year 2 .....	60,948
	Year 3 .....	45,900
	Year 4 .....	60,500
	Year 5 .....	67,000
	Year 6 .....	77,358
	Year 7 .....	82,205
	Year 8 .....	77,384
	Year 9 .....	60,466
	Year 10 .....	50,896
	Year 11 .....	45,723
	Year 12 .....	63,764
	Year 13 .....	80,106
	Year 14 .....	78,072
	Year 15 .....	73,982
	Year 16 .....	73,974
	Year 17 .....	76,159
	Total.....	\$1,124,437

**Participants** Texas A&M University System serves as Lead Institution, with Dr. Michael Masser as Project Leader. Participants in this project include authors and co-authors from all states in the region as shown in the listing of publications.

### **PROJECT OBJECTIVES**

1. Review and revise, as necessary, all SRAC extension printed and video publications.
2. Establish an ongoing project location to develop and distribute new SRAC educational publications and videos for Southern Region aquaculture industries. This project will be responsible for preparation, peer review, editing, reproduction, and distribution of all Extension and popular-type publications for all SRAC projects.
3. Place current, revised, and new publications in electronic format (e.g., Internet or compact disk) for more efficient use, duplication, and distribution.

## ANTICIPATED BENEFITS

The direct benefit from this project to the aquaculture industry is the widespread and ready availability of detailed information on production and marketing of aquacultural products. SRAC fact sheets, videos, and other publications are distributed worldwide to a diverse clientele. All SRAC publications are based on research conducted within the region or in surrounding areas.

**Extension Specialists.** When this project was initiated, fewer than half the states had educational materials covering the major aquacultural species in their state. The concept of using the SRAC program to produce timely, high-quality educational materials is based upon the benefits of centralizing the production process while using a region-wide pool of expertise to develop materials. Distribution is then decentralized through the nationwide network of Extension Specialists and County Agents. This process assures an efficient publication process that makes use of the best available talent in specific subject areas. The result is widespread availability of high-quality educational materials for scientists, educators, producers, and the general public.

**Educators.** Many high schools, colleges, and universities in the United States and around the world, use SRAC technical fact sheets as reference materials in aquaculture and fisheries courses. Educational institutions use SRAC extension materials in the classroom to make students aware of aquaculture production and associated trades as a possible vocation.

**Consumers.** Information is readily available for consumers who are seeking background information on aquaculture.

**Producers.** Information on the use of therapeutants, pesticides, methods of calculating treatment rates, and possible alternative crops and marketing strategies is in constant demand by aquaculturists.

Videos that demonstrate such techniques are a ready source of “how-to” information.

**Potential investors.** Detailed information on production and marketing constraints and ways to alleviate or manage those constraints are particularly helpful to people making decisions about entering the aquaculture business. Economic information is used by lending agencies and potential investors, as well as established producers who use the information to help make day-to-day decisions on farm management.

**Internet access.** Availability of SRAC publications via the Internet and makes access faster and easier, facilitates searching for needed information, and reduces storage space requirements for printed documents.

### *Results at a glance...*

- *Over 242 authors have contributed to SRAC publications since the project's inception.*
- *Sixteen new fact sheets and one project summary were completed this year. Seven more fact sheets and one DVD are in some stage of review.*
- *Twenty-four scientists from across the Southern Region contributed to completed publications this year.*

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

During this current project year, 16 new fact sheets were completed. The Aquaplant web site was also updated. All publications have been distributed throughout the Southern Region and to interested Extension Specialists in other regions. Eighteen fact sheets are in some stage of writing, production, or

revision. Ten fact sheets currently do not have drafts submitted. Research funding from universities within the region, as well as funding from private sources, has been used to support the work on which the fact sheets are based.

## WORK PLANNED

The next project year is in development and specific publications have not been established. The SRAC Publications Steering Committee meets during the

annual IAC/TC meeting to develop the following year's project.

## IMPACTS

This is a highly productive project with significant regional, national, and international impact. Fact sheets and videos are requested and used by clientele in all 50 states on a regular basis. Fact sheets generated within the Southern Region are also widely distributed by RACs and extension personnel in other regions. In addition to direct requests for printed material, fact sheets and other informational materials are accessed daily from the SRAC web site by people searching for technical information. In the period from September 2011 through August 2012, 23,934 visitors with 16,648 unique visitors came to the SRAC Publications web site and accessed 108,418 pages. These visitors came from 160 countries/territories. Since the fact sheets are also accessible through numerous other university research and extension web sites, the total usage and impact is undoubtedly several times greater. The AQUAPLANT web site from September 2011 through August 2012 had 250,494 visitors with 206,735 unique visitors that accessed 934,362 pages. These visitors came from 191 countries/territories.

Publications and videos produced by SRAC are increasingly used in educating high school and college

students about aquaculture. In recent years there has been a rapid expansion of aquaculture curricula in high schools. These programs heavily utilize our publications and videos for educational purposes

### *Results at a glance...*

- *In the period from September 2011 through August 2012, 23,934 visitors with 16,648 unique visitors came to the SRAC Publications website and accessed 108,418 pages.*

but usage is impossible to measure because many people access the information from Internet sites. Aquaculture and fisheries courses taught at many colleges and universities also use SRAC technical fact sheets as part of their course reference material.

Another important impact is the education of local, state, and federal regulators about the aquaculture industry. This impact is difficult to measure but

feedback from personnel in two states indicates that the fact sheets are recommended reading for all new employees dealing with aquaculture water quality, exotic species, and other permitting duties. This should be a positive influence toward making aquaculturists better understood and the development of more enlightened regulations.

The impact on consumers of aquaculture products is also likely significant, although it has not been

quantified. Consumers are primarily interested in a wholesome, safe, and inexpensive product, and it has been reported that the consumer-oriented fact sheets and videos developed within SRAC have generated more interest than the producer-directed materials. The fact sheets are in demand in both the English and Spanish versions and, as more information becomes available, extension materials on food safety will be in increased demand by health conscious consumers.

## **PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED**

### **Fact Sheets Completed (9/1/11 - 8/31/2012)**

*Soft Shell Crab Shedding Systems*, by Albert Gaude and Julie A. Anderson, SRAC Publication Number 4306

*Mycobacterium Infections of Fish*, by Ruth Francis-Floyd, SRAC Publication Number 4706

*Species Profile: Black Sea Bass*, by Wade Watanabe, SRAC #7207

*Species Profile: Pigfish*, by Cortney L. Ohs, Matthew A. DiMaggio and Scott W. Grabe, SRAC Publication Number 7209

*Species Profile: Pinfish, Lagodon rhomboids*, by Cortney L. Ohs, Matthew A. DiMaggio and Scott W. Grabe, SRAC Publication Number 7210

*Production of Hybrid Catfish*, by Rex Dunham and Michael P. Masser, SRAC Publication Number 190 (Revision)

*Non-Commercial Oyster Culture or Oyster Gardening*, by Michael Osterling and Christopher Petrone, SRAC Publication Number 4307

*How to Start a Biofilter*, by Dennis P. DeLong and Thomas M. Losordo, SRAC Publication Number 4502

*Herpesviruses in Fish*, by Andy Goodwin, SRAC Publication Number 4710

*Aquaculture and the Lacey Act*, by Elizabeth R. Rumley, SRAC Publication Number 5005

*Introduction to Financial Management of Aquaculture Businesses*, by Carole R. Engle, SRAC Publication Number 4400

*Assessing the Financial Position of an Aquaculture Business: Using Balance Sheets*, by Carole R. Engle, SRAC Publication Number 4401

*Determining the Profitability of an Aquaculture Business: Using Income Statements and Enterprise Budgets*, by Carole R. Engle, SRAC Publication Number 4402

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*A Spreadsheet Tool for the Economic Analysis of a Recirculation Tank System*, by Matthew Parker, Dennis DeLong, Rebecca D. Dunning, Thomas M. Losordo and Alex O. Hobbs, SRAC Publication Number 456 (Revision)

### **Final Project Summary**

*Management of Aquaculture Effluents from Ponds*, by Robert P. Romaine, SRAC Publication Number 6004

### **Manuscripts in Review**

*Aquatic Weed Management*, by Michael P. Masser, SRAC Publication Number 361 (Revision)

*Nutritional Aspects of Seafood*, by Elizabeth Reames, SRAC Publication Number 7300

*Cranfish-One Page Bulletin* by Elizabeth Reames

*Farmed Hybrid Striped Bass-One Page Bulletin*, by Harry Daniels

*Prebiotics and Probiotics: Definitions and Applications*, by Delbert M. Gatlin, III

*Sorting and Grading Warm Water Fish*, by Anita Kelly, SRAC Publication Number 391 (Revision)

*Heterotrophic/Biofloc Systems*, by John Hargreaves

### **DVD in Review**

*Safety for Fish Farm Workers*, by Nathan Stone

### **On-going project**

Updating of the AQUAPLANT web site on aquatic weed management – Michael P. Masser.

**All fact sheets completed by this project to date are available on the Internet  
at <http://www.msstate.edu/dept/srac> and <http://srac.tamu.edu>**



## IMPROVING REPRODUCTIVE EFFICIENCY OF CULTURED FINFISH

### Reporting Period

February 1, 2009 - August 31, 2012

<b>Funding Level</b>	Year 1 .....	\$222,530
	Year 2 .....	\$193,402
	Year 3 .....	\$78,041
	Total.....	\$493,973

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	USDA/ARS/Stuttgart National Aquaculture Research Center .....	Adam Fuller, Jerry Ludwig

### PROJECT OBJECTIVES

1. Improve broodfish management protocols for increased reproductive efficiency through:
  - a. Developing pre-selection methods of potential broodfish to be included in the broodstock population.
  - b. Improving conditioning and preparation of broodfish.
  - c. Final identification of broodstock for spawning.
  
2. Improve spawning protocols to increase reproductive efficiency through:
  - a. Managing spawning conditions.
  - b. Improving the collection and handling of fertilized eggs.

## ANTICIPATED BENEFITS

Captive-bred finfish rarely experience all aspects of natural spawning conditions, and thus dependence on natural reproduction is often unreliable. Consequently, reproductive efficiency is often less than desired, frequently requiring creative management or compensatory protocols to overcome the failure to reproduce spontaneously and at full potential. This project will improve

reproductive efficiency of commercially cultured finfish of immediate importance to the Southern Region. Management protocols will be established that address reproductive bottlenecks and result in improved protocols that increase reproductive efficiency for the target species and have the potential for use with other similarly cultured finfish species.

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

**Objective 1.** *Improve broodfish management protocols for increased reproductive efficiency.*

**Sub-Objective 1a.** *Develop pre-selection methods of potential broodfish to be included in the broodstock population.*

### USDA-ARS Catfish Genetics Research Unit

Channel catfish farming is the largest sector of the U.S. aquaculture industry. Production of catfish fry relies on random spawning of mature male and female catfish in brood ponds. Spawning success is highly variable, but research and farm data indicate that on average about 40% of females produce a spawn. Little is known about males' contribution to spawning, but most farmers stock a ratio of 1:1 or 2:1 female to male broodfish. In addition to spawning incidence, spawning time (early to late in the year) is important to farmers since early spawning allows earlier stocking of fry and production of larger fingerlings by the end of the first growing season. The inability to identify parentage of pond-spawns has hindered determination of factors influencing spawning in catfish. However, the advent of molecular marker techniques for parentage determination allows evaluation of factors influencing spawning in pond-spawned catfish. Understanding factors influencing spawning could lead to development of improved fish and management techniques for more efficient reproduction of farm-raised catfish.

One hundred channel catfish spawns were collected from eight commercial catfish farms in the spring of 2006 as part of a project to establish a diverse population of catfish for selection of an improved catfish line. Full-sib families were maintained in separate tanks until fish were large enough to be individually tagged (> 4 inches), and then tagged fish were reared communally in ponds. Fish were fed a 32% protein commercial catfish diet throughout the study. The largest 5 to 9 females and 3 to 7 males from each family (~ 800 females and 500 males) were selected during the fall of 2007 to be used as future broodfish and blood samples were collected from each fish for DNA marker analysis. Each broodfish was scored for a series of highly polymorphic microsatellite loci and inheritance patterns at these loci were used to determine parentage of spawns. Broodfish were weighed and blood samples were drawn for determination of estrogen and testosterone levels in females and testosterone levels in males in the late winter (February to March) of 2008 and 2009. Ultrasound images of maximum cross-sectional area of ovaries were also



**Figure 1. Ultrasound image of catfish ovary.**

recorded for females at this time. Following sampling, broodfish were stocked into 0.25-acre ponds at a 2:1 female to male ratio in the spring of 2008 (2-year-old broodfish, 24 ponds) and again in 2009 (3-year-old broodfish, 20 ponds). Eight spawning cans were placed in each pond in early April and checked for spawns through the end of August each year. Data were analysed to determine relationships among spawning incidence and time; and broodfish weight, farm-of-origin, family-of-origin, spawning pond, hormone levels, and estimated ovary size.

In March of 2010, 4-year-old broodfish were categorized based on their previous spawning history: spawning males, spawning females, non-spawning males and non-spawning females. Two, 0.1-acre ponds were stocked with each possible combination of males and females (previously spawning males with previously spawning females, previously spawning males with previously non-spawning females, previously non-spawning males with previously spawning females, and previously non-spawning males with previously non-spawning females) to determine if spawning history was predictive of future spawning success for either gender. Broodfish were stocked at 1.5:1 female to male ratio and six spawning cans were placed in each pond in early April and checked for spawns through the end of August. Data were analyzed to determine

effects of previous spawning history and farm of origin on spawning incidence and farm of origin on spawning time.

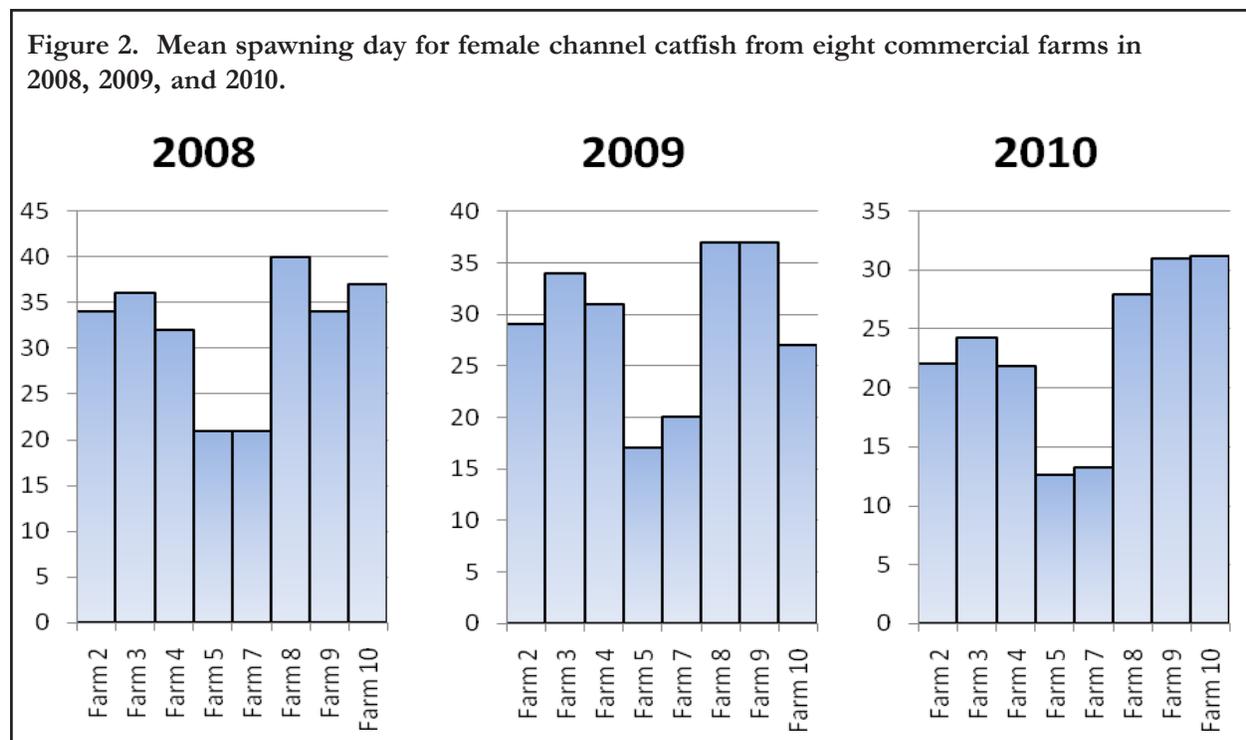
Spawns were collected over a 103-day period in 2008, a 98-day period in 2009, and a 75-day period in 2010; however, over 60% of the spawns were collected within 35 days of the first spawn each year. Spawning percentages were 27.4%, 48.3%, and 60.3% for 2-, 3-, and 4-year-old females, respectively; and 25.7%, 37.7%, and 46.6% for 2-, 3-, and 4-year-old males, respectively. Due to frequent multiple spawning by males, over 60% of spawns were attributed to fewer than 15% of the males each year.

Spawning incidence was influenced by fish weight and spawning pond; but farm-of-origin, family-of-origin, plasma estrogen and testosterone, and ultrasound estimates of ovary size were not predictive of spawning incidence. As 2-year-old fish, spawning females (1.8 lbs) were larger than non-spawning females (1.6 lbs), but there was no difference in weight of spawning and non-spawning 3-year-old females (3.2 lbs). Spawning males and non-spawning males were not different for weight as 2-year-olds (2.1 lbs), but spawning males were larger than non-spawning males as 3-year-olds (3.5 lbs and 3.0 lbs, respectively) and 4-year-olds (4.7 lbs and 4.1 lbs, respectively). Previous spawning incidence was not

predictive of future spawning incidence for males or females. There was no difference in spawning percentage of 4-year-old fish for the various stocking combinations based on previous spawning success.

Our data suggest variation in female spawning date has a genetic component. Average spawning date of females from the same two farms-of-origin (farms 5 and 7) was significantly earlier than other farms by 9 days in both 2008 and 2009, and 12 days in 2010 (Figure 2). Farm-of-origin and family-of-origin were significant predictors of female spawning date; combined these factors accounted for 26% and 16% of variation in female spawning date in 2008 and 2009, respectively. There was a positive correlation among spawning date for individual females across years, and for mean spawning date of full-sib sisters across years for the 2008 and 2009 data. There were insufficient numbers of females per family in 2010 to provide accurate estimates for effects of family-

of-origin on female spawning date or correlation of female spawning date across years. Analysis and interpretation of male spawning date was precluded due to the high proportion of males that spawned multiple times over each spawning season. We also observed multiple spawning by female channel catfish. Approximately 20% of spawning females produced more than one egg mass during a spawning season each year. These were not interrupted spawning events as the egg masses were collected greater than 7 days apart and typically the second egg mass was collected 20 to 50 days after the first egg mass. Most multiple-spawning females produced 2 separate egg masses, although 3 females produced 3 separate egg masses. To our knowledge, this is the first report of female channel catfish producing multiple egg masses over a spawning season. Plasma hormone levels and ultrasound estimates of ovary size were not predictive of female spawning date for 2008 and 2009.



Spawning incidence of females appears to be primarily under environmental control suggesting that future work should focus on identification of environmental factors that influence spawning incidence. Management of environmental factors to promote spawning would reduce the number of broodfish needed and reduce costs. A relatively small proportion of males did the majority of spawning, indicating that the number of males typically used by farmers (1:1 or 1:2 male to female ratio) is probably excessive. Reducing the number of males could reduce broodfish costs substantially but it will be important to determine how few males can be stocked without reducing spawning incidence. Selection for early spawning date appears to be feasible and could allow farmers to stock fry earlier and potentially produce larger fingerlings at the end

of the first growing season.

## Results at a glance...

- *Timing of female spawning (early or late season, for example) has a genetic component that can be exploited in breeding programs to expand the spawning season. On the other hand, the incidence of females spawning is primarily under environmental control, suggesting that spawning success can be improved by identifying and managing appropriate environmental factors affecting spawning success.*

### University of Arkansas at Pine Bluff and USDA-ARS Stuttgart National Aquaculture Research Center

Some female white bass do not respond to changes in the duration of the reproductive cycle (i.e. compression, shifting, or expansion). Females may fail to spawn after being included in groups that are projected to spawn when seed stock is required. Ultrasonography has the potential to guide decisions regarding which females to include in a production cycle. Quantification of characteristics of the images collected with an ultrasound machine could be used to determine which females will be most likely to spawn following photothermal manipulation. This would reduce the number of female white bass necessary to hold under controlled photothermal regimes for use in hybrid striped bass seed stock production.

A Tela-Vet portable ultrasound system (Classic Medical, Tequesta, FL) equipped with a 5-8 MHz linear transducer was used for this study. During 2010-2011 we captured digital images from 170 fish cataloging more than 6000 digital images. We have begun the process of collecting information regarding

ovary characteristics (diameter, cross sectional area, perimeter) using image analysis software (Image-Pro Plus Version 4.5.1.22, Media Cybernetics, Inc., Silver Spring, Maryland). Our initial efforts have included developing a standardized assessment for determining cross sectional surface area. Multiple technicians are involved with this process. Quality control steps include the evaluating agreement between data collected from different personnel. The consistency of the 'rule-set' for interpreting the image and defining the perimeter of each ovary is evaluated for each worker evaluating images. To further optimize training we have collected ultrasound images from three white bass that were then sacrificed. The animals were then frozen and cross sections of the peritoneal cavity were exposed. Photographs of the cross section of the fish are being compared to data gathered using ultrasound. Finally, during April-May, 2011, 24 wild female white bass were also collected during their spawning migration in Caney Bayou, a tributary of the Arkansas River. Nine of these fish had mature ovaries and the remaining fish

were in varying post-spawning stages. Ultrasound images were captured from each of these fish. Ovaries of each fish were removed and length and diameter measurements were recorded so that a cross sectional area of the ovary was determined. The average cross-sectional area determined for the nine gravid fish was  $1078 \pm 74 \text{ mm}^2$  (average  $\pm$  standard error). Cross sectional areas were also determined using the image analysis program. Mean cross sectional area determined using ultrasound and image analysis was  $966 \pm 104 \text{ mm}^2$ .

A standardized approach to collecting images has been developed. During 2010 and 2011 images were collected over 16 different dates from female white bass. Fish were anesthetized and held upright, submerged in a holding tank. Images were collected from the body region between the posterior insertion of the pelvic fin and the anterior insertion of the anal fin. Whether or not the female responded to hormone injection, and the time to ovulation (if spawning occurred) was recorded. Multiple images of cross sections of the peritoneal cavity were captured. Length and weight of each fish was recorded and age data were gathered from hatchery passive integrated transponder (PIT) tag records. The female

white bass were held for about 24 hours before initial inspection for ovulation. The eggs were visually inspected to determine readiness for spawning. Eggs were then expressed from 'ripe' females into plastic containers. Fish not ready for spawning initially were inspected at several intervals over a 24-hour period. The total mass of eggs expressed was recorded for the females screened using ultrasound imaging. Three small samples of eggs (0.1-0.2 g) were weighed. The number of eggs in each of these samples was tallied to determine the number of eggs/g. Female fecundity (eggs/kg fish weight) was then determined by multiplying eggs/g by the total mass of eggs expressed. The digital images of these gonads are being currently being examined. Depth, power, gain, frequency and decibel settings and the quality of each image collected by the of the ultrasound system are being summarized. This procedure does have the potential to guide hatchery decisions and improve reproductive efficiency during production of hybrid striped bass seed stock.

No new images were captured in 2012. We continue to assess images to determine ovary characteristics (diameter, cross sectional area, perimeter) using image analysis software.

### **Texas A&M University-Corpus Christi and Auburn University**

Steroid analysis was conducted for channel catfish and blue catfish. The objective was to determine if the steroid analysis can be used as a predictor for sexual maturity and ripeness of the fish or as a predictor for future reproductive performance. Steroids were also measured as an indicator of the relative effectiveness of different diets supplemented with vitamin C when fed to broodfish.

Blood and serum samples were collected during three periods corresponding to spring (March-prior to visual spawning activity), early summer (June-during spawning activity), and after spawning (July). Additional studies included exposure studies of blue

and channel catfish to vitamin C treatments, and establishment of baseline data for immature 2-3 year-old blue catfish males. Serum samples were immediately frozen and stored at -112 degrees F until processed. Samples were purified, concentrated, and analyzed by high-performance liquid chromatography (HPLC). Eight steroids were targeted: estradiol (E2), 11-ketotestosterone (11-KT), 11 $\beta$ -hydroxyandrostenedione (11 $\beta$ -OHA), 11 $\beta$ -hydroxytestosterone (11 $\beta$ -HT), 17, 20 $\beta$ -dihydroxypregn-4-en-3-one (17, 20 $\beta$ -P), estrone (E1), testosterone (T), and 17 $\alpha$ -hydroxyprogesterone (17-OHP).

Whole blood samples yielded inconsistent extraction results compared to sera samples. Fish fed diets supplemented with vitamin C had significantly higher overall steroid concentrations relative to fish fed a control diet. Several trends were evident from comparing female blue versus channel catfish. Estradiol and testosterone were more strongly expressed and accumulated in blue catfish compared to channel catfish, whereas estrone was found in higher concentration in channel catfish post-spawn (June). Male catfish had a larger number of steroids represented in the samples than females. Blue catfish males had higher total steroid content than channel catfish during pre- and spawning periods. 11-Ketotestosterone was highest in pre-spawning for both catfish species and decreased over the study period. T was below detection threshold for most

of the study period in both species, peaking in channel catfish post-spawn.  $17\alpha$ -Hydroxyprogesterone concentrations peaked during spawning period for both channel and blue catfish. 11bP was not present in male catfish of either species. Steroids of female blue and channel catfish show several expected observations. Peaks in estradiol and testosterone occurred during spawning, whereas  $17, 20\beta$ -dihydroxypregn-4-en-3-one was most abundant after this period.

Species differences were observed for steroid levels in both and female fish. This may help explain the differences in the success of open pond spawning and for induced spawning observed between these two species.

**Sub-Objective 1b.** *Improve conditioning and preparation of broodfish.*

**University of Arkansas at Pine Bluff, University of Tennessee, Texas A&M University**

To achieve maximum Atlantic croaker production efficiency, larvae must be available throughout the year. In addition, nothing is known of nutritional requirements for Atlantic croaker broodfish, which must be known to ensure quality spawns. Four studies were undertaken to resolve these problems. Study 1 examined the feasibility of conditioning and inducing Atlantic croaker to spawn during the spring/summer utilizing 90- or 120-day abbreviated conditioning cycles and hormones. Due to failure to spawn in suitable quantities during study 1, study 2 was conducted to determine the feasibility of delayed spawning through photoperiod and temperature manipulation. Study 3 examined the effects of dietary lipid source and inclusion rate on reproductive performance of Atlantic croaker. Study 4 examined the effects of fish meal replacement and alternative protein sources, as well as dietary lipid percentage and protein source interactions on reproductive performance of Atlantic croaker.

*Study 1: Accelerated spawning cycles*

The biology of Atlantic croaker dictates they spawn during the autumn months, but baitfish production relies on availability of small fish throughout the year, especially during spring and summer. Atlantic croaker broodstock were spawned in November, 2009, and then maintained under static winter conditions. Broodstock were measured (mean = 11.9 inches), gender determined (male:female ratios of 3:5 or 4:4) and fish were stocked into eight experimental tanks, in two systems on March 1, 2010. Each system underwent either 90- or 120-day abbreviated cycles that condensed annual photoperiod/water temperatures into the experimental duration. Treatments ended during autumn conditions optimal for spawning of Atlantic croaker (10 hours light/14 hours dark, water temperature 66 degrees F). The broodfish were then injected with a 75-microgram salmon

gonadotropin-releasing hormone analogue implant (sGnRHa; Ovaplant®). Fish were allowed to spawn within the tanks and eggs were collected. No spawning occurred during the 90-day cycle treatment and only two small spawns were collected from fish in the 120-day cycle. In the 120-day cycle, a 4,300 egg spawn and a 2,700 egg spawn were collected 4 and 6 days after implantation, respectively. Fertilization was less than 5% for both spawns, and eggs were atypically small and discolored. Egg incubation was not attempted due to poor egg quality. Two plausible explanations for low reproductive output are 1) annual cycles compressed to 90 or 120 days were too short a duration to allow adequate deposition of nutrients to gametes; and 2) spawning twice in a 6-month period does not allow proper physiological preparation through environmental cues. Abbreviated 90- or 120-day cycles are ineffective for out-of-season conditioning and spawning in Atlantic croaker that have previously spawned.

#### *Study 2: Effects of delayed spawning*

Due to the poor reproductive success experienced during study 1, a second study was conducted that examined the effects of delayed spawning on the reproduction of Atlantic croaker. Atlantic croaker broodstock were *not* spawned during their natural spawning season in November of 2010, and instead maintained under static summer conditions of 82-86 degrees F and a photoperiod of 15 hours light and 9 hours dark. Histological examination revealed approximately 80% of females developed immature ova during this period, but only 10% demonstrated advanced stage ova development. In February, 2010, the un-spawned broodstock were measured (mean = 12.8 inches), gender determined (male:female ratios of 3:5 or 4:4), and stocked into eight tanks in two systems. Each system underwent a 90-day cooling period with a reduction in photoperiod simulating natural autumn conditions. After the water temperature and photoperiod reached conditions conducive to spawning for Atlantic croakers, the fish in one system were injected

with Ovaprim® (sGnRHa and a dopamine inhibitor; 0.23 cc/pound), followed by a second injection 2 days later. The fish in the second system received a single 75 µg sGnRHa implant (Ovaplant®), and all fish were allowed to spawn within the tanks. No spawning occurred from fish administered the implant although egg hydration occurred and several females died due to over-hydration of eggs. All males receiving the 75-µg implant did not express milt upon application of pressure to the abdomen. Although spawning was irregular among the tanks administered the aqueous Ovaprim® injections, 1.1 million eggs were produced. The mean number of eggs produced per tank was 275,530 with fertilization rates ranging from 42 to 88%. At the conclusion of spawning, all males receiving the injection expressed milt upon application of abdominal pressure. Although both treatments resulted in egg production by females, only the injection treatments resulted in viable spawns. The limiting factor of successful spawning appears to be milt production by the males. Only males receiving the Ovaprim® injections successfully produced milt, indicating that a dopamine blocker is required for males to produce milt outside on the natural spawning season. The results of this study indicate Atlantic croaker can be spawned out-of-season for year round production.

### **Results at a glance...**

- *Atlantic croaker can be spawned out-of-season for year-round production. Hormone implants (sGnRHa) improved spawning success, egg production, fecundity, and synchronized spawning events for commercial production. Only males receiving sGnRHa and a dopamine inhibitor successfully produced milt, indicating that a dopamine blocker is required for males to produce milt outside of the natural spawning season.*

*Study 3: Lipids in broodfish diets*

No information is currently known on the nutritional requirements of Atlantic croaker broodstock. Broodstock nutrition is vital to producing good quality eggs in sufficient quantities to support commercial production while keeping costs down. Atlantic croaker broodstock (4 males:4 females) were stocked into each of 12 tanks in three experimental systems in September, 2010. Four experimental diets were formulated and manufactured to contain 45% protein and either 6 or 10% lipid. Lipids sources and contents were 10% menhaden fish oil, 6% menhaden fish oil, 10% poultry fat, or 10% soybean oil. In November 2010, all fish received a single 75 microgram sGnRHa implant (Ovaplant®), and all fish were allowed to spawn naturally within the tanks. Fish fed the 6 and 10% fish oil diets produced more spawns per tank

**Results at a glance...**

- *These studies provide a good basal diet for producers wanting to undertake Atlantic croaker production. Fish oil cannot be entirely removed from broodstock diets, but it can be reduced to an inclusion rate of 6%, or possibly lower.*

(3.0-3.7 per tank), a greater percentage of floating eggs (64.3-73.2%), larger egg diameters, greater number of eggs produced (879,320-1,470,215 eggs), greater fertilization rates (37.5-65.3%), and better hatching rates (15.3-29.3%) than fish fed the 10% soybean oil or poultry fat diets. While fish fed the 10% soybean oil or poultry fat diets did produce eggs, fertilization rates were extremely poor (< 4.9%) and no egg hatching occurred. While it appears that fish oil cannot be entirely removed from broodstock diets, it can at least be reduced as the 6% fish oil diet produced more spawns, more eggs, and greater

fertilization and hatch rates than the 10% fish oil diet. This study provides the first step in determining the nutritional requirements of Atlantic croaker to support commercialized production, and it provides a good basal diet for producers wanting to undertake Atlantic croaker production immediately.

*Study 4: Protein in broodfish diets*

Broodstock nutrition is vital to producing good quality eggs in sufficient quantities to support commercial production. Now that some information is known on lipid sources and inclusion rates to sustain Atlantic croaker production, more information is needed on the protein requirements for broodstock. Proteins and free amino acids (FAA) are important energy sources and structural elements for embryo and larval development. This study examined the effects of fish meal replacement and alternative protein sources, as well as dietary lipid percentage and protein source interactions on reproductive performance of Atlantic croaker. Atlantic croaker broodstock (2 males: 4 females) were stocked into each of 12 tanks in three experimental systems in October, 2011. Four experimental diets were formulated and manufactured to contain 45% protein and either 6 or 10% lipid. Lipid concentrations were either 10% or 6% menhaden fish oil. Protein sources included combinations of menhaden fish meal, poultry by-product meal, meat, bone and blood meal, and soybean meal. All experimental diets proved to be un-palatable to Atlantic croaker, likely due to the reduced total fish content (12% fishmeal and 6 or 10% fish oil) of the diets compared to diets used in study 3 (30% fishmeal and 6 or 10% fish oil). Feed consumption was less than 0.2% of body weight/day, and the broodstock in all dietary treatments rapidly lost weight. Disease and parasite related mortalities quickly escalated resulting in a 47% loss of total broodstock numbers despite treatment efforts with antibiotics and Paracide and Dimilin. No reproductive response was induced through the use of sGnRH and domperidone. Little information

on protein requirements for reproduction can be ascertained from the study, other than certain protein

## University of Florida

Ornamental fish production, like all of aquaculture, relies heavily on the successful spawning, hatching, and survival of larval fish. The complexity of this industry, which produces hundreds of species of fish, makes reproduction a critical challenge to improve efficiency. Very little information is available to formulate science-based recommendations for producers and their suppliers.

Research has shown that inclusion of highly unsaturated fatty acids (HUFA) in fish diets can lead to increases in hatch and survival of larval fish. To provide HUFAs in the diet to ornamental fish, current practices rely heavily on feeding a wide variety of foods, often at an extremely high cost which include frozen feeds (e.g. ground beef heart, adult *Artemia*, blood worms, shrimp, and squid). Formulation of an artificial diet which provides proper levels of HUFAs, and is designed to feed smaller broodstock typical to the ornamental industry, could lead to considerable savings in production costs and increased performance of broodstock.

Redtail black sharks, *Epalzeorhynchus bicolor*, are popular ornamental freshwater fish that have been in production in Florida since the late 1980s. This fish represents a variety of similar species which are spawned in hatcheries using induced spawning procedures. Annual sales of redbtail black sharks from Florida farms is estimated to be in excess of 1,000,000/year, with a farm-gate value of \$0.25 per fish. Mono sebae, *Monodactylus sebae*, are an ornamental fish species commonly captured from the wild along the western coast of Africa. In recent years their popularity in the ornamental industry has prompted interest in the development of culture methods. No information is available on their nutritional requirements.

sources or reduced fish meal content can lead to palatability issues in Atlantic croaker broodstock.

Producers identified broodstock nutrition to be a major bottleneck in commercialization and thus served as the impetus for this experiment. These studies were designed to evaluate the effects of altering the fatty acid profiles of diets fed to brood fish on egg and larval quality in these two commercially important ornamental fishes.

### *Mono Sebae*

Brood mono sebae were stocked into three independent systems and held for one year. Spawning was induced by increasing the salinity by 5 g/L every three days until a salinity of 25 g/L was attained. When a salinity of 25 g/L and 75 degrees F was attained, natural volitional spawning initiated. Eggs were collected in an air lift floating egg collector. Fish were fed the various experimental diets daily to apparent satiation. The three formulated diets included an ornamental fish industry standard formulation, a diet fortified with docosahexaenoic acid (DHA) and a diet fortified with DHA plus arachadonic acid (ARA). The formulation of the DHA and DHA+ARA diets was altered by adding commercially available algal additives Algamac 3050 Flake and Algamac-ARA to increase the n-3 and n-6 fatty acid contents, respectively.

The effects of feeding the experimental diets on spawning performance was measured by quantifying the number of spawns, egg quantity, fertilization percent, hatch percent, egg morphology (egg diameter, oil droplet diameter), larval survival at 24 and 48 hours post-hatch, larval morphology (oil droplet diameter, yolk volume, and notochord length) at hatch, and larval morphology (notochord length) at 24 and 48 hours post-hatch using standard methods. The fatty acid composition of the eggs was determined using standard methods.

A total of 49, 33, and 67 spawning events were recorded over the 88-day experimental period for the control, DHA, and DHA+ARA diets, respectively. The total number of eggs spawned was 758,282 for the control, 521,211, and 1,260,255 for the DHA+ARA diets. The mean total egg production per female was 2160 for the control, 1959 for the DHA diet, and 2813 for the DHA+ARA diet. The mean floating egg percent fertilization were all greater than 93.6% and the mean sinking egg percent fertilization ranged from 70.6 to 88.4%. Mean hatching percentage was 57.4% for the control diet, 55.2% for the DHA diet, and 47.5% for the DHA+ARA diet. The mean 24 hour survival was 62.0% for the control diet, 42.8% for the DHA diet, and 46.4% for the DHA+ARA diet with the control diet being significantly greater than the DHA and DHA+ARA diets. The mean 48-hour survival was 45.3% for the control diet, 30.5% for the DHA diet, and 30.3% for the DHA+ARA diet with the control diet being significantly greater than the DHA and DHA+ARA diets.

The mean egg and oil globule diameters were significantly different among diets with those in the DHA+ARA diet being smaller. At hatch, mean notochord length and oil droplet diameter were significantly different among diets with the DHA+ARA diet being smaller, however, the yolk volume was not significantly different among diets.

At 24 hours post-hatch, the notochord length was significantly different among treatments with the DHA+ARA diet being significantly smaller. At 48 hours post-hatch, the notochord length was significantly different among treatments with the DHA+ARA diet being significantly smaller than the control but not different from the DHA diet.

#### *Redtail Black Sharks*

Seven female broodfish were stocked into each of nine 1,000-L concrete tanks in a greenhouse at the University of Florida Tropical Aquaculture Laboratory. Each experimental diet was fed to fish in three tanks. Females were individually weighed at the beginning of the experiment and feed portions were weighed for each feeding. All female fish were fed the brood diets for 27 days at 5% body weight divided into a morning and evening feeding. Males were kept in a tenth tank on the same system and fed the control diet at 5% of their body weight per day. On day 28 the fish were not fed and all female fish were administered Ovaprim injections at a dosage of 1 mL/kg body weight. The injections were divided into a 10% priming dose at midnight and a 90% resolving dose at 6 a.m. Female fish were injected with a 20-minute interval between each tank to allow for the timing of spawning and egg sampling.

Fish were hand-stripped when ovulation occurred using a dry method, mixing eggs and sperm in a bowl and then adding water to initiate fertilization. A combined sample of non-fertilized eggs was taken from females in each tank and placed in a -112 degree F freezer until fatty acid analysis. Fertilization percent was determined by examining a subsample of eggs at the onset of gastrulation, and hatching percent was based on a random subsample taken from spawns from each tank. Survival of larvae was based on a 50 fry sample at day 2 (fully developed gut and functional mouth) and a 100 fry sample at day 30 following standard feeding protocol.

## Results at a glance...

- *Mono sebae* broodfish fed increased diets fortified with DHA and ARA produced the greatest number of eggs and greatest number of spawns. Redtail black shark larval survival at 2-days post hatch was significantly lower for the control diet which indicates a possible benefit from incorporation of DHA and ARA in brood diets.

Redtail Black Sharks represent several cyprinid species in commercial production and therefore were a good model candidate for this study. However, due the length of time required to fully mature eggs in their gonad and the seasonality of maturation, we were limited on the number of replications in this trial. Added to the problem were early failures of all treatment eggs to hatch due to elevated water temperatures in the hatchery. Fatty acid profiles of the eggs were determined for both trial but the percent hatch and larval survival data was only for one spawning event in year 2.

Percent fertilization ranged from 89-95%, percent hatch ranged from 82-87.3%, 2-day post-hatch survival ranged from 44-97.3%, and 30-day survival ranged from 42-73.3%. Percent fertilization, percent hatch, percent survival and percent deform at hatch, 2 and 30 days post-hatch were not significantly different among fish fed the different diets. The only statistically significant difference was survival at 2 days post-hatch, which was significantly lowest for eggs for fish fed the control diet. Egg diameters at the onset of the blastomere were not significantly different.

**Sub-Objective 1c.** *Final identification of broodstock for spawning.*

#### **USDA-ARS Catfish Genetics Research Unit**

Results of studies in Objective 1a showed that ultrasound is not accurate for predicting spawning success of female channel catfish. Therefore, this

objective was not addressed, given the labor and time required with no evidence indicating a successful outcome.

#### **University of Arkansas at Pine Bluff and USDA-Stuttgart National Aquaculture Research Center**

Often, injection of white bass females with hormone does not result in spawning during the next 24 to 48 hours. Females may fail to spawn at all, or they may spawn later than 48 hours, rendering a spawning effort less successful. Ultrasonography has the potential to guide decisions during the spawning process. Quantifying characteristics of the ultrasound images collected with an ultrasound machine could be used to determine which females will be most likely to spawn in a fixed period following hormone injection. This would substantially improve the efficiency of hybrid striped bass seed stock production.

corresponding indices of reproductive success has been developed and images continue to be assessed using image analysis software. Image analysis software (Image-Pro Plus Version 4.5.1.22, Media Cybernetics, Inc., Silver Spring, Maryland) is being used to determine several morphometric measures (diameter, cross sectional area, perimeter) to characterize ovaries of fish as they approach ovulation. Statistical models that include categorical variables such as ovulating/non-ovulating and continuous responses such as fecundity, as well as fertilization success, are being used to determine if variation in ovary images predicts higher or lower reproductive output.

Standardized imaging techniques for female white bass were developed in the first year of the project. A database of over 6,000 ultrasound images and

**Figure 3. Ultrasound image of pre-spawning white bass female collected during June 2010 spawning activities. Outline indicates area containing the ovary.**



**Objective 2.** *Improve spawning protocols to increase reproductive efficiency*

**Sub-Objective 2a.** *Manage spawning conditions*

**University of Arkansas at Pine Bluff and USDA-ARS Stuttgart National Aquaculture Research Center**

Domestication of striped bass and white bass allows greater control over the reproductive cycle and spawning conditions. Domestication also allows choices related to age, size, and the duration of the reproductive photothermal period. The choices made may affect the success of induced spawning efforts. For example, choosing older or larger individuals might affect fertilization or hatching rates, or size of individuals at hatch or at yolk absorption. Quantifying the importance of these factors should lead to improvements in hatchery efficiency during production of hybrid striped bass.

A combination of 3-, 4-, and 5-year-old white bass were subjected to a 12-month photothermal regime. During the 12-month period, fish were fed a 45% protein diet twice daily to satiation. At the end of the 12-month period, fish were induced to spawn with hormone injections. Weights and lengths of females were determined prior to hormone injection. Fish were injected with 330 IU HCG per kg body weight. The eggs were treated with tannic acid and povidine, and maintained in McDonald hatching jars at 66 to 75 degrees F. During year 1, egg development ceased after approximately 19 hours.

It appears that povidine treatment of moronid eggs is lethal. During the second year, hatching percentages were determined. On the day of hatch, approximately 40 larvae from each cross were preserved in 4% buffered formalin. At 5 days post-hatch, approximately 40 larvae from each cross were preserved in 4% buffered formalin. Preserved larvae were photographed individually and larval total length was determined. The effect of female age on length at hatch was examined using an analysis of covariance with female weight utilized as the continuous covariate. The same statistical approach was used to examine the effect of female age on length at 5 days post-hatch.

This study was repeated during spring year 3 of the project. The procedures for year 3 were similar to those of year 2. Brood stock were subjected to a 12-month photothermal regime, fed a 45% protein diet twice daily to satiation, and induced to spawn with injections of 330 IU HCG per kg body weight. The eggs were treated with tannic acid and maintained in McDonald hatching jars at 70 degrees F. Larvae were sampled at hatch and at 5 dph, preserved in 4% buffered formalin, and later individually photographed. Lengths of larvae were determined. Statistical analyses were conducted as before, with the effects of female age and weight on length at hatch and length at 5 days post-hatch examined using analyses of covariance.

The study was repeated a third time during summer of year 3. The procedures for the summer year 3 study were similar to those of the previous two studies. Brood stock were subjected to a 12-month photothermal regime, fed a 45% protein diet twice daily to satiation, and induced to spawn with injections of 330 IU HCG per kg body weight. The eggs were treated with tannic acid and maintained in McDonald hatching jars at 70 degrees F. Larvae were sampled at hatch and at 5 dph, preserved in 4% buffered formalin, and later individually photographed. Lengths of larvae were determined. Statistical analysis consisted of examination of the effect of age on

length at hatch and length at 4 days post-hatch. Age was not a factor in the third study because all the fish were age 2.

Altogether, three 3-year-old, thirteen 4-year-old, and two 5-year-old females were used during the year 2 study. Female weights averaged  $614 \pm 146$  g (mean  $\pm$  SD) and ranged from 400 to 890 g. Fertilization rates averaged  $6 \pm 6\%$  and ranged from 0% to 25%. Hatch rates were fairly low ( $< 10\%$ ) for most crosses. A total of 11 females had enough hatching to collect adequate sample sizes for length at hatch estimates. Larvae from one of those 11 females all died before 5 days post-hatch. Length at hatch averaged  $2.40 \pm 0.27$  mm TL. Length at 5 days post-hatch averaged  $3.07 \pm 0.31$  mm. Neither female weight nor female age significantly influenced length at hatch (Table 1). Likewise, neither female weight nor female age significantly influenced length at 5 days post-hatch (Table 1).

During the spring year 3 study, six 2-year-old, nine 4-year-old, and three 6-year-old females were used. Female weights averaged  $581 \pm 170$  g and ranged from 370 to 1090 g. Hatch rates varied considerably during the study. Length at hatch averaged  $2.66 \pm 0.15$  mm TL. Length at 5 days post-hatch averaged  $3.80 \pm 0.21$  mm TL. In this study, female weight and age both significantly influenced length at hatch (Table 2), while female age alone significantly influenced length at 5 days post-hatch (Table 2). The relationship between length at hatch and female weight was positive, suggesting that heavier females produced slightly larger larvae at hatch, but we note that this relationship was no longer significant at 5 days post-hatch. At hatch and at 5 days post-hatch, larvae from 2-year-old females were larger than larvae from 4-year-old or 6-year-old females. The magnitude of the difference in length between larvae from 2-year-old females and 4 or 6-year-old females was a few hundredths of a millimeter at hatch, but was 0.3 mm at 5 days post-hatch.

During the summer year 3 study, seventeen 2-year-

**Table 1. Output from statistical examinations of the effect of female weight and female age or dam on length at hatch and length at 5 days post hatch during the 2010 study (year 2).**

Response variable	Effect	Type III SS	F	df	P	R <sup>2</sup>
Length at Hatch (mm)	weight	0.071	0.94	1	0.332	0.008
	age	0.182	1.21	2	0.301	
Length at 5 dph (mm)	weight	0.109	1.10	3	0.295	0.005
	age	0.126	0.64	2	0.531	
Length at Hatch (mm)	dam	2.34	3.33	10	0.0004	0.098
Length at 5 dph (mm)	dam	0.710	0.79	9	0.622	0.027

**Table 2. Output from statistical examinations of the effect of female weight and female age or dam on length at hatch and length at 5 days post hatch during the 2011 study (year 3).**

Response variable	Effect	Type III SS	F	df	P	R <sup>2</sup>
Length at Hatch (mm)	weight	1.671	93.05	1	<0.001	0.192
	age	0.266	7.41	2	<0.001	
Length at 5 dph (mm)	weight	0.001	0.00	1	0.986	0.058
	age	0.842	10.40	2	<0.001	
Length at Hatch (mm)	dam	3.811	19.45	15	<0.001	0.430
Length at 5 dph (mm)	dam	5.463	12.32	15	<0.001	0.334

old females were spawned. Female weights averaged  $669 \pm 124$  g and ranged from 440 to 856 g. Length at hatch averaged  $2.57 \pm 0.13$  mm TL. Length at 5 days post-hatch averaged  $3.34 \pm 0.21$  mm TL. As in the spring year 3 study, the relationship between

female weight and length at hatch was positive and significant (Table 3). However, the relationship between female weight and length at 5 dph was not significant. These results are also consistent with the spring year 3 study.

**Table 3. Output from statistical examinations of the effect of female weight or dam on length at hatch and length at 5 days post hatch during the 2011 summer study (year 3). Note: all fish in this spawn were age 2.**

Response variable	Effect	Type III SS	F	df	P	R <sup>2</sup>
Length at Hatch (mm)	weight	.	7.62	1	0.006	0.026
Length at 5 dph (mm)	weight	.	0.00	1	0.944	0.001
Length at Hatch (mm)	dam	2.848	18.56	16	<0.001	0.518
Length at 5 dph (mm)	dam	1.710	4.02	12	<0.001	0.285

Earlier work suggested that there was a maternal effect influencing size at hatch and size at 5 days post-hatch. This earlier work was not designed to ascertain whether the maternal effect was genotypic or phenotypic. The results of our year 2 study point to a genotypic effect, since phenotype (i.e. age and weight of female) did not influence size at hatch or size at 5 days post-hatch. To further examine this possibility, we ran a one-way analysis of variance using female as the independent variable. The effect of female was statistically significant (Table 1) for length at hatch, but not for length at 5 days post-hatch. For example, larvae from females 3 and 11 were significantly larger than larvae from females 4 and 7. The results of the spring year 3 study also indicate a maternal effect, though it is less clear that the effect is genotypic, since age and weight significantly influenced length at hatch and age affected length at 5 days post-hatch. Statistical analysis indicated that females affected size at hatch and at 5 days post-hatch (Table 2). Larvae from two females in particular were larger at hatch than larvae from most other females. However, by 5 days post-hatch, larvae from three completely different females had caught and surpassed the average lengths of larvae from the two stand-out females identified by data collected at hatch. It appears that larvae from the latter three

females were more efficient at utilizing endogenous energy reserves (available from yolk). This efficiency could conceivably be carried into latter life stages and might be a characteristic targeted by a selective breeding program. As in the spring year 3 study, statistical analysis indicated that females affected size at hatch and at 5 days post-hatch (Table 3).

The difference in results of the studies is noteworthy. In year 2, ages ranged from 3 to 5, but in spring year 3, ages ranged from 2 to 6. Hence, the age range examined was greater in the second study. Likewise, the weight range observed was greater in the spring year 3. The converse conclusions from the first two studies, regarding the importance of age and weight to length at hatch and length at 5 days post-hatch, could be due to the greater age and size ranges of the second study. The positive relation between female weight and larval characteristics supports earlier research on striped bass, which showed that larger females tended to produce larger larvae. However, our results indicate that younger females produce larger larvae at hatch. This result should be considered tentative. It is confounded by the fact that a few of the 2-year-old females from spring year 3 happened to be among the heaviest females in the study. To examine this observation further, we limited the data

analysis of larval length at 5 days post-hatch to females weighing between 450 and 650 g. This included five 2-year-old, three 4-year-old, and three 6-year-old females. Even when the weight range is reduced, we still observed that age significantly affected length at 5 days post-hatch, and that larvae from 2-year-old females were larger than larvae from 6-year-old females.

Several consistencies exist among the three studies. Regardless of whether female weight significantly affected length at hatch, in all three studies weight no longer significantly influenced larval length at 5 days post-hatch. The effect of female age on length at 5 days post-hatch could only be tested in two of the three studies. In only one of those two studies did female age significantly affect length at 5 days post-hatch. These facts taken together suggest that female

weight and female age have limited influences on length at 5 days post-hatch. In all three studies, statistical analysis indicated that female had a significant effect on length at 5 days post-hatch. The preponderance of evidence suggests that the maternal effect on length at 5 days post-hatch is mostly genotypic.

If heritability of length at 5 days post-hatch is sufficient, then selection for this trait could increase the size of larvae and consequently, their gape. If gape is large enough, hybrid striped bass might be able to consume *Artemia* nauplii at first feeding, eliminating the current requirement for rotifers at first feeding. This might significantly change the economics of tank culture of fingerling hybrid striped bass and lead to year-round availability of fingerlings.

#### **USDA-ARS Catfish Genetics Research Unit**

Yearling USDA-403 fingerlings were randomly divided into two groups. Group one was fed to satiation and group two was fed half the amount fed to group one. Both groups were exposed to a compressed annual temperature cycle of 4 months at 79 degrees F and 2 months at 55 degrees F. Exposure to three complete temperature cycles was done over two calendar years and 30 females and 20 males from each group were stocked separately into two, 0.1-acre ponds with 10 spawning containers in April. Male fish fed to satiation weighed 2.2 lbs and females weighed 1.7 lbs. Male fish fed to half-satiation weighed 1.6 lbs and females weighed 1.3 lbs. Spawning cans were checked through the summer, however, there were no spawns produced from either group.

An experiment was designed to determine if fish exposed to extreme compressed cycles would spawn when they were 1 year old. One group of fish was USDA-103 and the other group was created from an industry pool and designated as Delta Select.

Both groups of fish were grown 4 months at 79 degrees F then exposed to 55 degrees F for 1 month. A temperature cycle of 2 months at 79 degrees F followed by a month of cold temperature was repeated until the fish had been exposed to three cycles of cold and warm temperatures. The fish were then stocked into 0.1-acre ponds with spawning containers and the cans checked regularly through the summer. No spawns were produced in either group.

Another experiment was performed to determine if fish could be spawned after 18 months of alternating temperature cycles. Fish from an industry pool, designated as Delta Select, were raised in the hatchery at 79 degrees F until October 13, 2009. Four groups of 150 fish from an industry pool, mean weight 27.0 g, were stocked into each of four, 300-gallon tanks equipped with chillers. Another group of 300 fish was stocked into a 0.1-acre pond. Two tanks were fed to satiation and two tanks were fed to one-half satiation. Fish were exposed to three cycles of

2 months of 55 degree F water and three cycles of 79 degree F water. In early October, 2010, 30 females and 20 males from each group were stocked in each of two, 0.1-acre ponds with 10 spawning containers. Female fish from the fish fed to satiation weighed an average of 0.25 lbs and males weighed 0.51 lbs; female fish fed one-half-satiation weighed 0.13 lbs and male fish weighed 0.18 lbs. Female fish from the ponds weighed 0.74 lbs and males weighed 1.4 lbs. A sample of eight fish from each treatment were weighed, the gonads dissected and weighed and a blood sample taken. Gonadal development was reported as the gonadosomatic index (GSI). Fish fed to satiation were twice as heavy as those fed to half-satiation; however fish fed in the ponds were over twice as heavy as those fed in tanks. The GSI from both groups fed in tanks were larger than fish from the pond. Spawning cans were checked during October, however, no spawns occurred.

Although the October water temperature was warm enough to support spawning, no spawning occurred suggesting that age of the fish may be an important component of reproductive maturation and that there is a limit on the effectiveness of temperature cycles to advance spawning. Some maturational events may have been advanced suggested by the larger GSI in cycled female fish compared to pond raised fish. However, both female satiation fed and half-satiation fed fish had similar GSIs. In all three groups, males were heavier than females. Pond raised fish were heavier than both cycled groups and the group fed to satiation were about twice as heavy as fish fed the one-half satiation ration.

Fish from the pond raised group and the cycled

fish fed to satiation were held through the winter in ponds and 10 males and ten females from each group were stocked in 0.1 acre ponds with spawning containers in April of the following spring. There were not enough fish from the one-half satiation group to attempt spawning in this group. Males in both groups were heavier than females and fish raised in ponds were heavier than the cycled fish. Only one spawn (10%) occurred in the pond with cycled fish and four spawns (40%) occurred in the pond raised fish.

Altering the temperature cycle was not effective in inducing spawning after 12 months or 18 months of age, in spite of their having experienced three cold cycles, the number of cold cycles thought to be necessary to induce precocious puberty. The cold cycles in the 12 month experiment was only one month in length compared to a 2 month cold exposure in previous experiments. Further, even fish exposed for 2 months and attempted to spawn in the fall failed to show any gonadal development or spawning. The only appreciable spawning (40%) occurred in pond raised fish when they were 2 years old and had been exposed to 2 winters in the pond. Fish exposed to 3 artificial (in tanks) cold periods and one winter in a pond only had 1 out of 10 fish spawning.

These data suggest that artificially inducing precocious puberty may be more difficult than originally thought. Regardless of the feeding or temperature regime male fish were heavier than females, which support earlier reports of differential growth in the sexes of channel catfish.

**University of Arkansas at Pine Bluff, University of Tennessee,  
Texas A&M University**

Atlantic croaker display asynchronous spawning with a prolonged spawning season, which limits the potential to reproduce this species on a scale capable

of sustaining commercial culture. Therefore, a study was conducted to determine if 1) Atlantic croaker could be spawned naturally in captivity; 2) hormone

implants could induce spawning or improve fecundity; and 3) temperature, photoperiod, and hormone implants could synchronize spawning.

Atlantic croaker broodstock (average total length = 11.3 inches) were captured from Trinity Bay in August, 2009. Two males and three females were stocked into each of twelve, 300-gallon tanks in three recirculation systems with temperature/photoperiod controls. Natural photoperiod and temperature mimicked seasonal temperature fluctuations in Trinity Bay. Tanks were assigned to four treatments; 1) natural spawning (NAT); 2) pre-optimal temp (77 degrees F) hormone implant (PRE); 3) optimal temp (73 degrees F) hormone implant (OPT); or 4) post-optimal temp (70 degrees F) hormone implant (POST). Implants used were Ovaplant<sup>®</sup> 75- $\mu$ g sGnRH $\alpha$ . Egg samples were taken for determination of egg diameter, fertilization rate, and hatch rate. Egg samples from each spawning event were placed into conical, 25-gallon hatching tanks to determine hatch rates at 27 to 30 hours.

Total egg production was 2.9 million from all treatments (36 females; 24 males). Parameter means were: water temperature at spawning, 67.8 degrees F; photoperiod at spawning, 10.1 hours daylight; eggs/spawn, 97,417; fertilization rate, 42%; hatch rate, 19%; and 3-day larval survival, 37%. The POST treatment produced the greatest quantity of eggs and spawns per tank. Spawning events were highly synchronized for hormone treatments compared to NAT. The shortest to longest latency occurred in the following order: 1) POST; 2) OPT; 3) PRE. The total egg per spawn was greater in the POST treatment than PRE or OPT. The quantity of eggs per spawn was greater from POST than

from fish in PRE or OPT, while the quantity of eggs per spawn from NAT was not different from other treatments. Egg fertilization was greater in the NAT and POST treatments than for PRE or OPT. Overall fecundity for all treatments in the study (36 females) was 81,180 eggs per female. The mean fecundity for females in the POST treatment was greater than fecundity of the NAT, PRE, or OPT treatments.

The results of this study demonstrate that Atlantic croaker can be spawned passively in a captive environment, but 75- $\mu$ g sGnRH $\alpha$  hormone implants used to actively induce maturation and spawning in Atlantic croaker can improve spawning success, egg production, fecundity, and

## Results at a glance...

- *Atlantic croaker can be spawned naturally in captivity, but a single 75-microgram sGnRH $\alpha$  implant injected at 10 hours of daylight and water temperature of 69 degrees F will control, improve, and synchronize reproduction of Atlantic croaker for commercial production.*

synchronize spawning events for commercial production. Optimal spawning of captive Atlantic croaker occurs at a photoperiod consisting of 10 hour daylight/14 hour dark and a water temperature of 66 degrees F. A single 75- $\mu$ g sGnRH $\alpha$  implant should be injected at 10 hours of daylight and water temperature of 68 to 70 degrees F in order to control, improve, and synchronize reproduction of Atlantic croaker.

## **Sub-Objective 2b.** *Improving the Collection and Handling of Eggs*

### **USDA-ARS-Catfish Genetics Research Unit**

Channel catfish were first spawned in captivity nearly a century ago and the methods used have changed little. Egg masses are placed in hatchery troughs in baskets made with 0.25-inch-mesh hardware cloth or plastic screen, and are agitated with paddles located between the baskets. The paddles are attached to a shaft running the length of the hatchery trough, and either rotate 360 degrees or oscillate back and forth. Normally 10 to 12 spawns (roughly 18 lbs or 250,000 eggs) are held in each 100-gallon hatchery trough, with a water flow of 5 gallons per minute at 78 to 82 degrees F. This incubation system has proved functional, but it has limitations. If egg loading is increased, as normally happens during the peak of the spawning season when facilities are limited, water circulation between and through the spawns is greatly restricted resulting in a low dissolved oxygen concentration and dead eggs in the center of the spawns. Those areas may serve as foci for fungal and bacterial infection, greatly reducing the hatch rate in the entire trough. We believed that a new incubation system, one in which water (and oxygen) was more thoroughly and efficiently forced through the egg masses, would increase the efficiency of commercial catfish hatcheries.

The new incubator, called the “See-Saw” by collaborating farmers, utilizes an angle aluminum frame slightly smaller the standard hatchery troughs. Three baskets made with 0.25-inch PVC-coated hardware cloth contain the spawns and are held in place by the frame. The baskets have cross-partitions to evenly distribute the egg masses within the baskets, and hinged lids to hold the spawns in place during operation. Agitation is accomplished by raising and lowering the frame up and down through the water. A prototype of the new incubator underwent preliminary testing during the 2007 and 2008 spawning seasons. The first trial (2007) determined

the appropriate cycle interval to be approximately 10 seconds. In the second trial (2008) the See-Saw was tested with twice the egg density as is recommended. Although a thoroughly replicated comparison with standard incubators was not conducted, the See-Saw operated flawlessly. Those preliminary studies were published and describe the construction and operation of the prototype incubator in more detail. With the initiation of this SRAC project, a non-funded cooperative agreement was initiated with Needmore Fisheries LLC, Glen Allen, Mississippi, to more thoroughly compare the See-Saw with conventional paddle-type incubators and to test and quantify several operational parameters. All studies reported here were conducted at that commercial hatchery using experimental incubators fabricated and operated by the hatchery employees.

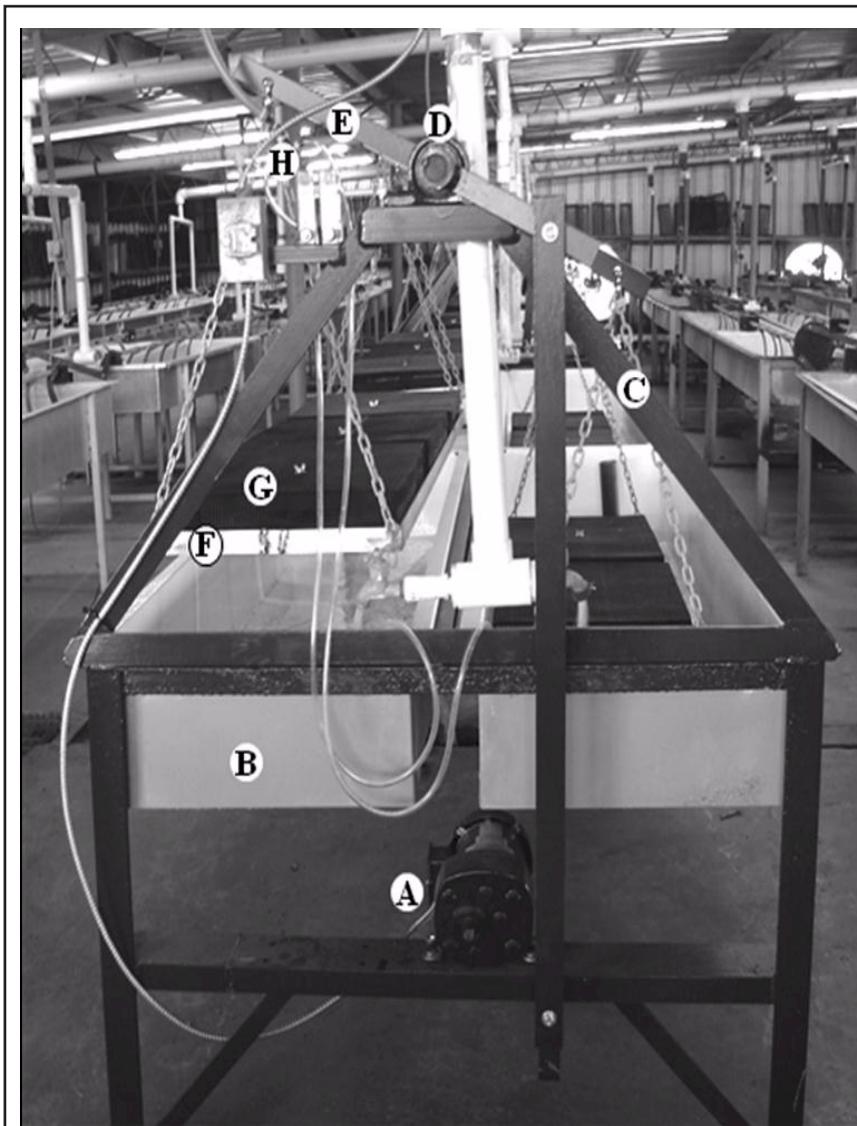
### ***Results at a glance...***

- *A novel catfish egg incubator has been designed and tested on two commercial farms. More eggs can be incubated using less water exchange than with conventional incubators, while achieving increased survival to swim-out stage.*

Most of the first year (2009 spawning season) was used to design the system, purchase motors and material for fabrication, and preliminary stress-testing of the system without live eggs. Near the end of the spawning season the first comparative trial was conducted. Pairs of troughs (one control and one See-Saw, with four troughs for each treatment) were loaded with approximately 26 egg masses per trough (approximately 475,000 and 473,000 eggs per trough, respectively). Water quality was measured in the

water supply and in each trough daily. Sac fry were measured volumetrically and sub-sampled to determine total number, then transferred to rearing troughs. When the fry reached swim-up stage, they were measured volumetrically and sampled to determine total number before transfer to rearing ponds. Survival to swim-up stage averaged 54% in the See-Saw and 23% for the control troughs, a 2.3-fold difference.

In Year 2 of the project (2010 spawning season) we measured the effect of egg loading density in See-Saw incubators on survival to hatch and swim-up. Further comparisons with the paddle-type incubators were not conducted. We loaded See-Saws (five troughs for each treatment) with approximately 15 lbs (220,000 eggs), 30 lbs (447,000 eggs), 45 lbs (670,000 eggs), and 60 lbs (893,000 eggs) of spawns. Water flow into the troughs averaged 2.1 gallons/



**Figure 4. See-Saw incubator prior to loading eggs. Note that as the left rack is up in the air, the right rack is down in the water. Each rack contains three hatching baskets that are secured to the rack. The water supply for these two troughs is in the foreground and the drain is at the far end in each trough. The following components are labeled: (A) 6 rpm motor, (B) hatching trough, (C) angle-aluminum frame supporting the See-Saw, (D) steel shaft running the length of the troughs, (E) crossbars, (F) angle-aluminum rack that holds the baskets, (G) hatching baskets, and (H) oxygen supply used in Year 3 of this study.**

minute, roughly half of the rate recommended for commercial hatcheries. The 15, 30, and 45 pound troughs produced an average of 132,700, 263,800, and 429,400 swim-up fry (survivals from egg of 60, 59, 64%, respectively, similar to values reported in commercial hatcheries). However, the 60-pound treatment produced only 417,200 swim-up fry (survival of 46%). The results of this year's study indicate that both hatchery space and water use would be maximized with See-Saw incubators loaded at the 45-pound rate.

In 2011 (Year 3 of the project) we examined the effect of oxygen supplementation on troughs loaded with 45 lbs of eggs. Fifteen troughs were incubated using no oxygen supplementation and had a mean oxygen saturation of 82.4%; 17 troughs were incubated using additional oxygen added through ceramic diffusers at an average rate of 0.12 liters/min resulting in an average oxygen saturation of 124.1%. Mean swim-up fry production overall was 462,363 fry/trough (10,327 fry/pound of eggs), for a survival from egg to swim-up of 71.2%. There were no significant differences between treatments,

## IMPACTS

Atlantic croaker display asynchronous spawning during a prolonged spawning season, which limits the potential to reproduce this species on a scale capable of sustaining commercial culture. This project has developed reliable hatchery methods to induce and synchronize Atlantic croaker spawning for production creating a new market for farm-raised marine baitfish. These methods could be implemented immediately at several hatcheries in the southern United States. At least two commercial redfish production facilities in Texas have acquired Atlantic croaker broodstock and started the first attempts at commercial production.

Delayed spawning combined with aqueous Ovaprim® injections results in successful spawning

confirming that 45 lbs of eggs can be incubated per See-Saw trough without additional oxygen if the hatchery water supply is near air saturation.

We believe that even higher loading densities could be incubated using supplemental oxygen with no impact on hatch rate or survival to swim-up stage. Even without a pure oxygen supplement, the See-Saw incubator can incubate 3 to 4 times as many eggs as traditional paddle-type incubators using half the water, a tremendous savings in both floor space and energy use.

The use of this incubator across the commercial industry would result in considerable savings, particularly for those hatcheries that need to heat their well water. This incubator may have even greater application in the numerous state and federal hatcheries which are tasked with hatching a growing number of fish species. The See-Saw can reduce both the space and water flow needed to meet their channel catfish production quota, making those resources available for other priority species.

of Atlantic croaker out-of-season for year round production. This is a major breakthrough for a marine baitfish industry that relies upon the availability of specific sizes of bait throughout the year. Atlantic croaker is a high value marine baitfish species that can retail for more than \$1 for a 2 to 5 inch fish. Current markets for this species rely upon wild-captured juveniles. During the off-season (April to September) Atlantic croaker are subject to limited availability which increases demand and price significantly. This project has provided a means to produce ideal-sized baitfish year round to meet consumer demand and create new markets.

This project has produced the first information on the dietary requirements for Atlantic croaker

broodstock in order to improve reproductive performance. While fish oil could not completely be eliminated from the diets to improve sustainability and reduce costs, production was improved by using lower inclusion rates (6% fish oil) than in diets previously used for Atlantic croaker. This will increase profitability over using higher lipid diets while still making moderate advances toward sustainability of the fish feed. The fish oil diets provide good basal diets for producers wanting to undertake Atlantic croaker production immediately while meeting or exceeding the reproductive

performance of wild fish.

Over 100 million catfish eggs have been incubated thus far in on-farm trials. Next spring at least 16 four-trough see-saw units will be in commercial operation. Publication of blueprints and assembly instructions is planned. To speed transfer of the technology, a collaborating farmer is considering the manufacture and sale of single four-trough units so potential users can both test the unit in their hatchery and have a physical model to guide fabrication of additional units.

## PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

### Publications

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## USING NATIONAL RETAIL DATABASES TO DETERMINE MARKET TRENDS FOR SOUTHERN AQUACULTURE PRODUCTS

### Reporting Period

June 1, 2009 – August 31, 2012

<b>Funding Level</b>	Year 1 .....	\$123,930
	Year 2 .....	\$124,605
	Year 3 (Supplement) .....	\$ 73,683
	Year 4 (Supplement) .....	\$ 76,322
	Total.....	\$398,540

<b>Participants</b>	Mississippi State University .....	Jimmy Avery (Project Leader)
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	University of Florida .....	Sherry Larkin, Charles Adams

### PROJECT OBJECTIVES

1. Compile historical data on retail price and sales volumes of major aquaculture species and competing products in key cities and regions in the United States.
  - a. Convene a project planning meeting.
  - b. Procure store-level scanner data for the most recent 5 years and household-based scanner data for the two most recent years available from 18 markets covering all nine U.S. census regions.
  - c. Analyze trends on sales price (dollar per pound), volumes (pounds) and market shares of major aquaculture products.
  - d. Prepare fact sheets that summarize price and sales volume data for catfish, crawfish, clams, and shrimp as well as for competing products for distribution to industry stakeholders through extension mechanisms.
  
2. Identify factors affecting a) trends in prices and sales volumes and b) consumption of fresh and frozen farm-raised catfish, crawfish, clam, and shrimp products.
  - a. Estimate retail pricing models for various fresh and frozen farm-raised catfish, crawfish, clam, and shrimp products based on store level data.
  - b. Estimate retail sales response models for various fresh and frozen farm-raised catfish, crawfish, clam, and shrimp products based on store level data.
  - c. Estimate disaggregated demand functions for various fresh and frozen farm-raised catfish, crawfish, clam, and shrimp products using discrete-choice models based on panel data.

3. Measure competitive position and substitutability of frozen farm-raised catfish, clam, and shrimp products with other seafood products, with an emphasis on imported products.
  - a. Estimate cross price elasticities of various seafood products consumed in the U.S.
  - b. Model consumer demand for type, form, and package size
  - c. Construct policy analysis matrices (PAM) and estimate domestic resource costs (DRC) for various aquaculture products of the southern region of the U.S.

## ANTICIPATED BENEFITS

Seafood retailing in the United States (U.S.) is carried out via two chief outlets: restaurants and grocery stores. There has been no major study attempting to understand the seafood grocery sector at the national level in the U.S. The grocery sector needs to be analyzed in greater depth in order for seafood businesses to succeed in these times of rapid market change. Increased understanding of trends in supermarket sales of seafood and fish has potential to assist the U.S. aquaculture industry to refine marketing strategies and targets. National scanner data are being used for this study since they contain

details of the quantity, price, and hence expenditure, on products sold in a grocery market. They also provide information on characteristics of the products sold per daily/weekly/monthly/or higher frequency and consumers' characteristics, and therefore are a rich source of market information. The Project team has procured scanner data from the A. C. Nielsen Company, and has been analyzing these data and providing customized market reports to seafood companies in the U.S. The total value of these reports provided to industry (at no cost to them) over the past two years was \$5.4 million.

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

**Objective 1.** *Compile historical data on retail price and sales volumes of major aquaculture species and competing products in key cities and regions in the U.S.*

**Sub-Objective 1a.** *Convene a project planning meeting.*

**University of Arkansas at Pine Bluff, Texas Tech University, Auburn University, and University of Florida**

A meeting was held on November 17, 2009 with all project participants attending. Project stakeholder's expectations, project objectives and methods, sources of national scanner data and characteristics of data, matching data requirements and sources, project methodology, and work plan were discussed. The project team decided to purchase a national database from A.C. Nielsen for the recent five years, and considered Information Resources Inc. as an

alternative source. Household consumption data using Consumer Expenditure Survey of the Bureau of Labor Statistics, and the USDA National Nutrient Database for Standard Reference were identified as sources for matching data. The University of Arkansas at Pine Bluff was entrusted with the task of obtaining these data.

**Sub-Objective 1b.** *Procure store-level scanner data for the most recent 5 years and household-based scanner data for the two most recent years available from 18 markets covering all nine U.S. census regions.*

### The University of Arkansas at Pine Bluff

Year 1

The University of Arkansas at Pine Bluff (UAPB) procured the household consumption data from the Consumer Expenditure Survey (CES) of the Bureau of Labor Statistics (BLS). The CES data for the years from 2004 to 2008 was obtained in the form of compact discs (CDs) from the BLS. The CES is the most comprehensive and detailed U.S. data source for analyzing demographic effects on household consumption. It collects data on expenditure, income and various household characteristics. It includes two types of survey procedures: the quarterly Interview survey and the weekly Diary survey. Information from approximately 5,000 households is available in each of these surveys. The interview survey collects information pertaining to expenditures on housing, household durables, apparel, transportation, health care, insurance and entertainment. The Diary survey collects information on weekly expenditures on frequently consumed goods like food and beverage, tobacco, personal care products and nonprescription drugs and supplies. In addition, demographic and family characteristics of each consumer unit (CU) are collected. There are five main data files in CES. These are the Consumer Unit Characteristics and Income (FMLY) file, the Monthly Expenditures (MTAB) file, the Detailed Expenditures (EXPN) file, the Income (DTAB) file and the Imputed Income (DTID) file. Overall these files provide information such as age, gender, race, marital status, education and relationships amongst the members of the CU.

Next, the nutritional characteristics of the relevant products were collected from the USDA National Nutrient Database for Standard Reference. Among

## Results at a glance...

- Overall, fish expenditure per household has grown over the past 3 years.
- Unbreaded shrimp is the most dominant product frozen products.
- Tilapia marketers diversified their products into entrée products over the years.
- Crawfish in the unbreaded form consistently saw drops in share, while shares in entrée crawfish increased.
- Clams had decreased sales over the last five years, but the reduction in sales was slowing down.
- The retail sales modeling study indicates that both basa/tra and tilapia frozen products are substitutes for catfish frozen products.
- For future market planning to increase sales in U.S. supermarkets, U.S. aquaculture industry needs to consider a broader regional approach regarding substitute products. The catfish industry needs to include other major whitefish products, particularly basa/tra and tilapia products.
- Average expenditure per household on all non-finish has decreased over the period among all income groups due to a significant decrease in the consumption of unbreaded forms of products.
- Salmon and tilapia have remained the two most popular unbreaded frozen/chilled finfish products among all income groups (“higher-income”, “lower-income” and “poverty-threshold”).

the four aquaculture species under study in this project, only the information on crawfish is not available. This will be collected from scientific references and other possible public databases.

UAPB procured store-level scanner data from the A. C. Nielsen Company following an opening bidding process; the dataset is composed of weekly data covering 52 U.S. markets for the last 5 years, ending on June 12, 2010. The A.C. Nielsen data category reflects the “department” or physical layout of the typical supermarket and divides food-at-home items into different departments: dry grocery, frozen foods, dairy, deli, packaged meats, and perishables. The store-level database purchased from A.C. Nielsen Inc. contains information for all fish/seafood products in “dry grocery” and frozen food categories. The fish and seafood within the “dry grocery” items include products in canned, shelf-stable, and paste forms. The frozen seafood includes all frozen and chilled fish and seafood available in both prepared and unprepared forms found in refrigerated and frozen sections. The term ‘frozen’ as defined by A.C. Nielsen Inc. includes all chilled/frozen products having Universal Product Codes (UPC) but does not include random weight (or loose) fresh products that have no UPC codes.

## Year 2

The UAPB team analyzed the A.C. Nielsen homescan dataset covering five markets, namely Chicago, Houston, Miami, Memphis, New Orleans-Mobile for a five year period from 2007-2008 to 2009-2010. The dataset was comprised of information on buyers’ socio-economic and demographic characteristics (such as household income, age of household head and number of household member), and all the fish/seafood purchases they made during a year (including quantity of product purchased, price paid, date of purchase, and various product attributes). The UAPB team was able to group the households into three income categories (“higher-income”, “lower-income” and “poverty-threshold”)

based on the yearly weighted average poverty threshold measured by the U.S Census Bureau. The “lower-income” group refers to households with income at or below the 130% of poverty line, while the “higher-income” group with income above the 130% of poverty line. The total number of households included in the dataset for 2007-2008, 2008-2009 and 2009-2010 are 3427, 3487 and 3655, respectively. The average household size did not vary much over the period and across the markets, with an average of 2.53, 2.83 and 2.65 persons for the “higher-income”, “lower-income” and “under-poverty-threshold” groups, respectively.

Results show that the average household expenditure on fish/seafood increased over the period of 2007-2008 to 2009-2010 in all the five markets studied, with the highest increase found in the “lower-income” group (approximately of 20%) followed by the “higher-income” group (9%) and the “poverty-threshold” group (6%). Frozen/chilled finfish has become more popular among the households within the surveyed markets. In particular, the average household expenditure on unbreaded finfish products increased substantially, by 75% in the “poverty-threshold” group, 21% in the “lower-income” group and 22% in the “higher-income” group. Top five unbreaded finfish products based on average household expenditure on fish products are shown in Table 1, along with information on number of purchasing households and average expenditure per household on the listed products. As expected, the average fish/seafood consumption increases consistently with increase in income. Salmon and tilapia have remained the two most popular unbreaded frozen/chilled finfish products among all income groups. Consumption of unbreaded frozen/chilled catfish has increased among all income groups. It was a mixed result for breaded finfish products; the average household expenditure increased by 7% for the “lower-income” group, while it decreased for both the “poverty-threshold” group (by 3%) and the “higher-income” group (by 4%).

**Table 1. Top five preferred unbreaded species in Chicago, Houston, Miami, Memphis, New Orleans-Mobile markets, A. C. Nielsen data, 2007-08 to 2009-10.**

Income group	Rank	Year 2007/2008			Year 2008-2009			Year 2009-2010		
		Species	% of HH consumed the species	Expenditure (\$ per HH per annum	Species	% of HH consumed the species	Expenditure (\$ per HH per annum	Species	% of HH consumed the species	Expenditure (\$ per HH per annum
Higher-income	1	SALMON	12.58	20.58	SALMON	13.51	20.37	SALMON	13.68	20.82
	2	TILAPIA	12.02	16.57	TILAPIA	15.08	18.12	TILAPIA	16.25	16.21
	3	ORANGE ROUGHY	2.83	19.95	CATFISH	3.15	19.57	CATFISH	3.01	20.67
	4	CATFISH	2.48	20.01	COD	2.95	14.82	COD	3.56	13.65
	5	COD	2.80	14.35	ORANGE ROUGHY	2.06	20.67	FLOUNDER	4.40	10.49
Lower-income	1	TILAPIA	3.30	15.47	TILAPIA	3.36	18.46	TILAPIA	4.95	14.36
	2	SALMON	2.63	14.03	SALMON	2.29	16.29	SALMON	3.17	13.53
	3	CATFISH	0.79	20.25	CATFISH	0.86	19.21	CATFISH	0.93	24.51
	4	WHITING	0.44	26.50	ORANGE ROUGHY	0.46	15.65	COD	0.79	15.72
	5	FLOUNDER	0.70	10.97	FLOUNDER	0.75	9.27	FLOUNDER	1.07	10.02
Poverty-threshold	1	TILAPIA	0.67	11.42	TILAPIA	0.83	14.80	SALMON	0.71	24.73
	2	SALMON	0.35	11.92	SALMON	0.40	18.40	TILAPIA	1.01	13.97
	3	WHITING	0.18	15.44	WHITING	0.11	34.23	CATFISH	0.30	13.44
	4	CATFISH	0.06	28.63	FLOUNDER	0.26	7.40	COD	0.16	14.18
	5	BASA/TRA	0.03	54.18	COD	0.09	15.65	MAHI MAHI	0.08	27.43

The average consumption of breaded non-fish products has increased among the “higher-income” (by 10%) and the “lower-income” (by 16%) groups. But the average expenditure per household on all non-fish has decreased over the period by approximately 20% in each of the income groups due to a significant decrease in the consumption of

unbreaded forms of products. Shrimp is the most preferred species – in both non-fish and overall fish/seafood categories – across the markets; however, the expenditure per household on unbreaded shrimp has decreased over the period among the “higher-income” group by approximately 18%.

**Sub-Objective-1.b. (Supplemental).** *Procure store-level scanner data for the most recent 2 years covering 52 U.S. metro markets (exclude Walmart) and an additional most recent 3 years data covering 10 metro markets with Walmart included.*

UAPB procured an additional two years of store-level scanner data from the A. C. Nielsen Company; the dataset is composed of weekly data covering 52 U.S. markets from July 2010 ending on June 12, 2012. Recently, A.C. Nielsen Company has started

to cover Walmart in their database. Hence the UAPB team also procured weekly data covering 10 U.S. markets that includes Walmart and other outlets for the last three years, ending on September 1, 2012.

**Sub-Objective 1.c.** *Analyze trends on sales price (dollar per pound), volumes (pounds) and market shares of major aquaculture products.*

## The University of Arkansas at Pine Bluff

### Overall Trends in Sales

From 2005-06 to 2009-10, frozen seafood sales in supermarkets increased by an average of approximately 6% per year. Frozen finfish accounted for a significant part of total frozen seafood sales during this period with its share increasing from 39% in 2005-06 to 42% in 2009-10. From 2005-06 to 2009-10, frozen finfish sales in supermarkets increased by 35%. This indicates that frozen finfish sales (in value) are increasing faster than total frozen seafood sales. A list of the top ten best-selling frozen fish over the past five years is provided in Table 2. Tilapia has been the top seller in supermarkets since 2006-07. The position of catfish has improved over the years from eighth place in 2005-06 to fourth place from 2008-09 onwards.

Results indicate that catfish and shrimp saw consistent increasing sales over the last five years (2005-06 to

2009-10), with the former registering high increases; clams had decreased sales in comparison to 2005-6, but the reduction in sales was slowing down; and crawfish sales increased last year (2009-10). Unbreaded shrimp is the most dominant product amongst all the finfish and shellfish frozen products.

### Trends in Prices

Breaded catfish products were cheaper than most others, since most of them were sold as breaded nuggets. Shrimp prices remained stable over the years, while there was a marginal increase in breaded clam prices. It is interesting to note that breaded clams and crawfish are priced higher than shrimp.

Catfish, as well as crawfish, clams and tuna, were amongst lower-priced entrée products. Entrée clam prices increased in price over the years and there was decrease in prices of entrée shrimp that could be

**Table 2. Top ten best-selling frozen finfish species from scanner data from 2005-06 to 2009-10.**

Rank	2005-06	2006-07	2007-08	2008-09	2009-10
1	Whiting	Tilapia	Tilapia	Tilapia	Tilapia
2	Tilapia	Whiting	Salmon	Salmon	Salmon
3	Salmon	Salmon	Whiting	Whiting	Whiting
4	Tuna	Tuna	Tuna	Catfish	Catfish
5	Cod	Flounder	Catfish	Flounder	Flounder
6	Flounder	Catfish	Flounder	Cod	Cod
7	Pollock	Cod	Cod	Pollock	Haddock
8	Catfish	Pollock	Pollock	Tuna	Tuna
9	Haddock	Haddock	Haddock	Haddock	Pollock
10	Perch	Orange Roughy	Orange Roughy	Perch	Perch

explained by the introduction of cheaper types of products (like Alfredo) and cheaper package sizes (like 21oz regular entrée). Results also show that average price of entrée crawfish products decreased over the years.

Prices of unbreaded finfish fillets with or without promotion showed that tilapia, basa/tra, pollock, and whiting were less expensive than catfish. Price differences between catfish and unbreaded tilapia fillets became wider after price promotion, but between catfish and basa/tra the differences became narrower after promotion. Crawfish was priced higher than shrimp, while clam prices fell sharply over the last two years (2008-09 and 2009-10).

One key observation from price trends is that the sales performance of the product in a market is positively correlated with the degree of promotional pricing given to that product. This behavior was seen for many products in many markets. Another observation is that there was a tendency to price larger packages at lower unit prices, thereby indicating the presence of “quantity discounts”, for example “economy packs” in the retail sales.

### Trends in Product-Specific Sales

#### *Catfish Products*

Breaded catfish sales rose in general over the last five years (2005-6 to 2009-10). Breaded nuggets constitute about 88 to 94% of breaded catfish sales with the rest shared between fillet and strips. The most popular packaging size of breaded nugget was 80 oz, followed by 32 oz. For fillet and strips, they were 8 oz and 13 oz respectively.

Catfish in entrée form was not able to penetrate the market. There was a decline in sales of about -25% to -35% annually with respect to 2005-06, with a corresponding decline in market share. It also received a much lower degree of promotion. Amongst nine types of entrée catfish available, only Cajun catfish registered consistent increases in year-to-year sales, raising its share amongst catfish entrées to almost 62% in 2009-10 from 4% in 2005-06.

Unbreaded catfish sales showed impressive year-to-year growth. Nuggets and fillet constitute about 99% of unbreaded catfish sold. Nuggets still formed

the largest selling product form, though their share decreased by around 15% over the years. The share of fillet consistently increased during the same period by approximately 14%. Amongst nugget package sizes, 80 oz (5 lb) and 32 oz (2 lb) were the most prevalent, capturing 40 to 48% of unbreaded catfish sales. Amongst fillet packages, there was an increase in share of 40 oz packages, having replaced 64 oz packages as the most popular package size in the last two years (2008-09 to 2009-10).

For breaded catfish products, New Orleans/Mobile is the largest market and accounts for about 11 to 14% of the sales during 2005-06 to 2008-09, while its share increased sharply to 44.6% during 2009-10. San Antonio and Memphis are important markets for breaded catfish strips and fillets respectively. New Orleans/Mobile is the only city where entrée catfish sales are on the rise with Cajun catfish mostly sold there.

Memphis is also the largest market for unbreaded catfish, and there are three Pacific cities, Los Angeles, Sacramento, and San Francisco, in the top-5 markets. Southern markets are still important as demonstrated by the presence of cities like Little Rock, San Antonio, Houston, Dallas and New Orleans/Mobile.

#### *Crawfish Products*

Breaded crawfish is not a major product in terms of sales volume, and its sales mainly take place in the Southern cities like San Antonio, Houston and Dallas. There are about 20 different types of entrée crawfish products of which the top-5 account for about 65 to 86% of all crawfish entrées. The share of entrée crawfish in total entrée seafood market has almost doubled over the last five years.

Amongst unbreaded crawfish products, crawfish tail meat constitutes 76 to 94% of all unbreaded crawfish sales. Sales of crawfish tail meat dropped over the last five years, while whole unbreaded crawfish and crawfish pieces increased during the

last two years. Amongst package sizes, crawfish tail meat packages of 12 oz (decreasing share) and 16 oz (increasing share) account for about 90 to 94% of total unbreaded crawfish sales.

Amongst the major markets for entrée crawfish, New Orleans/Mobile tops the list but its share of total entrée crawfish sales declined considerably over the years from about 40% in 2005-06 to 14% in 2009-10. Sales in Houston, Dallas, Tampa and Washington D.C. increased during the same period. The top-20 cities account for about 90 to 92% of sales. It is interesting to note that entrée crawfish products, though sold mostly in southern U.S. cities, saw a sales growth in non-traditional crawfish markets like New York, Detroit, Philadelphia, New England cities, and cities in Ohio.

New Orleans/Mobile is the most dominant market for unbreaded crawfish with about 70% consumption. Houston has about 14% of the share, followed by the other two Texas cities of Dallas and San Antonio with about 3 to 4% market share each. Memphis has about 1.5 to 2% share. Thus, these five cities together account for about 90 to 95% of all crawfish consumed through retail supermarkets.

#### *Clam Products*

There are five product types that are popular-Crunchy clam, regular clam, Crispy tender, Stuffed and Fried Crunchy. The first two types accounted for about 65%, and the first three types accounted for about 85% of the breaded clam products sales. Smaller package sizes (less than 12 to 14 oz) were observed to be more popular. Amongst 12 types of entrée clam products, “Regular clam entrée” have the largest share of 87 to 90%. Amongst unbreaded clams, whole unbreaded clams account for about 76 to 94% of these products, thus being the most prevalent product form. Whole clams packed in 50 oz packages have become the most important package sizes, with their shares rising to almost 70% of the total unbreaded clams sold.

For breaded clams, New York and Boston are the largest markets, each accounting for about 10 to 15% of sales. Top-5 cities account for about 40 to 45% of sales, including Hartford/New Haven and Boston amongst. New York alone consumes about 30 to 33% of entrée clam products, thus making it largest market for the products. In the last two years, Philadelphia alone consumed about 50% of all the unbreaded clams sold. Sales in Raleigh/Durham and Charlotte have also increased in the last two years.

#### *Shrimp Products*

Whole breaded shrimp were observed to be the most prevalent products amongst breaded shrimp. In terms of sales growth, whole breaded shrimp in 10 oz, 10.5 oz, 14 oz, 20 oz, and 32 oz (2 lb) packages

have sold well. There are more than 600 different types (with respect to dressing style, method of pre-treatments, etc) of unbreaded shrimp products available in the U.S. market. Uncooked types and cooked types have almost equal share.

For breaded shrimp, there are no single markets with a very high share of total sales. The 15 largest markets together have only about 25% of total sales, with the top-2 cities (i.e., Washington, DC and Chicago) consuming about 5% each. New York is the top market for entrée shrimp products, with a share of 7.5 to 8.4% of all entrée shrimp sold. New York and Philadelphia are the largest two markets for unbreaded shrimp products, while Miami, Tampa and Orlando have also registered high growth amongst the top-10 cities.

**Sub-Objective 1.d.** *Prepare fact sheets that summarize price and sales volume data for catfish, crawfish, clams, and shrimp as well as for competing products for distribution to industry stakeholders through extension mechanisms.*

#### **The University of Arkansas at Pine Bluff**

Upon advice of key industry representatives, the decision was made not to form a single industry advisory panel, particularly given the competitive nature of processors who will make best use of the results of this project. Instead, a combination of formal presentations to trade associations and individual meetings and consultations has been held. Formal presentations have been made at the Catfish Farmers of America annual convention and at the annual meeting of the U.S. Trout Farmers Association at the Aquaculture America 2011 meeting. These presentations focused on explaining to industry

members what the data are, what the possibilities for using the data are, and offering to run customized reports for individual companies. The U.S. Trout Farmers Association has also requested a follow-up presentation at their annual fall meeting on September 29, 2011. Individual meetings were held at the request of 6 catfish processing companies to further discuss the data and the type of reports that can be run with the data. Written reports have been sent to 19 catfish processing companies and detailed follow-up reports to 6 catfish processing companies at their request.

**Objective 2.** *Identify factors affecting a) trends in prices and sales volumes and b) consumption of fresh and frozen farm-raised catfish, crawfish, clam, and shrimp products.*

**Sub-Objective 2.a.** *Estimate retail pricing models for various fresh and frozen farm-raised catfish, crawfish, clam, and shrimp products based on store level data.*

### University of Florida

The goal of this sub-objective was to generate information from revealed preference data (i.e., observed market behavior) on individual product attributes for various types of fish and shellfish products sold in the U.S., with an emphasis on information on products sourced from the Southeast and from culture operations.

A retail price response model and sales response model were previously developed for catfish products only. The effects of product characteristics (product type, form, packaging size, brand identity) and product promotion were modeled. To augment this analysis (e.g., for other species or species groups and to consider other product attributes), an approach was utilized that assumes that retail prices paid by consumers reflect the total of the values of individual product attributes. The approach estimates “implicit prices” or market values of the attributes or characteristics that are commonly used to generate market information. These implicit attribute prices identify premiums and discounts associated with specific attributes and can be beneficial to harvesters/growers, processors and retailers as they make production, processing, packaging and pricing decisions.

Implicit retail prices were estimated for frozen unbreaded finfish and shellfish sold in the U.S. Southeast. The “frozen” category included all frozen and chilled seafood available in both prepared and unprepared forms that are usually found in refrigerated and frozen sections of supermarkets. “Seafood” includes 84 freshwater and marine finfish and shellfish species (or species groups). The analysis excludes shrimp, a large volume and relatively generic

product, to better focus on lesser studied species and product attributes.

The product characteristic and form attributes include variables that identify species, flesh color, geographic origin and level of processing. The packaging attributes include package size, whether it is a store branded product (versus name brand), and whether the product was registered in the U.S. The promotional information indicates whether the promotion involved a change in price (e.g., price increase, small price decrease, or large price decrease).

Two hedonic models were initially estimated: one for finfish and one for shellfish (separate models are necessary since some of the attributes are different). To better understand the markets for these products, the data are disaggregated to allow for the inclusion of attributes that are only available for a subset of products. For example, approximately 70% of the observations in the finfish model are fillets and approximately 50% are categorized as “regular.” By examining the data and models for these groups separately, we are able to explain the seafood sales in the market and obtain more robust estimates of implicit prices and promotional strategies.

In addition to the hedonic analyses, the monthly AC Nielson data is available five years (July 2005 through June 2010) with seafood products grouped into 19 product categories. Four of these product categories include clam products: canned, entrees, breaded and unbreaded products. In 2010 (July 2009 through June 2010), these clam products

accounted for over \$59 million in sales at the retail level (approximately 2% of total seafood product sales). By category, canned, entree, breaded and unbreaded clam products sold \$40, \$12, \$4, and \$3 million in 2010, respectively. The number of clam products included in each product category was 137 canned products, 37 unbreaded products, 27 entrée products and 16 breaded products. The unbreaded product category included the most detail, especially with respect to species. As a result, this summary will focus on hard shell, little neck and mahogany clam products, as those types of clam products are of importance to the Florida industry.

Findings revealed expected premiums and discounts for traditional attributes (size, species, and form). In general, large discounts were estimated for low-valued products versus non-price promotions for higher-valued species, with interesting implications for distributors and retail outlets.

#### Frozen Unbreaded Fish and Shellfish (weekly, June 2007 through June 2010)

- In the U.S. finfish dataset, 11 groups of fish species had some products labeled as “wild.” Of those species, 14% of the weekly observations were products with “wild” on the label and the wild label was found to command price premiums of 5.9% to 43.7% depending on the species.
- In the shellfish dataset, nearly one-third of the weekly observations were products labeled as “imported” and the imported label was found to increase price 5.9% in the case of one species (lobster) but decrease price for four other species groups from 11.0% to 34.1%.
- Across both product types (i.e., unbreaded frozen fish and shellfish), promotional activities resulted in products being sold at a discount, from 15.2% to 29.7% when all products were on promotion.
- The retail prices for only two species groups

(scallops and lobster) declined after the Deepwater Horizon oil spill, which occurred in late April 2010, while the prices for 10 others increased, but only marginally (i.e., from 3.1% to 9.6%).

- Products sold under a retailers’ own label (grocery store brand) were found to substantially reduce the price of shellfish products but slightly increase the price of finfish products; only the latter result concurs with previous studies.

#### Hard Shell, Little Neck and Mahogany Clam Products (monthly, June 2005 through June 2010)

- Eight products sold by six different companies are labeled as either “hard shell,” “little neck” or “mahogany.” Only one company, J.P.’s Shellfish Company, sells mahogany clam products. Two companies, Cherrystone Aqua Farms and Ocean Rich, sell little neck clam products. The Cherrystone Aqua Farms clams are labeled as “shell-on.” Three companies, Mar-lees Seafood Inc., Pana Pesca and Sysco sell hard shell clam products.
- Clam products labeled as mahogany or little neck were introduced during 2008. Since then, little neck products have experienced the most growth, selling 5,000 units in 2009, and over 50,000 units in 2010. The majority of this growth is from the sale of a one pound package by Ocean Rich Distributors (not indicated, but believed to be shell-off), with 1,900 units sold in 2009 and 47,000 units sold in 2010. Mahogany clam product sales have grown by 27% during the same time period, which indicates strong market demand for clam products similar to those cultured in Florida.
- Price comparison is difficult due to different package measurements. All comparison must be done at the package level because some products are sold in counts, while others are sold in ounces. For example, Cherrystone

Aqua Farms sells shell-on Little Neck clams in counts of 50. These clam products have declined in price from \$23 per package in 2008, to \$14 per package in 2010. However, at \$14 per package, this product still sells at a price nearly triple that of hard shell and mahogany clam products. When J.P.'s Shellfish Company introduced mahogany clam products in 2008, they sold at an average price of \$8 per package. Since then, this

product has declined in price to around \$5 per package, much better positioned to compete with hard shell clam products with an average package price of \$5. However, all hard shell clam products are sold in a 16 oz package, while mahogany clam products are sold in a 32 oz package. The mahogany products cost approximately half that of hard shell products, when measured in dollars per ounce.

**Sub-Objective 2.b.** *Estimate retail sales response models for various fresh and frozen farm-raised catfish, crawfish, clam, and shrimp products based on store level data.*

### The University of Arkansas at Pine Bluff

A retail price response model and sales response model were developed for catfish products. The effects of product characteristics (product type, form, packaging size, brand identity), consumer characteristics (average family size, racial/ethnic background, household income, food-related expenditures, region), and product promotion were modeled.

As expected, the price of unbreaded catfish had significantly negative effects on sales volume. Effects of prices of unbreaded tilapia, basa/tra, salmon, cod, and pollock on unbreaded catfish volume varied with the region (Table 3). In the South,

Northeast and Mid-West regions, tilapia had positive cross-price elasticities. Thus, in these regions, catfish sales would rise with increases in tilapia prices. This relationship is a characteristic of substitute products. However, in the West, tilapia has negative cross-price elasticities indicating that tilapia is a complement of catfish. These findings imply that frozen tilapia and catfish may be in the same market in these regions. Cross-price elasticity of tilapia in the South region was positive, but was negative in the West, Mid-West, and Northeast regions. This result implies that, in the South region, breaded products of catfish and tilapia are substitutes.

**Table 3. Retail price and retail sales models: cross-price elasticities for unbreaded catfish products in different regions.**

	Northeast	South	West	Mid-West
Tilapia	0.499	0.499	-0.051	0.481
Basa/tra	-0.165	-0.165	0.506	-0.165
Salmon	-0.902	-0.902	-0.902	-0.836
Cod	0.000	0.017	0.000	0.000
Pollock	-0.274	-0.274	-0.550	-0.274

The model indicated catfish retail price and sales vary with region; so, marketing efforts should take this variation into consideration. Substitutability and complementarity for catfish with other finfish products also vary with the region. Retail level non-

price competition strategy should be considered as an effective tool to increase sales in U.S. supermarkets. The model also indicated the positive influence of promotional pricing on sales volume but a negative influence on retail price.

**Sub-Objective 2.c.** *Estimate disaggregated demand functions for various fresh and frozen farm-raised catfish, crawfish, clam, and shrimp products using discrete-choice models based on panel data.*

### The University of Arkansas at Pine Bluff

Factors affecting market demand for these aquaculture products were estimated using an Inverse Almost Ideal Demand System (IAIDS). The IAIDS model was modified to account for the effects of season, deepwater horizon oil spill (DHOS), and lagged consumption. The distribution of tilapia and catfish is not similar across the markets in the U.S. and studying substitutability of tilapia for catfish in markets where catfish is having significant market shares (e.g., South and Midwest region of the U.S.) can produce different results. Catfish, tuna, flounder, cod, clam, scallop and squid show seasonality in their marginal consumption value (price).

Region-wise market interaction between different white fish modeled suggested many marketing strategies for the domestic catfish industry. In the Midwest region, catfish is a quantity-substitute to tilapia, and tilapia is a quantity-complement to catfish. The other white fish products (cod, flounder, Pollock, and whiting) are weaker quantity substitutes for catfish in the Midwest region as compared to catfish. Therefore, offering customers high value at a low price and/or short-term reduction in the product's price in the Midwest region of the U.S. can benefit the catfish industry. In the West region cod and whiting are substitute products for catfish in all seasons. Catfish substitutes for flounder in all seasons, and for cod, tilapia and pollock from November to April. In the Northeast region, catfish demand is own-price inelastic with no stronger substitutes except for tilapia from November to January.

Therefore, the catfish industry can increase its revenue by increasing supply of unbreaded frozen catfish products to the Northeast region. Own- and cross-price flexibility estimates show that in the Southern

## Results at a glance...

- *In monthly retail sales of frozen unbreaded finfish, the top three products were salmon (19%), tilapia (12%), and whiting (10%). By comparison, only 5% were catfish products. Saltwater products accounted for 58% of sales.*
- *The regional analysis revealed that the average price in the Southern region was the highest at \$3.35 per pound indicating higher-valued finfish products are sold in the South. Products sold in the South were also discounted less. However, the price of salmon was \$0.56 per pound less in the Southern region than the price of catfish.*
- *In monthly retail sales of frozen unbreaded shellfish products (excluding shrimp), the top three products were: scallop (30%), squid (25%), and lobster (17%). By comparison, only 7% were clam products.*
- *Shellfish products in tail form received a retail premium of \$4.01 per pound.*

region, price strategies would not help the U.S. catfish industry. However, non-price marketing strategies like non-price promotion (e.g. product placement in the stores), country-of-origin labeling, and generic advertisement would benefit the catfish industry.

Catfish demand is the highest in the Southern region followed by the Midwest, and is the lowest in the Northeast region of the U.S. Catfish demand is the highest during the months of November to January. Tilapia demand is the highest in the West region, while demand for pollock is the lowest in the

Midwest region. Demand for frozen whiting is the highest in the Northeast and is the lowest in the South region.

Amongst unbreaded products shrimp and crab, and crab and crawfish were quantity substitutes. Seafood consumers who consumed a product in the previous week would prefer to consume its substitute product (for example crawfish and crab, squid and perch, lobster and squid, and scallop and clam) in the current week. Clam products exhibit seasonality in sales.

**Objective 3.** *Measure competitive position and substitutability of frozen farm-raised catfish, crawfish, clam, and shrimp products with other seafood product with an emphasis on imported products.*

**Sub-Objective 3.a.** *Estimate cross price elasticities of various seafood products consumed in the U.S.*

### **Texas Tech University and Auburn University**

One of the biggest challenges facing American aquaculture industries remains the high levels of fish and shellfish imports. According to NOAA, the U.S. imports approximately 84% of its seafood needs, up from 63% ten years ago. Most consumers want processed fish/shellfish products and not live product, putting domestically produced and processed products in direct competition with less expensive imported aquaculture and wild-caught fish products. Therefore, accurate knowledge of demand and substitution among domestic and imported aquaculture products is critical to the U.S. aquaculture industry as well as to public policy evaluation. At the production, processing and marketing levels, understanding consumer demand is a helpful decision-making tool in determining investment and production capacity planning, production allocation, sales, and advertising and promotional activities.

An Almost Ideal Demand System (AIDS) approach

was used to estimate the substitutions between fish and shellfish products and assess the impact of promotional activities on sales of fish and shellfish products in the U.S. The objective was to shed light on the substitutability between specific aquatic products produced in the U.S., namely catfish, crawfish, and clams with other potentially competing seafood products, such as imported shrimp, tilapia, and salmon. Additionally, another objective was to provide promotional elasticities that can be used to assess the impact of the promotional activities and developing future marketing strategies.

The estimation of the AIDS model was used to derive demand price elasticities, which are the responsiveness of retail buyer's change in quantity demanded to changes in product price, for aquaculture products (Table 4). For the price elasticities, all the own-price elasticities are negative, as expected, and statistically significant, except for the case of crawfish. In addition, the demands for the

considered fish/seafood categories are inelastic. Price inelasticity means total revenue would increase when increasing the price of the product. The results indicate the demand for salmon is the more elastic of the inelastic species, followed by shrimp, clams, tilapia, and catfish. A 10% increase in salmon price would decrease its sales by 9.8%; while for catfish, the same price increase would decrease the sales by only 4.8%. This indicates that American consumers show some “loyalty” towards domestically produced catfish compared to the imported categories, such as shrimp, tilapia, and salmon.

In terms of substitution, it is worth noting that shrimp has all other products as its complement, though they are weak complements. In contrast, shrimp is a strong complement for crawfish (-0.89),

tilapia (-0.65), and catfish (-0.59). In fact, a 10% increase in the price of shrimp will reduce the sales of crawfish, tilapia, and catfish by 8.9%, 6.5%, and 6.0%, respectively. Results show that American consumers consider catfish as a strong substitute for tilapia, probably because both have white flesh coloration and similar texture; while catfish and salmon are complements as salmon flesh being pink/red colored is seen in a different light from white colored catfish flesh. Hence, an increase of 10% in the price of tilapia will increase catfish sales by 7.2%; while an increase of 10% in the price of salmon will reduce catfish sales by 3.8%. In contrast, a 10% increase in the catfish price will increase tilapia sales by only 1.5%, suggesting tilapia is not a strong substitute for catfish.

**Table 4. Marshallian own-price, promotion and expenditure elasticities\*.**

	Catfish	Crawfish	Clams	Shrimps	Tilapia	Salmon
Catfish	-0.4804 (-3.26)	-0.0590 (-0.79)	-0.3283 (-4.27)	-0.5985 (-5.55)	0.7229 (7.29)	-0.3784 (-2.51)
Crawfish	-0.1788 (-0.77)	-0.1443 (-0.60)	0.5081 (3.03)	-0.8884 (-5.65)	0.3898 (2.27)	-0.6939 (-2.45)
Clams	-0.2900 (-4.20)	0.1489 (3.04)	-0.7901 (-5.16)	0.0803 (0.58)	-0.6601 (-4.91)	0.6029 (3.89)
Shrimps	-0.0217 (-5.15)	-0.0121 (-5.80)	-0.0031 (-0.51)	-0.8728 (-28.16)	-0.1324 (-6.68)	-0.0629 (-5.83)
Tilapia	0.1463 (7.36)	0.0249 (2.23)	-0.1498 (-4.97)	-0.6472 (-6.22)	-0.6905 (-8.79)	0.2817 (5.28)
Salmon	-0.0501 (-2.13)	-0.0313 (-2.17)	0.1198 (4.45)	0.1242 (3.13)	0.2704 (6.72)	-0.9797 (-16.85)
Expenditure	1.1216 (15.40)	1.0076 (10.47)	0.9079 (9.90)	1.1050 (14.61)	1.0345 (13.83)	0.5466 (19.90)
Promotion	0.0007 (0.50)	0.0117 (4.04)	0.0082 (12.55)	0.0014 (14.61)	-0.0029 (-6.77)	-0.0051 (-12.66)

\*Figures in parenthesis are t-test values.

The results for the expenditure elasticities show that, on average, American consumers consider catfish, crawfish, shrimp and tilapia as luxury goods (elasticity > 1); while they consider clams and salmon as necessity goods (elasticity < 1). For instance, an increase of 10% in the consumer's income would increase consumption of catfish, crawfish, shrimp, and tilapia by 11.2%, 10.1%, 11.1%, and 10.4%, respectively. The same income increase would induce a 9.08% increase in the consumption of clams and only a 5.47% increase in the consumption of salmon.

On average, shrimp are the most promoted product by retailers, followed closely by tilapia. In fact, more than 58% of the sales of shrimp are realized using some sorts of promotion efforts such as price reduction, feature, and display. The other imported fish category that is heavily promoted is tilapia, with more than 57% of its sales having been realized in association with promotional activities. For salmon, there were more than 41% of sales occurring with some type of promotion. Catfish promotion level was similar to salmon, at more than 38% of sales having promotions. Promotion elasticities are positive and statistically significant for crawfish, clam, and shrimp. For instance, a 10% increase in the volume sold under any type of promotion would increase budget share by 0.12%, 0.08%, and 0.01% for crawfish, clam, and shrimp, respectively. For tilapia and salmon, which are primarily imported products, promotional activities have a negative and statistically significant impact on the budget share. For catfish, promotional activities have a positive but not statistically significant impact on budget share.

Overall, results indicate that the demand for all categories considered are inelastic, suggesting that consumers are less responsive to price changes than previously thought. Interestingly, the consumer price responsiveness for catfish is lower compared to the imported categories of shrimp, tilapia, and salmon. This implies that American consumers can "tolerate" an increase in the catfish price. In addition, American

consumers consider catfish as a strong substitute for tilapia; while tilapia, though a substitute for catfish is not a strong one. One strategic implication for catfish producers/processors is to survey tilapia prices and react appropriately. For instance, if tilapia prices increase by 10%, all else held constant, catfish producers/processors could keep the prices unchanged and "enjoy" more than 7% increase in the sale of catfish. In contrast, if they match the price increase, the catfish sales will drop due the own-price effect (4.80% in this case) and loss of sales to tilapia (about 1.50%). However, any price decrease

## Results at a glance...

- *A 10% increase in the price of catfish, will reduce the sales of catfish by 4.8%; while a 10% increase in the price of family size breaded catfish nugget will decrease the sales by 44.2%.*
- *We cannot say that the demand for catfish is more price sensitive than demand for shrimp or vice versa.*
- *Demand for entrée products is more price sensitive than the other forms, regardless of the category of fish or seafood.*
- *U.S. consumers are more price sensitive for catfish fillets than for catfish nuggets.*
- *Consumers do not yet consider value-added products to be strong substitutes for the less-prepared unbreaded products, whereas the reverse is not true. In other words, unbreaded products are stronger substitutes for value-added products than the reverse.*

in tilapia should be matched by catfish producers/processors. In fact, if tilapia prices decrease by 10% and catfish producers/processors do not react, catfish sales will be reduced by more than 7.23%. If they match the price decrease (10%), their sales will increase by more than 4.80% due to own-price responsiveness plus an additional 1.50% due to substitution from tilapia. This would bring the

### University of Arkansas at Pine Bluff

Frozen and chilled seafood marketing in grocery stores has undergone substantial transformation as a result of the introduction of value-added and convenient products into the category. However, it is not yet clear whether consumers perceive these value-added seafood products to be substitutes for the traditional unbreaded products. We have modeled demand for frozen seafood in the U.S. within the linear approximate almost ideal demand system (LA-AIDS) framework using market-level monthly retail scanner panel data. We have estimated the own price elasticity (percentage change in demand for a product due to one per cent change in price of the same product), cross-price elasticity (percentage change in demand for a product due to one percent change in price of another product) and expenditure elasticities (percentage change in demand due to one percent change in expenditure) of demand for various fish/seafood categories. Our emphasis has been on the demand for the three aggregate frozen seafood categories, namely breaded products, seafood entrées and unbreaded products, and also on the demand for these categories when disaggregated as finfish and shellfish.

Using market-level retail scanner data we find that the unbreaded products, which are less value-added products, have a dominant share of about 65% of total frozen seafood. Unbreaded shellfish alone account for about 47% of all frozen/chilled seafood. Table 5 presents responsiveness of demand for seafood products due to a) changes in expenditure

decrease in sales to only 0.93%. In addition, this study shows that even though shrimp and salmon have the largest market values in the U.S. seafood market, tilapia is the species that has the most negative effect on domestic aquaculture products, e.g. the US farm-raised catfish industry. Data show that imported products are heavily promoted by retailers; while domestic products lag behind.

(expenditure elasticities), and b) changes in price (price elasticities) with and without taking into account the income effect of price change. Uncompensated price elasticity covers both the income and substitution effects of price change, while the compensated elasticity only captures the substitution effect of price change. The responsiveness to changes in own-price of unbreaded shellfish decreased in absolute value due to the removal of the income effect; this result indicates a substantial expenditure/income effect of the price changes of these products. Overall, although the degree of substitution decreased between most products after income compensation, but still indicate net substitutability. Considering the dominant share of unbreaded products in the frozen seafood section, their higher responsive to expenditure, and the prediction of increased seafood consumption, these products have strong potential for further market dominance.

The two value-added product categories (breaded and entrée) were mutual substitutes. Results imply that consumers do not yet consider value-added products to be strong substitutes for the less-prepared unbreaded products, whereas not vice versa. Therefore, processors and retailers of value-added products may attempt to differentiate their products with respect to unbreaded products to reduce competitive effects.

**Table 5. Demand elasticities for disaggregated finfish and shellfish products**

Price of *	Expenditure elasticity	Uncompensated price elasticity						Compensated price elasticity (without income effect)					
		Dependent variables*						Dependent variables*					
		BFF	BSF	EFF	ESF	UFF	USF	BFF	BSF	EFF	ESF	UFF	USF
BFF	0.634	-1.216	0.629	0.327	0.313	0.018	-0.082	-1.257	0.621	0.307	0.289	0.008	0.022
BSF	0.766	0.199	-1.882	0.123	0.114	0.010	-0.009	0.150	-1.891	0.098	0.114	-0.002	0.117
EFF	0.461	0.100	0.118	-1.002	-0.018	0.008	-0.051	0.070	0.112	-1.017	-0.017	0.001	0.024
ESF	0.413	0.101	0.115	-0.023	-0.898	-0.074	-0.035	0.074	0.110	-0.036	-0.898	-0.081	0.033
UFF	0.904	0.064	0.057	0.100	-0.111	-1.030	-0.017	0.005	0.045	0.071	-0.109	-1.045	0.132
USF	1.347	0.118	0.197	0.014	0.187	0.164	-1.153	0.031	0.180	-0.030	0.186	0.141	-0.932

\* BFF- Breaded finfish, BSF- Breaded shellfish, EFF- Finfish entrée, ESF- Shellfish entrée, UFF- Unbreaded finfish, USF- Unbreaded shellfish

**Sub-Objective 3.b.** *Modeling consumer demand for type, form, and package size*

**Texas Tech University**

Manufacturers/processors often offer food products in more than one package size, allowing consumers more choices on a given shopping experience. This has implied an expansion of the number of fish and seafood products in the last two decades. For example, in the shrimp category, there are more than 400 unbreaded shrimp brands sold under different package size. In addition, considerations such as health issues, family size, and the number of children in the household have triggered more product differentiation. In the case of fish and seafood products, this differentiation operates in three dimensions:

1. Type: Fish and seafood products come under different types: entrée products, breaded fish and seafood, unbreaded fish and seafood, and canned products;
2. Form: Fish and seafood come under different forms: regular, fillets, nuggets, pop-corn, ...;
3. Size: Fish and seafood products are sold in different packaging sizes from few ounces to 5 lb or more.

This product differentiation is always accompanied with difference in pricing (Table 6). According to a previous study, between 16 and 34% of products available in two or more package found in retail grocery outlets exhibit a quantity surcharge. The hypothesis here is that consumers do not expect the price for various types, forms, and package sizes of fish and seafood products to be the same. In other words, consumers view these products as imperfect substitutes, that is, shrimp packaged in a 32 oz package may not cost the double of a 16 oz package.

However, when many products are involved, the number of parameters to be estimated in order to analyze demand increases. Traditional demand estimation, such as the linear expenditure system (LES), the almost ideal demand system (AIDS), and the Rotterdam model are inappropriate to cope with the dimensionality problem. Researchers in applied demand analysis have devised techniques to overcome the dimensionality problem. One of these techniques relies on the assumption of weak separability and multi-stage budgeting, allowing the researchers to concentrate on a single group, and sidestepping the effect of important factors such as the type, the form, and the size.

The development of discrete choice methods offers a very practical alternative to solve the dimensionality problem and estimate demand parameters at more disaggregate levels. In our context, the use of discrete

**Table 6. Average Prices for Catfish Products.**

Product	Average Price (\$/lb)
Catfish (entire category)	3.0890
Catfish Entrée Breaded	4.4209
Catfish Unbreaded	2.4975
Catfish Unbreaded	3.1578
Catfish fillet medium size (between 1 and 3 lb)	4.6570

choice approach allows estimating taste parameters for product characteristics such as the form, the type, and the package size of the product. For example, unbreaded tilapia fillet, sold in 48 oz pack is treated as a different product than unbreaded tilapia, fillet, sold in 16 oz pack. In doing so, we are able to identify consumers' preferences for the product (tilapia vs. catfish, for example), the coating (breaded vs. unbreaded), the form (fillet vs. nugget), and the package size.

In this study, the weekly purchase of fish and seafood products from June 2008 to June 2010 in United States was studied. Six categories of frozen fish and seafood products are considered: catfish, clams, shrimp, salmon, tilapia, and tuna. Results from this study are:

- Overall, the more the product is narrowly defined, the bigger is the magnitude of the own-price elasticity. For instance, a 10% increase in the price of catfish, will reduce the

sales of catfish by 4.8%; while a 10% increase in the price of family size breaded catfish nugget will decrease the sales by 44.2%.

- At the type level (catfish, shrimp,...), the results show no pattern. We cannot say, for instance, that the demand for catfish is more price sensitive than demand for shrimp or vice versa.
- In regard to the form, demand for entrée products is more price sensitive than the other forms, regardless of the category of fish or seafood.
- U.S. consumers are more price sensitive for catfish fillet than for catfish nuggets.
- For the package size, out of 33 products analyzed, 31 have their price sensitivity decreasing as the package size increases, implying quantity discount.
- For the cross-price elasticities, they are all positive and of small magnitude, which is consistent with highly differentiated products theory.

**Sub-Objective 3.c.** *Construct policy analysis matrices (PAM) and estimate domestic resource costs (DRC) for various aquaculture products of the southern region of the US.*

### **Auburn University**

The U.S. farm-raised catfish industry has contributed significantly to the economic development of rural economies in the southern U.S. The industry enjoyed a long period of continuous growth for several decades until the early 2000s. Since 2003 the industry has been experiencing reductions in production acres, output, and sales volume and values. The declines are attributed primarily to the competition with substitute catfish-like imports and increases in production input costs, especially for feed and fuel. Knowledge of the comparative advantage and disadvantages of the U.S. catfish industry will help improve policy interventions and provide catfish producers with science-based decisions on ways to improve their profitability.

This research objective constructed an industry budget using both market and shadow prices. The resulting industry budgets are employed to build the policy analysis matrix (PAM), and derive domestic resource cost (DRC) and other economic indicators, such as nominal protection coefficients of inputs and outputs, to evaluate the comparativeness, and efficiencies of resources used in the U.S. farm-raised catfish industry. Data used in this analysis include costs and revenues of catfish farm production in four regions, a black belt soil region in west Alabama and one in east Mississippi, and in the lower and upper delta regions of western Mississippi. Data covered a 5-year production span from 2005 to 2009.

Results presented in Table 7 show that catfish producers received market prices that were close to its social/shadow prices. Over the study period, U.S. catfish producers faced an implicit tax on their output sale in 2005 as shown by the nominal protection coefficient of output being less than one. However, the situation improved in the following years. In terms of input use, U.S. catfish producers received small indirect supports from U.S. farm policy through subsidies provided to certain crops such as corn, soybean, and wheat that are ingredients in catfish feed. The industry used to have a comparative advantage in 2005 and 2006 when the domestic resource cost (DRC) ratio was less than one, but due to increases in feed price in later years, U.S. catfish production has demonstrated a comparative disadvantage to foreign competitors.

The U.S. farm-raised catfish industry has become comparatively disadvantaged recently due to low prices paid to producers, both in terms of private and social prices, for the years 2005 through 2009 (in 2011 pond bank prices rose by 66%). The private price paid to catfish producers were determined by interactions in the domestic market and little public price intervention. However, the social price paid to producers could be improved by reducing competition with inexpensive imported aquaculture products through import tariffs. Simulations of different tariff levels on catfish-like imported products were conducted and suggested that a 25% increase in tariff level would have helped the U.S. farm-raised catfish industry in its worst year, 2008, have a comparative advantage.

**Table 7: Domestic resource cost (DRC) and protection coefficients of the US catfish.**

Ratios	2005	2006	2007	2008	2009
Domestic resource cost (DRC)	0.84	0.71	1.31	14.57	1.76
Nominal protection coefficient on tradable output (NPCO)	0.96	1.00	1.05	1.01	1.02
Nominal protection coefficient on tradable inputs (NPCI)	0.95	0.91	0.92	0.93	0.93
Effective protection coefficient (EPC)	0.96	1.17	1.56	6.42	1.62

**Sub-Objective 3.d.** *Preparing fact sheets based on previous results and provide information on how to improve competitive position of aquaculture producers and processors in the southern region of the U.S.*

### **Auburn University**

This is being done now based upon results presented in presentations to the Aquaculture America 2013 and Food Distribution Research Society meetings.

## IMPACTS

- The project has acquired store level scanner data for 12,898 seafood products. The data includes information on 84 seafood species (or species groups) of unbreaded frozen products, 30 species of breaded frozen products, 40 species of entrées, and 5 species of canned products. The data covers 209 marketing chains over 52 U.S. cities and all U.S. Census divisions. The data period is from four weeks (cumulative) ending on 07/16/2005 to four weeks (cumulative) ending on 06/16/2007 and from week ending on 06/23/2007 to week ending on 06/12/2010 (total 156 weeks).
- The cost of an individual report developed from scanner data begins at \$200,000. Summaries of market trends in 52 cities across the U.S. for the past 5 years have been sent to 19 catfish processing companies, and detailed customized reports have been sent to 6 catfish processing companies at their request. Given the 25 reports that have been prepared for individual companies, the total value of these reports provided to industry (at no cost to them) over the past year is \$5 million.
- Supermarket and household level scanner data have potential to provide guidance to seafood marketers on trends of specific products, product forms, and product substitutes in specific markets.
- The results have been communicated among various farmer and processor groups throughout the country. Several aquaculture farmers/processors have used the project results in designing or redesigning their marketing plans.

## PUBLICATION, RESEARCH PAPERS SUBMITTED, AND PRESENTATIONS

### Publications in Print

- Chidmi, B., T. Hanson, and G. Nguyen. 2012. Substitutions between U.S. and imported fish and seafood products at the national retail level. *Marine Resource Economics* 27(4):359-370.
- Gold, G. C. 2012. The implicit prices of finfish and shellfish attributes and retail price promotion strategies: hedonic analysis of weekly scanner data in the U.S. M.S. Thesis, Food and Resource Economics Department, University of Florida, Gainesville, FL.

### Manuscripts in Preparation

- Chidmi, B., T. Hanson, and G. Nguyen. Effect of promotional activities on substitution pattern and market share for aquaculture products. *Journal of the Food Distribution Research Society* (in review).
- Chidmi, B., T. Hanson, and G. Nguyen. Substitutions between U.S. and imported fish and seafood products at the national retail level. *Marine Resource Economics* (in review).
- Hanson, T.R., G. Nguyen, and C. Jolly. 2012. Comparative advantage of the U.S. farm-raised catfish industry: a cross-regional domestic resource cost analysis. *Journal of Aquacultural Economics and Management* (in press).

Singh, K. and M. M. Dey. Retail level demand for canned seafood in the U.S: estimates from almost ideal demand system using scanner data. *Journal of Agricultural and Applied Economics* (in review).

Singh, K.; M. M. Dey, and P. Surathkal. Analysis of demand system for unbreaded frozen seafood in the U.S. using store-level scanner data. *Marine Resource Economics*, 27(4) (in press).

Singh, K.; M. M. Dey, and P. Surathkal. Price and scale flexibilities of white fish products in the United States: a seasonal and regional analysis based on store-level scanner data. *Canadian Journal of Agricultural Economics* (in review).

Surathkal, P. and M. M. Dey . Consumer demand for frozen and chilled seafood products in the United States: an analysis using market-level retail scanner panel data (in review).

### Abstracts

Dey, M.; K. Singh, C. R. Engle, A. Rabbani, and P. Surathkal. 2011. Trends in seafood markets in the United States: reflections from store level scanner data. p. 58 in NAAFE Forum 2011 Program, University of Hawaii at Manoa and NOAA Fisheries, Silver Spring, MD.

Hanson, T.R. and G. V. Nguyen. 2012. Crawfish market overview for the southeastern U.S. Book of Abstracts of Aquaculture America 2012 meeting, Las Vegas, NV.

Larkin, S., M. Dey, C. Roheim, B. Chidmi, P. Surathkal, and F. Asche. 2011. Hedonic analysis of seafood scanner data: an application to southern U.S. Markets. p. 63 in NAAFE Forum 2011 Program, University of Hawaii at Manoa and NOAA Fisheries, Silver Spring, MD.

Nguyen, G.V. and T.R. Hanson. 2012. Comparative advantage of the Alabama farm-raised catfish industry. Book of Abstracts of Aquaculture America 2012 meeting, Las Vegas, NV.

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Singh, K. and M. Dey. 2011. Demand for seafood in the United States: econometric analysis for major seafood categories and seafood canned products. p. 208 in 16<sup>th</sup> Biennial Research Symposium 2011, Association of Research Directors, Atlanta, GA.

Singh, K., M. M. Dey, and P. Surathkal. 2011. Inverse almost ideal demand system for unbreaded frozen seafood in the United States: estimation of demand flexibilities using full system of equations with theoretical Restrictions. p. 57 in NAAFE Forum 2011 Program, University of Hawaii at Manoa and NOAA Fisheries, Silver Spring, MD.

Surathkal, P., M. Dey, C. Engle, and K. Singh. 2011. Demand for catfish, crawfish, clam, and shrimp in the United States: effects of demographic and product characteristics. p. 59 in NAAFE Forum 2011 Program, University of Hawaii at Manoa and NOAA Fisheries, Silver Spring, MD.

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## Presentations

- Adams, C. January 2011. Florida Clam Industry Workshop, Cedar Key, FL.
- Chidmi, B. 2011. Modeling consumer demand for type, form, and package size in the seafood and fish industry. INFORMS Marketing Science Conference, Houston, TX.
- Chidmi, B., T. Hanson, and G. Nguyen. 2011. Effect of promotional activities on substitution pattern and market share for aquaculture products. 52<sup>nd</sup> Annual Conference of the Food Distribution Research Society-FDRS, Portland, OR.
- Chidmi, B., T. Hanson, and G. Nguyen. 2011. Substitutions between U.S. and imported aquaculture products at retail market level. NAAFE Forum 2011, Honolulu, HI.
- Dey, M. M. 2012. Retail markets for trout in the USA: an analysis of store level scanner data. Presented at the U.S. Trout Farmers Association 2012 Fall Conference in Denver, CO.
- Dey, M. M. 2012. Supermarkets sales trends for aquaculture products. Aquaculture Business Management and Marketing Workshop organized by the National Aquaculture Association at the University of South Florida, St Petersburg, FL.
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- Dey, M., K. Singh, C.R. Engle, A. Rabbani, and P. Surathkal. 2011. Trends in seafood markets in the United States: reflections from store level scanner data. NAAFE Forum 2011, Honolulu, HI.
- Dey, M.M. and K. Singh. 2012. Regional and seasonal variation for catfish and its substitutes in the United States: implications for developing marketing strategies. 2012 Catfish Research Symposium, Catfish Farmers of American Annual Convention, Savannah, GA.
- Engle, C.R. 2011. National supply and demand for trout, supermarket sales data, and economic tools for survival. U.S. Trout Farmers Annual Convention, Twin Falls, ID.
- Engle, C.R. 2011. Supermarket sales of catfish and competing products: what does recent scanner data tell us? Annual Convention of the Catfish Farmers of America, Mobile, AL.
- Engle, C.R. and M. Dey. 2011. Supply models for trout: industry wide effects of changing costs, prices and international exchange rates. Aquaculture America 2011, New Orleans, LA.
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- Gold, G., S. Larkin, and C. Adams. 2012. The value of a word: estimating the implicit prices of popular finfish and shellfish labeling practices in the U.S. International Institute of Fisheries Economics and Trade Biennial Conference, Tanzania, Africa.

- Hanson, T. and G. Nguyen. 2012. Supermarket sales of crawfish and competing crustacean products. 53<sup>rd</sup> Annual Conference of the Food Distribution Research Society-FDRS, San Juan, PR.
- Hanson, T. and G. Nguyen. 2011. Comparative advantage of the U.S. catfish aquaculture sector. NAAFE Forum 2011, Honolulu, HI.
- Hanson, T.R. and G. V. Nguyen. 2012. Crawfish market overview for the Southeastern US. Aquaculture America 2012 meeting, Las Vegas, NV.
- Larkin, S., M. M. Dey, C. Roheim, B. Chidmi, P. Surathkal, and F. Asche. 2011. Hedonic analysis of seafood scanner data: an application to southern U.S. markets. NAAFE Forum 2011, Honolulu, HI.
- Larkin, S.L. and C.M. Adams. 2012. Using retail-level scanner data for a revealed preference analysis fish and seafood products sold in the U.S. W2004 (Marketing, Trade, and Management of Aquaculture and Fishery Resources) 2012 Workshop, Cedar Key, FL.
- Nguyen, G.V. and T.R. Hanson. 2012. Comparative advantage of the Alabama farm-raised catfish industry. Aquaculture America 2012 meeting, Las Vegas, NV.
- Rabbani, A.G., M. Dey, K. Singh, and P. Surathkal. 2011. Market trends of frozen catfish products at the supermarkets of the USA: an application of national scanner data. NAAFE Forum 2011, Honolulu, HI.
- Singh, K. and M.M. Dey. 2011. Demand for seafood in the United States: econometric analysis for major seafood categories and seafood canned products. 16<sup>th</sup> Biennial Research Symposium 2011, Association of Research Directors, Atlanta, GA.
- Singh, K., M. M. Dey, and P. Surathkal. 2011. Inverse almost ideal demand system for unbreaded frozen seafood in the United States: estimation of demand flexibilities using full system of equations with theoretical restrictions. NAAFE Forum 2011, Honolulu, HI.
- Surathkal, P., A. Rabbani, M. Dey, and K. Singh. 2011. Recent trends in marketing of aquaculture products in the United States: results from store-level scanner data. 16<sup>th</sup> Biennial Research Symposium 2011, Association of Research Directors, Atlanta, GA.
- Surathkal, P., M. Dey, C. Engle, and K. Singh. 2011. Demand for catfish, crawfish, clam, and shrimp in the United States: effects of demographic and product characteristics. NAAFE Forum 2011, Honolulu, HI.



## EVALUATION OF IMPACTS OF POTENTIAL “CAP AND TRADE” CARBON EMISSION POLICIES ON CATFISH, BAITFISH, AND CRAWFISH FARMING

### Reporting Period

January 1, 2011 – August 31, 2012

<b>Funding Level</b>	Year 1 .....	\$59,952
	Year 2 .....	\$60,000
	Total.....	\$119,952

<b>Participants</b>	Auburn University .....	Claude Boyd, C. Wesley Wood
	Louisiana State University .....	Ray McClain, Robert Romaine
	University of Arkansas at Pine Bluff .....	Carole Engle, Nathan Stone, Madan Dey

### PROJECT OBJECTIVES

1. Estimate net carbon balance for channel catfish, bait minnow, and crawfish ponds and propose science-based management practices that may increase net carbon capture by ponds.
2. Estimate the economic effects on U.S. catfish, bait minnow, and crawfish farms of alternative policy options under consideration to reduce carbon emissions, including cap-and-trade programs and carbon taxes.
3. Disseminate results for Objectives 1 and 2 through a general fact sheet that explains the impact of the carbon emissions issue on southern aquaculture, and specific fact sheets for the three species. A special session at a major, national, aquaculture meeting will also be organized.

### ANTICIPATED BENEFITS

The study will estimate the carbon emissions balance at the farm level for catfish, baitfish, and crawfish farming in the southern United States. This information will allow an assessment of the possible effects of potential “cap and trade” carbon emissions policies on these three types of aquaculture. It will be possible to ascertain if production ponds can be

operated as carbon sinks to develop carbon credits or if farms will be sources of carbon emissions. In addition to allowing predications about effects of possible, future, carbon emissions rules, the findings will allow an estimation of the farm-level carbon footprint of the three types of aquaculture.

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

**Objective 1.** *Estimate net carbon balance for channel catfish, bait minnow, and crawfish ponds and propose science-based management practices that may increase net carbon capture by ponds.*

### Auburn University

This component of the project is being conducted to determine the amounts of carbon emissions resulting from farm-level activities, and to evaluate the quantities of carbon sequestered in the sediment of ponds on catfish, bait minnow, and crayfish farms. The results will show the carbon emissions balance for the three types of aquaculture. In addition, production methodology will be evaluated and practices for increasing net carbon capture in ponds will be suggested.

Carbon dioxide emission balance on farms will be determined as the difference between annual carbon dioxide sequestration rates in pond sediments and amounts of annual carbon dioxide emissions from aquaculture production operations. Direct carbon emissions from fuels (including electricity) will be estimated for pond construction (amortized over expected service life of ponds) and annual management operations. This assessment will be for farm carbon emissions only – carbon emissions for hatchery operation and fingerling delivery, feed manufacturing and delivery, and processing and product delivery will not be included. Data on fuel use for channel catfish production at the farm level are available from several energy use studies done by various investigators. Enterprise budgets also are available for both golden shiner production and crawfish farming that contain estimates of fuel use for pond construction and farm operations. University of Arkansas at Pine Bluff (UAPB) and Louisiana State University (LSU) teams will provide any additional information and advice needed by the Auburn University (AU) team for estimating amounts of carbon emissions. Researchers at AU obtained core samples from 233 aquaculture ponds

### Results at a glance...

- *Researchers at Auburn University have shown that channel catfish aquaculture at the farm level is essentially neutral with respect to carbon dioxide emissions because considerable carbon dioxide is sequestered in pond sediment.*

in nine counties. Samples were subjected to physical and chemical analyses; the resulting database includes channel catfish ponds in Alabama and Mississippi and bait minnow ponds in Arkansas. Sediment accumulation rates and organic carbon concentrations also are available from previous research by Mississippi State University (MSU) in 45 channel catfish ponds in Mississippi. Data referred to above will be used to assess carbon sequestration rate in channel catfish and bait minnow ponds. LSU investigators are presently collecting bottom soil samples from crawfish ponds that will be sent to us for estimation of carbon sequestration rate.

Average carbon sequestration rates in catfish and bait minnow ponds were 517 g CO<sub>2</sub>/m<sup>2</sup>/yr and 209 g CO<sub>2</sub>/m<sup>2</sup>/yr, respectively. The farm-level carbon dioxide emissions were estimated for channel catfish (Table 1). Each kilogram of fish produced resulted in 0.9405 kg CO<sub>2</sub>. The largest contributor to carbon emissions at the farm-level (70.9%) was use of electric paddlewheel aerators. Average channel catfish production in 2010 was 5,553 kg/ha. Thus, carbon emissions were equivalent to about 5,223 kg

CO<sub>2</sub>/ha or 522 g CO<sub>2</sub>/m<sup>2</sup>. Thus, at the farm level, catfish ponds sequester roughly as much carbon dioxide as is emitted by production activities. In other words, farms are essentially carbon dioxide emissions neutral.

Soil samples from crawfish farms in Louisiana were provided by LSU researchers. These samples were analyzed for organic carbon concentrations. The averages provided in Table 2 reveal that soil carbon concentrations did not differ between crawfish ponds and agricultural fields.

Data collected on sediment chemical and physical characteristics in bait minnow ponds in Arkansas as

a component of an earlier study were used to estimate the annual carbon sequestration rate in bait minnow ponds (n = 9). The average rate of carbon sequestration was 57 g/m<sup>2</sup>/yr in the upper 20-cm layer – 570 kg/ha/yr.

The effort to assess the carbon dioxide emissions at the farm level for bait minnows and crawfish has nearly been completed.

**Table 1. Farm-level carbon dioxide emissions for channel catfish production.**

Activity	CO <sub>2</sub> emissions (kg CO <sub>2</sub> /kg fish)
Pond construction	0.0361
Electric paddlewheel aerator operation	0.6668
Tractor use – emergency aeration, feeding, mowing	0.1502
Light truck and boat use	0.0651
Harvesting	0.0223
Total	0.9405

**Table 2. Amounts of carbon in upper 0-20 cm soil layer in agricultural fields and crawfish ponds.**

System	Total C (kg/ha)
Continuous crawfish cropping	
Crawfish ponds (n = 8)	39,555 a
Agricultural fields (n = 4)	43,007 a
Rotational crawfish cropping	
Crawfish ponds (n = 5)	30,660 a
Agricultural fields (n = 5)	29,216 a

## Louisiana State University

The AU investigators met with their counterparts at LSU and explained the sampling requirements for the sediment samples from crawfish ponds. Eight single-crop permanent crawfish ponds in three Louisiana parishes and seven rice field-crawfish rotational production systems in four parishes were sampled for carbon analysis. Bottom soil core samples were collected from crawfish ponds and from nearby agriculture fields in each location. Samples were collected prior to the beginning of the respective production cycle for each scenario (August/September for permanent ponds and February – April for rice-crawfish rotational ponds).

**Objective 2.** *Estimate the economic effects on U.S. catfish, bait minnow, and crawfish farms of alternative policy options under consideration to reduce carbon emissions, including cap-and-trade programs and carbon taxes.*

## University of Arkansas at Pine Bluff

UAPB has developed a general framework for simulating potential effects of “cap and trade” carbon emission policies on U.S. aquaculture. Researchers have developed baseline models for catfish and crawfish referred to as Catfish-CapTrade Policy Model and Crawfish-CapTrade Policy Model, respectively. The researchers have compiled baseline data (production/supply, consumption, producer and market prices, input quantity and prices, net carbon balance, etc.) and some of the parameters of these models (own and cross price elasticity of demand and supply).

The general framework of the model developed for simulating effects of “Cap and Trade” carbon emission policies on U.S. aquaculture is given in Fig. 1. The models postulate that the carbon offsets/taxes depend on a) net carbon supply of the aquaculture farms and b) government policies to distribute the revenue generated through the

Bulk density determinations were made for soil cores from 5-cm depth increments within the 20-cm plow-depth of fields and additional soil cores from 5-cm depth increments were sent to Auburn University for determination of organic carbon concentration. Data will be used to estimate carbon accumulation in sediment.

Researchers at LSU also worked with colleagues at AU to compile and define production data needed to ascertain customary carbon expenditures during the production phase of crawfish production, both under a single-crop farming practice as well as under the typical rice-crawfish field rotational approach.

program. The model captures the potential effects of alternative policies on farmers as well as consumers through changes in input prices, output and market prices, and in real per capita income.

Net carbon balance of existing farm practices will

## Results at a glance...

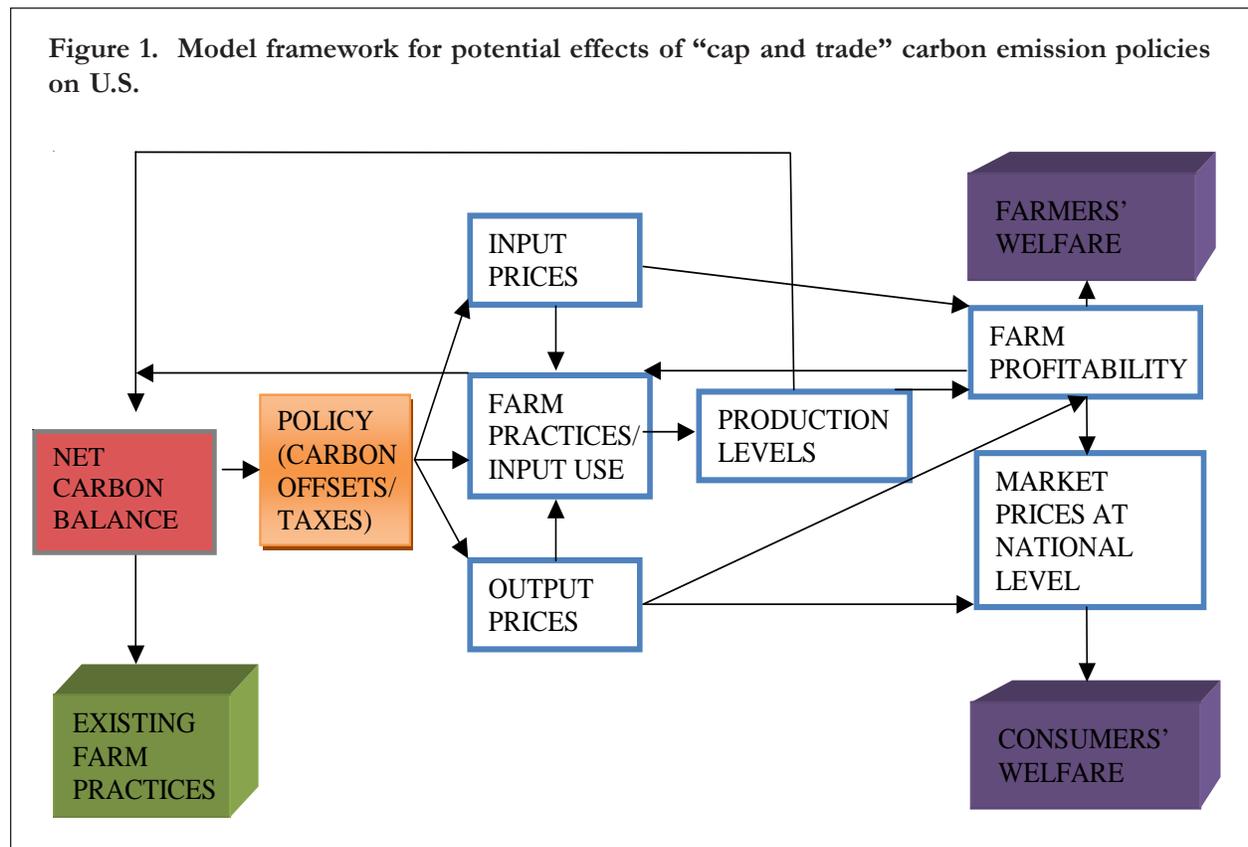
- *UAPB has developed a general framework for simulating potential effects of “cap and trade” carbon emission policies on U.S. aquaculture. The University has developed baseline models for catfish and crawfish called as Catfish-CapTrade Policy Model and Crawfish-CapTrade Policy Model, respectively.*

determine carbon emission policies (rates of carbon offsets/taxes). The policy will affect input and output prices. Farmers may adjust their farm practices and/or input use according to these policies. Changes in input and output prices can influence the existing farm practices/input usage. Changes in input and output prices and in farm practices will affect farm profitability, which will potentially affect farmers’ as well as consumers’ welfare. Changes in farm production levels and farm practices/input usage will result in new net carbon balances.

The UAPB team has conducted some simulations under baseline and different alternative “carbon sequestration efficiency” scenarios in exploring the potential impacts of possible “cap and trade” programs and carbon tax policies. Recently, a globally

coordinated carbon charge of US \$25 per ton of carbon dioxide (CO<sub>2</sub>) emission had been proposed in the G20 meeting. But there is no fixed carbon pricing instrument recommended for implementation; the selection of carbon-related fiscal instruments lies with the individual countries. The question on whether to impose carbon tax on final consumption goods or on inputs (e.g., fuels) remained unsolved (IMF, 2011; B20, 2012).

As there is no specific tax policy instruments agreed for a possible “cap and trade” program and carbon taxes in the U.S., we made the following assumptions in the simulation exercise: (i) an increase in cost of carbon-based products, namely fuel/diesel and electricity, by 6 percent (CBO 2009); (ii) an increase in feed price by 6 percent; (iii) carbon-trading is



## Results at a glance...

- *“Cap and trade” programs have the potential to affect catfish, baitfish and crawfish industries negatively. This negative impact of carbon tax could be reduced if farms can improve the net carbon sequestration without incurring additional cost.*

exercised through subsidy (net carbon sequestration) and/or tax (net carbon emission), which will then influence farmers’ decision making in farming based on the net carbon supply of their farming activities; (iv) net zero carbon-balance under baseline scenario for all the three species, as suggested by the Auburn University team for catfish farming. In general, net carbon-balance varies across species, weather and farming practices covering inputs and management skills. Under a “cap and trade” program, farmers may change their farming practices to higher carbon sequestered techniques and/or lesser carbon emitted practices. Therefore, we analyzed the potential impacts of “cap and trade” policy under the following alternative scenarios:

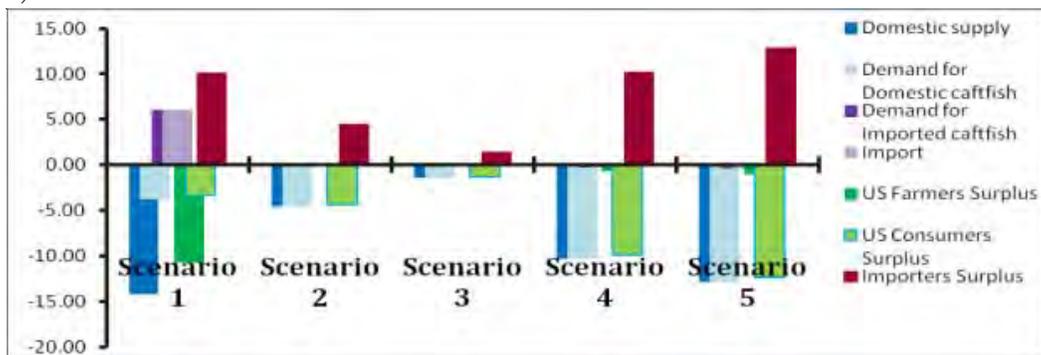
- Scenario 1: 6% increase in the price of carbon based inputs, such as fuel and electricity; and 6% increase in feed prices, with net zero carbon balance.
- Scenario 2: 6% increase in the price of carbon based inputs, such as fuel and electricity; and 6% increase in feed prices, with 5% increase in carbon sequestration efficiency.
- Scenario 3: 6% increase in the price of carbon based inputs, such as fuel and electricity; and 6% increase in

- Scenario 4: feed prices, with 10% increase in carbon sequestration efficiency. 6% increase in the price of carbon based inputs, such as fuel and electricity; and 6% increase in feed prices, with 5% decrease in carbon sequestration efficiency or 5% increase in net carbon emission.
- Scenario 5: 6% increase in the price of carbon based inputs, such as fuel and electricity; and 6% increase in feed prices, with 10% decrease in carbon sequestration efficiency or 10% increase in net carbon emission.

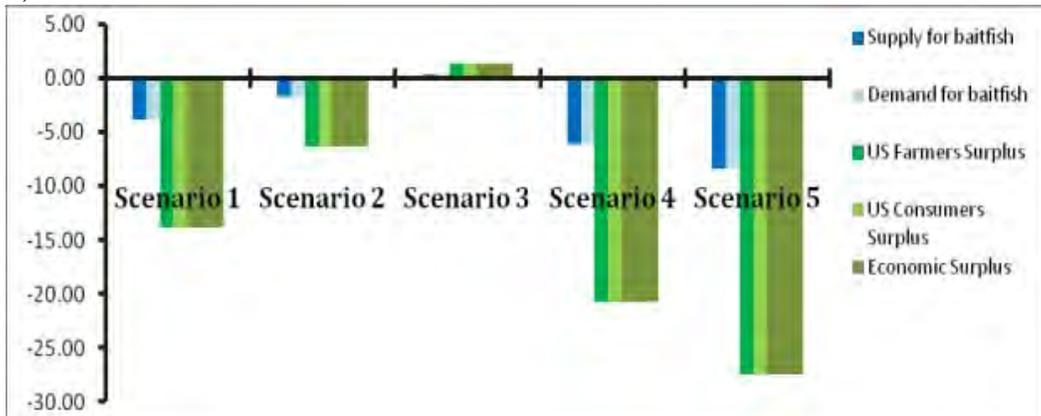
The results generated from the simulation exercises under baseline and alternative scenarios are presented in Fig. 2. The findings show that under Scenario 1 where carbon based inputs and feed prices increased by 6% with zero net carbon balance, the carbon tax policies will negatively affect the domestic aquaculture industry, which will reduce aquaculture productions and will reduce welfare of producers and consumers (as measured by producer and consumer surpluses) across all the aquaculture industries studied (catfish, baitfish and crawfish sectors). This negative impact of carbon tax could be reduced if farms can improve the net carbon sequestration without incurring additional cost (Scenario 2 and 3). Tax on inputs distorts production decisions and increases production cost. Even with substantial improvement in carbon sequestration (Scenario 3), taxes on inputs (such as, fuel and feed) might create negative impact on the welfare of catfish, crawfish and baitfish farmers. The negative effects may be mitigated with an increase in practices that favors net carbon sequestration; the higher the net carbon sequestration, the lesser will be the negative impact of carbon tax.

Fig. 2. Simulations results under baseline and different alternative scenarios (Axis Y – deviation from baseline (%); Axis X – different scenarios)

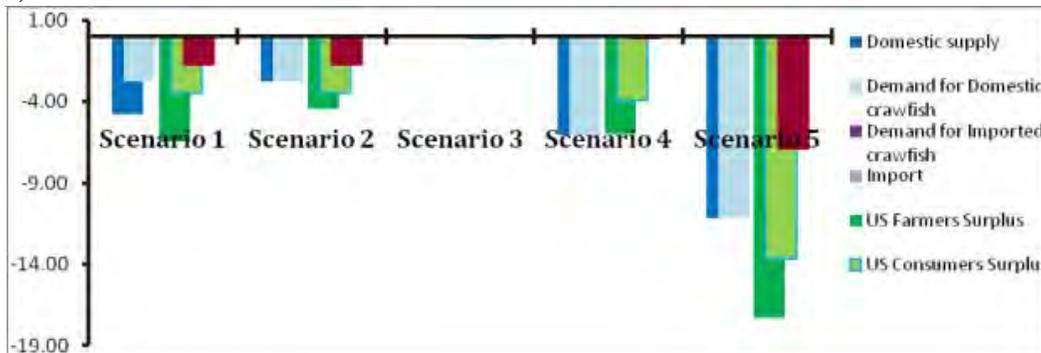
a) Catfish



b) Baitfish minnow



c) Crawfish



**Objective 3.** *Disseminate results for Objectives 1 and 2 through a general fact sheet that explains the impact of the carbon emissions issue on southern aquaculture, and specific fact sheets for the three species. A special session at a major, national, aquaculture meeting will also be organized.*

**Louisiana State University, University of Arkansas at Pine Bluff,  
and Auburn University**

This objective cannot be addressed until we are nearer completion of the work outlined in Objectives 1 and 2.

## **IMPACTS**

Channel catfish farming appears to be carbon dioxide emissions neutral with respect to farm-level operations, i.e. ponds sequester as much carbon dioxide as farming activities emit. Models for evaluating the likely economic effects of possible, future, carbon emissions regulations are still being

developed. However, the fact that catfish farming is carbon neutral and the likelihood that bait minnow and crawfish farming will contribute less to carbon dioxide emissions than channel catfish farming suggest that the economic impacts of carbon emissions regulations would probably be minor.

## **PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED**

### **Manuscripts**

Boyd, C. E. 2010. Aquaculture ponds hold carbon. *Global Aquaculture Advocate* 13(2):23-24.

Boyd, C. E., C. W. Wood, P. Chaney, and J. F. Queiroz. 2010. Role of aquaculture pond sediments in sequestration of annual global carbon emissions. *Environmental Pollution* 158:2537-2540.

### **Presentations**

Dey, M. M., K. Singh, and C. Engle. 2009. Analysis of catfish supply, demand and trade in USA: baseline model, estimation strategy and preliminary results. Paper presented in: NAAFE (North American Association of Fisheries Economists) Forum 2009, May 17-20, 2009, Newport, Rhode Island (U.S.).

Dey, M. M., K. Singh, and C. Engle. 2010. Impact of marketing, trade and exchange rate policies on U.S. catfish and trout markets: results from disaggregated fish sector models. Paper presented in: Aquaculture 2010 – Meeting Abstracts, Page 506.



## DEVELOPMENT AND EVALUATION OF COOL-WATER CRAWFISH BAITS

### Reporting Period

January 1, 2011 – August 31, 2012

<b>Funding Level</b>	Year 1 .....	\$37,595
	Year 2 .....	\$43,503
	Year 3 .....	\$43,798
	Total.....	\$124,896
<b>Participants</b>	Louisiana State University Ag Center .....	Ray McClain, Robert P. Romaine, Charles Gregory Lutz
	Texas A&M University .....	Delbert M. Gatlin, III
	Auburn University .....	D. Allen Davis

### PROJECT OBJECTIVES

1. Identify attractants, bait formulations, or potential manufacturing processes that increase the efficacy of formulated crawfish bait for use at water temperatures below 70 degrees F.
2. Compare the efficacy of experimental formulated baits or processes with fish baits for increasing crawfish catch and profits under simulated commercial conditions at water temperatures below 70 degrees F.

### ANTICIPATED BENEFITS

Crawfish are harvested in over 185,000 acres of aquaculture ponds using baited wire-mesh traps that are lifted 3 to 5 days a week beginning as early as November and continuing through May to July of the following year. Traps are typically baited with manufactured formulated bait in warmer weather but, because formulated baits are inferior at cooler water temperatures, fresh-frozen cut fish is used. Fish for crawfish bait has become expensive, costing twice that of commercially formulated bait, and fish bait is frequently in short supply. More than half of

the annual crawfish harvesting effort occurs during cool-water periods (December through late March), and with availability and price issues with fish, as well as the need to transport and store fish baits in a frozen state, this bait has become problematic for the crawfish industry. Development of an effective and economical cool-water formulated crawfish bait will address not only some of the cost and handling/storage issues with fish baits, but also will help conserve the fishery for many of these species.

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

**Objective 1.** *Identify attractants, bait formulations, or potential manufacturing processes that increase the efficacy of formulated crawfish bait for use at water temperatures below 70 degrees F.*

### Louisiana State University Agricultural Center

A review of the scientific literature for identified attractants to carnivorous crustaceans revealed that amino acids and related biochemical compounds tend to elicit chemoattraction responses and may act as feeding stimulants for these crustaceans. Although some studies have shown that a feeding response can be elicited by single components, most have shown the response to be greatest with specific mixtures of amino acids or other compounds. Although detection does not equal attraction and an elicited feeding response may not equate to attraction over some distance to elicit entry of crawfish into a baited trap, this review provided creditable information.

Preliminary efforts were undertaken to establish an effective protocol for testing preferences of crawfish to attractants in the laboratory. Those efforts were not successful. The response of crawfish to field-proven attractants in a controlled laboratory environment, even at optimum temperatures with acclimated and/or starved captive stock, proved inconsistent and not predictable. Therefore, research was subsequently directed at developing a suitable technique “in the field” for effectively evaluating the efficacy of attractants in ponds that simulated commercial crawfish aquaculture. A gelatin-based matrix made without excessive heat or processing was found to be an effective medium to test attractants and could be used to evaluate potential attractants using commercial crawfish traps in experimental or commercial crawfish ponds. Several flesh-based attractants, including fish meal, when incorporated into the gelatin-based matrix, caught as many crawfish as cut gizzard shad (industry standard fish bait) when evaluated at temperatures from 51 to 63 degrees F. In contrast, concurrent

field studies evaluated single amino acids, an amino acid mixture, sugar, fish oil and fish solubles incorporated into solid blocks of plaster of Paris (calcium sulfate dehydrate), as attractants for crawfish. The attractants imbedded in the plaster blocks were not effective when compared to either cut shad or attractants incorporated into the gelatin matrix. Thus, the gelatin matrix proved to be best suitable for identifying and testing potential attractants in experimental baits for crawfish in earthen ponds.

Initial tests during the first year of the project involved baits composed of the gelatin medium and various proteinaceous ingredients as test attractants. The test attractants consisted of selected commercially available ingredients, ground flesh products, and proprietary mixtures of synthetic amino acids. The experimental trials were conducted in either a commercial crawfish production pond or small research pond managed to simulate commercial crawfish ponds. Trials were conducted from January

### **Results at a glance...**

- *Early testing of potential alternative attractants as crawfish bait revealed the limitations for several of the more promising high protein feedstuffs; yet valuable information was gleaned regarding which amino acids may be key for determining efficacious baits for use in cool-water trap harvesting of crawfish.*

to early March at water temperatures ranging from 47 to 77 degrees F, and consisted of baited wire mesh traps (standard commercial trap of the crawfish aquaculture industry) placed at 45-foot intervals at random locations within the ponds. Trap soak duration was 24 hours. The response variables consisted of average number and weight of crawfish captured per trap per treatment. Capture rates with experimental baits were compared to cut fish (pogy, an industry standard), no bait, and in one trial, a commercially formulated bait (Purina Mills, Shreveport, LA). Results of these trials are provided in Table 1.

Of the experimental attractants tested, only minced pogy caught as many crawfish as cut pogy, the industry standard for cool water use. While other baits generally yielded higher catches than traps without bait, few differences were observed among the other proteinaceous attractants.

In year 2 of the project, additional ingredients were tested within the gelatin matrix for their relative

### Auburn University

Dr. Allen Davis has provided technical support for the project. This has included direction of the project, recommendations for sources and types of test ingredients and has provided insight into possible manufacturing processes. Additionally, he has

**Objective 2.** *Compare the efficacy of experimental formulated baits or processes with fish baits for increasing crawfish catch and profits under simulated commercial conditions at water temperatures below 70 degrees F.*

### Louisiana State University Agricultural Center

While all tests conducted and planned involve a comparison with cut fish (the current industry standard for cool water) as the attractant, capture rate of non-baited traps is also included in every experimental trial. When effective alternative

effect on attracting crawfish to traps over five experimental trials. Catch results are presented in Table 2. Ingredients used for testing of attractant quality included several forms of fish products, various levels of fish product inclusion, a saccharide, an essential oil that exhibited potential for increasing catfish feed intake, and various commercially available high protein feed ingredients. Each trial also included treatments of cut pogy (menhaden), manufactured (warmwater) crawfish bait, and a non-baited control.

Results were mixed, with few test ingredients facilitating a catch rate nearing that of cut pogy. To determine if there were individual amino acids associated with better catch results, a correlation analysis was conducted. Individual amino acid concentrations (determined at Texas A&M University) for each test bait was correlated with the magnitude of that respective catch rate (standardized for the different trials by expressing catch in relation to cut pogy). Statistically significant correlation results are presented in Table 3.

provided several ingredients for testing. He will continue to participate in planning sessions for the next series of experiments and will assist in reviewing and analyzing research results as they become available.

attractants to fish baits are identified, and a suitable process determined for manufacture of those baits, a full scale trial will be conducted to compare the efficacy and cost effectiveness of those baits/processes under rigorous commercial conditions.

**Table 1. Average crawfish catch (by number and total weight of crawfish per trap), average weight of crawfish captured, and average catch (by number and by total weight) expressed as a percentage of that caught with cut menhaden (pogy) for experimental cool water attractants in 2011.**

Treatment	No. Trps	Avg catch (No/Trp)	Avg Catch (Lb/Trp)	Wt. (g)	% of Cut Pogy (by No.)	% of Cut Pogy (by Wt.)
<i>Trial 1 (temp = 47.2 min / 56.7 max / 52.4 average)</i>						
Cut frozen menhaden	3	4.8	0.20	17.4		
Gelatin-Minced Crawfish	3	0.5	0.02	15.0	10.4	10.0
Gelatin-Krill Meal	3	1.2	0.05	15.0	25.0	25.0
Gelatin-Squid Meal	3	2.3	0.07	14.1	47.9	35.0
Gelatin-Fish Meal	3	3.5	0.11	14.3	72.9	55.0
Gelatin-Minced Fresh Pogy	3	2.8	0.12	19.4	58.3	60.0
Gelatin-Minced Air-dried Pogy	3	5.5	0.22	17.6	114.6	110.0
No Bait	3	1.7	0.04	10.9	35.4	20.0
<i>Trial 2 (temp = 53.2 min / 66.3 max / 60.2 average)</i>						
Cut frozen menhaden	12	18.1	0.6	14.9		
Gelatin-Amino Acid Mix (1%)	12	10.3	0.31	13.5	56.9	51.7
Gelatin-No Attractant	12	9.3	0.32	15.5	51.4	53.3
Gelatin-Krill Meal	12	10.8	0.38	15.5	59.7	63.3
Gelatin-Squid Meal	12	11.1	0.38	14.8	61.3	63.3
Gelatin-Minced Crawfish	12	11.6	0.41	15.8	64.1	68.3
Gelatin-Fish Meal	12	13.6	0.47	15.9	75.1	78.3
Gelatin-Minced Fresh Pogy	12	21.8	0.69	14.3	120.4	115.0
Gelatin-Minced Air-dried Pogy	12	19.3	0.61	14.3	106.6	101.7
No Bait	12	5.3	0.16	13.8	29.3	26.7
<i>Trial 3 (temp = 67.7 min / 76.7 max / 71.3 average)</i>						
Cut frozen menhaden	12	24.9	0.96	17.6		
Gelatin-Amino Acid Mix (3%)	12	12.8	0.45	16.0	51.4	46.9
Gelatin-Minced Crawfish	12	15.8	0.62	17.9	63.5	64.6
Gelatin-Fish/Squid/Krill Meal	12	16.3	0.62	17.1	65.5	64.6
Gelatin-Minced Fresh Pogy	12	26.2	0.99	17.2	105.2	103.1
Purina Pellets-Southern Pride	12	27.1	1.04	17.4	108.8	108.3
No Bait	12	6.3	0.2	14.3	25.3	20.8

**Table 2. Average crawfish catch (by number and total weight of crawfish per trap), average weight of crawfish captured, and average catch (by number and by total weight) expressed as a percentage of that caught with cut menhaden (pogy) for experimental cool water attractants in 2012. Values within columns, by trial, with the same superscript were not significantly different ( $P > 0.05$ ). No significant differences were detected among treatments for mean size.**

Treatment (Attractant)	Avg Catch (No/Trp)	Avg Catch (Lb/Trp)	Avg Wt. (g)	% of cut Pogy (by No.)	% of cut Pogy (by Wt.)
Trial 1: n=24 traps; Water Temperature = 56.8 min / 63.5 max / 61.0 average					
Cut frozen menhaden	27.6 <sup>A</sup>	.89 <sup>A</sup>	14.1	-	-
Catfish feed with EO*	7.4 <sup>BC</sup>	.23 <sup>B</sup>	13.7	26.8	25.8
EO (at 3%)*	6.5 <sup>BC</sup>	.21 <sup>B</sup>	14.2	23.6	23.6
EO (at 6%)*	5.8 <sup>BC</sup>	.18 <sup>B</sup>	13.9	21.0	20.2
Purina bait	12.2 <sup>B</sup>	.37 <sup>B</sup>	13.6	44.2	41.6
No bait	3.9 <sup>C</sup>	.12 <sup>B</sup>	13.8	14.1	13.5
Trial 2: n=16 traps; Water Temperature = 58.2 min / 62.5 max / 60.8 average					
Cut frozen menhaden	32.1 <sup>A</sup>	1.23 <sup>A</sup>	17.3	-	-
Freeze-dried menhaden meal (100%)*	27.3 <sup>A</sup>	1.01 <sup>AB</sup>	16.8	85.0	82.1
Freeze-dried menhaden meal (50%)*	26.2 <sup>A</sup>	1.01 <sup>AB</sup>	17.4	81.6	82.1
Freeze-dried menhaden meal (10%)*	14.8 <sup>B</sup>	.59 <sup>C</sup>	17.8	46.1	48.0
Freeze-dried menhaden meal (2%)*	12.4 <sup>B</sup>	.47 <sup>CD</sup>	16.7	38.6	38.2
Purina bait	18.6 <sup>B</sup>	.70 <sup>BC</sup>	17.1	57.9	56.9
No bait	5.3 <sup>C</sup>	.19 <sup>D</sup>	16.1	16.5	15.4
Trial 3: n=16 traps; Water Temperature = 60.3 min / 64.1 max / 62.6 average					
Cut frozen menhaden	35.8 <sup>B</sup>	1.45 <sup>AB</sup>	18.2	-	-
Minced fresh fish*	51.2 <sup>A</sup>	2.1 <sup>A</sup>	18.4	143.0	144.8
Solvent extracted freeze-dried menhaden meal*	39.6 <sup>AB</sup>	1.61 <sup>AB</sup>	18.1	110.6	111.0
Freeze-dried menhaden meal*	32.3 <sup>B</sup>	1.39 <sup>B</sup>	19.4	90.2	95.9
Freeze-dried menhaden meal (heated)* <sup>1</sup>	32.9 <sup>B</sup>	1.36 <sup>B</sup>	18.6	91.9	93.8
Minced oven dried menhaden (low temp)* <sup>2</sup>	32.9 <sup>B</sup>	1.37 <sup>B</sup>	18.8	91.9	94.5
Minced oven dried menhaden (high temp)* <sup>1</sup>	24.9 <sup>BC</sup>	1.04 <sup>BC</sup>	18.7	69.6	71.7
Purina bait	31.6 <sup>B</sup>	1.31 <sup>B</sup>	18.8	88.3	90.3
No bait	11.5 <sup>C</sup>	.42 <sup>C</sup>	16.5	32.1	29.0
Trial 4: n=16 traps; Water Temperature = 52.8 min / 58.2 max / 55.9 average					
Cut frozen menhaden	50.9 <sup>A</sup>	2.04 <sup>A</sup>	18.2	-	-
Poultry by-products*	32.1 <sup>B</sup>	1.04 <sup>B</sup>	18.9	63.1	51.0
Dried grains w/solubles*	21.9 <sup>CD</sup>	.92 <sup>B</sup>	19.0	43.0	45.1
Fish meal 200%*	21.9 <sup>CD</sup>	.92 <sup>B</sup>	19.2	43.0	45.1
Fish meal 100%*	19.6 <sup>CD</sup>	.81 <sup>BC</sup>	19.0	38.5	39.7
Fish meal/soybean meal*	16.0 <sup>D</sup>	.63 <sup>C</sup>	17.8	31.4	30.9
Soy protein concentrate*	15.0 <sup>D</sup>	.62 <sup>C</sup>	19.0	29.5	30.4
Soybean meal in matrix*	13.8 <sup>DE</sup>	.54 <sup>C</sup>	17.9	27.1	26.5
Purina bait	26.6 <sup>BC</sup>	1.05 <sup>B</sup>	17.9	52.3	51.5
No bait	5.0 <sup>E</sup>	.20 <sup>D</sup>	17.9	9.8	9.8
Trial 5: n=14 traps; Water Temperature = 56.7 min / 61.7 max / 59.6 average					
Cut frozen menhaden	25.9 <sup>A</sup>	.94 <sup>A</sup>	17.7	-	-
Fish meal*	15.5 <sup>B</sup>	.54 <sup>B</sup>	17.0	59.8	57.4
Fish meal + sugar (20%)*	13.8 <sup>BC</sup>	.52 <sup>B</sup>	18.1	53.3	55.3
Sugar*	10.7 <sup>C</sup>	.38 <sup>BC</sup>	17.1	41.3	40.4
Purina bait	26.2 <sup>A</sup>	.96 <sup>A</sup>	17.7	101.2	102.1
No bait	5.9 <sup>D</sup>	.22 <sup>C</sup>	17.0	22.8	23.4

\*Indicates attractant was contained within the gelatin matrix

<sup>1</sup> Drying/heating temperature = 90 C.

<sup>2</sup> Drying temperature = 60 C.

**Table 3. Correlation coefficients (r value) for selected amino acid levels in test baits and corresponding relative catch values by number of crawfish caught per trap for those baits. Amino acid (AA) levels in cut menhaden (pogy) as well as freeze-dried pogy (*Best* showing category of test baits), fish meal (*Mediocre* showing category), and soybean meal (*Poor* showing category) are also included. Amino acid concentrations expressed as nmol/mg of wet weight.**

AA (or Derivative)	r value	P value	AA level in Cut Pogy	AA level in FD Pogy	AA level in Fish meal	AA level in Soy Meal
Serine	.88897	<.0001	1.69	5.52	2.52	.16
Tyrosine	.87619	<.0001	0.58	3.08	1.62	.13
Threonine	.87169	.0001	0.77	3.18	2.38	.10
Aspartate	.87139	.0001	0.07	2.88	1.34	1.24
Glutamine	.82951	.0005	0.65	5.06	1.02	0.00
Lysine	.82459	.0005	0.91	4.64	3.38	.17
Histidine	.81087	.0008	5.27	12.16	9.44	.21
Cystathionine	.78869	.0014	0.04	.19	.09	.03
Phenylalanine	.77874	.0017	0.71	2.57	2.60	.26
1-Methylhistidine	.76106	.0025	0.07	.32	.27	.08
Cystine	.76051	.0025	0.04	.25	.03	.02
Leucine	.72484	.0051	1.45	4.83	6.38	.12
Taurine	.72445	.0051	8.46	18.14	24.43	.01
Isoleucine	.69781	.0080	0.57	2.30	3.33	.12
Glutamate	.6815	.0103	0.84	5.06	2.19	1.00
Valine	.67707	.0110	1.02	3.55	5.51	.19
3-Methylhistidine	.66849	.0125	0.18	1.05	.86	.11
Glycine	.65795	.0145	1.34	4.34	5.38	.27
Methionine	.64992	.0162	0.33	.38	.72	.09
Alanine	.57872	.0382	2.86	9.42	12.82	.77

### Texas A&M University

A total of 16 different test baits for crawfish were analyzed for amino acid composition of their protein-bound and free-pool constituents. For most baits (Essential oil had none), 23 primary and 18 secondary amino acids were detected in the free pool; whereas, 18 amino acids were detected in the protein-bound form. Regression analysis showed that 23 amino acids significantly correlated with catch values obtained in earlier trials at Louisiana State University. Only two amino acids correlated with catch were in

the protein-bound form and only seven had correlation values above 0.8 (all free amino acids) (Table 3).

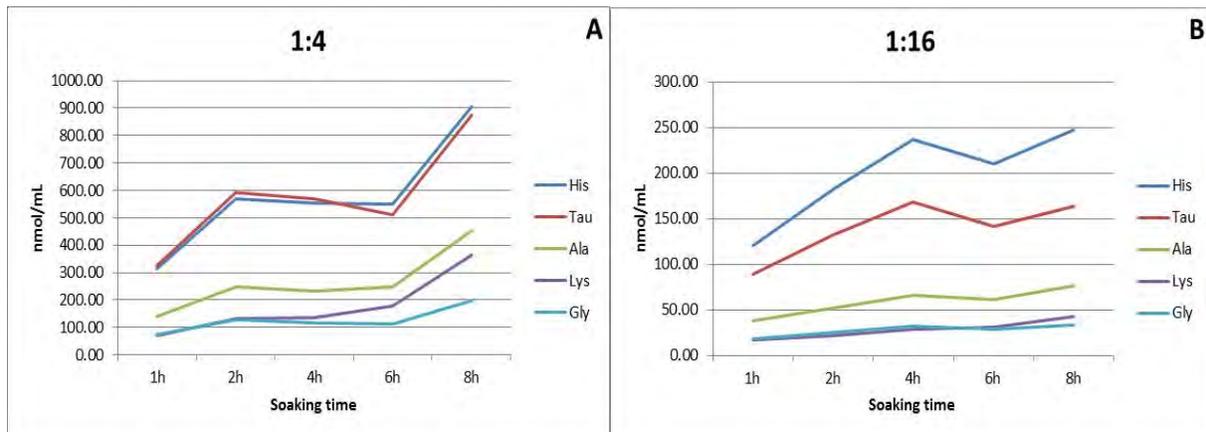
In addition, the correlations seem to point to a limited number of amino acids that are found in relatively high concentrations in the fresh pogy and the other highest ranking baits. These include taurine, histidine, alanine, lysine, serine, leucine, glycine, valine, and glutamate (Table 3).

On the other hand, most of these amino acids were in lower concentrations in the mediocre and poor ranking baits than in fresh pogy. Nevertheless, the finding of two mediocre showing baits, fish and poultry meals, having higher absolute values of all these amino acids than the fresh pogy, seems to point out that other non-detected attractant(s) may be present in the latter bait, ergo not present in the former two meals.

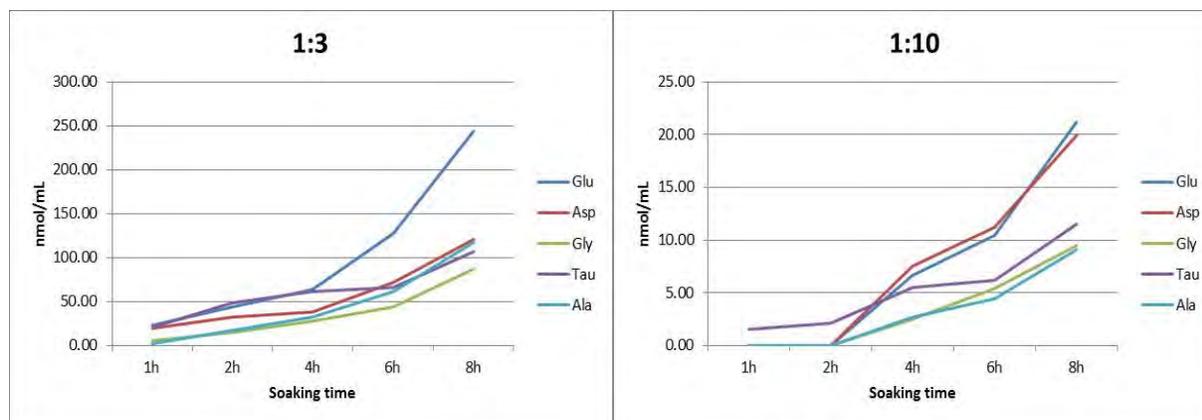
Based on these findings, a follow-up study was carried out with the fresh pogy – control bait with excellent showing – and the Purina bait – mediocre showing – to identify top-leaching amino acids

from soaked baits throughout an 8-h period. Results from this assay showed five prominent amino acids in water containing fresh pogy, which in the order of concentration were histidine, taurine, alanine, lysine and glycine (Fig. 1); however, histidine and taurine were in the range of 4- to 6-fold higher than the other three amino acids. On the other hand, the top five amino acids in water containing the Purina bait were glutamate, aspartate, taurine, alanine and glycine (Fig. 2). Interestingly, although both baits had similar amino acids within the top five (e.g., taurine, alanine and glycine), the release of them to the water was much slower in soaked Purina bait than in fresh pogy.

**Figure 1. Top five amino acid concentrations (nmol/mL) in water containing fresh pogy at different bait:water ratios, 1:4 (A) and 1:16 (B). Histidine and taurine were 4- to 6- fold higher in both ratios compared to alanine, lysine and glycine.**



**Figure 2. Top five amino acid concentrations (nmol/mL) in water containing Purina bait at different bait:water ratios, 1:3 (A) and 1:10 (B). Amino acid release was slower with this bait, and at the end of the assay glutamate was 2-fold higher than the other amino acids at 1:3 ratio; whereas, at 1:10 glutamate and aspartate were 2-fold higher than glycine, taurine and alanine.**



## IMPACTS

The primary impact of the results to date has been to provide scientists with valuable information regarding what direction to take the research in an attempt to identify methods and potential attractants for further testing. The recent findings have provided quantitative assessments of the value of limited substances and feedstuffs as potential crawfish attractants, and have provided valuable information regarding certain physical aspects needed for effective

crawfish baits. Specifically, this research suggests that key amino acids may play a vital role in determining the quality of a crawfish attractant and have identified several amino acids that may be most important. Moreover, it was determined that the rate and timings of key amino acid released from baits in water may affect the efficacy of different baits. This provides the impetus and possible direction for further research.

## PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED

McClain, W.R. and J.J. Sonnier. 2011. Investigation of test ingredients as potential attractants for crawfish in cool water. Ann. Res. Rpt., Rice Res. Stn., La. Agri. Exp. Stn., L.S.U. Agricultural Center, 103:257-262.



## IDENTIFYING DETERMINANTS FOR DEVELOPMENT OF LIVE-MARKET GRADING STANDARDS FOR CRAWFISH

### Reporting Period

January 1, 2011 – August 31, 2012

<b>Funding Level</b>	Year 1 .....	\$49,952
	Total.....	\$49,952

<b>Participants</b>	Louisiana State University Ag Center .....	Ray McClain, Robert P. Romaine
	University of Arkansas at Pine Bluff .....	Carole Engle, Madan Dey

### PROJECT OBJECTIVES

1. Survey major components of the supply chain for live procambriid crawfish to determine the desire for establishing grade standards, and to define the number of grades desired and potential size or weight limits (or other factors) for each grade.
2. Determine size distribution at harvest for live red swamp crawfish collected with standard commercial wire-mesh traps and graded using various bar spacing parameters over a December through June crawfish harvesting season.
  - a. Identify the body dimensions, weight, and maturity of the smallest crawfish captured by 0.75-inch and 0.875-inch coated square wire mesh traps.
  - b. Identify grader spacing parameters that will segregate crawfish (captured in 0.75-inch square mesh traps) into 3, 4, 5, and 6 different size grade categories.
  - c. Determine cross-sectional width dimensions and weight variances of immature and mature crawfish for each of the spacing parameters used in 2b.
3. Present findings of Objectives 1 and 2 to the crawfish aquaculture industry via workshops, educational programs, newsletters, etc. with recommendations for establishment of industry-wide adoptable grade standards.

### ANTICIPATED BENEFITS

A lack of industry adopted grading standards for crawfish sales potentially hampers market expansion and leads to tension among sellers and buyers of crawfish in regards to what constitutes “quality”. To improve and expand markets for live crawfish,

acceptable grading standards are needed. This research was initiated to ascertain (by industry survey) the desire among the various participants of the supply chain for establishing grade standards, and to determine the need for number of grades and

potential size classifications for those grades. The research will also resolve many of questions relating to potential grading standards because of the changing morphology of crawfish as they mature. Crawfish are currently graded by size (i.e., width of the carapace) but are noted by count per pound (as with grades of shrimp). However, the weight of crawfish for a given size is highly dependent on maturity (unlike with shrimp) because weight changes for a given size based on morphology associated with

maturity. Therefore, this research will not only identify grader bar spacings necessary for several potential grade classifications, it will determine whether any of these systems will be compatible with grading bar spacing requirements that will not change with seasonal changes in crawfish morphometry. Findings of the survey and field research will be presented to the crawfish industry, with recommendations for potential grading standards that could be adopted by industry.

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

**Objective 1.** *Survey major components of the supply chain for live procambriid crawfish to determine the desire for establishing grade standards, and to define the number of grades desired and potential size or weight limits (or other factors) for each grade.*

### University of Arkansas at Pine Bluff (UAPB)

**Activity-1.1:** *Identifying important crawfish markets through scanner data*

It is necessary to identify potential markets and the needs of the consumer before developing a standardized grading system. Increased understanding of trends in supermarket sales of crawfish has potential to assist the crawfish industry to refine grading strategies. The UAPB team has analyzed the market trends of the US crawfish retail market, including market shares, price fluctuations, promotional sales, and package size across several major regional cities. The analysis is based on store-level scanner data procured from the AC Nielsen Company. The dataset is composed of weekly data covering 52 US markets for the last 5 years, ending on June 12, 2010, for different seafood products that include freshwater crawfish. A.C. Nielsen data in the category of “frozen” were used to develop this descriptive analysis. The “frozen” category includes all frozen and chilled fish and seafood available in both prepared and unprepared forms usually found in refrigerated and frozen sections of supermarkets.

Crawfish products sold in the US supermarkets can be grouped into three frozen/chilled categories—entrée, breaded and unbreaded products. Breaded products do not show much product diversity, and their sales are mostly in Southern cities. Entrée products are the most diverse in terms of products sold, though they are sold in fewer cities than are unbreaded products. Cities with no sales of crawfish entrées during the last five years were: Des Moines, Las Vegas, Los Angeles, Omaha, Phoenix, Sacramento, Salt Lake City, San Diego, and San Francisco. Unbreaded products are sold by the largest number of brands, and have the largest regional distribution in terms number of cities with sales.

Unbreaded crawfish product is the main category, contributing about 85% of the total crawfish market in terms of volume and about 70% of the market in terms of value. The market for unbreaded crawfish is concentrated in the southern region of the country. Among the 52 markets we have analyzed, New Orleans/Mobile has the maximum share (about 70%) of unbreaded crawfish sales in

supermarkets (Table 1). Three Texas cities, Houston, Dallas and San Antonio, are the other major markets for unbreaded crawfish; with Houston having about 13-14% of sales share and the other two about 3-3.5% each. The only two cities out of 52 cities that did not show sales of unbreaded crawfish in the five-year period were Portland and Seattle. But the sales of crawfish in non-southern cities are very limited. Though crawfish products have been sold in cities other than the traditional markets, they are yet to become established in the Western and Midwestern US. Promotional sale is an important crawfish marketing strategy. About 35% of unbreaded crawfish products were sold under some sort of promotion during the during last five years

(Table 1). New Orleans/Mobile also had the highest degree of promotion, with about 28-33% of products sold under promotion.

In terms of grading requirements, the unbreaded crawfish category could present more potential applications than other categories. Tail meat is the most dominant product form, with about 90-96% share of unbreaded crawfish sales. However, in the last two years, there is a gradual increase in the share of whole crawfish. Direct applications of potential grading systems are evident in the case of whole crawfish.

The most popular packaging size is 60-oz packages,

**Table 1. Top cities for overall unbreaded crawfish sales**

Product/City	Proportion (%) out of Total Sales in Individual cities					Proportion (%) of Promotional Sales out of Total Sales (lb)				
	2005/6	2006/7	2007/8	2008/9	2009/10	2005/6	2006/7	2007/8	2008/9	2009/10
Crawfish						33.3	35.2	38.3	32.0	34.0
New Orleans/Mobile	68.3	70.9	71.9	70.9	69.9	29.2	32.6	33.2	27.5	28.8
Houston	13.5	13.7	13.1	13.8	13.7	1.4	1.0	2.4	2.1	2.6
Dallas	3.3	3.5	3.7	3.5	3.8	0.9	0.8	1.4	1.2	1.5
San Antonio	3.2	3.2	3.1	2.8	2.8	0.0	0.1	0.6	0.4	0.3
Memphis	1.5	1.8	1.8	1.7	1.6	0.4	0.2	0.1	0.2	0.1
Jacksonville	1.2	0.4	0.7	1.3	1.3	0.1	0.0	0.0	0.1	0.1
Little Rock	1.0	0.8	0.8	0.9	0.8	0.3	0.1	0.2	0.2	0.2
Birmingham	0.5	0.5	0.8	0.8	1.2	0.1	0.0	0.0	0.0	0.0
St. Louis	0.6	0.7	0.5	0.8	0.6	0.1	0.1	0.0	0.3	0.2
Atlanta	0.6	0.6	0.5	0.1	0.1	0.0	0.0	0.1	0.0	0.0
Raleigh/Durham	0.2	0.5	0.4	0.3	0.4	0.0	0.0	0.0	0.0	0.0
Chicago	0.5	0.3	0.1	0.3	0.4	0.1	0.0	0.0	0.0	0.0
Orlando	0.5	0.1	0.2	0.4	0.5	0.0	0.0	0.0	0.0	0.0
Tampa	0.4	0.2	0.2	0.2	0.5	0.0	0.0	0.0	0.0	0.0
Kansas City	0.3	0.2	0.2	0.3	0.3	0.0	0.0	0.0	0.0	0.0
West Texas	0.2	0.3	0.3	0.3	0.3	0.0	0.0	0.1	0.1	0.1
Nashville	0.4	0.3	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0
Detroit	0.3	0.3	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0
New York	0.9	0.1	0.1	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Oklahoma City/Tulsa	0.2	0.4	0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.0

and their share has increased over the years, to almost 90% of whole crawfish sales. The remaining sales are constituted by 80 oz, 48 oz and 20 oz. Among these 3 packages, only 80 oz packages have demonstrated consistent sales in all 5 years. Hence, it can be said that larger package sizes (3.75 lb or 60 oz, and 5 lb or 80 oz) form the most important package sizes.

Average price of whole products has fluctuated over the years, with a dip in the last two years. The

### University of Arkansas at Pine Bluff (UAPB)

**Activity 1.2:** (a) *Focus group discussion with the wholesale buyers and producers of the crawfish industry.*

The UAPB team conducted an organized focus group discussion with the crawfish wholesale buyers and producers in Crowley, Louisiana in October, 2011 to identify major markets for survey and to determine important issues that the industry is greatly concerned about. The focus group discussion revealed that the demand for live crawfish is increasing, local market of Louisiana prefers graded product, but the market trends and consumers' preference for crawfish outside the traditional market area are still relatively unknown. Some of the issues of concern for the crawfish industry are: grading is expensive; the smallest size becomes a waste product if they cannot mix them with other sizes for sale; if customers pay a premium of more than \$0.25 to \$0.50/lb for a graded product, then the standardized grading system would work well. The industry representatives are interested to know the price gaps between different size categories.

**Activity 1.2:** (b) *Conduct surveys of retail store and restaurant managers to know their perception and willingness to pay for the graded crawfish products.*

The study has followed a stakeholder driven approach. Based on the recommendation of the

cheapest pack is the 80 oz package. The most popular package, i.e. the 60 oz package, is priced considerably higher than the 80 oz package.

Fifty-five brands of unbreaded crawfish were sold in the market during the last five years, out of which 11 brands sold whole unbreaded crawfish products. Sales of crawfish entrées sales have been growing at a high rate. There were 25 crawfish entrée products in the markets, and they contributed almost 30% of total crawfish sold in 2009-2010.

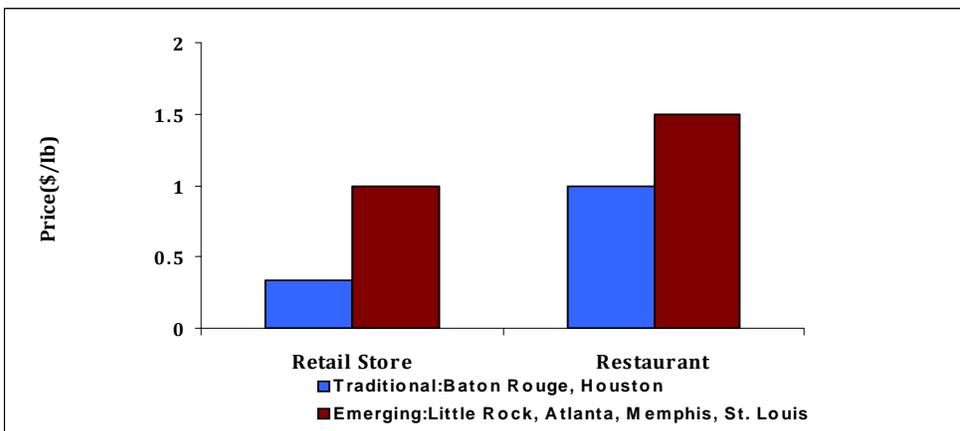
focus group discussion reported above, the UAPB team conducted surveys in two major crawfish markets (Houston and Baton Rouge) and in four emerging markets (Little Rock, Memphis, Atlanta and St. Louis). We used the list of seafood restaurants and retail stores in each of these cities as sampling frames, and randomly selected 21 seafood stores and 24 restaurants from these six selected cities. Surveys of store/restaurant managers were conducted through face to face interview using a pre-tested interview schedule.

We have found that January-June is the main crawfish selling season for retail stores, whereas February-May is the peak season for restaurants. There are various crawfish products available in the market. Crawfish whole boil followed by crawfish live are the dominant products in retail stores. In the restaurant sector, crawfish live is the most demanded product during the peak season. The survey results indicate that the demand for live crawfish has been increasing. Retail store/restaurants managers' most preferred packet size to buy is 25 to 35 lb/sac for live crawfish, 1 to 5 lb/pack for whole frozen, and 1 lb/box for tail meat.

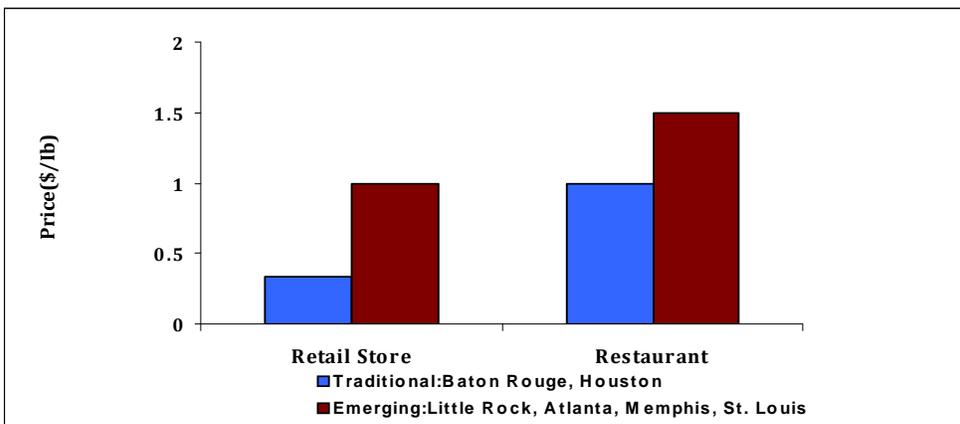
Grading of domestic live crawfish are done based on length and size (weight per count). There are two main grades based on length: category 1: (3 to

4 inch), and category 2: ( mixed). The main size-based grades are: 10 count/lb (jumbo), 10 to 15 count/lb (selected/medium), and 16 to 20 count/lb (mixed). Around 43% of store managers and 58% of restaurant managers indicate that the sales of live crawfish vary significantly with its size and length. The price difference between graded and mixed size category in retail stores ranges from \$0.34/lb to \$1.00/lb. In restaurants, the price varies from \$0.99/lb to \$1.50/lb (Fig. 1a). On average, 35% of retail store managers are willing to pay an extra \$0.38/lb, and 50% of restaurant managers are willing to pay an extra \$0.86/lb for obtaining the standard size graded crawfish (Fig. 1b). Fifty eight percent of stores' managers prefer the U.S product and are willing to pay higher prices for the domestic

(USA labeled) products. Crawfish consumers come from all major ethnic and income groups in the country. About 81% of the crawfish customers buy/consume crawfish on a regular basis. Most managers claim that consumers from areas outside the traditional markets are not very familiar with crawfish products, implying the importance of promotional/awareness activities for market expansion. A majority of the managers indicate that the price of crawfish is greatly influence by seasonality of supply. Survey results show that there is potential to expand the market for graded crawfish, but the feasibility of establishing a grading standard may be hampered due to seasonal variability in size and availability, related high cost, and unawareness of the consumers about the grade.



**Fig. 1a. Price difference between graded and mixed size crawfish.**



**Fig. 1b. Managers' willingness to pay for graded crawfish product.**

**Objective 2.** *Determine size distribution at harvest for live red swamp crawfish collected with standard commercial wire-mesh traps and graded using various bar spacing parameters over a December through June crawfish harvesting season.*

### Louisiana State University Agricultural Center

**Sub-objective 2a.** *Identify the body dimensions, weight, and maturity of the smallest crawfish captured by 3/4-inch and 7/8-inch coated square wire mesh traps.*

Daily harvest data, whereby crawfish were sorted into 3 grade categories (Large = <15 count/lb, Medium = 16-21 count/lb, and Small = >21 count/lb), was organized by trap type when traps were constructed of either 3/4-inch or 7/8-inch square mesh wire. The daily yield of smallest crawfish within the small grade category harvested with 3/4-inch traps occurred in February and averaged 37.5 count/lb or 12.1 grams (g) each. Yields of the smallest crawfish captured with 7/8-inch traps occurred in June and averaged 31 count/lb or 14.6 grams – a 21% increase in the minimum average size from those captured with the smaller mesh traps. Virtually all crawfish in both cases were immature and were composed of an approximate equal mixture of males and females. These average weights correspond to a carapace width and height of 17.1 and 20.0 mm for crawfish weighing 12 g and a carapace width and height of 17.7 and 21.1 for crawfish weighing 14.5 g. The total length (rostrum to

tail) for these two sizes of crawfish is approximately 7.7 and 8.0 cm, respectively.

## Results at a glance...

- *This research has provided valuable information regarding biological feasibility of establishing standard size grades in crawfish, and offers insight into possible grade categories for several potential systems of classifying graded crawfish for markets. Moreover, marketing surveys have determined that demand for USA-produced crawfish is increasing, with premiums of from \$0.34 to \$1.00 per pound warranted for the larger sizes in the marketplace.*

**Sub-objective 2b:** *Identify grader spacing parameters that will segregate crawfish (captured in 3/4-inch square mesh traps) into 3, 4, 5, and 6 different size grade categories.*

Two hundred and twelve crawfish representing various sizes (weights), maturity status, and both sexes were measured for carapace width and height and overall length. Preliminary grading of crawfish at various bar spacings with a variable fish grader (Fig. 2) revealed that carapace width was the best indicator for determining grader bar spacing. Arbitrary grade categories for each of 4 grading systems (6-, 5-, 4-, and 3-grade systems) were determined for this study (Table 2) based on

observations and discussions of previously used grading categories by some in the industry. These were established to represent a range of possible/practical standards and were used solely for testing purposes. Based on carapace width measurements and measurements of fixed grader bar spacing of the fish grader, settings were established that closely matched the biological data for each grade category within the chosen grade systems.



Figure 2. Size grading of crawfish was accomplished in the boat at harvest with a variable fish grader. Grader was adjusted for different size categories.

Table 2. Target crawfish grade (count per pound) and nearest settings on the fish grader (based on crawfish carapace width measurements) for the categories within each numbered grade system. Also presented are the average size (count per pound) and percentage maturity for crawfish harvested and graded according to the various grader bar spacings for each grade category within the 6-, 5-, 4-, and 3-grade system. Averages were determined for each month of harvest and for the season. Yellow highlight indicates averages that fall outside of the targeted range.

Number of Grades in System	Target Grade (Cnt/lb)	Nearest Setting (mm)	March		April		May		June		Seasonal	
			% Mat	Cnt/Lb	% Mat	Cnt/Lb						
6	< 10	< 26.3	71.7	8.7	84.9	9.8	47.2	9.6	88.9	10.8	72.6	9.6
	10 - 14	23.0 - 26.3	47.7	12.9	55.5	12.4	35.7	13.0	68.6	14.0	49.5	12.9
	15 - 19	20.8 - 23.0	44.1	17.8	63.0	17.1	35.1	16.6	50.7	16.9	47.2	17.1
	20 - 24	19.1 - 20.8	44.9	23.0	41.1	21.2	28.4	20.6	48.2	21.3	40.0	21.9
	25 - 29	17.8 - 19.1	25.9	28.7	41.4	27.7	43.2	24.9	42.3	24.8	39.6	26.5
	> 29	> 17.8	34.4	35.0	27.8	35.7	41.0	32.3	32.7	30.1	33.9	33.0
5	< 12	< 24.6	69.6	11.2	80.4	10.2	48.2	10.6	92.7	10.4	69.9	10.5
	12 - 17	21.5 - 24.6	39.7	17.3	48.4	15.2	60.1	15.1	61.1	14.6	51.4	15.6
	18 - 23	19.9 - 21.5	43.1	18.9	44.7	18.9	54.7	19.8	46.2	19.2	47.4	19.2
	24 - 29	17.8 - 19.9	35.3	25.8	41.0	25.8	44.6	24.1	49.3	23.6	41.5	25.2
	> 29	> 17.8	20.7	36.5	34.1	36.6	43.0	31.4	39.7	31.1	35.8	33.9
4	< 12	< 24.6	77.3	10.5	78.5	10.5	45.8	10.9	76.4	11.1	67.1	10.7
	12 - 18	20.8 - 24.6	39.8	17.3	46.4	17.4	47.1	15.7	53.3	15.7	46.2	16.6
	19 - 25	19.1 - 20.8	39.7	23.5	43.1	23.5	49.9	21.3	41.5	21.1	44.3	22.6
	>25	> 19.1	40.9	26.1	46.1	26.1	48.4	26.0	38.1	26.1	44.7	26.0
3	< 12	< 24.6	58.1	10.7	60.1	10.7	53.2	10.8	73.1	11.2	59.6	10.8
	12 - 21	19.9 - 24.6	50.2	17.0	52.9	16.8	52.7	16.1	43.5	17.0	51.1	16.6
	> 21	> 19.9	24.7	31.9	27.9	29.9	49.8	25.0	40.4	24.2	36.8	27.6

**Sub-objective 2c:** *Determine cross-sectional width dimensions and weight variances of immature and mature crawfish for each of the spacing parameters used in 2b.*

Crawfish were subjected to each grader bar spacing directly as they were emptied from traps over the course of a production season (Fig. 3). For the first part of this sub-objective, a random sample of the smallest crawfish retained by each grader setting and the largest crawfish passing through each setting was collected (on 2-week intervals) and weighed and assessed for sex and maturity. Data was summarized by month and a seasonal average was calculated. Using these data to represent crawfish sizes that fall at either end of the target category when graded, we can determine if a theoretical batch of crawfish, uniform in size and maturity but at the extreme ends of each grade category, might fall outside of the targeted grade. The results indicated that monthly

averages were smaller than the targeted minimum size for a category 5 times in the 6-grade system, 3 times in the 5-grade system, 3 times in the 4-grade systems, and 2 times in the 3-grade system. Likewise, there were 1, 4, 1, and 0 monthly category averages that represented averages larger than the targeted grade for the 6-, 5-, 4-, and 3-grade systems, respectively. Only 1 seasonal grade average was outside (under) the targeted size for a category. It should be noted that all averages outside of the targeted range in this exercise were outside of the range only by small fractions when expressed in count per pound – likely due to the slight difference in actual bar spacings from the desired nominal spacing.

**Figure 3.** Size grading in crawfish is accomplished according to width of the carapace.



For the second part of this sub-objective, crawfish were subjected at harvest to each grader bar setting every other week. Crawfish that passed through the grader at each setting were then placed in holding tanks and graded again after 3 hours. Each batch of crawfish within the holding tanks was subjected to the next grader setting corresponding to the appropriate target standard and no crawfish were subjected to more than the initial grading and one other after the 3 hour reprieve. Crawfish falling into each grading category encompassed a range of sizes (and maturity status) within the spectrum of the two grader settings at time of harvest. Average weight and % maturity were determined for each grade category and this is presented in Table 2 by month of harvest and as a seasonal average. While there were 3 monthly averages slightly outside of the target range, all of the seasonal averages were within the desired grade ranges for category within each grading system tested. Small adjustments in grader

bar settings could possibly reduce or eliminate the undesirable results. It should be noted though that resulting count per pound averages were all within the desired ranges for the 3 and 4 grade system categories. This is likely due to a wider range of individual crawfish sizes contained within each category, which tends to mitigate influences on weight per carapace width as maturity is reached. In conclusion, these results suggest that setting grader bar spacings to closely correspond with the carapace width of crawfish within the desired size ranges, within reason, can achieve suitable results for sorting crawfish by size, even when weight per given carapace width is influenced by physical changes as crawfish mature. Moreover, it is likely that grading standard results will be easier to achieve and less influenced by morphological differences in crawfish if grade categories encompass a wider range of individual sizes when contrasted to a narrow range.

**Objective 3.** *Present findings of Objectives 1 and 2 to the crawfish aquaculture industry via workshops, educational programs, newsletters, etc. with recommendations for establishment of industry-wide adoptable grade standards.*

Preliminary findings regarding market scanning data and crawfish grading biometrics were presented to interested individuals via office and farm visits, and were discussed during a workshop of principal crawfish wholesale buyers/brokers. A partial

summary of field grading results was made available to interested parties via a report published online and in print. Final comprehensive results will be distributed by a variety of means.

## IMPACTS

The impact of this research has provided confirmation that dimorphism associated with maturity in crawfish can impact results of grading operations based on exterior measurements (specifically carapace width) of the animal. However, tangible evidence is presented that suggests satisfactory results can be achieved in grading crawfish by size if precise grader bar spacings are used and if grade standards are kept to a manageable number while avoiding narrow ranges in the acceptable sizes within a grade category.

This is all predicated on grading crawfish at a time when they are less likely to grab and hold onto one another while grading, thereby giving the cleanest grades possible. This information, including the arbitrary target grade specifications used in this study, should be an asset and basis for discussion for the industry when deciding if and what grading standards are needed industry wide.

## **PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED**

McClain, W.R., and J.J. Sonnier. 2011. Key biometrics associated with size grading of live crawfish at harvest. Ann. Res. Rpt., Rice Res. Stn., La. Agri. Exp. Stn., L.S.U. Agricultural Center, 103:263-272.

Yasmin S, M. M. Dey, and K. Singh. 2012. Identifying determinants for development of live-market grading standard for crawfish. Presented at 25th Annual UAPB Research Forum organized by the University of Arkansas at Pine Bluff, on March 7, 2012 at University of Arkansas at Pine Bluff, Arkansas, U.S.A.

Yasmin S, M. M. Dey, and K. Singh. 2012. Perception of store and restaurant managers about crawfish, and their willingness to pay for graded crawfish products. Poster presented at the Field Day (stakeholders event) organized by the Aquaculture/Fisheries Center of the University of Arkansas at Pine Bluff, on October 4, 2012.



## POTENTIAL MARKETING STRUCTURES FOR THE CATFISH INDUSTRY

### Reporting Period

January 1, 2011 – August 31, 2012

<b>Funding Level</b>	Year 1 .....	\$124,996
	Year 2 .....	\$125,000
	Total .....	\$249,996

<b>Participants</b>	University of Arkansas at Pine Bluff.....	Carole Engle, Madan Dey
	Auburn University .....	Terrill Hanson
	Kentucky State University .....	Siddhartha Dasgupta
	University of California at Davis .....	Richard Sexton
	University of Missouri .....	Michael Cook

### PROJECT OBJECTIVES

1. Identify and characterize forms of market organization (including ownership and control of the processing/packing function) that have successfully resulted in higher farm-level prices and rank the forms of market organization that have the greatest likelihood of success for the U.S. farm-raised catfish industry.
2. Develop comprehensive economic analyses to evaluate likely impacts on the U.S. farm-raised catfish industry of implementing proposed structures identified under Objective 1. Results would measure effects on product price, product volume, product characteristics, size of the industry, and competitiveness with imports.

### ANTICIPATED BENEFITS

Policy makers in the United States have given producers of agricultural and aquaculture products the opportunity to engage in horizontal integration and undertake collective action. The fundamental pieces of authorizing legislation at the federal level are the Capper-Volstead Act of 1922 and the Agricultural Marketing Agreement Act (AMAA) of 1937. Capper Volstead authorizes farmers to market their products collectively through cooperative organizations, while the AMAA allows industries to self regulate through marketing orders. Many states

have passed legislation authorizing farmers within a state to self regulate (Lee et al. 1996). Regulations permissible under marketing order statutes include forms of volume control, setting of grades and quality standards, and collection of funds to support research and promotion.

Arguably the need for producer collective action is even more acute today than it was at the time the authorizing legislation was implemented, in light of substantial and rising consolidation in the food

manufacturing and retailing sectors. The increasing consolidation in the food processing and retailing sector and the increasing power of dominant food retailers has been documented by a number of authors including Franklin and Cotterill (1993), Kaufman (2000), Rogers (2001), and Harris et al. (2002). Producers of perishable food products are perhaps most vulnerable to the power of buyers because their products are not storable and must be marketed quickly upon harvest (Sexton and Zhang 1996).

Despite the opportunities afforded by the aforementioned legislation, the track record of farmer collective action in the U.S. is mixed. As this discussion indicates, tools for horizontal integration and collective action available to farmers are quite varied and flexible, with the potential for complementarities among them. Thus, careful consideration of the appropriate options is required if these tools are going to achieve maximum effectiveness for southern aquaculture producers.

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

The industry advisory panel (including representatives from Alabama, Arkansas, and Mississippi)

organized for the project has met with and advised project participants throughout the life of the project.

**Objective 1.** *Identify and characterize forms of market organization (including ownership and control of the processing/packing function) that have successfully resulted in higher farm-level prices and rank the forms of market organization that have the greatest likelihood of success for the U.S. farm-raised catfish industry.*

### University of Arkansas at Pine Bluff

*Responsiveness of unbreaded frozen catfish products over season and across different geographical setting*

Food consumption patterns generally do change over season and across geographical setting. Though the number of studies on seafood demand structure has increased considerably during the 1990's and the 2000's, little attention has been paid on the variability in consumers' responsiveness to changes in prices (product's own price and other product's price) and income/expenditure across species, season and across space (region/division). Hence, the University of Arkansas at Pine Bluff (UAPB) team has conducted an analysis focusing on the effects of season and geographical setting on the demand structure of unbreaded frozen catfish and its substitutes in supermarkets of the U.S.

The study used weekly market-level scanner data

acquired from A. C. Nielsen Inc. for 52 U.S. markets for the period of June 19, 2005 to June 12, 2010. We have extended a state-of-the-art market modeling tool (Almost Ideal Demand System model) by incorporating the seasonal and spatial dimensions, and have estimated the own price elasticity (percentage change in demand for a product due to one per cent change in price of the same product), cross-price elasticity (percentage change in demand for a product due to one percent change in price of another product), and expenditure elasticities (percentage change in demand due to one percent change in expenditure) of demand for 14 unbreaded frozen/chilled finfish products in the U.S. The finfish products considered in the study are salmon, tilapia, whiting, cod, flounder, pollock, catfish, halibut, orange roughy, mahi mahi, tuna, swordfish, perch, and other finfish. We divided the data into quarters to capture the effects of season on

demand: November to January, February to April, May to July, and August to October. We have considered U.S. census divisions to examine the spatial variations in the quantity demanded and the demand elasticities for different finfish products. The U.S. census divisions considered are: South Atlantic, East South Central, New England, East North Central, West South Central, Mountain, West North Central, Mid Atlantic, and Pacific.

The results show that own- and cross-price elasticities as well as expenditure elasticities of demand for different finfish products varied significantly across species and divisions. Seasonal variations are less important than spatial variations in the quantity demanded and the elasticities of demand for finfish products in the U.S. The cross-price elasticity estimates for different divisions show that one finfish product is substituting product for another finfish in one division and complementary to the same product in another division. The analysis further shows that not only the degree of competition among finfish products varies over the divisions, but also the competing products change. The analysis shows that demand for catfish varies considerably more across geographical areas than seasons. The spatial variations are considerably high for catfish. The highest demand for catfish is from West South Central division (145% higher), while the lowest in Mountain division compared to the Mid Atlantic division (base). The responsiveness of catfish demand to changes in its own and substitute products' prices vary over seasons and U.S. census divisions (Table 1). The demand for unbreaded frozen catfish products are own-price elastic (i.e., 1% change in catfish price will change catfish demand by more than 1%). Tilapia is a very strong substitute for catfish in all seasons, but not vice versa. However, their relationship varies across divisions between complementarity and substitutability. For example, tilapia is a substitute for catfish in the East North Central, New England, Pacific, while it is complementary for catfish in Mountain, West North Central and East South Central divisions. Overall,

important substitutes for catfish are perch, flounder, and cod in East South Central division, tilapia and whiting in New England division, salmon and tilapia in East North Central division, whiting in West North Central division, and tilapia in Pacific division.

The responsiveness of demand to a change in expenditure (expenditure elasticity) is greater than one for catfish all seasons. Parallel to own- and cross-price elasticities, the spatial effects on expenditure elasticities of demand for catfish products are more prominent as compared to seasonal effects. South Atlantic and West South Central divisions have expenditure elasticity of demand for catfish near to one; other divisions have expenditure elasticity of demand for catfish greater than one except for the West South Central division where it is as low as 0.39.

The seasonally and spatially varying responsiveness of catfish demand due to the changes in prices and income, denote the necessity to understand the consumer demand behavior across seasons and different geographical settings at species level. For future market planning to increase sales in U.S. supermarkets, the catfish industry needs to consider a market-specific approach regarding substitute products of catfish by including other major white fish products relevant for that market.

*Spatial price transmission and market integration among major markets for unbreaded catfish fillets of the U.S.*

The efficiency of any marketing system depends, among other things, on the perfect market integration and full price transmission with an instantaneous adjustment of any price changes. The UAPB team has investigated the causal and horizontal price transmission relationship among the top seven strategically important retail markets of catfish in the U.S. The study used monthly average retail price data of catfish unbreaded fillets for the period of June 19, 2005 to June 12, 2010. The unique dataset was acquired from the A.C. Nielson Inc. The markets

Table 1: Responsiveness of unbreaded frozen catfish to changes in its own price and other products prices.

	Season					Division							
	Nov-Jan	Feb-April	May-July	Aug-Oct	Mid Atlantic	South Atlantic	East South Central	New England	East North Central	West South Central	Mountain	West North Central	Pacific
Catfish	-1.10	-1.13	-1.22	-1.23	-1.10	-0.70	-5.00	-1.10	-2.03	-1.46	-1.16	-1.07	-1.54
Catfish own-price elasticities													
Salmon	-0.11	-0.07	-0.14	-0.10	-0.11	-0.13	-4.61	-0.39	0.67	-0.97	0.36	0.04	0.24
Tilapia	1.71	1.49	1.46	1.33	1.71	0.19	-0.41	0.89	1.14	0.27	-0.41	-0.57	0.45
Whiting	-1.45	-1.34	-1.43	-1.34	-1.45	-1.10	0.23	0.82	-0.05	1.32	0.13	0.22	0.28
Cod	0.32	0.32	0.38	0.36	0.32	0.24	2.09	0.21	0.05	0.19	0.32	0.37	-0.20
Flounder	-0.74	-0.64	-0.63	-0.63	-0.74	-0.26	2.08	0.04	-0.15	0.22	-0.02	-0.10	0.17
Pollock	-0.07	-0.16	-0.12	-0.08	-0.07	0.09	0.12	-0.22	0.21	0.16	-0.13	0.51	-0.29
Halibut	0.12	0.16	0.11	0.13	0.12	-0.01	0.28	0.23	-0.20	0.08	0.25	0.02	0.20
Orange roughy	0.19	0.19	0.12	0.18	0.19	0.42	0.75	0.07	0.14	-0.14	0.09	0.44	0.27
Mahi mahi	0.07	-0.01	0.28	0.20	0.07	0.24	-0.05	0.11	-0.09	-0.19	0.06	-0.20	-0.03
Tuna	0.02	0.00	0.08	0.09	0.02	0.10	0.23	0.03	0.01	0.15	0.07	-0.04	0.09
Swordfish	0.04	0.04	0.06	0.08	0.04	0.16	-0.17	0.28	0.03	-0.23	0.10	-0.01	0.04
Perch	0.07	0.02	0.09	0.13	0.07	0.09	0.93	-0.01	-0.31	0.00	0.08	0.23	0.04

covered in the study are Memphis, Little Rock, New Orleans/Mobile, Chicago, Los Angeles, Sacramento, and San Francisco.

The analysis shows that New Orleans/Mobile retail price influences the Sacramento and Chicago retail price. A significant causal relationship also exists between the Memphis and San Francisco retail market price. High degrees of integration and price transmission relationships have been observed between those pairs of market. The integration analysis has shown that a significant long-run equilibrium existed between the New Orleans and Sacramento prices, with a price transmission elasticity of 1.23, implying that a one percent change in New Orleans retail price will result in a greater than proportional change of 1.23 percent in Sacramento. Similarly, long-run equilibrium prevailed between New Orleans and Chicago market pair with the price transmission elasticity of 1.09 (significant at the 5%

level of significance). An overreaction of price changes has been depicted between the New Orleans-Sacramento and New Orleans-Chicago market pairs. In both cases, price transmission elasticity has been observed to be more than one. The catfish fillet price of Memphis and San Francisco market has also been found to move closely with the existence of long-run price equilibrium of 0.60, implying that 60% of the price changes in Memphis get transmitted to San Francisco. This probably is a result of the bulk movement of catfish fillets between these markets. Fast exchange of price information between the markets could also be one of the contributing factors behind the observed market integration. In spite of being a large retail market of catfish fillet, Little Rock has not been found significantly integrated with any other domestic market studied. It could be possibly because of the large volume of sales within the state.

### University of California at Davis

One of the fundamental purposes of this project is to study and recommend forms of producer collective action to the U.S. farm-raised catfish industry. If producers are able to act effectively together they will be better able to countervail market power exercised by processors, combat ills caused by international competitors, and build demand for their product. Enhanced demand in turn will enable U.S. farm-raised catfish farmers to receive higher prices and earn higher incomes.

Federal legislation in the U.S. affords producers of agricultural and aquaculture products the ability to act collectively to market their production. The Capper-Volstead Act of 1922 authorizes farmers to jointly market through cooperatives, while the Agricultural Marketing Agreement Act of 1937 allows producers of a specific commodity in a given geographic region of the country to establish and maintain a federal marketing order. Cooperatives

and marketing orders are not mutually exclusive; they can be used in concert to serve producers' marketing objectives.

The present focus of University of California at Davis is on federal marketing orders including (i) analyzing the tools and strategies available under marketing orders, (ii) evaluating the results of using marketing orders in other U.S. agricultural industries, (iii) assessing how establishing a marketing order may benefit the U.S. farm-raised catfish industry and (iv) determining what specific marketing-order provisions should be considered by the industry.

Under the auspices of federal marketing order regulation, producers have a variety of provisions at their disposal including, but not limited to: volume/supply control, generic promotion and advertising, and minimum quality standards. Our research to

date suggests that the minimum quality standard and generic promotion provisions offer the most potential to U.S. farm-raised catfish producers, especially given the increased international competition faced by the industry from China and Vietnam.<sup>1</sup>

These importers are supplying the U.S. market with catfish raised in suboptimal conditions that can directly affect the taste and quality of the fish marketed. Consumers' inability to distinguish quality of catfish *ex ante* constitutes an adverse selection problem wherein poor quality can drive good quality from the market and reduce consumer demand. Imposing minimum quality standards through a federal marketing order represents one option for U.S. farm-raised catfish farmers to address problems caused by inferior imported products. Under U.S. law, imports must meet the same standards that a domestic agricultural industry imposes upon itself.

Generic commodity promotion programs have an extensive history within U.S. agriculture, and numerous studies have documented their overall effectiveness. Economic evaluation of such programs reveals that they are most effective when commodity

markets involve relatively undifferentiated products that, in the absence of a mandatory industry program, would be under-promoted due to free riding. Such conditions are present in the U.S. farm-raised catfish industry. Two small promotion programs are in place presently for promoting U.S. farm-raised catfish. Both operate at the state level and one of them is voluntary, so free riding and under-promotion is a genuine concern under the present structure, creating the potential in our view to achieve producer benefits through a federal program encompassing all major U.S. producing areas and possibly also involving contributions from international competitors.

Appendix A to this Objective Report details the history and current use of marketing orders and reviews relevant literature with specific focus upon the minimum quality standard and generic promotion provisions. Table 2, Federal Marketing Orders and Quality Standards, shows that each extant federal marketing order contains provisions for generic commodity promotions and indicates the orders that utilize minimum quality standards and the types of provisions that are utilized.

## University of Missouri

Project activities of the University of Missouri to date include:

- Review of conceptual models of alternative marketing cooperatives utilized by agricultural and aquacultural producers before attending the initial August 16, 2011 meeting.
- Identification of several models and correlations with institutional environments where highest probability of success might emerge.
- Attendance at initial research meeting in Pine Bluff, Arkansas, August 16, 2011.
- Presentation at the initial meeting of a Life Cycle Approach to examining the intra-firm challenges to developing collective action among and within the U.S. catfish industry.
- Engaged in discussions with team researchers and industry participants regarding the advantages/disadvantages of alternative marketing options which included: state

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<sup>1</sup> Section 8e of the Agricultural Marketing Agreement Act of 1937 (amended in 1954) requires that importers comply with the same minimum quality standards, adopted by the marketing order, that domestic producers face.

**Table 2. Federal Marketing Orders and Quality Standards**

<u>Commodity</u>	<u>Promotion</u>	<u>Grade</u>	<u>Size</u>	<u>Quality</u>	<u>Section 8e</u>
Florida Citrus Fruit	X	E	E		X
Texas Oranges and Grapefruit	X	E	E		X
Florida Avocados	X	E	E		X
California Nectarines	X	E	E		
California Pears and Peaches	X	E	E		
California Kiwifruit	X	E	E		
Washington Apricots	X	E	E		
Washington Cherries	X	E	E		
Washington-Oregon Fresh Prunes	X	A	A		
California Desert Grapes	X	E	E		
Oregon-Washington Pears	X			X	
Cranberries-10 states	X	E*	E*		
Tart Cherries-7 states	X	A			
California Olives	X	E	E		
Idaho-East Oregon Potatoes	X	E	E		X
Washington Potatoes	X	E	E		X
Colorado Potatoes	X	E	E		X
Virginia-North Carolina Potatoes	X	E	E		X
Georgia Vidalia Onions	X				X
Walla Walla Onions	X	A	A		X
Idaho-Oregon Onions	X	E	E		X
South Texas Onions	X	E	E		X
Florida Tomatoes	X	E	E		X
California Almonds	X			X	
Oregon Hazelnuts	X	E	E		X
California Pistachios	X		E	X	
California Walnuts	X	E	A		X
Far West Spearmint Oil	X				
California Dates	X	E	E		X
California Raisins	X	E	E		X
California Dried Prunes	X	A	A		
Oregon and California Potatoes	X				

Source: USDA, 2007. E=In effect, A=Authorized but not in effect, E\*=In effect to withheld or reserve product.

marketing orders, federal marketing orders, marketing boards, traditional cooperatives, new generation cooperatives and Limited Liability Companies.

- Researched the history and evolution of bargaining associations in the U.S.
- Presented to the group overview of bargaining

### **Auburn University**

Attended project meetings and participated in the discussion of the implications of the Capper-Volstead Act of 1922 and the Agricultural Marketing Agreement Act of 1937 on agricultural producers and potentially for aquaculture producers. Auburn University's role in the discussion was primarily to

### **Kentucky State University**

A survey was conducted at several community supported agriculture (CSA) operations in Kentucky (N=60). Each CSA consumer was given two fresh catfish fillets, along with a simple recipe. The consumers were asked to eat the fish, and then answer a set of questions. A payment-card approach was used to determine their willingness to pay for fresh catfish fillets.

Demographics of the respondents were: 1) 97% of CSA consumers were Caucasian, only 1 consumer was Asian; 2) Education (proxy of income): 60% had either graduate degrees or professional degrees; 3) 53% of respondents were female; 4) 53% of respondents lived in a suburban area; 5) 60% of respondents were 50 years old or older; and 6) average household size = 2.68.

Preferences for fish and catfish identified included: 1) 93% indicated that they like to eat freshwater fish; 2) 13% ate catfish once per month; 3) 45% ate

associations, including the role of bargaining associations, the different types, potential benefits and challenges as well as specific issues for consideration for the catfish producers.

- Initiated search for and gathering of materials to prepare case study on aquatic and catfish cooperatives.

provide University of California at Davis (Richard Sexton) and University of Missouri (Michael Cook) information about the U.S. farm-raised catfish industry, current and historical and understand better agricultural cooperative option and their potential in the U.S. farm-raised catfish industry.

catfish a few times a year; and 4) 425 ate catfish less often than "a few times a year".

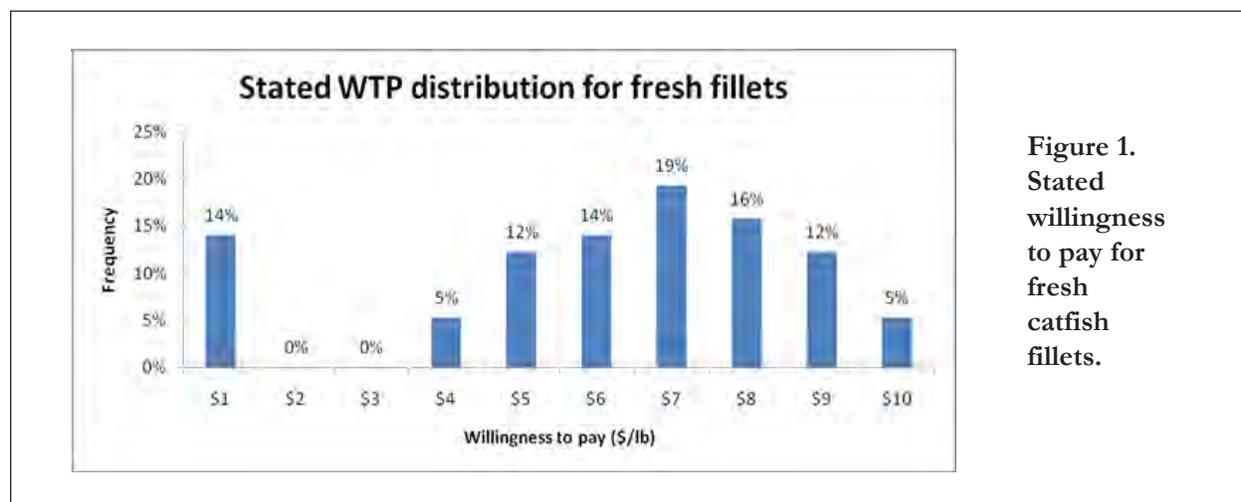
With respect to the sample of fresh fillets they received: 1) Taste: 92% either "loved it" or "liked it"; 2) Texture: 82% either "loved it" or "liked it"; 3) freshness: 97% either "loved it" or "liked it"; 4) 67% consider regular or year-round availability of a product is important; and 5) 75% want their CSA to offer fresh, locally-grown catfish fillets as a future protein.

Demand for fresh catfish fillets: 72% of CSA consumers indicated that they will be willing to buy 1 to 10 pounds of fillets per month, 17% of respondents said that they will not buy any catfish fillets, and 3% of respondents will buy more than 10 pounds of catfish fillets per month. Figure 1 shows the distribution of the willingness to pay responses for fresh catfish fillets.

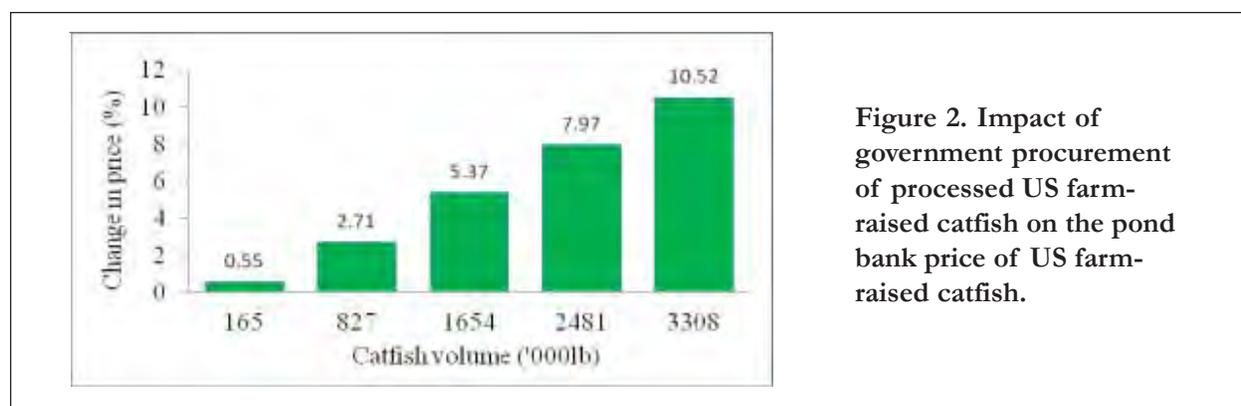
**Objective 2.** *Develop comprehensive economic analyses to evaluate likely impacts on the U.S. farm-raised catfish industry of implementing proposed structures identified under Objective 1. Results would measure effects on product price, product volume, product characteristics, size of the industry, and competitiveness with imports.*

The UAPB team has expanded “The U.S.-Catfish model”, recently developed as part of the SRAC Economic Forecasting project, to assess the impact of various marketing strategies and structure. The team has analyzed the likely effects of the impact of government procurement of processed U.S. farm-raised catfish on the pond bank price of U.S. farm-raised catfish. The results indicate that, if the U.S. government and/or other institutional buyers (such as U.S. military, hospital) purchase about 3 million pounds of processed catfish, pond bank

price of catfish would be increased by about 10% (Figure 2). This result has direct implications for the likely impacts of federal marketing orders on catfish. If provisions of federal marketing orders, such as commodity promotion and minimum quality standards, succeed in raising consumer demand for U.S. farm raised catfish by about 20%, we would expect similar impact (10% increase) on the pond-bank price of U.S. farm raised catfish.



**Figure 1.** Stated willingness to pay for fresh catfish fillets.



**Figure 2.** Impact of government procurement of processed US farm-raised catfish on the pond bank price of US farm-raised catfish.

### Kentucky State University

A survey was conducted in two cities in Kentucky with high Hispanic population concentrations, Lexington and Shelbyville (N=73). The Hispanic Consumer Survey focused on fresh, whole-gutted catfish (2011-2012). The demographics of the consumers surveyed were: Male respondents 58%; average household size = 4; Origin (Mexico 52%; Peru 39%); Age (77% <40 years old); Occupation (32% in “factory work” and 20% in agriculture).

Fish eating habits included: 1) 56% cook fish at home weekly; 2) 71% prefer freshwater fish; 3) 68%

like eating catfish “A Lot” + 20% like eating catfish but not “A lot”. Catfish preferences measured were: 1) live fish preferred by 25%; 2) fresh gutted fish preferred by 53%; 3) fresh fillets preferred by 15%; 4) they do not like frozen catfish (nor frozen fillets); and 5) “will you buy fresh gutted catfish?” YES (84% of respondents); NO (3%). Figure 3 shows the distribution of the willingness-to-pay responses for fresh unprocessed catfish on ice. The average weekly demand per store was 30 pounds (Fig. 4). Stores will pay \$1.50 to \$1.70/pound for unprocessed catfish on ice. Preferred size = 1.5 to 2 pound fish.

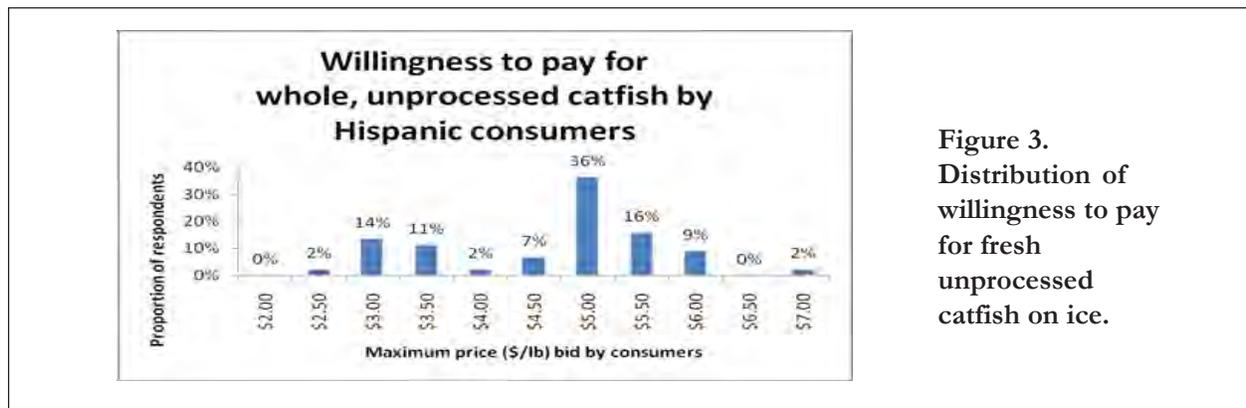


Figure 3. Distribution of willingness to pay for fresh unprocessed catfish on ice.

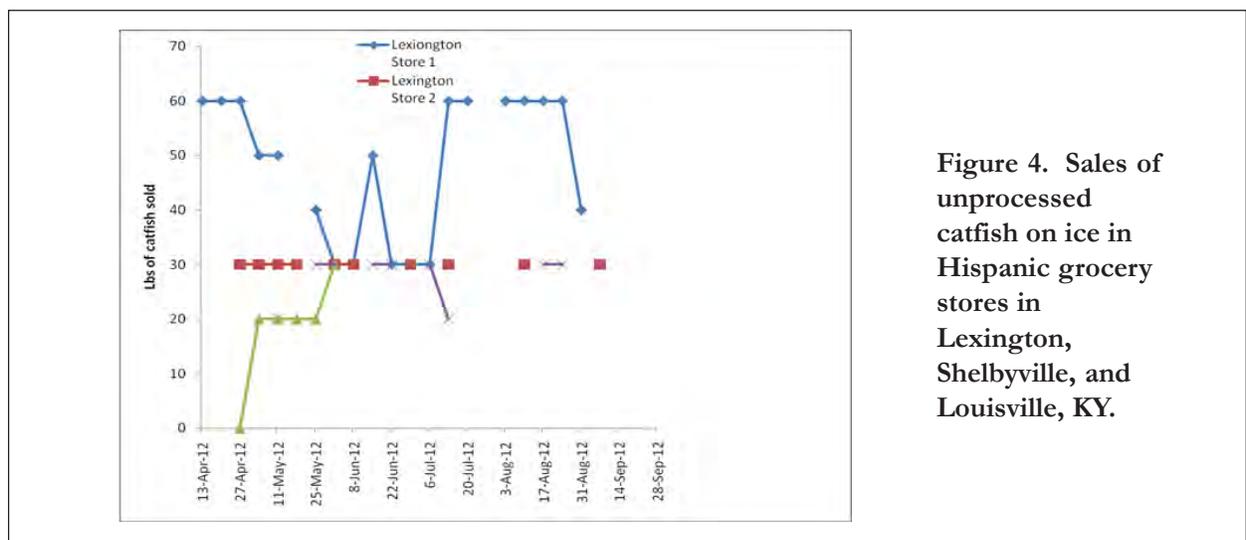


Figure 4. Sales of unprocessed catfish on ice in Hispanic grocery stores in Lexington, Shelbyville, and Louisville, KY.

## Results at a glance...

*A summary table of federal marketing orders and quality standards for various segments of U.S. agriculture has been developed as well as a narrative summary of the use of federal marketing orders in U.S. agriculture. Main highlights for catfish industry are:*

- *Not only does the degree of competition among finfish products vary considerably across markets, but substituting products change.*
- *Understanding of consumer demand behavior across seasons and different geographical settings at species level is essential.*
- *Many of the key markets for catfish fillet are highly integrated with other markets.*
- *New Orleans retail market of catfish fillet is highly integrated with Sacramento and Chicago retail markets, and is causing price changes of these markets.*
- *A significant causal relationship and integration also exists between the Memphis and San Francisco retail market price*
- *In spite of being a large retail market of catfish fillet, Little Rock has not been found significantly integrated with any other domestic market studied.*
- *If the U.S. government and/or other institutional buyers (such as U.S. military, hospitals) purchase about 3 million pound of processed catfish, pond bank price of catfish would be increased by about 10%. If provisions of federal marketing orders, such as commodity promotion and minimum quality standards, succeed in raising consumer demand for U.S. farm raised catfish by about 20%, we would expect a similar impact (10% increase) on the pond-bank price of U.S. farm raised catfish.*

## IMPACTS

The Project has identified alternative marketing structures with potential to provide economic benefits to the U.S. catfish industry. The interactive process between the researchers and industry leaders has led to a consensus that a marketing structure with significant potential to benefit the industry is a federal marketing order organized under statutory authority of the Agricultural Marketing Agreement Act, as amended. Similarly, a complementary cooperative structure can help catfish farmers to

improve their bargaining power.

We have communicated the results of this project to the catfish industry. The industry leaders (including catfish farmers, processors) are seriously considering our recommendation for market specific catfish marketing strategy. The catfish industry has constituted a technical committee to implement the recommendations of the project.

## **PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED**

Singh K., M. M. Dey, and P. Surathkal. 2012. Seasonal and spatial variations in demand for and elasticities of fish products in the United States: An analysis based on market-level scanner data. Under review in *Canadian Journal of Agriculture Economics*.

Singh K., M. M. Dey, and P. Surathkal. 2012. Seasonal and spatial variations in demand for frozen and chilled finfish products in the United States: An analysis of fourteen unbreaded products based on market-level scanner data. Poster presented at the Field Day organized by the Aquaculture/Fisheries Center, University of Arkansas at Pine Bluff, October 4, 2012.

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## APPENDIX A

### Marketing Orders: Commodity Promotion and Quality Regulations

Federal marketing orders are authorized under the Agricultural Marketing and Agreement Act (AMAA) passed by Congress in 1937 (7 U.S.C. § 601 *et seq.*). The AMAA and subsequent amendments provide for five general types of regulatory actions: *i*) restrictions on the quantity of a commodity that can be sold, either through marketing allotments or reserve pools, *ii*) limits on the grade, size, or quality of the commodity, *iii*) regulation of packaging and container sizes, *iv*) generic promotion and advertising, and *v*) production and health-related research.

The AMAA was amended by Congress in 1954 to include “marketing development projects” and in the 1996 farm bill (P.L. 104-127) wherein Congress granted to the U.S. Department of Agriculture (USDA) the ability to create promotion programs for any commodity if the producers wished to have such programs. This amendment stipulated that advertising conducted under a federal marketing order must be generic in nature and not a benefit to some producers and not others. Promotion programs created under this legislation thus “stand alone” and do not operate under the auspices of a federal marketing order.

Federal marketing order programs must be for specific commodities and in as small a region as possible to further the objectives of the order. The process to create a federal marketing order begins with a producer initiative to the USDA. The USDA will conduct hearings on the proposal and if the industry’s rationale seems consistent with the AMAA and subsequent amendments, the proposal is put to an industry referendum. If two-thirds of the producers in an industry (or producers representing two-thirds of the value of production) vote in favor of the regulation, it is set in place and its provisions are legally binding upon all who operate under the order’s auspices (a detailed overview of the establishment process is provided at the USDA Agricultural Marketing Service Website by linking to the subject “Marketing Orders and Agreements”). A vote by a simple majority vote by the producers can abolish an extant order.

Federal marketing orders operate under the control of an elected producer board whose appointments are approved by the Secretary of Agriculture. However, ultimate decision authority lies with the Secretary, who must approve board recommendations. An equivalent standard of government approval exists for marketing programs that have state authorization. In these cases approval of board decisions must come from the head of the state’s department of agriculture. Programs are funded by assessments on producers and sometimes on handlers of the commodity.

Among the possible functions that can be performed under federal marketing orders, we have focused to date upon two—commodity promotion and minimum quality standards—that seem especially relevant to the U.S. catfish industry. For example, in 1999 the Arkansas Catfish Promotion Board was created to promote growth and development of the state’s industry through research and promotion. The Board’s funding is collected by an assessment of one dollar per ton on all catfish feed purchased by commercial Arkansas catfish producers. A state-level program with multistate production of catfish and relative product homogeneity across states means that producers from other states benefit as free riders on benefits generated from Arkansas promotions. Volume control programs, although important at the inception of the AMAA (Sexton and Alston 2009), are utilized

actively today in only a few industries, and it is generally understood that the USDA will not approve new volume-control programs.

### *Commodity Promotion Programs*

The main justification for generic commodity programs is that agricultural products are, essentially, homogeneous (undifferentiated), and, because benefits of advertising by one firm inure to all firms, free-rider problems create little incentive for unilateral promotion. Opponents of commodity promotion programs have challenged this characterization, arguing that their products were differentiated from those of competitors. Thus, even if total demand increased with generic advertising, the effects would not be consistent among growers. Specifically, opponents have argued that generic promotion reduced the differentiation among products and, therefore harmed producers who had worked to establish a brand identity. These arguments, notably, would appear at present to have little resonance in the U.S. catfish industry where product differentiation seems unimportant. Agricultural industries in the United States spend about \$1 billion dollars annually on producer-funded, generic marketing programs. Ninety percent of all U.S. farmers pay assessments to support at least one commodity promotion program (Congressional Research Service 2005, p. 52).

The marketing programs that allow generic advertising and promotion exist under various state and federal statutes in addition to the AMAA, although the goals of the various programs are similar. So-called “stand-alone programs” are authorized by separate legislation or farm bill legislation, and are generally significantly larger in the amount of funding involved than those under marketing orders. Currently 51 agricultural industries are covered by federal programs and many others are promoted under state programs. However, only the federal programs can encompass inter-state industries. A list of federally authorized commodity marketing programs is provided in Table 1 of this annual report.

Nearly 250 research studies including 124 peer-reviewed journal articles and chapters in 14 books have examined the effectiveness of commodity promotion programs. The overwhelming majority of these studies have shown that the benefits outweigh the costs (Alston, Crespi, Kaiser and Sexton 2007). Most studies report a benefit-cost ratio wherein the producer benefit (additional profit) generated from the program is divided by the share of program costs borne by producers. Even if producers pay 100 percent of an assessment, the incidence upon producers will normally be less than 100 percent due to tax shifting. In nearly every study this benefit-cost ratio is higher (and often substantially higher) than one, meaning that the commodity program not only worked but worked very well because a dollar spent on it earned the industry greater than a dollar’s worth of revenue. See, for example, the summary of various studies provided in Alston, Crespi, Kaiser, and Sexton, 2007.

Given the general consensus on the overall effectiveness of commodity advertising programs, more recent research has focused on distributional issues. Is it true that the rising tide caused by a successful advertising program raises all boats? Scant research has addressed the claim that generic advertising can frustrate firms’ attempts to create product differentiation through their own advertising. This concern is of paramount importance given the growth in number of product varieties and amount of branded advertising in modern agricultural markets. One exception is Chakravarti and Janiszewski (2004) who in a lab experiment showed that generic advertisements could reduce consumers’ responsiveness to branded advertisements, thus making a firm’s own advertising less successful than if the generic program did not exist, just as program opponents have argued.

Another key trend in U.S. agricultural markets that impacts promotion effectiveness is consolidation and increased market concentration in food manufacturing, and retailing. Market power of retailers and food manufacturers, both as buyers from farmers and sellers to consumers, is a legitimate concern in many markets. Only a few commodity-promotion studies have taken these considerations into account when investigating the benefits of generic marketing programs. Norman, Pepall and Richards (2008) showed that when industry concentration is low, generic programs are welfare improving but when concentration is high, there may not be a good reason to have generic advertising. Suzuki and Kaiser (1997), Kawaguchi, Suzuki and Kaiser (1997), Chung and Kaiser (2000), and Wohlgenant and Piggott (2003) looked at the effect of generic advertising by size of firm and/or in imperfectly competitive markets and in markets with differing farm supply elasticities, finding various differential effects among producers.

These studies suggest that outside of the idealized market setting where goods truly are homogeneous and firms are unable to differentiate their products, the market structure of the industry is immensely important to understanding the potential of generic promotions to boost farmers' incomes. Further, while most studies examine what is happening at the production sector, Zhang and Sexton (2002) examined the entirety of the supply chain and showed that at least half of the benefits from an advertising program will not get to the farmers if either the processing or retailing sectors are imperfectly competitive, and will instead be captured by the players holding market power.

What if promotions succeed in raising consumer demand, but downstream sellers such as retailers and food-service establishments capture that demand shift in the form of higher prices? No more farm product is sold in such a case, and, thus, farmers derive no benefit from a program that "worked" in the sense of raising consumer demand. Little research has been conducted into such possibilities. One exception is work by Carman, Li, and Sexton (2009), which used retail-level scanner data to examine both price and quantity impacts of promotions conducted by the Hass Avocado Board. They found no evidence that retailers raised prices in response to avocado promotions.

Relevant directly to the U.S. catfish industry is the comparison of voluntary commodity promotion programs to mandatory programs. The Alabama Catfish Producers Association administers a voluntary catfish commercial feed assessment of 50 cents per ton of feed manufactured, bought, and sold in Alabama to fund the research and promotion activities of Alabama catfish producers. Whereas mandatory programs must have federal or state mandates and supervision, voluntary programs can operate entirely under industry auspices. Messer, Kaiser, and Schulze (2008) examined voluntary programs, noting that many of today's mandatory programs began as voluntary programs. They report that free riding on voluntary programs tends to increase over time, often causing producers to seek to establish mandatory programs. These authors conducted experiments with voluntary promotion programs that tended to replicate the progressive incidence of free riding in successive iterations of the experiment. However, the introduction of a "provision point mechanism", which is a threshold participation rate (70% in their base case), substantially reduced free riding. If participation falls below the provision point, all contributions are refunded and no expenditures take place, limiting the opportunity to free ride.

#### *Minimum Quality Standards*

Federal marketing orders enable producers to self-regulate the quality of their production with approval by the Secretary of Agriculture by choosing whether or not to impose minimum quality standards (MQS) and what

standards to set. Through amendments to the AMAA enacted in 1954 (section 8e) imports can be made subject to the same quality standards, regulations, and other provisions as are imposed upon domestic production by a marketing order. Thus federal marketing orders allow industries to influence international competition and protect the domestic market from being downgraded by the receipt of poorer quality product from abroad. Article III of the General Agreement on Tariffs and Trade (GATT) requires that imports not be held to higher standards than domestic production, therefore section 8e requirements can only be effect when domestic production is being produced, regulated, and shipped (USDA, 2007). Yet, since catfish are grown, processed, and sold on a year-round basis, section 8e requirements would apply to imports on a continuous basis.

MQS imposed through federal marketing orders are relatively common for fruit and vegetable commodities in the United States. Currently, 16 of the 31 commodities regulated under federal marketing order statutes are subject to section 8e requirements of the AMAA. A summary of these commodities and the specific product attributes regulated is provided in Table 1 *Federal Marketing Orders and Quality Standards*. Of the 31 marketing orders currently operating under the federal statutes, 29 have some combination of grade, size, quality, or maturity provisions authorized or in effect (USDA, 2007). Twenty-five of the federal marketing orders have minimum grade standards in place, 25 have size regulations in authorized or in effect, and 3 have general “quality” regulations in effect.

Increasing international competition faced by domestic farm-raised catfish producers and growing concerns of U.S. consumers surrounding the safety and quality of fish make the establishment of MQS worthy of U.S. catfish producers’ consideration. Since 2003 U.S. farm-raised catfish producers have faced increased international competition, primarily from Vietnam and China. From May 2010 to May 2011, the U.S. has seen a 64 percent increase in the amount of Siluriforme catfish imported (USDA 2011).

Catfish produced in Asian countries are typically raised in floating cages on rivers and ponds while being fed a diet of agricultural by-products consisting of rice bran, soy, and fish by-products (Orban et al. 2008). In addition to being subject to chemical contamination from high anthropic pollution, fish raised in these fresh-water, caged environments also often test positive for organochlorine pesticides and polychlorinated biphenyls (PCBs), which have been banned in the U.S. due to human health concerns (Orban et al. 2008).

Processed catfish cuts and fillets are relatively indistinguishable in terms of their origin. As such, without mandatory country-of-origin labeling and/or safety and quality standards in place, imported fish with inferior quality or taste may compromise domestic demand. The problem as described originally by Leland (1979) is one of adverse selection or Gresham’s law. When both high-quality and low-quality products are available on the market and are indistinguishable ex ante to consumers Gresham’s law states that the bad product will drive the good product out of the market. One potential solution to this problem is for individual farmers to attempt to voluntarily certify the quality of their production. Such certification is, of course, costly and often ineffective because consumers are generally skeptical as to the reliability, stringency, and credibility of voluntary certification. Thus, certification of quality at the industry level is often necessary to surmount the adverse selection problem. MQS maintain and/or enhance market demand for commodities by ensuring that the poorest quality product doesn’t reach consumers (Carmen and Alston, 2005) and, thus, deter them from making future purchases. MQS in this setting can at once mitigate imports, improve overall product quality, and stabilize or increase consumer demand. International shipments that do not meet the MQS may be (i) reconditioned for re-inspection, (ii) re-exported, or (iii) sent to an exempt (normally, nonhuman) use.

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## REPRODUCTION AND LARVAL REARING OF FRESHWATER ORNAMENTAL AND MARINE BAITFISH

### Reporting Period

January 1, 2011 – August 31, 2012

<b>Funding Level</b>	Year 1 .....	\$167,778
	Year 2 .....	\$169,429
	Year 3 .....	\$162,637
	Total.....	\$499,844

<b>Participants</b>	University of Florida (Lead Institution) .....	Cortney Ohs, Craig Watson
	Louisiana State University .....	Chris Green, Ronald Malone
	Mississippi State University .....	Louis D'Abramo

### PROJECT OBJECTIVES

1. Develop improved technologies for spawning and larval rearing of pinfish.
  - a. Evaluate efficacy of catfish pituitary extract on spawning induction of pinfish.
  - b. Evaluate dosing of catfish pituitary extract on spawning induction of pinfish.
  - c. Compare human chorionic gonadotropin and catfish pituitary extract on the spawning induction of pinfish.
  - d. Evaluate commercial rotifer enrichments and their effects on larval survival and growth.
  - e. Evaluate larval feeding regimes employing copepods and rotifers and their effects on larval survival and growth in pinfish.
  - f. Evaluate the effects of stocking density on survival and growth of larval pinfish.
2. Develop improved technologies for spawning and larval rearing of goggle eye.
  - a. Evaluate the efficacy of Ovaprim on spawning induction of goggle eye.
  - b. Evaluate larval feeding regimes employing copepods and rotifers and their effects on larval survival and growth.
  - c. Evaluate the effects of stocking density on survival and growth of larval goggle eye.
3. Evaluate spawning substrate preference for captive ballyhoo.
4. Develop improved technologies for egg hatching and larval rearing of *Fundulus grandis* and *Fundulus seminolis*
  - a. Evaluate air incubation of *Fundulus* sp. eggs.

- b. Identify a replacement of live feeds for *Fundulus*.
  - c. Determine relationship between larval density and performance in *Fundulus*.
5. Develop improved technologies for spawning and larval rearing of Bala shark
- a. Improve Bala shark broodstock maturation.
  - b. Develop technologies for induced spawning of Bala shark.
  - c. Develop improved technologies for larval rearing of Bala shark
  - d. Design water treatment technologies for commercial larval rearing of Bala shark
6. Publication, extension, and dissemination of results.

## ANTICIPATED BENEFITS

Baitfish culture has long been dominated by production of freshwater species. Culture of marine baitfish is a logical progression for the region and offers enterprise diversification and increased marketing opportunities. Pinfish, *Lagodon rhomboides*, will be induced to spawn with both HCG and catfish pituitary hormone. At the termination of the project, research results will provide knowledge about specific methods for induced spawning using an FDA approved hormone (HCG). Additionally, the results may provide the impetus for a potential INAD expansion for catfish pituitary extract. A larval feeding regime that includes the identification of optimal live feed organisms with proper enrichments will be characterized for pinfish. Goggle eye, *Selar crumenophthalmus*, will be spawned using

previously established methods. Optimal stocking density and larval feeding regimes, including live feed and enrichment selection, will be defined. The spawning substrate preference of ballyhoo *Hemiramphus* sp. will be investigated. Research with Gulf killifish, *Fundulus grandis*, and Seminole killifish, *Fundulus seminolis*, will address the development of protocols for air incubation of eggs which will optimize fry production, survival, and growth. These data will help to establish future recommendations to producers about the optimal methods of incubating eggs within a humid environment to delay hatch and better coordinate stocking of larger numbers of *Fundulus* fry. Feeding and density trials will identify efficient culture methods to produce *Fundulus* juveniles.

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

**Objective 1.** *Develop improved technologies for spawning and larval rearing of pinfish*

### University of Florida Indian River Research and Extension Center

Two trials were conducted to examine the effects of commercial rotifer enrichments on growth and survival of larval pinfish. Eggs were collected from two volitional spawns from brood held in a 2,000 L re-circulating system. Eggs were incubated in seawater (~32 g/L) at 77.6 degrees F. At 1 days post hatch

(dph), larvae were stocked into replicate tanks (14.75 L) at 100 larvae/L for each of the treatments (Non-enrichment, OriGo, AlgaMac 3050, and DHA Protein Selco) in each trial and fed rotifers twice daily beginning at 3 dph throughout 11 dph. Rotifer enrichment procedures adhered to manufactures'

recommendations. Tanks were flushed with continuous flow through seawater (~32 g/L; 73.4 degrees F) with a minimum daily water exchange of 200%. Tanks were also inoculated daily with microalgae (*T-iso*) at approximately 200,000 cells/mL. Larvae were sampled at 6 and 11 dph and photographed for growth measurements (notochord length (NL). Survival and percent swim bladder inflation were determined from all larvae

harvested at 11 dph.

Pinfish larvae fed rotifers enriched with OriGo had higher survival and growth at 11 dph. Larvae fed rotifers enriched with DHA Protein Selco had higher swim bladder inflation rates (Table 1). Additional trials will be conducted to confirm these results.

**Table 1. Mean notochord length (NL) of larval pinfish fed different enrichments via rotifers. Survival and swim bladder inflation as a percentage at 11 DPH.**

Run	Enrichment	NL (µm) at Stocking	NL (µm) at 6 DPH	NL (µm) at 11 DPH	Survival (%)	Swim Bladder inflation (%)
1	Non-enriched	281.07 ± 1.09	288.54 ± 3.56	349.42 ± 6.07	12.78 ± 3.29	16.99 ± 1.20
	Ori-Go	281.07 ± 1.10	287.27 ± 4.32	369.46 ± 6.89	17.36 ± 7.53	14.79 ± 4.26
	Algamac 3050	281.07 ± 1.11	285.79 ± 4.77	344.78 ± 7.21	9.97 ± 1.42	2.27 ± 2.27
	DHA Protein Selco	281.07 ± 1.12	305.41 ± 4.85	364.95 ± 7.19	14.81 ± 0.17	18.93 ± 1.07
2	Non-enriched	283.12 ± 1.65	294.67 ± 2.50	352.72 ± 8.63	0.86 ± 0.11	22.86 ± 19.48
	Ori-Go	283.12 ± 1.66	292.52 ± 2.01	341.53 ± 9.103	3.66 ± 2.69	17.92 ± 16.29
	Algamac 3050	283.12 ± 1.67	296.71 ± 3.03	349.22 ± 14.44	0.57 ± 0.21	27.27 ± 27.27
	DHA Protein Selco	283.12 ± 1.68	294.18 ± 2.61	355.29 ± 6.06	1.72 ± 0.54	25.51 ± 13.69
Mean	Non-enriched	282.10 ± 0.99	293.02 ± 2.08	350.36 ± 4.95	3.84 ± 2.05	21.18 ± 13.49
	Ori-Go	282.10 ± 0.10	291.21 ± 1.86	356.63 ± 5.80	7.09 ± 3.31	16.87 ± 10.38
	Algamac 3050	282.10 ± 0.10	292.88 ± 2.65	345.67 ± 6.39	2.92 ± 1.57	14.77 ± 13.30
	DHA Protein Selco	282.10 ± 0.10	296.99 ± 2.35	359.58 ± 4.64	4.99 ± 2.18	23.63 ± 9.53

**Objective 2.** *Develop improved technologies for spawning and larval rearing of goggle eye.*

Objective 2 will be addressed in year 3.

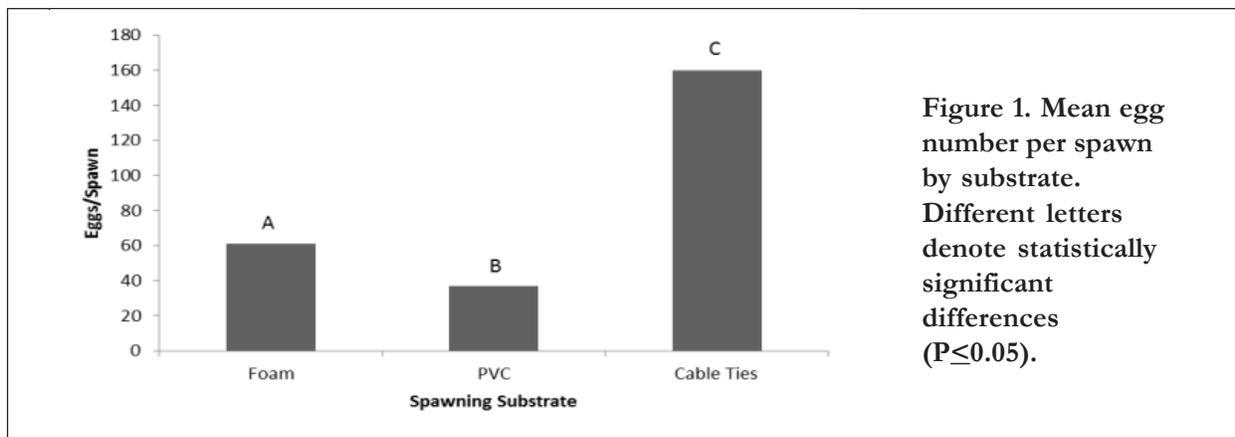
**Objective 3.** *Evaluate spawning substrate preference for captive ballyhoo.*

Ballyhoo are a high valued marine baitfish commonly used by anglers to target multiple species of pelagic game fish. In the wild, ballyhoo attach their adhesive eggs to marine substrate (*Sargassum*, sea grasses, flotsam etc.). Broodfish were collected from the wild and have been cultured in tanks for over two years at University of Florida Indian River Research and Education Center. Volitional spawning was observed in tanks and eggs were collected from filters and substrate placed within the tanks. Little is known about captive spawning and substrate preference.

Substrate preference experiments were conducted with two populations of *H. balao* (21 and 22 fish), maintained in 6000 L tanks at a salinity of 35 g/L and temperature of 67.1 to 85.3 degrees F. Three substrates (foam, PVC, and plastic cable ties) were

placed within the tanks and left undisturbed for a minimum of 18 hours. Substrates were subsequently removed and spawned eggs were enumerated for each substrate material. Additionally, the tank bottom, bag filter, and submerged airlines, were also monitored daily for any egg deposition. Substrate position was rotated daily to eliminate any positional bias within the tank. Spawned eggs were quantified five times per week (Monday-Friday) and water quality was monitored daily.

A total of 266 spawning events were recorded from ballyhoo cultured in both experimental tanks from January 23, 2012 through August 7, 2012, with 142,822 eggs collected. Analysis of substrate preference revealed cable ties were the preferred spawning substrate with a mean of 160 eggs collected from each spawning event (Figure 1).



**Objective 4.** *Develop improved technologies for egg hatching and larval rearing of *Fundulus grandis* and *Fundulus seminolis*.*

**Sub-objective 4a.** *Evaluate air incubation of *Fundulus sp.* eggs.*

**Louisiana State University**

For Gulf killifish and some related coastal species, spawning events are timed to semilunar tidal cycles

where embryos are deposited at the high water mark of marsh grasses during spring tide and are

exposed to air once the tide recesses. During this period, commonly referred to as terrestrial or air incubation, embryogenesis occurs at an accelerated rate compared to incubation in typical aquatic conditions. Air incubation appears to be a common occurrence in wild Gulf killifish. Females are known to lay their eggs among the marsh grass during maximum high tides where they develop fully exposed to the humid air when the tide recedes. The eggs then hatch when they are flooded by the next maximum high tide, approximately 13-15 days later. This situation can be replicated in an aquaculture setting. Air incubation encourages all of the eggs to hatch at the same time yielding uniform sized larvae and subsequently uniform adult minnows. This reduces the likelihood of larger older minnows eating the newly hatched larvae. In addition, air incubation provides the opportunity for easy transport of eggs to grow out facilities or other locations.

#### Year 1

Embryos were manually removed from the spawning substrate material and dead and pigmented embryos were discarded. Live embryos were quantified and treatments consisted of approximately 1,300 embryos sandwiched between two pieces of polyurethane hobby foam in triplicate for each respective temperature treatment. A solution of saline water (7.6 g/L) was mixed using artificial sea salts and was used to moisten the foam. Embryos and hobby foam were then covered with plastic to prevent desiccation while in the incubation chambers. Incubation chambers were set to nominal values of 68, 73, 79, and 86 degrees F with adjustable thermostats.

Time required for embryos to progress through five stages of development was recorded to determine the rate of embryogenesis. Staging was based upon descriptions detailed for the mummichog. Twelve embryos were randomly selected from each temperature-treatment triplicate to determine stage of development. If more than

75% of embryos were at a target stage, treatments were sampled for heart rate and ammonia, urea, and lactate concentrations. Embryos began terrestrial incubation for this study at stage 15. Stage 35 marked the stage at which embryos attain the ability to hatch when placed in an aqueous medium and therefore the transition into delayed hatch. Replicates were sampled in 48-hour delayed hatch intervals after reaching stage 35 until embryos could no longer be sampled due to mortalities. Embryos were sampled at 48-hour intervals for heart rate, morphometric parameters at hatch, and ammonia, urea, lactate and ATP concentrations.

Temperature did not have a significant influence on percent of viable embryos at stage 25. Percent of viable embryos were  $59 \pm 2\%$  at 68 degrees F,  $62 \pm 3\%$  at 73 degrees F,  $58 \pm 8\%$  at 79 degrees F, and  $75 \pm 1\%$  at 86 degrees F. Temperature had a significant effect on the period of time that delayed hatch embryos remained viable. Embryos began to hatch spontaneously on the substrate beginning at 96 delayed hatch hours in the 79 degrees F and 86 degrees F, but did not hatch on the substrate in the 73 degrees F and 68 degrees F treatments. The longest extent of delayed hatch was 320 hours post stage 35 for the 68 degrees F treatment, followed by 272, 224, and 176 hours for 73, 79, and 86 degrees F treatments, respectively. Hours of delayed hatch was significantly related to the total length (TL) of the embryo upon hatch. Size at hatch (TL) and body cavity area were not significantly related to temperature.

An accelerated rate of embryogenesis was observed during air incubation relative to aquatic incubation of this species. Temperature associated stresses were also observed in addition to stresses caused by air incubation. Embryogenesis for the 86 degrees F treatment was relatively brief compared to lower temperatures and first hatch occurred at 96 delayed hatch hours, although embryo viability began to decrease upon the initiation of delayed hatch and high urea concentrations were observed with delayed

hatch. Temperature can likely be modified during incubation to custom delay or accelerate embryo development based on the specific need of the culturist to time the hatching of different batches of eggs.

## Year 2

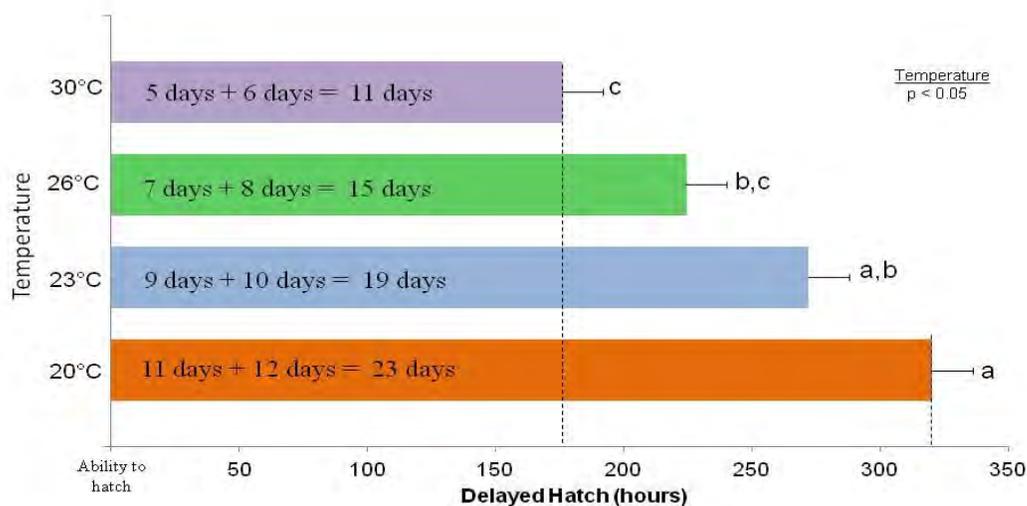
Incubators were constructed from small dormitory style refrigerators and each was fitted with an external thermostat. Incubation temperatures were set at 68, 73, 79, and 86 degrees F. Sheets of synthetic foam (Expanded Polystyrene) or soft hobby foam were soaked in clean saline water at a salinity of 10 g/L. These foam sheets were wet to the touch and not overly saturated or dripping with water. Sheets of foam were placed in a shallow plastic storage container. Newly fertilized Gulf killifish eggs were placed in a monolayer across the foam and gently covered with another moist foam sheet of the same size. The lids on the containers were secure but did

not form an airtight seal. Temperature data loggers were placed in each incubator to record humidity and temperature for the duration of incubations.

Embryo viability and ability to hatch at treatment temperatures was monitored once daily. A sample of embryos from each temperature treatment was placed in water to observe if they hatched and determine the minimum number of incubation days required at each temperature treatment. If larvae hatched within five hours of immersion, they were preserved in 10% buffered formalin for morphometric analysis. Throughout incubation, egg mortalities were monitored to determine the maximum number of incubation days allowed for viable embryos to be extended.

The earliest or minimum number of incubation days required for hatch occurred at a temperature of 86 degrees F at 5 days (Figure 2). At this high temperature

**Figure 2. The maximum number of hours incubation can be extended in Gulf killifish, *Fundulus grandis*, reared humid environment across a range of temperatures. The minimum number of incubations days are listed first in each temperature treatment followed by the number of days incubation can be extended. Temperature significantly influenced incubation and significant differences among temperature treatments are denoted with different letters.**



the maximum number of days allowable for viable hatch is approximately 11 days. Past 11 days at 86 degrees F the embryos utilize all of their yolk volume and expire. At the lowest treatment temperature (68 degrees F) the minimum number of days required to obtain hatch is 10 days, while the maximum number of incubation days is approximately 23 days.

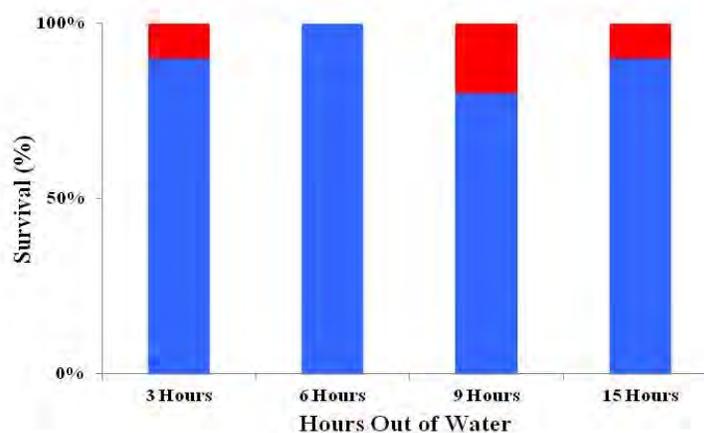
During periods of stranding many fish species will undergo a buildup of metabolites that can lead to their demise. In order for the Gulf killifish to survive it must respire while managing the accumulation of toxic metabolites that it usually removes through the gills. We have previously demonstrated that eggs can be held in moist environments and have been contacted by many stakeholders due to their interest in transporting small numbers of these baitfish out-of-water.

For this study Gulf killifish were wrapped in moist cheesecloth, placed inside a plastic container and then kept in a temperature controlled incubation chamber. Surviving fish were sampled after terrestrial

stranding periods of 0, 3, 6, 9, and 15 hours. Respirometry was used to measure standard metabolic rate in fish during an aquatic recovery period immediately following stranding. Remaining survivors were sampled for plasma and gill tissue. Plasma samples were used in assays to determine urea, ammonia, and lactic acid concentrations. Urea and ammonia are nitrogenous wastes that build up in plasma as a result of protein utilization. Lactic acid is produced when undergoing anaerobic (lacking in oxygen) conditions and can cause things like cramping while jogging. All of these metabolites can normally be processed at the gills but if not they may prove fatal when high concentrations occur in the blood.

In many species, terrestrial stranding proves lethal relatively quickly, possibly due to critical increases in high concentrations of lactate, urea, and ammonia. Survival was independent of stranding which highlights the remarkable ability of the Gulf killifish to withstand extended periods of terrestrial stranding (Figure 3). Under normal conditions the fish would filter waste through the gills but absence of a significant increase in plasma concentrations of

**Figure 3. The percent survival of Gulf killifish, *Fundulus grandis*, adults held out of water for 3, 6, 9 and 15 hours.**



ammonia and urea may indicate that the metabolites are being processed in alternative ways throughout stranding.

Respirometry data showed a significant decrease over time, which indicates that the fish undergo metabolic changes dependent on stranding. It is possible that an accumulation of mucus on the gills prevents them from drying and this would be reflected in the recovery because it may take time to

remove the mucus and begin respiring normally. This data may also indicate a change in heart rate known as bradychardia that would be used to slow down the respiration and build up of metabolites. It is clear that both Gulf killifish embryos and adults possess the rare ability to sustain terrestrial stranding. Culturists can take advantage of these unique attributes with both fry production and the transport of embryos and adults.

**Sub-objective 4b.** *Identify a replacement of live feeds for Fundulus.*

### University of Florida Indian Research and Education Center

Use of a microparticulate diet saves time, space, and labor associated with live feeds, eliminates the potential of disease introduction from the live feeds, and ultimately should reduce the cost of production of juvenile fish. The microparticulate microbound diet used was previously proven to be an effective and complete substitute for live *Artemia* nauplii in the culture of two species of crustaceans, *Macrobrachium rosenbergii* and *Litopenaeus vannamei*, and zebrafish (*Danio rerio*). The diet served as a partial *Artemia* replacement for 20-days post-hatch pinfish larvae and is being tested with larvae of several different marine fishes.

Year 1

Larval Gulf killifish were cultured in 15L circular fiberglass tanks with flow-through water providing an exchange of approximately 2 tank volumes/day. Upon hatching, 50 larvae were randomly stocked into a tank and randomly assigned one of three diet treatments: microbound microparticulate diet exclusively for 15 days (MICRO), *Artemia* nauplii exclusively for 15 days (ART), or *Artemia* nauplii for 5 days followed by a mix of *Artemia* and microparticulate diet for 5 days followed by the microparticulate diet exclusively for the remaining 5 days (MIX). There were 5 replicates assigned for each treatment.

The microparticulate diet was developed by L. R. D'Abramo at Mississippi State University. On a dry weight basis, the proximate composition of the microparticulate diet was 46.1% crude protein and 37.4% crude lipid. The microparticulate diet was stored frozen at -4 degrees F. Prior to every feeding, a portion of the microparticulate diet was removed from storage and added to a small volume of culture water. This was done to prevent it from clumping and floating, and to achieve a homogeneous particle size. Larvae were fed the microparticulate diet in excess twice daily.

The proximate composition of *Artemia* nauplii on a dry weight basis was 53.8% crude protein and 16.2% crude lipid. *Artemia* cysts were disinfected prior to hatching by exposing to a 2% hypochlorite

## Results at a glance...

- *This was the first study to show that microparticulate diets can be used to culture Fundulus larvae. Survival of fish fed the microparticulate diet alone from 0 to 15-days post-hatch was 95%.*

solution for 10 minutes and aerated. *Artemia* cysts were disinfected and hatched daily at a salinity of 3g/L. After harvesting, the concentration of hatched *Artemia* was determined by counting a subsample so each treatment received the same amount of *Artemia*. Larvae were fed in excess twice daily. Before feeding, uneaten microparticulate diet and dead *Artemia* were removed from the bottom of each tank. Excess uneaten live *Artemia* were removed from the surface of the water with a fine-mesh net.

At 0, 5, 10, and 15 days post-hatch, five larvae were removed from each tank. Photographs of larvae were taken using a stereo microscope outfitted with a digital camera to measure total length (TL) of each larva.

There were significant differences in total length of larvae among diet treatments at 5, 10, and 15 days post-hatch (Table 2). ART larvae were the largest during the 15-day experiment. Survival among the treatments was significantly different. The MIX diet larvae had no mortalities during the experimental period. The growth of larvae in the MICRO and MIX treatments were 71.5% and 83.9%, respectively, of that of the MICRO treatment after 15 days. However, the feeding schedule used in this experiment most likely affected growth in the treatments which received microparticulate diet

because live *Artemia* nauplii were available in the water column for a longer period of time than the microparticulate diets. *Artemia* nauplii were present in the appropriate tanks at the next feeding. If the microparticulate diet would have been available in the water column for a longer period of time, the larvae may have been able to increase consumption, thereby increasing growth. If the feeding of the microparticulate diet had been split into more feedings or placed in an automatic feeder, the results may differ. Feeding schedules need additional investigation.

## Results at a glance...

- *This study was the first evaluation of diets to be used for culturing Seminole killifish larvae. A 95% survival was achieved in larvae fed the MICRO diet exclusively for 15 days. While the survival was statistically significantly less than the survival in the MIX and ART treatments, survival was very high and can be considered a success in the larval culture of Seminole killifish. This study demonstrated that Seminole killifish larvae can be cultured exclusively on a microparticulate diet from 0 to 15 dph.*

**Table 2. Mean TL ± SE of larvae at 0, 5, 10, and 15 days post hatch (dph) and mean survival at the conclusion of the study. Within a row, different letters denote significant differences in TL and survival (Pd±0.05).**

	MICRO	ART	MIX
Total length (mm)			
0 dph	8.36 ± 0.09 z	8.36 ± 0.07 z	8.39 ± 0.09 z
5 dph	9.17 ± 0.06 y	9.77 ± 0.34 yz	10.29 ± 0.09 z
10 dph	10.00 ± 0.12 y	13.80 ± 0.20 z	13.29 ± 0.24 z
15 dph	11.58 ± 0.16 x	16.20 ± 0.17 z	13.59 ± 0.15 y
Survival (%)	95.20 ± 0.02 y	99.20 ± 0.01 z	100.00 ± 0.00 z

Year 2

Larval Gulf killifish are characterized as precocial larvae with well developed mouths and eyes upon hatch. Previous work with *Fundulus spp.* indicates that species within this genus can accept a powdered or microparticulate diet upon first feeding. Currently the default strategy in rearing killifish is to provide the larvae with *Artemia* nauplii because few studies are available to indicate the performance of powdered or microparticulate diets at this early-life stage. The ability to avoid or at least reduce the use of *Artemia* in the culture of *Fundulus spp.* has the potential to reduce cost, simplify labor, and reduce pathogen transfer.

This study was designed to compare larval growth and survival of larval Gulf killifish fed *Artemia* nauplii, a microparticulate diet, and a third treatment group consisting of a combination of these two diets. Embryos were harvested from spawning mats at the LSU AgCenter Aquaculture Research Station and shipped to the UF Indian River Research and Education Center where they were subsequently hatched after approximately 12 days of incubation. Five replicates of each treatment were stocked at a density of 5 larvae per liter at a salinity of 7.5 g/L. Larvae were fed twice daily (9am and 3pm) equivalent amounts by volume of either *Artemia* or

microparticulate diet. At 5, 10, and 15 days post hatch (DPH) survival was determined as well as a subsample of the larvae from each replicate tank was photographed for morphometric analysis. Standard length (SL) was determined from digital images captured at the UF Indian River Research and Education Center and sent to the LSU AgCenter Aquaculture Research Station.

Mean SL among the three treatments did not differ at 5 and 10 dph. At 15 dph the dry feed treatment SL was significantly smaller (REGWQ post hoc) (Table 3). Using a two-way ANOVA, time and treatment was significant while interaction (time\*treatment) was not. Mean survival among the *Artemia*, Dry, and Mixed feeding groups was 89.6, 87.7, and 93.8%, respectively. Using an arcsin square-root transformation and Tukey-Kramer post-hoc, the mixed feeding group had significantly higher survival. While SL of larvae between the *Artemia* and Mixed feeding groups was not different there was a potential benefit seen from increased survival. Although a microparticulate feed resulted in reduced length compared to the other groups at 15 dph the similar survival indicates that an artificial diet would work under culture conditions for Gulf killifish in a recirculating system. Two additional trials have been conducted but data has not been analyzed yet.

**Table 3. Mean SL  $\pm$  SE of larvae at 5, 10, and 15 days post hatch (DPH) and mean survival at 15 DPH for Gulf killifish, *Fundulus grandis*, fed a microparticulate diet (MICRO), *Artemia* (ART), or a mixture (MIX) of the two diets from first feeding. Within a row, different letter denote significant differences in SL and survival.**

	MICRO	ART	MIX
Standard length (mm)			
5 DPH	5.4 $\pm$ 0.13 $\zeta$	5.9 $\pm$ 0.20 $\zeta$	5.7 $\pm$ 0.12 $\zeta$
10 DPH	5.9 $\pm$ 0.10 $\zeta$	6.1 $\pm$ 0.18 $\zeta$	6.0 $\pm$ 0.13 $\zeta$
15 DPH	6.6 $\pm$ 0.08 $\zeta$	7.4 $\pm$ 0.14 $y$	7.1 $\pm$ 0.14 $y$
Survival (%)	87.7 $\pm$ 0.8 $\zeta$	89.6 $\pm$ 2.0 $\zeta$	93.8 $\pm$ 0.7 $y$

**Sub-objective 4c.** *Determine relationship between larval density and performance in Fundulus.*

There is little information available on *Fundulus* spp. culture in recirculation systems. Previous research with this species group has been pond based, where larvae are placed in fertilized ponds and allowed to feed on natural zooplankton. Densities of killifish fry and juveniles were estimated by weight within a specific pond area. Our research seeks to investigate growth performance and survival of larvae and juveniles within recirculation systems. Compared to the traditional systems, the ability to culture *Fundulus* spp. fry at high densities with a control over the culture environment in recirculation systems will enable aquaculturists raise and market more fishes per unit volume of water. Fry rearing utilizing recirculation capabilities will further increase the numbers of juveniles for grow-out phase within a production system and hence the numbers of adults and broodstock.

An 8 week study was conducted in four separate recirculating systems with newly hatched Gulf killifish. Salinity in all four systems was maintained between 9.5-10 g/L with synthetic marine salt. Each system consisted of eight 50-L aquaria, four aquaria were stocked at 7 larvae per liter, and the remaining four were stocked at 18 larvae per liter to represent high and low larval stocking densities. Larvae were

sampled at 0, 1, 2, 7, 10, 14, and 28 days post hatch for dry weight. Survival, wet weight, and length from each density treatment was determined at the end of the 8 week study.

Both mean length and weight were significantly different between the two larval rearing densities at the end of the 8 week study period (Table 4). Larvae reared at 18 per liter were significantly smaller than larvae reared at 7 per liter with the lower density having twice the survival. Dry weights of larvae from hatch to 28 days post hatch at regular intervals indicated that the lower density had greater weight gain beginning between 14 and 21 days post hatch.

Based on the results of the density study completed at 7 and 18 larvae per liter, Gulf killifish larvae were stocked in triplicate 40-L tanks within a large joined recirculating system at densities of 2, 5, 8, and 11 larvae per liter. The salinity of the system was maintained at 10 g/L using synthetic marine salt. Each tank was fed a commercially available feed that was ground and sieved with a 500-um mesh (40% crude protein, 9% crude fat, 4% crude fiber; Burris Mill and Feed, Franklinton, Louisiana). Individuals were fed daily at 10% body weight divided into three (3) feeding times, 9 am, 12 noon and 3 pm.

**Table 4. Mean SL and weight ( ± SE) of Gulf killifish, *Fundulus grandis*, juveniles initially stocked at 7 and 18 per liter and reared from hatch to eight weeks. Within a row, different letter denote significant differences in SL, weight, and survival.**

	7 larvae/L	18 larvae/L
Standard length (mm)	22.4 ± 0.48 <i>z</i>	17.5 ± 0.43 <i>y</i>
Wet weight (g)	0.15 ± 0.03 <i>z</i>	0.07 ± 0.01 <i>y</i>
Survival (%)	63.5 <i>z</i>	30.2 <i>y</i>

Quantity of feed given to the fry was adjusted biweekly according to body weight of the killifish. The wet weight (nearest 0.0001 g), and SL (nearest 0.1 mm) was determined from a sample of individuals ( $n = 20$ ), while survival was determined every four weeks for this 16 week study.

After two weeks of stocking, fry stocked at 5/L and 11/L had attained a significantly greater mean weight compared to individuals stocked at 2/L and 8/L. After six weeks in culture, fish stocked at 8/L had the highest weight, although not statistically different from the 11/L. From week 10 to the completion of the study (week 16), the fry stocked at 11/L had the highest mean weight and hence ended with the highest mean weight. There was a negative relationship between stocking density and survival, a majority of which could be attributed to cannibalism.

The onset of cannibalism was observed between the 6<sup>th</sup> and 8<sup>th</sup> week of the study and progressed till the completion of the 16 week study. Removal of cannibals was not conducted so the study results show a severe impact of cannibalism at densities of 5, 8, and 11 fish per liter.

These results indicate that optimum stocking densities of Gulf killifish in recirculation systems may be below 5 per liter after 6 to 8 weeks of growth, coinciding with significant increases in the incidence of cannibalism (Table 5). Although the lowest density (2 fish/L) had the lowest growth, it had the highest survival (slightly above 82%) at the end of the 16 week study period. One possible solution would be to decrease rearing densities as the fish progress from larvae to juveniles.

**Table 5. Mean Final weight ( + SE), Specific Growth Rate (SGR, and survival of Gulf killifish, *Fundulus grandis*, juveniles initially stocked at 2, 5, 8, and 11 per liter and reared for 16 weeks. Within a row, different letter denote significant differences in final weight.**

	2 larvae/L	5 larvae/L	8 larvae/L	11 larvae/L
Final weight (g)	0.33 ± 0.13 <i>z</i>	0.51 ± 0.01 <i>y</i>	1.16 ± 0.11 <i>x</i>	1.43 ± 0.10 <i>w</i>
SGR	1.68	2.06	2.79	2.98
Survival (%)	82.8	28.3	10.7	6.7

**Objective 5.** *Develop improved technologies for spawning and larval rearing of Bala shark*

**Sub-objective 5a.** *Improve Bala shark broodstock maturation.*

Subobjective 5a will be addressed in year 3.

**Sub-objective 5b.** *Develop technologies for induced spawning of Bala shark.*

**University of Florida Tropical Aquaculture Laboratory**

Year 1

Bala sharks are a high value and popular freshwater ornamental species but are only available from farms in Asia. Bala sharks have presented unique challenges in broodstock development, spawning techniques, and larval rearing for the U.S. ornamental aquaculture industry.

Bala sharks (2 g mean weight) were purchased from a local importer. Fish were stocked directly into two outdoor ponds. Pond water temperature was 84.2 degrees F. Six fish were sampled for dissection and histological examination of gonadal development. Fish were removed from the ponds in October and placed in recirculating water tank systems in a heated greenhouse. Gonadal samples were taken once a month since May 2011 to determine gonad maturation. At each sampling, twelve fish were anesthetized, weighed and measured, and an attempt was made to express sperm or extract eggs. For males, sperm maturation is determined by manually expressing sperm. For female sexual maturation, we are looking for fish that appear to be “fat.” When a fish is suspected as a female, a small catheter tube is inserted into the genital opening in an attempt to

extract eggs. Subsamples of fish were used to determine the maturational stage on December 23, 2011. Mean weight of the fish was 26.5 g and mean length was 14.1 cm. To date, no eggs have been collected and no viable sperm has been expressed. The fish will be returned to open ponds in late March or early April, 2012.

Year 2

Monitoring of the development of viable broodstock was conducted. In May 2012, 50% of the bala sharks were returned to open ponds and the remaining fish were retained for conditioning indoors in a recirculating water tank system.

Samples have been taken once a month throughout the year to determine sexual maturation and growth. The first eggs in females were detected in February 2012. The mean weight was 31.1 g and mean length was 14.4 cm. The first sexually mature males were detected April 2012. By July 2012, most of the fish were exhibiting gonadal development, and although the males are producing viable sperm, to date none of the females have produced mature eggs.

**Subobjective 5c.** *Develop improved technologies for larval rearing of Bala shark*

Over 50 older bala sharks were acquired which were capable of production of viable eggs. Mature eggs were first noted in June 2012 and spawning trials were begun at that time. The fish were successfully induced to ovulate in July 2012. A series of trials have been conducted to determine the optimum water quality parameters for hatching the eggs. Eggs were placed in hatching jars with water hardness ranging from 34 to 170 ppm, total alkalinity ranging from 34 to 68 ppm and pH ranging from 6.5 to 8.0. Successful hatching of the eggs occurred in water

that was 140 ppm hardness, 52 ppm alkalinity, and a pH of 8.0. Newly hatched fry were 4 mm in length and grew to 6 mm by day five at which time they were ready to feed on newly hatched *Artemia*. The fry are currently 6 weeks old and range from 1.5 to 2 cm.

In addition, several batches of eggs produced were frozen and shipped Louisiana State University to be used in subobjective 5d.

**Subobjective 5d.** *Design water treatment technologies for commercial larval rearing of Bala shark.*

**Louisiana State University Agriculture Engineering, University of Florida  
Tropical Aquaculture Laboratory**

Eggs of fish are commonly collected and are incubated in a variety of hatching tanks and systems. There is no consistent design and most tanks and filters used are not capable of handling large inputs of ammonia and other compounds released from hatching and decaying egg masses. A design of a water treatment strategy appropriate for use in commercial larval production systems capable of handling shock loading of these compounds is necessary and this type of system has application for many species of fish.

**Year 1**

Water treatment components were designed which are capable of responding to shock loading of total ammonia nitrogen and organic matter when a proportion of the egg mass decays in a recirculating system. This effort has been divided into two steps.

The first step was to identify a surrogate to Bala shark eggs that would permit the research team to conduct shock loading response experiments in the LSU laboratory. The research team has also acquired supplies necessary for conducting analysis defining the organic and nitrogen loading for a variety of egg types. Since Bala Shark eggs are not yet available to the team, techniques for freeze drying eggs were developed utilizing trout egg masses. Student workers were trained to conduct the chemical analysis. Freeze dried egg matter from the trout eggs was then used in a preliminary chemical analysis.

The second step was to initiate the design of treatment components for evaluation. In support of this goal, visits between the LSU and University of Florida research teams were made to observe current breeding practice and establish system size. The LSU

team has constructed and is evaluating appropriately sized prototype floating bead, fluidized sand, and moving bed reactors. Formal testing of the units will be conducted once the waste characterization work is complete.

**Year 2**

Eggs of bala sharks, speckled trout (*Cynoscion nebulosus*), snapper (*Lutjanus campechanus*), tilapia (*Oreochromis niloticus*), and channel catfish (*Ictalurus punctatus*) were collected for comparative purposes, as a precursor for further bala shark eggs studies. Eggs were weighed, freeze-dried, and powder-crushed to increase the surface area of each egg particle, thereby ensuring accurate biochemical oxygen demand (BOD<sub>5</sub>) readings. Each measurement was done in triplicate. Blank samples were also analyzed to ensure consistency. Averages (all samples) were: 0.72 mg/g BOD<sub>5</sub>, 10.49% nitrogen and 67.86% proteins. A statistical analysis indicated that channel catfish BOD<sub>5</sub> is the most representative of the multi-species lot. Analysis of six other species, including bala sharks is continuing and should be completed by year's end.

A theoretical rationale was developed for evaluating the filters used to mitigate shock loading experienced during spawning events. A time dependent model was developed in the Stella™ modeling environment using Monod kinetics to simulate shock loading of ammonia-nitrogen and BOD<sub>5</sub> in recirculating aquaculture systems (RAS). The timely reduction of ammonia-nitrogen was found to be mostly governed by the half saturation constant. A literature review that defined half saturation nitrification values identified the fine fluidized bed as the best treatment option. However, the ability to remove heterotrophic

growth might be a key issue that needs to be looked at, which could make a floating bead filter the best option. The research team has constructed a floating bead filter, a fine sand bed, and a moving bed reactor that will be subject to shock loading experiments in the upcoming months. Each filter holds four liters of media.

The recirculation capabilities of small scale airlifts were investigated. Water delivery as a function of air input was determined by replicated studies for 1, 1.5, and 2 inch airlifts at a variety of lift to submergence

## IMPACTS

First report of repeated volitional spawning of ballyhoo in captivity and the optimal egg collection method has been defined.

Gulf killifish embryos can be easily air incubated in moist foam and developmental times can be controlled by incubation temperature indicating the potential for coordinated hatching of multiple spawns at one time. This will increase efficiency and reduce production costs of hatcheries and growout facilities. Additionally, packaging and transportation of embryos on foam between producers is possible and increases efficiency.

## PUBLICATIONS AND PRESENTATIONS

### Publications

Brown, C.A., and C.C. Green. Metabolic and embryonic responses to terrestrial incubation of gulf killifish embryos across a temperature gradient. *Journal of Fish Biology*. Accepted for publication in a special issue in 2013.

Coulon M. P., C. T. Gothreaux, and C. C. Green. 2012. Influence of substrate and salinity on air incubated Gulf killifish embryos. *North American Journal of Aquaculture* 74:54-59.

### Presentations

Alt D., J. Wagner, and R. F. Malone. 2011. Verification of an airlifted polygeyser design for a warmwater marine RAS fingerling application., *Aquaculture America 2011*, New Orleans, February 28-March 3, 2011.

ratios (S:L) – 2:1, 3:1, 4:1, and 5:1. Optimal S:L was determined to be 4:1. Water flow for these units is predictable by the relationship:

$$Q_w = 4.3 * Q_g$$

where  $Q_w$  is water flow (in gpm) and  $Q_g$  is the air input to airlift (in cfm). The relationship was constant across all pipe sizes tested. It is anticipated that these results will be used in the design generated by the research team in the next year of the project.

*Fundulus* spp. larvae can be cultured exclusively on a microparticulate diet from 0 to 15 days post-hatch and this will reduce the cost of hatchery production and reduce the likelihood of pathogen introduction from live feeds.

Results indicate new handling techniques and spawning protocols have significantly reduced historic mortalities of brood bala sharks post spawning.

Three producers have acquired brood bala sharks based on the results indicating successful captive maturation and spawning.

- Alt, D. and R. F. Malone. 2012. Mitigation of shock loading in ornamental fish hatcheries, 9<sup>th</sup> International Conference on Recirculating Aquaculture, Roanoke, Virginia, August 24-26.
- DiMaggio, M.A., C.L. Ohs, A.T. Palau, and J.A. Broach. 2013. Evaluation of culture techniques and spawning substrates for ballyhoo *Hemiramphus balao*. Aquaculture 2013, Nashville, Tennessee.
- Fisher, C., and C. Green. 2012. Effect of stocking density and potassium ion concentration on growth, survival, and ion regulation in gulf killifish (*Fundulus grandis*). Aquaculture America 2012. Las Vegas, Nevada.
- Greensword, M. and R. F. Malone. 2012. Use of airlift design guidelines for ornamental fish and baitfish systems, 9<sup>th</sup> International Conference on Recirculating Aquaculture, Roanoke Virginia, August 24-26.
- Fisher, C., and C. Green. 2012. Effect of stocking density and potassium ion concentration on growth, survival, and ion regulation in Gulf killifish *Fundulus grandis*. American Fisheries Society, Louisiana Chapter Annual Meeting, Lafayette, Louisiana (poster presentation).
- Malone, R. F., R. Tabor, D. Alt, and C. Cristina. 2011. An examination of the simplified kinetic assumptions underlying the analysis of airlifted polygeyser designs. Aquaculture America, New Orleans, February 28-March 3.
- Malone, R. F. 2012. Airlift supported recirculating aquaculture systems. Aquaculture America 2012, Las Vegas, Nevada, February 29-March 3.
- Ohs, C.L., L.M.V. Onjukka, M.A. DiMaggio, and J.S. Broach. 2012. Current status of the culture of the Seminole killifish *Fundulus seminolis* as a freshwater and marine baitfish. Aquaculture America 2012. Las Vegas, Nevada.
- O'Malley, P., C. Brown, J. Patterson, and C. Green. 2012. Physiological effects of terrestrial stranding on *Fundulus grandis*. American Fisheries Society, Louisiana Chapter Annual Meeting, Lafayette, Louisiana (poster presentation).
- Onjukka, L.M.V., J.S. Broach, and C.L. Ohs. 2011. Spawning success of *Fundulus seminolis* in tanks at various densities and salinities. Aquaculture America 2011. March 1-3, 2011, New Orleans, Louisiana.



## EFFECTS OF MOSQUITO ABATEMENT PESTICIDES ON VARIOUS LIFE STAGES OF COMMERCIALY IMPORTANT SHELLFISH AQUACULTURE SPECIES IN THE SOUTH

### Reporting Period

March 1, 2011 – August 31, 2012

**Funding Level**    Year 1 ..... \$39,973  
                          Total..... \$39,973

**Participants**      College of Charleston ..... Craig Plante  
                          Florida Atlantic University ..... Loren D. Coen

### PROJECT OBJECTIVES

1. Review the various mosquito abatement pesticides utilized in the Southern U.S. near the major shellfish hatchery and nursery facilities and select a larvicide and adulticide of most concern, based on application data and available toxicity data, for further bioassay testing.
2. Use standard toxicity testing protocols to assess the potential impacts of the mosquito control larvicide and adulticide pesticides of most concern on larval and post-set (1-3 mm) hard clams, *Mercenaria mercenaria*, and Eastern oysters, *Crassostrea virginica*.
3. Disseminate information to shellfish hatchery operators and agencies responsible for mosquito abatement through SRAC Fact Sheets and meetings.

### ANTICIPATED BENEFITS

Developing (larval and juvenile) shellfish may be subject to many environmental and biological stressors, including predation, disease, abiotic variables (temperature, dissolved oxygen, salinity, pH), and chemical contamination. Mosquito control pesticide applications often coincide with both the location of shellfish hatcheries and nurseries, as well as the season(s) when sensitive early life stages are occurring. Shellfish aquaculture facilities are concerned with mosquito spraying activities as they may impact their source water and thereby their larval or juvenile offspring or

their algal cultures, potentially causing significant mortality. Currently, toxicity data for mosquito control chemicals and larval clams and oysters are limited (Table 1).

This Southern Regional Aquaculture Center (SRAC)-supported project established the concentrations required to cause mortality in early molluscan life stages, and enables us to predict whether these chemicals will have adverse effects on coastal shellfish operations or for that matter, native field populations. The potential impacts of

this work are increased knowledge of the effects of mosquito control chemicals on molluscan shell-

fish, as well as improved management strategies for competing uses of coastal resources.

**Table 1. Summary of existing organophosphate and pyrethroid toxicity data for *M. mercenaria* and *C. virginica*.**

Active Ingredient	Chemical Class	Species	Life Stage	Toxicity (µg/L)	Source
Chlorpyrifos	Organophosphate	<i>C. virginica</i>	Juvenile	270 (96h EC50)	Mayer, 1987
Chlorpyrifos	Organophosphate	<i>C. virginica</i>	Embryo	2000 (48h EC50)	Mayer, 1987
Malathion	Organophosphate	<i>C. virginica</i>	Juvenile	>1000 (48h EC50)	Mayer, 1987
Malathion	Organophosphate	<i>C. virginica</i>	Juvenile	>1000 (96h LC50)	Mayer, 1987
Naled	Organophosphate	<i>C. virginica</i>	Juvenile	590 (96h EC50)	Mayer, 1987
Dichlorvos	Organophosphate	<i>C. virginica</i>	Juvenile	>1000 (96h EC50)	Mayer, 1987
Dichlorvos	Organophosphate	<i>C. virginica</i>	Adult	89,000 (96h LC50)	Jones and Davis, 1994
Dichlorvos	Organophosphate	<i>C. virginica</i>	Adult	31,620 (96h LC50)	Bolton-Warberg <i>et al.</i> , 2007
Permethrin	Pyrethroid	<i>C. virginica</i>	Embryo	>1000 (48h EC50)	Mayer, 1987
Cypermethrin	Pyrethroid	<i>C. virginica</i>		370 (96h EC50)	Werner and Moran, 2008
Bifenthrin	Pyrethroid	<i>C. virginica</i>	Embryo	285 (48h EC50)	Werner and Moran, 2008
Cyfluthrin	Pyrethroid	<i>C. virginica</i>		2.69 (96h LC50)	Werner and Moran, 2008
Deltamethrin	Pyrethroid	<i>C. virginica</i>		8.2 (96h EC50)	Werner and Moran, 2008
Tralomethrin	Pyrethroid	<i>C. virginica</i>		0.006 (EC50)	EPA EFED database

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

**Objective 1.** *Review the various mosquito abatement pesticides utilized in the Southern United States near the major shellfish hatchery and nursery facilities and select a larvicide and adulticide of most concern, based on application data and available toxicity data, for further bioassay testing.*

### College of Charleston and Florida Atlantic University

Organophosphates used in adult mosquito control include malathion, fenthion, naled, and chlorpyrifos. Some of the pyrethroids used in mosquito control include: permethrin, resmethrin, sumithrin, lambda-cyhalothrin, esfenvalerate, tralomethrin, deltamethrin, cyfluthrin, bifenthrin, cypermethrin, and etofenprox. Commercial and government applicators decide which chemical to use based on several factors such as efficacy (determined by field trials), mosquito species sensitivity, safety, and cost. The pesticide choice is made by each individual mosquito control agency and varies with location because of differences in mosquito species and application requirements. Mosquito species may develop resistance to a given insecticide over time, rendering it less effective and necessitating a change in the chemical used.

To collect information regarding what types of chemicals are being used to control adult and larval mosquitoes, we conducted a survey (internet and phone) of the following southeastern coastal counties: Charleston, Beaufort, and Georgetown counties of SC; Cataret County, NC; Chatham County, GA; Brevard, Charlotte, Collier, Lee, Manatee, Sarasota, Citrus, St. Lucie, and Seminole counties of FL.

The survey revealed that permethrin, resmethrin, sumithrin, and naled accounted for the majority of chemicals used to control adult mosquitoes. The same survey revealed that methoprene-based products (Altosid) were the most common larvicide

applied. Eleven of the 14 southeastern counties surveyed reported using naled for mosquito control. Permethrin was applied by 50% of the 14 counties surveyed. The extensive use of resmethrin to control mosquito populations in New York City coincided with massive lobster mortality in Long Island Sound, and concerns over resmethrin toxicity to estuarine organisms remains (Zulkosky et al., 2005).

We selected permethrin, resmethrin, and naled (dibrom) as the adult mosquito control compounds for toxicity evaluation based on their frequency of use in our coastal county survey (Table 2), number of registered products, and preliminary testing data. These chemicals represent two different classes of mosquito control compounds (permethrin and resmethrin are pyrethroid insecticides, whereas naled is an organophosphate insecticide). By including two different classes of chemical, we were able to compare organism sensitivity across chemical groups and identify the compounds of most concern to shellfish species. Methoprene, a juvenile growth hormone mimic, was tested as the larvicide in this project. In target invertebrates, the principal mechanism of action of pyrethroids is disruption of sodium channel function in the nervous system, whereas the toxicity of OPs is produced through the inhibition of certain cholinesterase enzymes of the nervous system (Klaassen, 1996). Juvenile growth hormone interferes with insect maturation and prevent the insect from reaching the adult stage (Klaassen, 1996).

**Table 2. Survey (June, 2011) of mosquito control chemicals applied in southeastern coastal counties.**

County	State	Adulticides Used	Active Ingredient	Larvicides Used	Active Ingredient	
Beaufort	SC	Anvil 10+10 ULV	sumithrin	Agnique MMF	monomolecular film	
		Biomist 30+30 ULV	permethrin	Altosid Briquets	methoprene	
		Evoluer 30-30 ULV	permethrin	Altosid Liquid Conc.	methoprene	
		Suspend SC	deltamethrin	Altosid Pellets	methoprene	
			Altosid SBG	methoprene		
			Altosid XR Briquets	methoprene		
			Altosid XR-G	methoprene		
			Aquabac XT	Bti		
			Aquabac 400-G	Bti		
			Bactimos Pellets	Bti		
			GB-1111	petro-hydrocarbons		
			Summit Bti Briquets	Bti		
			VectoBac 12AS	Bti		
		Charleston	SC	Zenivex	etofenprox	Agnique MMF
Kontrol 30-30	permethrin			VectoBac AS	Bti	
Anvil 10+10	sumithrin			Altosid XR Briquets	methoprene	
	Trumpet			naled (dibrom)	Altosid SR20	methoprene
	5 EC			malathion	30-day Altosid Pellets	
	Georgetown			methoprene		permethrin
Trumpet		naled (dibrom)	Bti			
Chatham	GA	Fyfanon	malathion	Altosid	methoprene	
		Scourge	resmethrin		Golden Bear	petro-hydrocarbons
Brevard	FL	Trumpet	naled (dibrom)	mineral oil	petro- hydrocarbons	
				Vectolex CG	Bti	
Charlotte	FL	Trumpet	naled (dibrom)	GB-1111	petro-hydrocarbons	
				Altosid SBG	methoprene	
Collier	FL	Trumpet	naled (dibrom)	VectoBac 12AS	Bti	
				Altosid XR-G	methoprene	
St. Lucie	FL	Zenivex	etofenprox	Aquabac XT	Bti	
		Biomist 3+15	permethrin	Altosid Briquets	methoprene	
		Permanone 30+30	permethrin	Agnique MMF	monomolecular film	
		Anvil 10+10	sumithrin	Altosid 30-d	methoprene	
		Duet	prallethrin+ sumithrin	Altosid XR Briquets	methoprene	
		Scourge 4+12	resmethrin	Altosid XR-G	methoprene	
		Zenivex	etofenprox	Summit Bti Briquets	Bti	
St. Johns	FL	Suspend SC	deltamethrin	Vectobac G & CG	Bti	
				Natular T30	Spinosyn	
				Vectolex WSP	Bti	

**TABLE 2, continued.**

Seminole	FL	Permanone - RTU	permethrin	Agnique MMF	monomolecular film
		Aqua-Reslin	permethrin	Altosid 30-d	methoprene
		Trumpet	naled (dibrom)	Altosid Liquid Conc.	methoprene
				Altosid Pellets	methoprene
				Altosid XR Briquets	methoprene
				Aquabac 200-G	Bti
				Aquabac XT	Bti
				Natular T30	Spinosyn
				Natular XRG	Spinosyn
				Natular XRT	Spinosyn
				VectoBac 12AS	Bti
				Vectobac G& CG	Bti
				Vectolex WDG	Bti
		Lee	FL	Trumpet	naled (dibrom)
Fyfanon	malathion			Vectolex CG	Bti
				GB-1111	petro-hydrocarbons
Manatee	FL	Trumpet	naled (dibrom)	Altosid	methoprene
			permethrin	Abate 4E	temephos
Sarasota	FL	Anvil 10+10	sumithrin	Altosid	methoprene
		Anvil 10+10	sumithrin	Vectolex CG	Bti
		Trumpet	naled (dibrom)	5% Skeeter Abate	temephos
Citrus	FL			Agnique MMF	monomolecular film
				GB-1111	petro- hydrocarbons
				Altosid Pellets	methoprene
				Altosid XR Briquets	methoprene
				Altosid XR-G	methoprene
				Vectolex CG	Bti
				Aquabac XT	Bti
				Aquabac 200-G	Bti
				Abate 4E	temephos
				Vectolex CG	Bti
				Zenivex	etofenprox
Carteret	NC	Trumpet	naled (dibrom)	Natular XRG	Spinosyn
		Anvil 10+10	sumithrin	Natular XRT	Spinosyn
		Zenivex	etofenprox	VectoBac 12AS	Bti
		Duet	prallethrin+ sumithrin	Vectobac G& CG	Bti
Carteret	NC	Aqua-Reslin	permethrin	Altosid XR Briquets	methoprene
				Altosid Pellets	methoprene
				Altosid XR Briquets	methoprene

**Objective 2.** Use standard toxicity testing protocols to assess the potential impacts of the mosquito control larvicide and adulticide pesticides of most concern on larval and post-set (1-3 mm) hard clams, *Mercenaria mercenaria*, and Eastern oysters, *Crassostrea virginica*.

### College of Charleston and Florida Atlantic University

Shellfish aquaculture facilities are concerned that environmental risk assessments of mosquito control pesticides do not include enough larval and small post-set molluscan shellfish information in the overall data available for assessments. The Eastern oyster, *Crassostrea virginica*, forms living subtidal and intertidal reefs in many Atlantic and Gulf coast estuaries. Hard clam populations also are critical species in the ecosystems where they naturally occur. At present, hard clam (*Mercenaria mercenaria*) aquaculture occurs in more Eastern U.S. states than any other native bivalve species under culture. Hence these two species are ideal candidates for focused research relating to impacts from spraying for mosquitoes. Specifically, there are few data available regarding the effects of mosquito control compounds on larval and juvenile forms of clams and oysters.

#### Larval Clams and Oysters

Newly hatched *M. mercenaria* were supplied by Bay Shellfish Co., Terra Ceia, FL; and newly hatched *C. virginica* were supplied by Fishers Island Oyster Farm (Fishers Island, NY). Larvae were transferred to 1 L flasks and acclimated to laboratory conditions (25 degrees C temperature, 20 ppt salinity, 16h light:8h dark photoperiod) over a 72 h period. The larvae were fed *Isochrysis galbana* daily, and natural seawater changes were made every 48 h.

Aqueous 96 h static bioassays were performed with the larval and juvenile life stages of each test species (US EPA, 1996; Mayer, 1987). Test conditions were 25 degrees C, 16h light:8h dark photoperiod, and 20 ppt salinity. Seawater used in the experiments was supplied to the laboratory from the Charleston Harbor estuary, filtered (5 µm), UV-sterilized, activated carbon filtered, and diluted with deionized water to adjust salinity.

Larval shellfish exposures to the mosquito control pesticides were conducted based on methods of Finnegan et al. (2009). Veliger larvae were exposed in 24-well plates pre-coated with hydrophilic gel to minimize chemical binding. The plates were soaked overnight in deionized water before use. One 7 day-old larva (approximate size 100-150 µm) was added per well in 2 mL test solution. The larvae were fed 12,000 cells/mL *I. galbana*. The plates were sealed with Parafilm to minimize evaporation, and placed on orbital shakers (150 rpm) in an environmental chamber. Pesticide stock solutions were prepared in 100% HPLC grade acetone, and the doses were administered to obtain a final acetone concentration of 0.1% in each treatment and control. The upper insecticide concentration used for testing was set at 10 mg/L (approximate limit of solubility). There were three replicate well plates per treatment (n=72 larvae per treatment), and there were five pesticide treatment concentrations plus a control.

Each day of the 96 h static-renewal exposure, the larvae were examined using a dissecting microscope. Mortality was noted and water quality parameters were measured (salinity, temperature, pH, and dissolved oxygen). Larvae were transferred to new plates containing fresh exposure media and *I. galbana*. Median lethal concentrations (LC50) with 95% confidence intervals were determined using the Trimmed-Spearman-Kärber method (Hamilton et al., 1977).

#### Post-Set Juvenile Clams and Oysters

Acute (96 h) tests were conducted using 1-3 mm post-set clams and oysters. Static renewal toxicity tests were performed using the same pesticide stock preparations as described above. Tests consisted of a control and 5 treatment concentrations, with three

replicates per treatment. Juveniles were exposed in 16-oz glass jars with Teflon-lined lids containing 180 mL of test media. Thirty juvenile clams or oysters were added to each replicate jar. Aeration was supplied through a glass pipette inserted through a hole in the lid. The bioassays were conducted at 20-ppt salinity (5  $\mu$ m filtered seawater), 25 degrees C, and a 16-h light: 8-h dark photoperiod in an environmental chamber. Temperature, salinity, dissolved oxygen, and pH were measured and the test media was renewed daily. Juveniles were not fed during the 96 h exposure. Mortality (indicated by gaping, lack of response to stimuli, and/or shell closure for more than two minutes) was determined and a 96 h LC50 was calculated. Significant differences

( $p < 0.05$ ) between LC50 values for different species, life stages, and test chemicals were determined using the LC50 ratio test (Wheeler, 2006).

In order to assess effects of these insecticides in juvenile clams and oysters over a longer exposure, we conducted 21 d chronic static renewal tests with each chemical. Every three days, test solutions were renewed, the organisms were fed, and water quality and mortality were assessed. After 21 d, an LC50 was calculated, and wet and dry weights (dried 48 h at 60 degrees C) were determined. The shell maximum length, maximum width, and shell area were then determined using an Olympus dissecting microscope, a digital camera, and Image Pro Plus image analysis software.

## RESULTS

### Acute testing

Larval clams (7 d old) were most sensitive to the pyrethroid, resmethrin, with a 96 h LC50 of 1.92 mg/L. The next most toxic compound was the juvenile growth hormone mimic, methoprene, with a 96 h LC50 of 2.40 mg/L. The pyrethroid, permethrin, yielded a 96 h LC50 of 6.81 mg/L, whereas the organophosphate chemical, naled, had a 96 h LC50 of 8.03 mg/L. Larval clams were significantly more sensitive to methoprene than the other species and life stages tested.

Larval oysters (7 d old) were less sensitive to resmethrin, permethrin, and methoprene than larval clams, with 96 h LC50 values greater than the highest concentration tested (10 mg/L). They showed similar sensitivity to the organophosphate chemical, naled, with a 96 h LC50 of 6.75 mg/L.

Juvenile clams were significantly less sensitive to resmethrin and methoprene compared to larval clams, with 96 h LC50 values of 7.79 mg/L and  $>10$  mg/L, respectively. The juvenile clam 96 h LC50 values for permethrin and naled were 9.66 mg/L and 3.44 mg/L, respectively.

Juvenile oysters were similar in sensitivity to oyster larvae and clam larvae for the organophosphate compound, naled (96 h LC50 of 6.80 mg/L). Juvenile oysters had 96 h LC50 values greater than the highest concentration tested (10 mg/L) for resmethrin, permethrin, and methoprene.

A summary of acute toxicity in larval and juvenile clams and oysters is shown in Tables 3 and 4. In general, larval clams were the most sensitive species and life stage tested. Larval clams were significantly more sensitive to resmethrin and methoprene than juvenile clams. Larval clams were significantly more sensitive to resmethrin, permethrin, and methoprene than larval or juvenile oysters.

### Results at a glance...

- *The insecticides, resmethrin, permethrin, and naled, caused decreases in larval and juvenile clam and oyster survival and growth, but at higher concentrations than they would be likely to encounter in the environment as a result of mosquito control spraying applications.*

**Table 3. Acute toxicity in clams. Values are LC50 (mg/L) with 95% confidence interval. Asterisks indicate significant differences between larval and juvenile values for same species determined using LC50 ratio test ( $\alpha = 0.05$ ).**

	Resmethrin	Permethrin	Naled	Methoprene
Larval	1.59* (0.97 – 2.58)	6.81 (4.88 – 9.51)	8.03 (3.73 – 17.28)	2.40* (1940 – 2980)
Juvenile	7.79* (6.46 – 9.40)	9.66 (7.07 – 13.20)	3.44 (0.83 – 14.36)	>10*

**Table 4. Acute toxicity in oysters. Values are LC50 (mg/L) with 95% confidence interval. Asterisks indicate significant differences between larval and juvenile values for same species determined using LC50 ratio test ( $\alpha = 0.05$ ).**

	Resmethrin	Permethrin	Naled	Methoprene
Larval	> 10	> 10	6.75 (4.17 – 10.94)	> 10
Juvenile	> 10	> 10	6.80 (5.93 – 7.79)	> 10

### Chronic testing

Juvenile clams and oysters did not exhibit much additional mortality after 21 d exposure to resmethrin than the acute 96 h exposure. Clam and oyster acute values (7.79 mg/L and >10 mg/L, respectively) were similar to 21 d chronic values (7.77 mg/L and 9.06 mg/L, respectively). The chronic permethrin exposure had a significant effect on mortality for both clams and oysters. The toxicity values decreased from 9.66 mg/L to 0.80 mg/L for clams, and from >10 mg/L to 4.06 mg/L for oysters. Methoprene also exhibited an increase in toxicity with chronic exposure, with clam LC50 values decreasing from

>10 mg/L to 0.83 mg/L and oyster LC50 values decreasing from >10 mg/L to 1.30 mg/L.

A summary of acute toxicity in larval and juvenile clams and oysters is shown in Tables 5 and 6. In general, increased length of exposure led to an increase in mosquito control insecticide toxicity to juvenile clams and oysters. The only compound that did not cause much increase in toxicity from 96 h to 21 d was resmethrin. The compound that had the most increased toxicity was methoprene; approximately 12 fold more toxic after 21 d than 96 h.

Juvenile clam and oyster growth was also affected by chronic exposure to most of the insecticides tested. In general, clam shell area was smaller after exposure to the mosquito control pesticides tested, with the exception of resmethrin (Figure 1). Clam shell weight was reduced by chronic exposure to the mosquito control pesticides tested, with resmethrin

having the smallest effect on weight and methoprene having the largest effect (Figure 2). Oyster shell area was significantly reduced by all the compounds tested (Figure 3) and oyster shell dry weight was also significantly reduced by all the compounds tested (Figure 4).

**Table 5. Acute vs. chronic toxicity in juvenile clams. Values are LC50 (mg/L) with 95% confidence interval. Asterisks indicate significant differences between acute and chronic values determined using LC50 ratio test ( $\alpha = 0.05$ ).**

	Resmethrin	Permethrin	Naled	Methoprene
Acute (96 h)	7.79 (6.46 – 9.40)	9.66* (7.07 – 13.2)	3.44 (0.83 – 14.36)	>10*
Chronic (21 d)	7.77 (5.03 – 12.01)	0.80* (0.63 – 1.01)	2.32 (1.31 – 4.10)	0.83* (0.63 – 1.09)

**Table 6. Acute vs. chronic toxicity in juvenile oysters. Values are LC50 (mg/L) with 95% confidence interval. Asterisks indicate significant differences between acute and chronic values determined using LC50 ratio test ( $\alpha = 0.05$ ).**

	Resmethrin	Permethrin	Naled	Methoprene
Acute (96 h)	> 10*	> 10*	6.80* (5.94 – 7.79)	>10*
Chronic (21 d)	9.06* (7.40 – 11.09)	4.06* (3.26 – 5.07)	1.01* (0.57 – 1.79)	1.30* (1.05 – 1.61)

Figure 1. Effect of insecticide exposure on juvenile clam shell area after 21 d exposure. Data are average and standard error for each treatment expressed as percent of control.

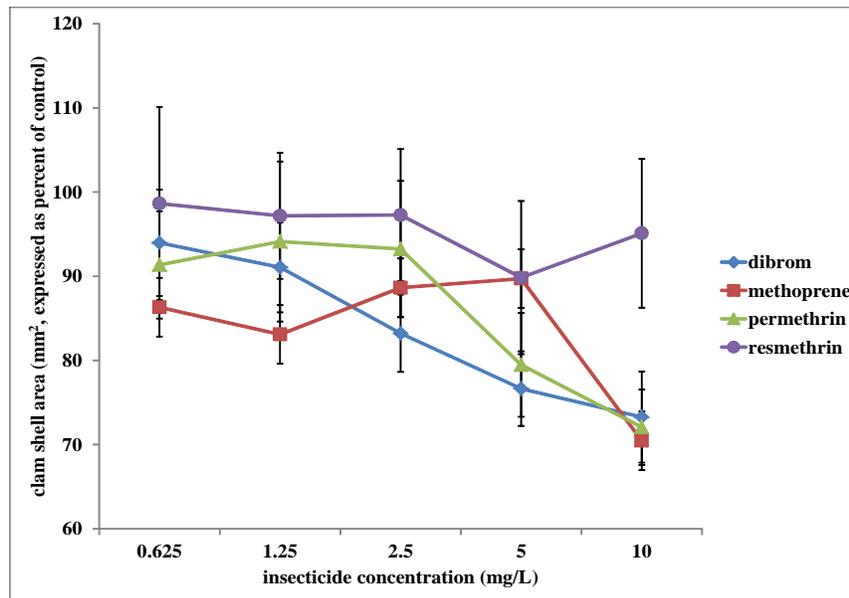


Figure 2. Effect of insecticide exposure on juvenile clam dry weight after 21 d. Data are average and standard error for each treatment expressed as percent of control.

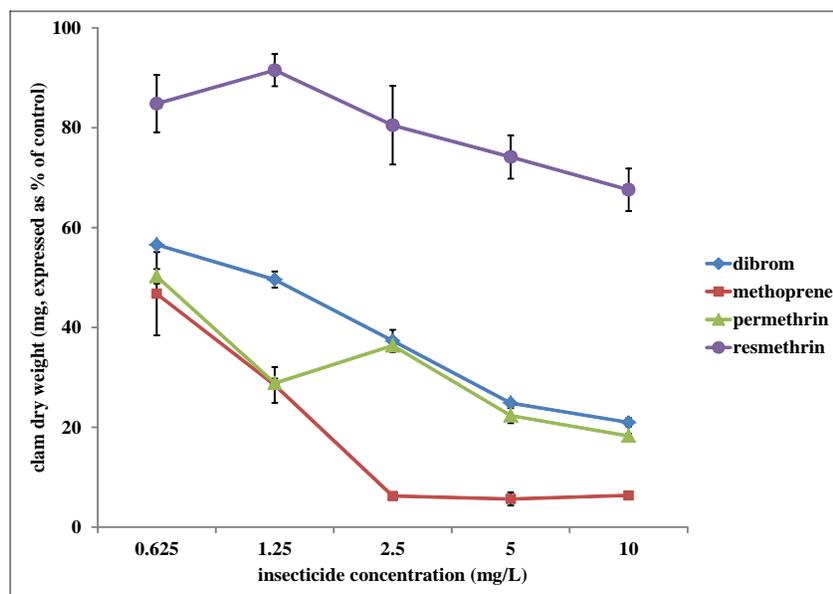


Figure 3. Effect of insecticide exposure on juvenile oyster shell area after 21 d exposure. Data are average and standard error for each treatment expressed as percent of control.

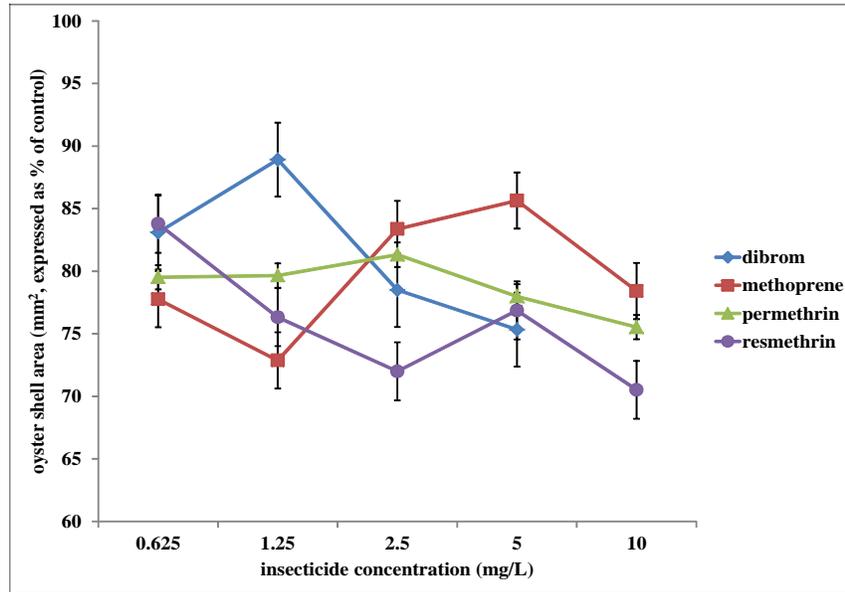
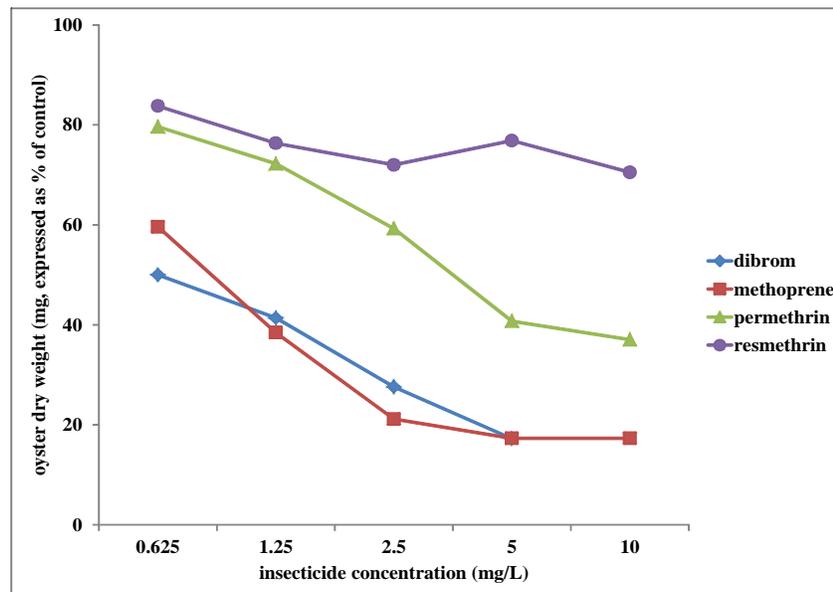


Figure 4. Effect of insecticide exposure on juvenile oyster dry weight after 21 d. Data are average and standard error for each treatment expressed as percent of control.



Resmethrin significantly decreased clam weight ( $p=0.0230$ ), but only at the highest concentration (10mg/L). Clam shell area was not affected ( $p=0.0665$ ). Resmethrin concentrations  $\geq 1.25$  mg/L significantly decreased oyster weight ( $p<0.0001$ ). The shell surface area was significantly smaller at all resmethrin concentrations compared to the control ( $p<0.0001$ ).

Clam shell weight was significantly reduced at all permethrin concentrations tested ( $p<0.0001$ ). A significant decrease in clam shell area was also detected ( $p=0.0002$ ), at 5 mg/L and 10 mg/L permethrin. Permethrin significantly decreased oyster weight and shell area at all concentrations tested ( $p<0.0001$ ).

Naled had a significant effect on oyster weight ( $p=0.0004$ ), with all concentrations showing reduced weight compared to the control. Naled concentrations  $\geq 2.5$  mg/L had significantly smaller shell area than the control ( $p<0.0001$ ) for both oysters and clams. Clam weight was significantly reduced at naled concentrations  $\geq 1.25$  mg/L ( $p=0.0032$ ).

Methoprene caused a significant decrease in oyster weight at all concentrations tested ( $p<0.0001$ ), and oyster shell area was significantly smaller than the control ( $p<0.0001$ ) at all concentrations except for 5 mg/L methoprene. Clam weight was reduced at methoprene concentrations  $\geq 0.625$  mg/L ( $p=0.0055$ ). The effect of methoprene on clam shell area was variable, with a significant effect detected ( $p<0.0001$ ), but only at concentrations of 0.625, 1.25, and 10 mg/L.

### **Risk assessment**

The data generated from the toxicity tests can be used to assess the risk to shellfish populations. Using the threshold concentrations established for the four insecticides tested, we prepared an environmental risk assessment specifically for clams and oysters and the selected mosquito control applications.

Application data were obtained from the pesticide

product labels, which were then used to calculate the maximum potential concentration ( $\mu\text{g/L}$ ) in 12 inches of surface water. This is a worst-case scenario estimate, and assumes all the product enters the water and is uniformly mixed. We then calculated a risk quotient, which is determined as the estimated water concentration/96 h LC50 value. In this simplified assessment if the risk quotient is greater than one, then a toxic effect is expected to occur (Suter, 1995).

The maximum application rate of resmethrin in a mosquito control product (Scourge<sup>®</sup> 18 + 54) is 0.007 lbs. of active ingredient (AI)/acre, yielding a maximum potential concentration of resmethrin in 12 inches of surface water of 0.00257 mg/L. Using the lowest 96h LC50 value found in this study (1.59 mg/L, larval clams), the risk quotient is 0.0016.

For permethrin, the maximum application rate of a mosquito control product (Permanone<sup>®</sup> 30-30) is 0.007 lbs. AI/acre, which equates to a maximum potential concentration of permethrin in 12 inches of surface water of 0.00257 mg/L. Using the lowest 96h LC50 value found in this study (6.81 mg/L, larval clams), the risk quotient is 0.0004.

Naled is used at a maximum application rate of 0.1 lbs. AI/acre in the mosquito control product Trumpet<sup>®</sup> EC, which equates to a maximum potential concentration of naled in 12 inches of surface water of 0.03677 mg/L. Using the lowest 96h LC50 value found in this study (3.44 mg/L, juvenile clams), the risk quotient is 0.0107.

The maximum application rate of methoprene in a mosquito control product (Altosid) is 0.13 lbs AI/acre, yielding a maximum potential concentration of methoprene in 12 inches of surface water of 0.0478 mg/L. Using the lowest 96h LC50 value found in this study (2.40 mg/L, larval clams), the risk quotient is 0.0199.

The estimated risk quotients for resmethrin, permethrin, naled and methoprene are all less than

one, indicating that use of these compounds for mosquito control is unlikely to cause direct mortality to *M. mercenaria* and *C. virginica*. The risk quotients are

at least 100 times below the predicted effects thresholds for the most sensitive species and life stage identified in this study, larval clams.

**Objective 3.** *Disseminate information to shellfish hatchery operators and agencies responsible for mosquito abatement through SRAC Fact Sheets and meetings.*

A summary of these findings has been presented at various regional scientific meetings and research community forums. In addition to this report, the data are also part of a College of Charleston graduate student thesis, and will be prepared for publication in a peer-reviewed scientific journal.

A summary of these findings was presented to members of the South Carolina Coastal Pesticide Advisory Committee (includes representatives from

the SC Department of Natural Resources, US Fish and Wildlife Service, SC Department of Health and Environmental Control, National Ocean Service, US Environmental Protection Agency, SC Sea Grant, Clemson University Department of Pesticide Regulation, The Citadel, Charleston, Georgetown and Beaufort county mosquito control programs, South Carolina Coastal Conservation League, US Department of Agriculture, and local golf course resort community managers).

## IMPACTS

While we were able to demonstrate significant effects of mosquito control insecticide exposure on molluscan growth and survival, the insecticide concentrations that were found to be detrimental to clams and oysters in this study are much higher than concentrations they are likely to encounter in the field. Environmental factors such as turbidity, sunlight, and microbial interactions all serve to enhance chemical breakdown and decrease bioavailable fractions of these compounds.

National monitoring data by the U.S. Geological Survey detected permethrin in 12% of surface water samples collected, with a maximum concentration of  $5.60 \times 10^{-4}$  mg/L (USGS 2005). Targeted monitoring of the Florida Keys National Marine Sanctuary after mosquito control spraying detected permethrin in canal surface water at a maximum concentration of  $9.4 \times 10^{-3}$  mg/L, naled was detected once at  $1.9 \times 10^{-4}$  mg/L, and dichlorvos (breakdown product of naled) was detected at concentrations up to  $5.6 \times 10^{-4}$  mg/L (Pierce et al. 2005). Bolton-Warberg et al. (2007) did not detect naled in water samples follow-

ing two spray events in Charleston, South Carolina, but detected dichlorvos at 0.21 mg/L. Zulkosky et al. (2005) detected resmethrin in 50% of the samples taken from Long Island, New York within an hour of spray events with a maximum concentration of  $9.8 \times 10^{-4}$  mg/L.

The results of this study provide toxicity threshold values for commonly-used mosquito control insecticides to early molluscan life stages. These data enable us to predict that the use of these chemicals in mosquito control operations should have a low risk of adverse effects on coastal clam and oyster operations and native bivalve populations. However misuse of the products or combined application of multiple products could lead to greater risk to these estuarine species. Furthermore, additional research has demonstrated that other estuarine organisms such as fish and shrimp are more sensitive to insecticides than molluscs; therefore, in order to protect all coastal resources, careful use of mosquito control insecticides is recommended.

## **PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED**

- Garcia, R. 2012. Effects of hypoxia and low pH on mosquito pesticide toxicity in two commercial shellfish species. M.S. Thesis, College of Charleston Graduate Program in Marine Biology, Charleston, SC.
- Garcia, R., and M.E. DeLorenzo. 2012. Effects of hypoxia and low pH on mosquito pesticide toxicity in two commercial shellfish species. June 11, 2012. Harbor Branch Oceanographic Institution, Fort Pierce, FL.
- Garcia, R., K. W. Chung, P.B. Key, L.E. Burnett, L.D. Coen, and M.E. DeLorenzo. 2012. Effects of hypoxia and low pH on mosquito pesticide toxicity in two commercial shellfish species. Southeastern Estuarine Research Society Meeting, April 13, 2012, Beaufort, NC.
- Garcia, R., K.W. Chung, P.B. Key, L.E. Burnett, L.D. Coen, and M.E. DeLorenzo. 2012. Effects of hypoxia and low pH on mosquito pesticide toxicity in two commercial shellfish species. Carolinas Society of Environmental Toxicology and Chemistry Meeting, March 30, 2012, Aiken, SC.
- The graduate student funded by this project presented the results of this study at two regional scientific society meetings; Carolinas Society of Environmental Toxicology and Chemistry and the Southeastern Estuarine Research Society. The data are being incorporated in to this student's College of Charleston master's thesis, and will be prepared for publication in a peer-reviewed scientific journal.

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## DEVELOPMENT OF BAITFISH, GOLDFISH, AND ORNAMENTAL FISH HATCHERY METHODS

### Reporting Period

January 1, 2012 – August 31, 2012

<b>Funding Level</b>	Year 1 .....	\$59,957
	Total.....	\$59,957

<b>Participants</b>	University of Arkansas at Pine Bluff .....	Anita Kelly, Nathan Stone
	Louisiana State University .....	Chris Green
	University of Florida .....	Cortney Ohs

### PROJECT OBJECTIVES

1. Develop goldfish and ornamental fish hatchery methods that result in cost-effective methods to de-stick adhered goldfish and ballyhoo eggs from spawning substrate within 24 hours of deposition.
  - a. Determine the composition of the egg matrix of goldfish and ballyhoo.
  - b. Identify the compounds that will de-stick goldfish and ballyhoo eggs from spawning substrate.
  - c. Perform embryo viability and hatch assays to determine toxicity thresholds for selected de-sticking agents.
  
2. Develop baitfish hatchery methods that result in cost-effective methods to maximize egg collection within 1 to 2 days of fertilization of fathead minnows.
  - a. Determine the optimal nest site to male fathead minnow ratio that will maximize egg number and collection efficiency (egg per unit of substrate).
  - b. Determine the effect of pond water temperature, dissolved oxygen, and depth on fathead minnow egg production in earthen ponds.

### ANTICIPATED BENEFITS

Removal of eggs from spawning substrate will enable producers to hatch eggs in a controlled environment. They will be able to obtain more accurate counts of the number of fry that are stocked into ponds. Increased production costs

have severely affected the profitability of bait, feeder and forage fish farming. New hatchery methods have the potential to reduce production costs and keep farms profitable.

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

**Objective 1.** *Develop goldfish and ornamental fish hatchery methods that result in cost-effective methods to de-stick adhered goldfish and ballyhoo eggs from spawning substrate within 24 hours of deposition.*

**Subobjective 1.** *To determine the composition of the egg matrix of goldfish and ballyhoo.*

### Louisiana State University

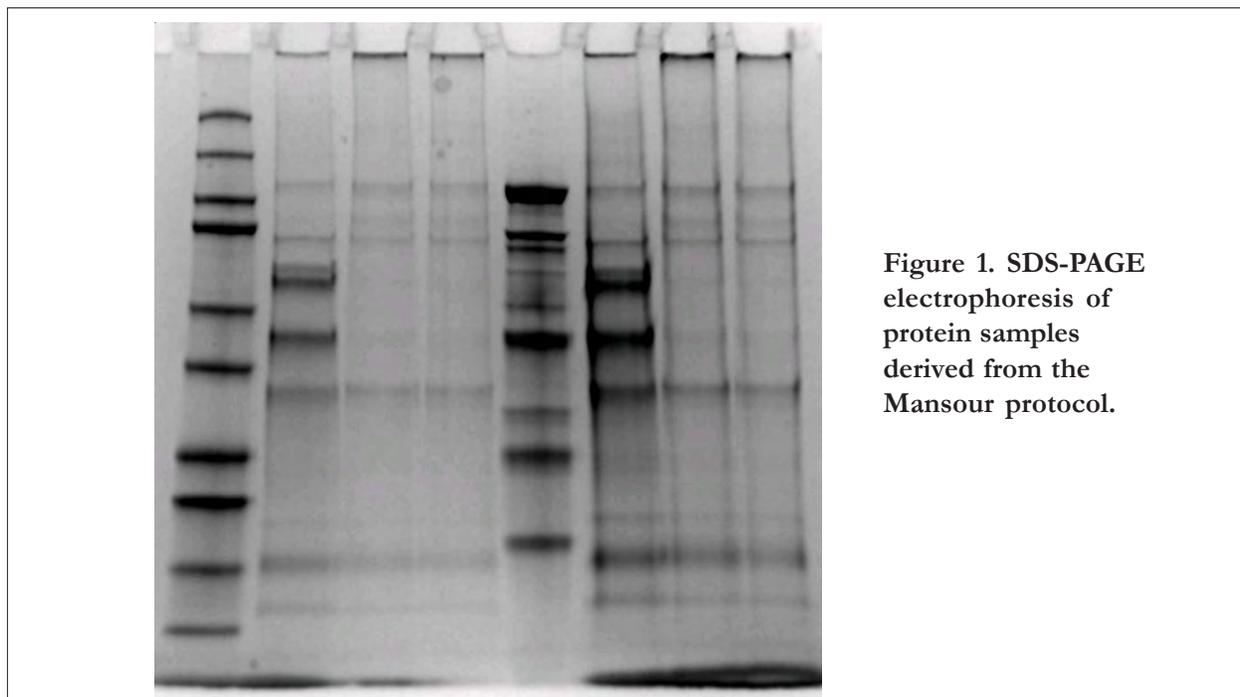
Glycoproteins are large molecules composed of proteins and sugars that are attached to the outer membranes of fish eggs. Glycoproteins are also responsible for the “sticky” nature of eggs developed after fertilization, allowing eggs to adhere to a substrate and preventing river and tidal currents from dislodging eggs (Riehl and Patzner 1998). In aquaculture settings, adhesive eggs prevent a speedy harvest of eggs and increase labor. The purpose of this sub-objective is to characterize the nature of goldfish (*Cyprinus carpio*) and ballyhoo (*Hemiramphus brasiliensis*) egg membrane glycoproteins to better identify appropriate de-sticking compounds.

Goldfish eggs were received from the University of Arkansas at Pine Bluff (UAPB). Isolation of glycoproteins was first attempted using a protocol derived from Mansour et al. 2009. The protocol was created with the assistance of Dr. Ted Gauthier and Tamara Chouljenko of the Louisiana State University (LSU) H.D. Wilson Laboratories Protein Center. The protocol called for removal of the egg membrane using forceps in an isotonic saline solution seated in ice. Egg membranes were then ground in a container that was seated in ice to release the glycoproteins. The saline solution was removed and frozen at -4 degrees F for later analysis and the ground egg membranes were transferred to a new container. The egg membranes were then incubated in a urea and bicarbonate solution for six hours at 39 degrees F. The solution was centrifuged to pellet the egg membranes and the supernatant was tested using SDS-PAGE gel electrophoresis for the presence of proteins. Low concentrations of proteins were

detected and were determined insufficient to proceed with glycoprotein staining. A new protocol was developed from Scapigliati et al. 1995 with the assistance of Dr. Gauthier and Mrs. Chouljenko. The new protocol called for the chorion removal over ice and 15 minute incubation in a solution containing Tris-HCl buffer, NaCl, EDTA, PMSF, and Triton X-100 at 39 degrees F. The egg membranes were transferred to a solution similar to the one listed previously, but with added BME, where it was homogenized. The mixture was centrifuged to pellet the chorions and the supernatant was collected.

The Mansour protocol produced four protein bands at the highest concentration of 20  $\mu$ L of sample (Fig. 1, lanes 1-3). Bands were detected at approximately 125, 105, 70, and 22 kDa. Lanes 5-7 (Fig 1.) represent a lower concentration of 5  $\mu$ L of sample. Lanes 8-10 (Fig. 1) represent the saline solution in which the egg membranes were homogenized. The fourth lane (Fig. 1) represents the Precision Plus Protein Standard from Biorad. Proteins isolated using the Scapigliati protocol have been measured at total protein concentrations (mean  $\pm$  SD) of  $1,775 \pm 62.21$ ,  $454.12 \pm 41.37$ , and  $1,426.22 \pm 573.87$  mg/L. These samples have been submitted to the H. D. Wilson protein lab for electrophoresis analysis and glycoprotein staining.

With future processed samples the resulting bands from SDS-PAGE will be excised and purified using C-18 columns. These glycoproteins will then be analyzed for composition using mass spectroscopy.



**Figure 1. SDS-PAGE electrophoresis of protein samples derived from the Mansour protocol.**

The current and future results of this portion of the project are ongoing and will develop as we continue to extract and characterize these proteins.

We received frozen ballyhoo embryos from Florida State University in September 2011, however, did not initially extract chorion proteins as we had to modify our extraction protocols from Mansour et al. (2009, *Animal Reproduction Science* 114:256-268) to Scapigliati et al. (1995, *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 112:169-175). The Scapigliati protocol resulted in greater amounts of egg shell proteins when compared to the Mansour method and as a result we continued protein extractions with the Scapigliati protocol. We utilized Gulf killifish egg shells as surrogate for method development due to the availability of eggs at our home institution.

Glycoprotein isolation from extracted egg shell proteins was performed using a Thermo Scientific Pierce Glycoprotein Isolation Kit (model # 89804).

This method uses lectins con canavilin to bind ligands with specific classes of glycoproteins from a sample with several types of proteins. After several extractions with this kit and modifying the extraction protocol, the use of this isolation method was discontinued due to a significant loss of glycoprotein in comparison to glycoprotein staining. Glycoprotein staining was used in subsequent examinations to prepare and isolate for mass spectrometry.

The first large band from the egg shell protein samples was extracted and purified in December 2011. This protein was submitted for mass spectroscopy and subsequent comparison analysis against a protein library. To accomplish this we used Mascot, search engine software that uses mass spectrometry data to identify proteins from sequence databases. This software was developed by Matrix Science Ltd. and is available on: ([www.matrixscience.com](http://www.matrixscience.com)). The two databases used for this search were SwissProt and the National Center for Biotechnology Information. Mascot software indicated that the

first band in our gel had an 84% similarity to vitellogenin, a yolk precursor protein from *Fundulus heteroclitus*. This is a sister species to the Gulf killifish and indicates that our first protein band was contamination from the yolk or embryo and not a protein that originates in the chorion.

New batches of koi and Gulf killifish egg shells were extracted using the Scapigliati protocol in the spring and summer of 2012 yield low and/or no proteins on gels. An updated protocol was obtained from Scapigliati. The updated protocol utilizes an S-TNE buffer with Urea rather than the previous Triton X-100, which is more efficient at extracting a greater number of proteins in comparison to Triton. In order to increase the quality of this initial egg shell homogenization and protein extraction method we investigated several urea concentrations and homogenization methods.

The egg shell proteins extracted using 3M urea were

### **University of Arkansas at Pine Bluff**

Newly laid goldfish eggs were manually removed from spawning substrate using tweezers. Eggs shells were removed and glycoproteins were extracted

**Subobjective 2.** *Identify the compounds that will de-stick goldfish and ballyhoo eggs from spawning substrate.*

### **University of Arkansas at Pine Bluff**

The inability of producers to remove goldfish eggs from spawning substrate does not allow for the producer to accurately count the number of fry that are stocked into a pond. Mats are currently placed into ponds where eggs hatch. The number of eggs per mat is usually a guess and the number of fry hatched and produced in the pond is often an inaccurate estimate of the true population. De-sticking eggs from spawning material is also a bottleneck for the commercial efficiency for ballyhoo.

different in molecular weight when compared to samples from the previous year. Figure 1a shows lane 1 as a molecular marker, lane 2 as the proteins from a 3M urea extraction, and lane 3 as proteins extracted from the previous year. When stained for glycoproteins the new samples did not indicate that any of the extracted proteins were glycoproteins in comparison to previously extracted samples. It is possible that the previous protein samples were contaminated with remnants of yolk proteins or embryos as seen in the mass spectroscopy results.

Work on protein extraction and isolation continues in samples of both goldfish and killifish egg shells with a majority of the work centered on protocol refinement for glycoprotein isolation. We have ballyhoo eggs frozen for future use, but due to the small numbers of eggs we plan to work with these egg shells only after the goldfish and killifish egg glycoproteins are successfully extracted and characterized.

following the protocol developed by the LSU researchers. Extracted glycoproteins were sent to LSU for isolation and identification.

Another bottleneck with ballyhoo is the hatching rates of the eggs have been low. Removal of ballyhoo eggs from the spawning substrate would enable producers to efficiently collect and hatch eggs. By hatching eggs in a controlled environment, hatching rates in goldfish and ballyhoo may also be increased.

Spawning mats containing freshly laid (<12 h) goldfish eggs were obtained from a local fish farm.

Mats were cut into 3 × 3 inch squares and placed into 1 pint beakers containing one of the following concentrations of a selected solution: sodium sulfite, 1.5%, 2.0%, and 3.0%; tannic acid 75%, and urea 3%, 4%, 6%, and 8%; control in hatchery water; fresh squeezed pineapple juice 1%, 3%, 5%, and 10%; bromelain 1%, 3%, 5%, and 10%; papaya 1%, 3%, 5%, and 10%; papain 1%, 3%, 5%, and 10%; 4M urea; lithium chloride 1 mg/L, 3 mg/L, 5 mg/L, and 10 mg/L; cadavarine 1 mg/L, 3 mg/L, and 5 mg/L; lysozyme; acetone; sodium bicarbonate; sodium hydroxide; ammonium chloride; ethylene glycol mono-butyl ether; propylene glycol n-butyl ether; citric acid; and alcalase, 20 mL/L, 40 mL/L, and 80 mL/L. Eggs were placed into treatment solutions for 2 minutes and removed. Loose eggs and eggs still attached to the substrate were counted and the percentage of eggs removed was calculated. The eggs were then placed into a tank and allowed to hatch. Newly hatched fry were counted and the percent survival was calculated.

Significant removal of eggs was only accomplished with the alcalase enzyme. The 20 mL/L alcalase treatment removed 10% of the eggs from the mat, the 40 mL/L treatment removed 78% and the 80 mL/L treatment removed 82% of the eggs in 2 minutes. To increase the number of eggs removed the alcalase was mixed in a 3% salt solution. Removal of eggs exposed to 20 mL/L alcalase with 3% salt was 36%, for 40 mL/L was 99%, and for the 80 mL/L was 100%.

Survival of the eggs to hatch was low with less than 2% surviving. This may have been due to the fact that the eggs were not placed into hatching jars but laid in a single layer on the bottom of an aquarium. Eggs did succumb to fungal infections. Once the egg membrane compounds are identified, other potential

compounds will be tested and eggs will be hatched in a hatching jar.

Since the matrix of the egg shells is still unknown, we tested different types of spawning substrate to determine if eggs could be removed more easily from a smooth substrate versus the grass-shaped spawning mats typically used. Spawning mats were constructed of geoweb material, strips of which were connected to make a 2 ft × 3 ft mat, and from corrugated PVC board that was weighted down with wire frames. Spawning mats were placed into commercial goldfish spawning vats and left overnight. Spawning mats containing freshly laid (<12 h) goldfish eggs were cut into 3 × 3 inch squares and placed into 1 pint beakers containing one of the following concentrations of a selected solution: sodium sulfite, 1.5%, 2.0%, and 3.0%; tannic acid 75%, and urea 3%, 4%, 6%, and 8%; control in hatchery water; fresh squeezed pineapple juice 1%, 3%, 5%, and 10%; bromelain 1%, 3%, 5%, and 10%; papaya 1%, 3%, 5%, and 10%; papain 1%, 3%, 5%, and 10%; 4M urea; lithium chloride 1 mg/L, 3 mg/L, 5 mg/L, and 10 mg/L; cadavarine 1 mg/L, 3 mg/L, and 5 mg/L; lysozyme; acetone; sodium bicarbonate; sodium hydroxide; ammonium chloride; ethylene glycol mono-butyl ether; propylene glycol n-butyl ether; citric acid; and alcalase, 20 mL/L, 40 mL/L, and 80 mL/L. Eggs were placed into treatment solutions for 2 minutes and removed. Loose eggs and eggs still attached to the substrate were counted and the percentage of eggs removed was calculated. The eggs were then placed into a tank and allowed to hatch. Newly hatched fry were counted and the percent survival was calculated.

No significant removal of eggs was accomplished with any of the treatments tested.

## University of Florida

Ballyhoo were collected from the wild and held in quarantine for 21 days. They were treated with five one hour formalin baths (100 mg/L) on alternating days and were fed mysid shrimp soaked in an antibiotic for 10 days. Thereafter, they were fed a combination of mysid shrimp, krill, and pelleted feed.

Ballyhoo maintained in tanks readily spawned onto substrate constructed of PVC and plastic zip ties during late May and early June. However, only small batches of eggs were spawned daily. Three compounds at three concentrations were exposed to egg samples in triplicate. These included sodium sulfite 1.5, 3.0, 6.0%; trypsin 0.05%, 0.25%, 0.5%; and alcalase 20, 40, 80 mL/L of water each for 15 minutes each. Additionally, samples of unexposed eggs were collected to determine the composition of the egg membrane and associated microfibrils which serve to adhere the eggs to substrate.

None of these compounds has been successful at the tested concentrations and exposure time in breaking up the microfibrils which adhere the eggs to substrate and to each other. Continued evaluation of other concentrations, exposure times, and compounds will be conducted when more eggs are naturally spawned. All eggs including those unexposed, and exposed to the various chemicals are stored in a -112 degrees F freezer. The composition of the egg matrix of ballyhoo eggs will be identified in the next few months.

Adult ballyhoo were collected from the wild and held in quarantine for 21 days. They were treated with five one hour formalin baths (100 mg/L) on alternating days and were fed mysid shrimp soaked in an antibiotic for 10 days. Thereafter, they were fed

a combination of mysid shrimp, krill, and pelleted feed.

Two populations of ballyhoo were maintained in tanks for one year. Ballyhoo spawned repeatedly onto substrate provided in the tanks which was constructed of PVC and plastic zip ties. Eggs were gently removed from the substrate. Egg masses consisting of 25 to 100 eggs were added to beakers containing solutions of saltwater and one of three compounds each at various concentrations. Each combination of compound and concentration was replicated three to five times. A small stir bar was added to the beakers to gently move the egg masses. Treatment compounds and concentrations included sodium sulfite 1.5%, 2.0%, 3.5%, sodium sulfite 1.5%, 3.0%, 6.0%; trypsin 0.05%, 0.25%, 0.5%; trypsin 1.0%, 1.5%, 2.0%, and alcalase 20, 40, 80 mL/L of water, each with an exposure time of 15 minutes. Additionally, samples of unexposed eggs were collected to determine the composition of the chorion and associated microfibrils which serve to adhere the eggs to substrate and to each other.

None of these compounds has been successful at the tested concentrations and exposure time in breaking up the microfibrils which adhere the eggs to substrate and to each other. Additional evaluation of different concentrations, exposure times, and compounds is warranted.

All eggs including those unexposed and exposed to the various compounds and concentrations were stored in a -112 degrees F freezer until shipped on dry ice to LSU for analyses of composition and change in composition following exposure to the various compounds.

**Subobjective 3.** *Perform embryo viability and hatch assays to determine toxicity thresholds for selected de-sticking agents.*

This objective will be completed once the egg membrane compounds have been identified.

**Objective 2.** *Develop baitfish hatchery methods that result in cost-effective methods to maximize egg collection within 1-2 days of fertilization of fathead minnows.*

**Subobjective 2a.** *Develop baitfish hatchery methods that result in cost-effective methods to maximize egg collection within 1-2 days of fertilization of fathead minnows.*

### University of Arkansas at Pine Bluff

A new hatchery method for fathead minnow is based on the collection of eggs from brood ponds for indoor hatching. Egg collection is costly, given the required spawning substrate and labor. The purpose of this study was to improve the efficiency of egg collection by evaluating resulting egg production from four different ratios of substrate area to male fish.

Approximately 121 rosy red fathead minnows (200 g total per pool; average weight per fish of 1.65 g) were stocked into each of 16, 35.5 ft<sup>2</sup>, 3-ft deep, outdoor plastic pools in June 2011. A male:female sex ratio of 1:2 was determined by visual inspection of 100 fish. Treatments consisted of 1, 2, 3, or 4 sections of 3 in-wide textured plastic geoweb material (Envirogrid® EGA20), each with an underside surface area of ~ 4.4 ft<sup>2</sup>, resulting in substrate area to

male fathead minnow ratios of 0.01, 0.02, 0.03, and 0.04. Water from a reservoir replenished by a shallow well was added to each pool at a rate of 6 L/min, and constant aeration was provided using a low pressure blower. Fish were fed once daily at 3% of initial body weight with extruded commercial catfish feed (32% protein). Temperature was recorded every four hours and dissolved oxygen concentration and mortalities were recorded twice daily. The number of nests and eggs were determined every two to three days and eggs were removed with 1.5% sodium sulfite. Eggs were collected a total of eight times. Mean temperature was 85 degrees F, close to the upper limit for reproduction. Less than half of the males were actively nesting at any one time and there was no difference in the number of nests collected between treatments (Table 1).

**Table 1. Mean (SEM) number of nests for each collection event, mean (SEM) number of eggs per male per day, and number of eggs per kg of fish per day. Means within a row with different letters were significantly different (P < 0.05).**

	Substrate Area (m <sup>2</sup> ): Male			
	0.01	0.02	0.03	0.04
Mean (SEM) nests/collection	9.8 ± 4.3	9.7 ± 3.7	10.9 ± 3.2	9.2 ± 4.1
Mean (SEM) eggs · male <sup>-1</sup> · day <sup>-1</sup>	31 ± 23ab	37 ± 25a	36 ± 17ab	26 ± 16b
Mean eggs · kg fish <sup>-1</sup> · day <sup>-1</sup>	6,115ab	7,366a	7,130ab	5,231b

Fathead minnows are traditionally propagated using the spawning-rearing pond method, where brood fish are stocked, spawning substrate is added, and resulting young are reared together with the adults. Unfortunately, using this method, diseases are passed from adults to the young, there is little control over fish density, and undesirable fish such as mosquitofish may be transferred together with brooders. A new hatchery method to overcome the limitations of the spawning-rearing pond method involves collection of fathead minnow eggs from brood ponds, indoor hatching of eggs, and stocking of resulting fry at known densities. However, spawning substrate and labor for egg collection are costly. A research study was designed to compare the numbers of fathead minnow eggs that could be collected when brood fish were supplied with differing amounts of spawning substrate. If the quantity of supplied substrate can be reduced without decreasing egg numbers, this will reduce the costs of this method.

Equal weights (0.44 lb) of rosy red fathead minnow brooders (3.6 lbs per 1,000) were stocked into each of 16, 800-gallon outdoor pools. The sex ratio of the broodfish was 2 females to 1 male. As male fathead minnows use the undersides of submerged materials as nest sites, sections of 3-inch deep textured plastic geoweb material (Envirogrid® EGA20) were supplied as spawning substrate. Geoweb material was added to pools at four different rates (four replicates per treatment); 13, 26, 40, or 53 inches of under-surface area of substrate per male fathead minnow. Water from a reservoir replenished by shallow wells was added to each pool at a rate of

1.6 gpm, and constant aeration was provided using a low pressure blower. Fish were fed once daily at 3% of initial body weight with extruded commercial catfish feed (32% protein). Temperature was recorded every four hours and dissolved oxygen concentration and mortalities were recorded twice daily. The number of nests and eggs on spawning substrates were determined every two to three days and eggs were removed with 1.5% sodium sulfite. Eggs were collected a total of eight times. Average water temperature was 85 degrees F, close to the upper limit for reproduction.

Total egg numbers (estimated from egg volume) per collection event did not differ among the three lowest substrate area to male ratios. Egg numbers for the treatment with 53 square inches of substrate per male was less than for the middle two rates, and equal to that of 13 square inches of substrate per male. Based on nest numbers, less than half of the males were actively nesting at any one time.

Providing too little spawning substrate for fathead minnows encourages males to select other surfaces in a brood pond as nesting sites, such as sticks, vegetation, and aerators, and would reduce egg numbers that could be collected. Providing too much substrate increases costs. Results from this study show that providing only 13 square inches of spawning substrate per male fathead minnow did not reduce egg numbers that could be collected, and suggest that even lower amounts of substrate area could be supplied with no decrease in egg numbers.

**Subobjective 2b.** *To determine the effect of pond water temperature, dissolved oxygen, and depth on fathead minnow egg production in earthen ponds.*

#### **University of Arkansas at Pine Bluff**

Placing spawning substrates within brood ponds in favorable locations for egg deposition should improve egg harvest and reduce the cost per million fry. This study was designed to examine nest location

and egg number on spawning substrates in relation to depth, temperature and early morning dissolved oxygen (DO).

Two 0.04-ac earthen ponds were fertilized and each stocked with 46.85 lbs ( $1.9 \pm 0.6$  g/fish) of adult rosy red fathead minnows with a visually determined male:female sex ratio of 7:10. The fish were fed once daily to satiation with extruded commercial catfish feed (32% protein). Spawning substrate consisted of three, 11-ft<sup>2</sup> sections of 3 in-wide Envirogrid® EGA20 geocell per pond, each suspended between a floating pipe at the surface and a weighed pipe at a depth of 3.3 ft.

Thermographs recorded temperature every 4 h at 10-in intervals from the surface to a depth of 3.3 ft, and DO was recorded at 10-cm intervals every morning. The number of nests and eggs were determined every two days and eggs were removed with 1.5% sodium sulfite and collected nine times. Temperature, DO and other water quality parameters were similar in both ponds and within acceptable ranges for this species. Cumulatively, the number of eggs was greatest on rows 2 and 4. Depth, temperature, DO, and date all had interactive effects on the number of nests per day and the total number of eggs produced per day. Data analysis is continuing.

For a new hatchery method for fathead minnows to be cost-effective, egg collection efficiency should be optimized. Placing fathead minnow spawning substrates within brood ponds in favorable locations for egg deposition should improve egg harvest and reduce the cost per million fry. This study was designed to examine nest location and egg number on spawning substrates in relation to depth, temperature and early morning dissolved oxygen (DO).

Two, 0.1-acre earthen ponds were fertilized and each stocked with 46.8 lbs of 4.2 lbs per 1,000 adult rosy red fathead minnows. The sex ratio was 7 males to 10 females. Fish were fed once daily to satiation with extruded commercial catfish feed (32% protein). Three, 10.8 square foot sections of 3-inch-deep Envirogrid® EGA20 geoweb were placed into each pond as spawning substrate.

Each honeycomb-like section of geoweb was suspended between a floating pipe at the surface and a weighed pipe at a depth of 3.3 ft. Thermographs recorded temperature every 4 hours at 10-inch intervals from the surface to a depth of 3.3 ft, and DO was recorded at 10-inch intervals every morning. The number of nests and eggs at 5-inch depth intervals (corresponding to each set of horizontal cells of the geoweb material) on each substrate were determined every two days for a total of nine collection events. Eggs were removed with 1.5% sodium sulfite.

Fathead minnow egg production peaked at the third collection event (May 16), and then declined sharply, even though water temperatures at this time were similar to initial conditions in the ponds. Differences in temperature with depth were found in both ponds. In general, water temperature declined linearly with depth and was 9 degrees F cooler at a depth of 3.3 feet as compared to the surface. Overall, the greatest numbers of eggs were collected at depths of 10 and 20 inches. Fewer eggs were found at depths below 30 inches, except for collections on May 16 and 18, when relatively greater numbers of eggs were found at the bottom of substrates. On May 14 -16, a cold front caused surface waters of the ponds to be up to 7 to 8 degrees F colder than at 3.3 feet, albeit for a relatively short period, and this temperature inversion apparently caused fish to seek out this deeper, warmer water for nest sites. Early morning dissolved oxygen levels were also lower with depth, with typical early morning concentrations in the range of 0.1 to 0.2 mg/L at 3.3 feet. Despite these low levels, eggs were found at the bottom of the substrates early in the study, when egg production was greater overall. We observed that the ends of the substrates were favored as nest sites, suggesting that narrow vertical sections of geoweb might be best.

Results from this study confirmed that fathead minnows will spawn over a broad range of

temperatures, minimum dissolved oxygen levels, and water depths. No specific favorable locations (conditions) for substrate placement could be

identified. As such, substrates can be placed in locations that are convenient for workers to collect.

## **IMPACTS**

Identification of goldfish and ballyhoo egg membrane compounds will aid in the identification of substances that will break the adhesive bond between the egg and spawning substrate.

production of goldfish and ballyhoo by enabling producers to utilize a smaller hatchery space. Current costs to run an indoor goldfish hatchery could save 90% of the costs per day.

Desticking goldfish and ballyhoo eggs will improve

## **PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED**

### **Publications in Print**

Weldon, D. B., N. Stone, and J. Sun. 2012. Effect of spawning substrate to male ratio on fathead minnow egg production. *North American Journal of Aquaculture* 74:419–423.

Stone, N., and D. Weldon. 2012. Potential fathead minnow spawning substrate for indoor egg incubation. *Arkansas Aquafarming* 29(1):1-2.

### **Presentations**

Kelly, A. M., S. Kumaran, and N. Stone. 2011. A potential method of desticking goldfish eggs from spawning mats. *Aquaculture America*, San Diego, California.

Kelly, A. M., S. Kumaran, and N. Stone. A potential method of desticking goldfish eggs from spawning mats. Poster presentation. Annual Meeting of the Arkansas Bait and Ornamental Fish Growers Association. Lonoke, Arkansas.

Stone, N. 2012. The latest in new baitfish technology. UAPB Rural Life Conference, Pine Bluff, Arkansas.

Sun, J., C. Lee, and N. Stone. 2012. Effects of egg mimic number and sphere diameter on fathead minnow egg production. Poster presentation, *Aquaculture America 2012*, U.S. Aquaculture Society, Las Vegas, Nevada.

Sun, J., C. Lee, and N. Stone. 2012. Effects of egg mimic number and sphere diameter on fathead minnow egg production. Poster presentation, *UAPB Aquaculture/Fisheries Field Day*, Pine Bluff, Arkansas.

Weldon, D. B., and N. Stone. 2012. Effect of removal from substrate on fathead minnow egg hatching success. Poster presentation, *UAPB Aquaculture/Fisheries Field Day*, Pine Bluff, Arkansas.

Weldon, D. B., N. Stone, and J. Sun. 2012. Effect of spawning substrate to male ratio on fathead minnow *Pimephales promelas* egg production. Aquaculture America 2012, U.S. Aquaculture Society, Las Vegas, Nevada.

Weldon, D. B., N. Stone, J. Sun, and L. Xie. 2012. Effect of depth on fathead minnow *Pimephales promelas* egg production. Poster presentation, Aquaculture America 2012, U.S. Aquaculture Society, Las Vegas, Nevada.

Weldon, D. B., N. Stone, J. Sun, and L. Xie. 2012. Effect of temperature and dissolved oxygen on fathead minnow *Pimephales promelas* egg production in ponds. Poster presentation, Lonoke Aquaculture Workshop, Lonoke, Arkansas.



## IMPROVING CATFISH BROODSTOCK MANAGEMENT BY MANIPULATING DIET, STOCKING DENSITIES AND SEX RATIOS

### Reporting Period

January 1, 2012 – August 31, 2012

<b>Funding Level</b>	Year 1 .....	\$115,860	
	Year 2 .....	\$127,400	
	Year 3 .....	\$145,125	
	Total.....	\$388,385	
<b>Participants</b>	University of Arkansas at Pine Bluff .....	Rebecca Lochmann, Carole Engle, Alf Haukens	
	Texas A&M University .....	Alejandro Buentello, Delbert M. Gatlin, III	
	USDA-ARS Catfish Genetics .....	Brian Bosworth, Sylvie Quiniou Research Unit	Geoff Waldbieser

### PROJECT OBJECTIVES

1. Identify diet formulations to improve reproductive performance (egg biochemical composition, fecundity, egg and fry quality) of catfish and determine associated effects on production costs.
  - a. Manipulate dietary protein concentration and lipid sources and assess reproductive performance and diet effects on production costs (Tank Trial 1).
  - b. Refine dietary protein and lipid sources, add nucleotides, and assess reproductive performance and diet effects on production costs (Tank Trial 2).
  - c. Conduct concurrent pond trials at UAPB and TAMU to assess reproductive performance and diet effects on production costs using with a high-performance diet and an economical diet (based on results of subobjectives I a and I b, above) and a standard commercial diet.
2. Determine effects of sex ratios, stocking densities, and post-spawning brood fish consolidation on catfish reproductive success and determine associated costs.
  - a. Effects of broodfish sex ratios.
  - b. Effects of broodfish stocking rates.
  - c. Effects of post-spawning broodfish consolidation.

## ANTICIPATED BENEFITS

Development of diets or supplements specifically designed to meet the requirements of catfish broodstock should optimize spawning performance and ensure the production of high-quality eggs and fry. Information on the effects of dietary lipids, proteins, and nucleotides on gamete and fry production and quality could reduce the number of broodfish needed to meet production goals. Altering

traditional management protocols by using different sex ratios or stocking densities could improve economic efficiencies of fry production and improve profitability for farmers. Research in catfish has just begun to yield data sufficient to support detailed economic analysis of broodstock and hatchery performance and management practices in the U.S.

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

**Objective 1.** *Identify diet formulations to improve reproductive performance (egg biochemical composition, fecundity, egg and fry quality) of catfish and determine associated effects on production costs.*

**Subobjective 1a.** *Manipulate dietary protein concentration and lipid sources and assess reproductive performance and diet effects on production costs (Tank Trial 1).*

### University of Arkansas at Pine Bluff; Texas A&M University

Industry wide, only 30 to 40% of female catfish spawn each year. The reasons for the poor spawning performance are not clear, so producers maintain an excess of broodstock to ensure that egg production goals are met. Development of diets or supplements to diets specifically designed to meet the requirements of catfish broodstock are needed to optimize spawning performance and ensure the production of high-quality eggs and fry.

Four experimental diets (Table 1), with simple modifications to protein concentration and lipid sources, were formulated based upon results of earlier channel catfish broodstock nutrition research conducted by the participating universities. These diets were formulated to provide effective broodstock nutrition for catfish produced throughout the southern region and will incorporate economic considerations and dietary modifications based upon other research on this topic. The four diets were evaluated (4 replicates/diet; stocking ratio

of 1:3 male to female) using physical and biochemical methods including analyses of egg composition, egg production and enumeration, and egg and fry quality.

There were no differences in water temperature at the time of spawning (Mean  $\pm$  SE; 77.5  $\pm$  1.0 degrees F), individual egg weight (0.031  $\pm$  0.00 oz), quantity of eggs per milliliter (24.2  $\pm$  1.4 eggs), quantity of eggs per spawn (18,944  $\pm$  2,674 eggs), hatch rate (87.5  $\pm$  2.9 %), fry survival to 14 days (92.0  $\pm$  2.3 %), fry produced per 1,000 eggs (801  $\pm$  36), or feed required to produce 1,000,000 fry (643  $\pm$  233 lbs). Spawning success during the study was considered poor, but within the industry average (30-40%). Spawning success for females fed diets 1 and 3 (41.6%) was greater than those of females fed diets 2 and 4 (33.3%). Total egg mass weight (matrix intact) was greatest for fish fed diet 1, followed by diet 3, diet 2, and diet 4 (1.77, 1.61, 1.06, and 0.98 lbs, respectively). The total egg volume (matrix

**Table 1. Formulae of diets for Trial 1, subobjective 1a (2012) in the Southern Regional Aquaculture Center catfish broodstock management study (% as-fed). Diets were extruded at the Harry K. Dupree Stuttgart National Aquaculture Research Center.**

Ingredient (%)	Diet 1-control (36% protein)	Diet 2 (36% protein)	Diet 3 (32% protein)	Diet 4 (32% protein)
Menhaden fish meal	6.0	-	3.5	-
Poultry by-product meal eat/bone/blood meal, pork	10.0	6.0	7.5	4.0
Soybean meal	-	6.0	-	4.0
Cottonseed meal	49.0	54.0	38.0	41.0
Corn starch	6.0	6.0	8.0	8.0
Wheat middlings	20.0	19.0	18.8	18.8
Menhaden fish oil	0.0	0.0	16.0	16.0
Poultry fat	4.0	4.0	3.6	3.6
Vitamin premix <sup>a</sup>	4.0	4.0	3.6	3.6
Stay-C	0.48	0.48	0.48	0.48
Mineral premix <sup>a</sup>	0.02	0.02	0.02	0.02
	0.50	0.50	0.50	0.50

<sup>a</sup> Standard vitamin and mineral premixes used at UAPB.

removed) was greatest for fish fed diet 1 (65.5 in<sup>3</sup>) and lowest for diet 4 (33.7 in<sup>3</sup>), while diets 2 (37.1 in<sup>3</sup>) and 3 (54.3 in<sup>3</sup>) were intermediate. The total quantity of 14-day-old fry produced was greater for fish fed diet 1 (118,257 fry) than for fish fed diets 2 and 4 (43,173 and 47,579 fry, respectively), while fry from fish fed diet 3 were intermediate (78,438 fry). The total quantity of 14-day-old fry produced per female was greater for fish fed diet 1 (9,854 fry) than for fish fed diets 2 and 4 (3,597 and 3,964 fry, respectively), while fry from fish fed diet 3 were intermediate (6,536 fry). The total pounds of females needed to produce 1,000,000 fry were significantly lower for diets 1 and 3 (5,562 and 8,158 lbs, respectively) than for diets 2 and 4 (15,382 and 12,564 lbs, respectively). The pounds of feed needed to produce 1,000,000 fry were significantly lower

for diet 1 (3,392 lbs) than for diets 2 or 4 (9,381 and 7,665 lbs, respectively), while diet 3 was intermediate (4,976). There were no significant differences in amino acid composition of the eggs. Fatty acid analysis of the eggs is ongoing, and economic analysis of results is in progress.

Based on the results of the study, Diet 1 (with 36% protein from menhaden fish meal, poultry by-product meal, soybean meal, and cottonseed meal, and 10% lipid from menhaden fish oil and poultry fat) performed the best in terms of fry production. Industry use of diet 1 should reduce the amount of feed and the total pounds of females required to achieve the most efficient production of channel catfish fry compared to 32 % protein diets and diets that replace fish meal with other animal by-products.

**Subobjective 1b.** *Refine dietary protein and lipid sources, add nucleotides, and assess reproductive performance and diet effects on production costs (Tank Trial 2).*

**University of Arkansas at Pine Bluff; Texas A&M University**

The diet from subobjective 1a that resulted in the best reproductive performance and fry production (Diet 1; 36% protein- 6% menhaden fish meal, 10% poultry by-product meal, 49% soybean meal, 6% cottonseed meal; 10% lipid – 4% menhaden fish oil, 4% poultry fat, 2% endogenous) and the diet with the lowest production costs (\$/fry produced) will be carried through to this study, year 2 (tank trial 2) and evaluated against two new experimental diets. New diet formulations will be based on the best performing diets from subobjective 1a, but will contain less animal protein, more cost-saving plant proteins, and yeast nucleotide supplements.

The tank trial for subobjective 1b is on schedule to begin in February 2013.

**Results at a glance...**

- *A diet with 36% protein including 16% animal protein (fish and poultry) and 4% fish oil should reduce the amount of feed and the total pounds of females required for efficient production of channel catfish fry compared to diets with less protein and no fish meal.*

**Subobjective 1c.** *Conduct concurrent pond trials at UAPB and TAMU with a high-performance diet, an economical diet (based on tank trials), and a standard commercial diet, and assess reproductive performance and diet effects on production costs.*

**University of Arkansas at Pine Bluff; Texas A&M University**

On schedule to begin in February 2014.

**Objective 2.** *Determine effects of sex ratios, stocking densities, and post spawning broodfish consolidation on catfish reproductive success and determine associated costs.*

**Subobjective 2a.** *Effect of broodfish sex ratios.*

**USDA-ARS Catfish Genetics Research Unit**

Commercial catfish farming is the largest commercial aquaculture enterprise in the U.S. However, catfish production in the U.S. has decreased approximately 50% over the last 10 years due to increased production costs and competition from imported farmed catfish and *Pangasius*. In order to remain competitive in a global market, U.S. catfish farmers must reduce production costs. Refinement of catfish broodfish

management strategies could improve reproductive efficiency and reduce production costs. Currently most farmers use a 1:1 or 1:2 male to female stocking ratio in brood ponds. However, unpublished data from the USDA-ARS Catfish Genetics Research Unit has demonstrated that less than 10% of the males present account for over half the spawns, and about half of the males do not

spawn at all. Therefore, commercial producers could possibly improve spawning efficiency by reducing the biomass of males in brood ponds and replacing males with additional females. Costs of maintaining broodfish (feed, pond space, etc.) would be similar regardless of sex ratio if the biomass was constant. Previous published data demonstrated no differences in the percent of females spawning at 1:1 and 1:4 male to female ratios, although study ponds were unreplicated. The objective of this study was to determine the effects of broodfish sex ratios on channel catfish spawning success and associated costs.

Mature Delta Select strain channel catfish males and females (3 and 4 years old, respectively) were randomly stocked at about 1000 lbs per acre in 0.25 acre ponds during the last week of February, 2012. Individual broodfish had been marked previously with pit tags and sampled for DNA to be used for subsequent parentage determination. Two sex ratios were compared: a 1:1 male to female ratio (30 males and 30 females per pond) and a 1:4 male to female ratio (12 males and 48 females per pond) with six replicate ponds per treatment. Spawning cans were placed in ponds the second week of March at a rate of 2 cans for every 3 males (8 cans in the 1:4 ponds and 20 cans in the 1:1 ponds). One week after placing spawning cans in ponds, they were checked 2 to 3 times per week for spawns through mid-July. Spawns were removed, brought to the hatchery, and weighed. A sample of eggs was taken from each spawn, weighed and counted, and the counts were used to determine total eggs per spawn. Each spawn was hatched in a separate 20-gallon fiberglass tank provided with flow-through ground water (1 gal/min, 79 degrees F, 5 ppm D.O.). Eggs were treated with hydrogen peroxide once daily until the eyed stage. Sac fry were siphoned into a volumetric cylinder and number of fry was determined volumetrically. Ten to twenty fry were sampled from each spawn, preserved in ethanol, and used for DNA isolation for parentage determination. Parents and fry were genotyped for 2, multiplexed DNA

microsatellite panels to determine the individual male and female parent of each spawn. Details of protocols used for parentage determination are given by Waldbieser and Bosworth (Animal Genetics, accepted). In August 2012, ponds were seined and drained and remaining fish were counted and pit tags were recorded to allow determination of broodfish survival. At the time of this report, DNA determination of parentage was still underway; therefore, we do not present data on individual female and male spawning success. We assumed each spawn was produced by a single female in estimates of female spawning percentage. Information on individual spawning success and number of spawns produced by individual male and female broodfish will be included in the final report when the parentage analysis is complete. The broodfish sex ratios were compared by ANOVA for broodfish survival, percent of females spawning, spawning day (the first day a spawn was collected was defined as day 1 and all subsequent spawn dates were determined relative to day 1), spawn weight, percent hatch, number of spawns per acre of brood pond, weight of eggs per acre, number of eggs per acre, and number of fry per acre.

## Results at a glance...

- *A 1:1 male to female broodfish ratio improved reproductive efficiency and fry production compared to a 1:4 male to female ratio.*

Reproductive traits for 1:1 and 1:4 male to female channel catfish broodfish treatments are summarized in Table 2. Male broodfish had lower survival than female broodfish (65.5 versus 91.4%) but there was no effect of sex ratio on survival of males or females. Spawning date, spawn weight, and percent hatch were not affected by broodfish sex ratio. Percent of

**Table 2. Influence of channel catfish broodfish sex ratios (1:4 vs. 1:1 male to female ratio) on broodfish survival and reproductive traits.**

Treatment Male:Female	Broodfish Survival (%)		Females Spawning Incidence (%)	Spawning Date (Days)	Spawn Weight (lbs)	Hatch (%)	Spawns per acre (#)	Weight of eggs per acre (lbs)	Eggs per acre (#)	Fry per acre (#)
	Male	Female								
1:4	66.7	89.2	16.3 <sup>a</sup>	33.0	1.0	28.3	31 <sup>a</sup>	31.1 <sup>a</sup>	343130 <sup>a</sup>	102500 <sup>a</sup>
1:1	64.2	93.5	57.2 <sup>b</sup>	30.6	1.1	29.9	69 <sup>b</sup>	72.8 <sup>b</sup>	801840 <sup>b</sup>	232240 <sup>b</sup>
SE	7.5	6.3	6.9	4.5	0.12	6.1	10	9.7	110688	40543
P value	0.750	0.509	.002	0.590	0.510	0.800	0.024	0.013	0.011	0.047

females spawning was over 3 fold higher for the 1:1 male to female ratio compared to the 1:4 ratio (57.2% versus 16.3%). The much higher percentage of females spawning at the 1:1 male to female ratio resulted in the 1:1 ratio being superior to 1:4 even when reproductive traits were considered on a per acre basis. Relative to the 1:4 ratio, the 1:1 ratio resulted in more spawns per acre, greater weight and number of eggs produced per acre, and greater number of fry per acre.

The results demonstrate that a greater percentage of channel catfish females spawned at a 1:1 male to

female broodfish ratio than at a 1:4 male to female ratio. The differences in female spawning percentage were large enough that the 1:1 male to female ratio was superior to the 1:4 ratio even when reproductive output was considered on a per acre basis. The increased number of females per acre in the 1:4 ratio ponds would have had an advantage on a per acre basis if the percent of females spawning had been similar in the two treatments. Therefore, the results of this study indicate the reproductive efficiency and economics of channel catfish fry production at a 1:1 male to female broodfish ratio are superior to those of a 1:4 male to female ratio.

## IMPACTS

Both diet modification and use of a 1:1 ratio of male to female broodfish significantly increased reproductive efficiency of channel catfish compared

to standard commercial practices. Pending economic analysis will allow cost comparison of the different strategies.

## PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED

First year of study--nothing to report.



## SRAC RESEARCH AND EXTENSION PROJECTS

Project	Duration	Funding	Grant No.
*Analysis of Regional and National Markets for Aquacultural Products Produced for Food in the Southern Region. Dr. J. G. Dillard, Mississippi State University, Principal Investigator	04/01/88-06/30/90 <b>Project Total</b>	<b>\$346,038</b>	87-CRSR-2-3218
*Preparation of Southern Regional Aquaculture Publications. Dr. J. T. Davis, Texas A&M University, Principal Investigator	01/01/88-12/31/90 <b>Project Total</b>	<b>\$150,000</b>	87-CRSR-2-3218
*Performance of Aeration Systems for Channel Catfish, Crawfish, and Rainbow Trout Production. Dr. C. E. Boyd, Auburn University, Principal Investigator	03/01/88-10/31/90 <b>Project Total</b>	<b>\$124,990</b>	87-CRSR-2-3218
*Develop a Statistical Data Collection System for Farm-Raised Catfish and Other Aquaculture Products in the Southern Region. Dr. J. E. Waldrop, Mississippi State University, Principal Investigator	06/01/89-11/30/90 <b>Project Total</b>	<b>\$13,771</b>	88-38500-4028
*Immunization of Channel Catfish. Dr. J. A. Plumb, Auburn University, Principal Investigator	Yr. 1-05/02/89-04/30/90 Yr. 2-05/01/90-04/30/91 <b>Project Total</b>	\$50,000 <u>49,789</u> <b>\$99,789</b>	88-38500-4028 89-38500-4516
*Enhancement of the Immune Response to <i>Edwardsiella ictaluri</i> in Channel Catfish. Dr. J. R. Tomasso, Clemson University, Principal Investigator	Yr. 1-05/02/89-04/30/90 Yr. 2-05/01/90-10/31/91 <b>Project Total</b>	\$46,559 <u>51,804</u> <b>\$98,363</b>	88-38500-4028 89-38500-4516
*Effect of Nutrition on Body Composition and Subsequent Storage Quality of Farm-Raised Channel Catfish. Dr. R. T. Lovell, Auburn University, Principal Investigator	Yr. 1-05/02/89-04/30/90 Yr. 2-05/01/90-04/30/91 Yr. 3-05/01/91-12/31/92 <b>Project Total</b>	\$274,651 274,720 <u>273,472</u> <b>\$822,843</b>	88-38500-4028 89-38500-4516 90-38500-5099
*Project Completed			

Project	Duration	Funding	Grant No.
*Harvesting, Loading and Grading Systems for Cultured Freshwater Finfishes and Crustaceans. Dr. R. P. Romaine, Louisiana State University, Principal Investigator	Yr. 1-05/02/89-04/30/90	\$124,201	88-38500-4028
	Yr. 2-05/01/90-04/30/91	124,976	89-38500-4516
	Yr. 3-05/01/91-04/30/93	<u>124,711</u>	90-38500-5099
	<b>Project Total</b>	<b>\$373,888</b>	
*Preparation of Extension Publications on Avian Predator Control in Aquaculture Facilities. Dr. James T. Davis, Texas A&M University, Principal Investigator	05/01/90-12/31/92		
	<b>Project Total</b>	<b>\$15,000</b>	89-38500-4516
*National Extension Aquaculture Workshop. Dr. Carole Engle, University of Arkansas at Pine Bluff, Principal Investigator	10/01/91-09/30/92		
	<b>Project Total</b>	<b>\$3,005</b>	89-38500-4516
*Educational Materials for Aquaculturists and Consumers. Dr. J. T. Davis, Texas A&M University, Principal Investigator	Yr. 1-05/01/91-04/30/92	\$3,971	87-CRSR-2-3218
		<u>35,671</u>	88-38500-4028
	Total Yr. 1	\$39,642	
	Yr. 2-06/01/92-05/31/93	\$58,584	91-38500-5909
	Yr. 3-06/01/93-12/31/94	<u>34,500</u>	92-38500-7110
<b>Project Total</b>	<b>\$132,726</b>		
*Characterization of Finfish and Shellfish Aquacultural Effluents. Dr. J. V. Shireman, University of Florida, Principal Investigator	Yr. 1-05/01/91-04/30/92	\$45,131	88-38500-4028
		65,552	89-38500-4516
		<u>34,317</u>	90-38500-5099
	Total Yr. 1	\$145,000	
	Yr. 2-06/01/92-05/31/93	\$168,105	91-38500-5909
	Yr. 3-06/01/93-12/31/94	<u>\$128,937</u>	92-38500-7110
<b>Project Total</b>	<b>\$442,042</b>		
*Food Safety and Sanitation for Aquacultural Products: Microbial. Dr. J. L. Wilson, University of Tennessee, Principal Investigator	Yr. 1-04/01/92-03/30/93	\$12,649	89-38500-4516
		<u>71,608</u>	90-38500-5099
	Total Yr. 1	\$84,257	
	Yr. 2-06/01/93-05/31/94	\$213,106	92-38500-7110
	Yr. 3-06/01/94-05/31/95	<u>\$237,975</u>	93-38500-8393
<b>Project Total</b>	<b>\$535,338</b>		
*Project Completed			

Project	Duration	Funding	Grant No.
*Aquaculture Food Safety: Residues. Dr. George Lewis, University of Georgia, Principal Investigator	Yr. 1-09/11/92-09/30/93	\$99,393	91-38500-5909
	Yr. 2-10/01/93-09/30/94	\$44,631	90-38500-5099
		<u>107,050</u>	91-38500-5909
	Total Yr. 2	\$151,681	
	Yr. 3-10/01/94-09/30/95	\$89,463	93-38500-8393
	Yr. 4-10/01/95-09/30/96	<u>\$11,392</u>	93-38500-8393
	<b>Project Total</b>	<b>\$351,929</b>	
*National Coordination for Aquaculture Investigational New Animal Drug (INAD) Applications. (In cooperation with other Regional Aquaculture Centers and USDA)	Yr. 1-09/01/93-08/31/94		
	<b>Project Total</b>	<b>\$2,000</b>	90-38500-5099
*Improving Production Efficiency of Warmwater Aquaculture Species Through Nutrition. Dr. Delbert Gatlin, Texas A&M University, Principal Investigator	Yr. 1-01/01/94-12/31/94	\$28,148	90-38500-5099
		123,705	91-38500-5909
		<u>128,444</u>	92-38500-7110
	Total Yr. 1	\$280,297	
	Yr. 2-01/01/95-12/31/95	\$38,059	92-38500-7110
		175,450	93-38500-8393
		<u>32,397</u>	94-38500-0045
	Total Yr. 2	\$245,906	
	Yr. 3-01/01/96-12/31/96	\$23,907	93-38500-8393
	<u>210,356</u>	94-38500-0045	
	Total Yr. 3	<u>\$234,263</u>	
	<b>Project Total</b>	<b>\$760,466</b>	
*Delineation and Evaluation of Catfish and Baitfish Pond Culture Practices. Dr. Michael Masser, Auburn University, Principal Investigator	Yr. 1-04/01/94-03/31/95	\$75,530	92-38500-7110
		<u>43,259</u>	93-38500-8393
	Total Yr. 1	\$118,789	
	Yr. 2-04/01/95-03/31/96	\$113,406	94-38500-0045
	Yr. 3-04/01/96-03/31/97	\$28,517	93-38500-8393
		<u>72,281</u>	94-38500-0045
	Total Yr. 3	<u>\$100,798</u>	
	<b>Project Total</b>	<b>\$332,993</b>	
*Project Completed			

Project	Duration	Funding	Grant No.
*Optimizing Nutrient Utilization and Reducing Waste through Diet Composition and Feeding Strategies. Dr. Kenneth Davis, University of Memphis, Principal Investigator	Yr. 1-12/01/96-11/30/97	\$241,476	95-38500-1411
	Yr. 2-12/01/97-11/30/98	\$47,105	95-38500-1411
		<u>210,047</u>	96-38500-2630
	Total Yr. 2	\$257,152	
	Yr. 3-12/1/98-11/30/99	\$34,365	96-38500-2630
		<u>199,811</u>	97-38500-4124
	Total Yr. 3 Project Total	<u>\$234,176</u> \$732,804	
*Management of Environmentally-Derived Off-Flavors in Warmwater Fish Ponds. Dr. Tom Hill, University of Tennessee, Principal Investigator	Yr.1-06/01/96-05/31/97	\$29,349	93-38500-8393
		34,918	94-38500-0045
		<u>186,560</u>	95-38500-1411
	Total Yr. 1	\$250,827	
	Yr. 2-06/01/97-05/31/98	\$68,718	94-38500-0045
		97,393	95-38500-1411
		<u>84,031</u>	96-38500-2630
	Total Yr. 2	\$250,142	
	Yr. 3-06/1/98-05/31/99	\$154,621	96-38500-2630
		<u>74,645</u>	97-38500-4124
Total Yr. 3	\$229,266		
Yr. 4-06/01/99-05/31/00	\$80,900	98-38500-5865	
Yr. 5-06/01/00-05/31/01	<u>\$55,146</u>	<u>99-38500-7375</u>	
<b>Project Total</b>	<b>\$866,281</b>		
*National Aquaculture Extension Conference (In cooperation with other Regional Aquaculture Centers)	01/01/97-12/31/97	\$3,392	93-38500-8393
		<u>308</u>	95-38500-1411
	<b>Project Total</b>	<b>\$3,700</b>	
*Verification of Recommended Management Practices for Major Aquatic Species. Dr. Carole Engle, University of Arkansas at Pine Bluff, Principal Investigator	Yr. 1-01/01/97-12/31/97	\$31,410	95-38500-1411
	Yr. 2-01/01/98-12/31/98	\$7,186	95-38500-1411
		<u>58,928</u>	96-38500-2630
	Total Yr. 2	\$66,114	
	Yr. 3-01/01/99-12/31/00	<u>\$62,781</u>	99-38500-4124
<b>Project Total</b>	<b>\$160,305</b>		
*Project Completed			

Project	Duration	Funding	Grant No.
Publications, Videos and Computer Software. Dr. Michael Masser, Texas A&M University, Principal Investigator (Continuing project)	Yr. 1-04/01/95-03/31/96	\$50,000	94-38500-0045
	Yr. 2-04/01/96-03/31/97	\$13,405	93-38500-8393
		<u>47,543</u>	94-38500-0045
	Total Yr. 2	\$60,948	
	Yr. 3-04/01/97-03/31/98	\$45,900	96-38500-2630
	Yr. 4-04/01/98-03/31/99	\$60,500	97-38500-4124
	Yr. 5-04/01/99-03/31/00	\$67,000	98-38500-5865
	Yr. 6-07/01/00-06/30/01	\$77,358	2000-38500-8992
	Yr.7-07/01/01-06/30/02	\$82,205	2001-38500-10307
	Yr.8-01/01/03-12/31/03	\$77,384	2002-38500-11805
	Yr.9-04/01/04-03/31/05	\$916	2002-38500-11805
		<u>59,550</u>	2003-38500-12997
	Total Yr. 9	\$60,466	
	Yr. 10-03/01/05-02/28/06	\$50,896	2004-38500-14387
	Yr. 11-03/01/06-02/28/07	\$45,723	2005-38500-15815
	Yr. 12-03/01/07-02/29/08	\$63,764	2006-38500-16977
	Yr. 13-05/01/08-04/30/09	\$80,106	2007-38500-18470
Yr. 14-05/01/09-04/30/10	\$78,072	2008-38500-19251	
Yr. 15-05/01/10-04/30/11	\$73,982	2008-38500-19251	
Yr. 16-05/01/11-04/30/12	\$4,000	2007-38500-18470	
	<u>69,974</u>	2008-38500-19251	
Total Yr. 16	\$73,974		
Yr. 17-05/01/12-04/30-13	\$7,624	2008-38500-19251	
	<u>68,535</u>	2010-38500-21142	
Total Yr. 17	\$76,159		
<b>Project Total</b>	<b>\$1,124,437</b>		
*Control of Blue-green Algae in Aquaculture Ponds. Dr. Larry Wilson, University of Tennessee, Principal Investigator	Yr. 1-01/01/99-12/31/99	\$25,147	96-38500-2630
		105,167	97-38500-4124
		<u>177,260</u>	98-38500-5865
	Total Yr. 1	\$307,574	
	Yr. 2-01/01/00-12/31/00	\$975	96-38500-2630
		17,394	97-38500-4124
		158,608	98-38500-5865
		<u>98,993</u>	99-38500-7375
	Total Yr. 2	\$275,970	
	Yr. 3-01/01/01-12/31/01	\$26,186	97-38500-4124
	7,202	98-38500-5865	
	188,550	99-38500-7375	
	<u>24,277</u>	00-38500-8992	
Total Yr. 3	<u>\$246,215</u>		
<b>Project Total</b>	<b>\$829,759</b>		
*Project Completed			

Project	Duration	Funding	Grant No.
*Management of Aquacultural Effluents from Ponds. Dr. John Hargreaves, Mississippi State University, Principal Investigator	Yr. 1-04/01/99-03/31/00	\$100,000	97-38500-4124
		<u>127,597</u>	98-38500-5865
	Total Yr. 1	\$227,597	
	Yr. 2-04/01/00-03/31/01	\$221,146	99-38500-7375
	Yr. 3-04/01/01-03/31/02	<u>\$106,610</u>	2000-38500-8992
	<b>Project Total</b>	<b>\$555,353</b>	
*Development of Improved Harvesting, Grading and Transport Technology for Finfish Aquaculture. Dr. Ed Robinson, Mississippi State University, Principal Investigator	Yr. 1-01/01/01-12/31/01	\$287,053	00-38500-8992
	Yr. 2-01/01/02-12/31/02	\$14,259	98-38500-5865
		39,720	99-38500-5865
		14,757	00-38500-8992
		<u>189,955</u>	01-38500-10307
	Total Yr. 2	\$258,691	
	Yr. 3-01/01/03-12/31/03	\$47,937	00-38500-8992
	<u>139,390</u>	01-38500-10307	
	Total Yr. 3	<u>\$187,327</u>	
	<b>Project Total</b>	<b>\$733,071</b>	
*National Aquaculture Extension Conference-2007 (In cooperation with other Regional Aquaculture Centers)	11/01/05-10/31/06		
	<b>Project Total</b>	<b>\$5,000</b>	2002-38500-11805
*Identification, Characterization, and Evaluation of Mechanisms of Control of <i>Bolbophorus</i> -like Trematodes and <i>Flavobacterium columnaris</i> -like Bacteria. Dr. John Hawke, Louisiana State University, Principal Investigator	Yr. 1-03/01-03-02/28/04	\$28,029	2000-38500-8992
		126,778	2001-38500-10307
		<u>67,298</u>	2002-38500-11307
	Total Yr. 1	\$222,105	
	Yr. 2-03/01-04-02/28/2005	\$27,126	2000-38500-8992
		47,498	2001-38500-10307
		151,614	2002-38500-11805
		<u>778</u>	2003-38500-12997
	Total Yr. 2	\$227,016	
	Yr. 3-03/01/05-02/28/06	\$24,074	2001-38500-10307
	15,417	2002-38500-11805	
	<u>104,918</u>	2003-38500-12997	
	Total Yr. 3	<u>\$144,409</u>	
	<b>Project Total</b>	<b>\$593,530</b>	
*Project Completed			

Project	Duration	Funding	Grant No.
*Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry. Dr. Rex Dunham, Auburn University, Principal Investigator	Yr. 1-03/01/04-02/28/05	\$1,000	2001-38500-10307
		<u>114,935</u>	2002-38500-11805
	Total Yr. 1	\$115,935	
	Yr. 2 -03/01/05-02/28/06	\$99,000	2003-38500-12997
	Yr. 3-03/01/06-02/28/07	\$14,549	2002-38500-11805
		28	2003-38500-12997
		<u>100,423</u>	2004-38500-14387
	Total Yr. 3	\$115,000	
	Yr. 4-03/01/07-02/29/08	<u>\$112,128</u>	2005-38500-15815
	<b>Project Total</b>	<b>\$442,063</b>	
*Innovative Technologies and Methodologies for Commercial-Scale Pond Aquaculture. Dr. Claude Boyd, Auburn University, Principal Investigator	Yr.1-08/01/04-07/31/05	\$1,053	2000-38500-8992
		167,433	2002-38500-11805
		<u>145,923</u>	2003-38500-12997
	Total Yr. 1	\$314,409	
	Yr. 2-08/01/05-07/31/06	\$39	2002-38500-11805
		116,043	2003-38500-12997
		<u>151,234</u>	2004-38500-14387
	Total Yr. 2	\$267,316	
	Yr.3-08/01/06-07/31/07	\$120	2002-38500-11805
		69,310	2003-38500-12997
		38,919	2004-38500-14387
		<u>96,508</u>	2005-38500-15815
	Total Yr. 3	\$204,857	
	Yr.4-08/01/07-07/31/08	\$62,491	2004-38500-14387
	51,892	2005-38500-15815	
	<u>34,760</u>	2006-38500-16977	
Total Yr. 4	<u>\$149,144</u>		
<b>Project Total</b>	<b>\$935,726</b>		
*Feed Formulation and Feeding Strategies for Bait and Ornamental Fish. Dr. Rebecca Lochmann, University of Arkansas at Pine Bluff, Principal Investigator	Yr. 1-05/01/05-04/30/06	\$102,913	2003-38500-12997
	Yr. 2-05/01/06-04/30/07	\$107,198	2004-38500-14387
	Yr. 3-05/01/07-04/30/08	\$66,789	2004-38500-14387
		<u>58,163</u>	2005-38500-15815
	Total Yr. 3	<u>\$124,952</u>	
<b>Project Total</b>	<b>\$335,063</b>		
*Project Completed			

Project	Duration	Funding	Grant No.
*Development and Evaluation of Pond Inventory Methods. Dr. David Heikes, University of Arkansas at Pine Bluff, Principal Investigator	Yr. 1-05/01/07-04/30/08	\$1,648	2003-38500-12997
		18,463	2004-38500-14387
		<u>137,707</u>	2005-38500-15815
	Total Yr. 1	\$157,818	
	Yr. 2-05/01/08-04/30/09	\$12,917	2004-38500-14387
		6,225	2005-38500-15815
		<u>118,016</u>	2006-38500-16977
Total Yr. 2	\$137,158		
<b>Project Total</b>	<b>\$294,976</b>		
*Economic Forecasting and Policy Analysis Models for Catfish and Trout. Dr. Carole Engle, University of Arkansas at Pine Bluff, Principal Investigator	Yr. 1-08/01/07-07/31/08	\$53,577	2006-38500-16977
		<u>20,000</u>	2008-38500-19251
	Total Yr. 1	\$73,577	
	Yr. 2-08/01/08-07/31/09	\$42,502	2005-38500-15815
		<u>32,256</u>	2006-38500-16977
		<u>\$74,758</u>	
<b>Project Total</b>	<b>\$148,335</b>		
*Improving Reproductive Efficiency of Cultured Finfish. Dr. Brian Small, USDA/ARS, Principal Investigator	Yr. 1-02/01/09-01/31/10	\$34,044	2005-38500-15815
		178,135	2006-38500-16977
		<u>10,351</u>	2008-38500-19251
	Total Yr. 1	\$222,530	
	Yr. 2- 02/01/10-01/31/11	\$23,887	2006-38500-16977
		155,213	2007-38500-19251
		<u>14,302</u>	2008-38500-19251
	Total Yr. 2	\$193,402	
	Yr. 3-02/01/11-01/31/12	\$10,830	2007-38500-18470
		<u>67,211</u>	2008-38500-19251
Total Yr. 3	<u>\$78,041</u>		
<b>Project Total</b>	<b>\$493,973</b>		
*National Aquaculture Extension Conference-2011 (In cooperation with other Regional Aquaculture Centers)	11/01/10-09/20/11		
	<b>Project Total</b>	<b>\$5,000</b>	2007-38500-18470
*Project Completed			

Project	Duration	Funding	Grant No.
Using National Retail Databases to Determine Market Trends. Dr. Jimmy Avery, Mississippi State University Extension Service, Principal Investigator	Yr. 1-06/01/09-05/31/10	\$1,649	2005-38500-15815
		93,803	2006-38500-16977
		<u>28,478</u>	2007-38500-18470
	Total Yr. 1	\$123,930	
	Yr. 2-06/01/10-05/31/11	\$1,322	2006-38500-16977
		48,560	2007-38500-18470
		69,337	2008-38500-19251
		<u>5,386</u>	2010-38500-21142
	Total Yr. 2	\$124,605	
	Yr. 3-03/01/12-02/28/13	\$22,304	2008-38500-19251
	<u>51,379</u>	2010-38500-21142	
Total Yr. 3	\$73,683		
Yr. 4-03/01/13-02/28/13	<u>\$76,322</u>	2010-38500-21142	
<b>Project Total</b>	<b>\$398,540</b>		
*Evaluation of Impacts of Potential “Cap and Trade” Carbon Emission Policies on Catfish, Baitfish, and Crawfish Farming. Dr. Claude Boyd, Auburn University, Principal Investigator	Yr. 1-01/01/11-12/31/11	\$9,747	2007-38500-18470
		<u>50,205</u>	2008-38500-19251
	Total Yr. 1	\$59,952	
	Yr. 2-01/01/12-12/31/12	\$48,427	2008-38500-19251
		<u>11,573</u>	2010-38500-21142
Total Yr. 2	\$60,000		
<b>Project Total</b>	<b>\$119,952</b>		
Development and Evaluation of Cool Water Crawfish Baits. Dr. Ray McClain, Louisiana State University, Principal Investigator	Yr. 1-01/01/11-12/31/11	\$15,108	2007-38500-18470
		<u>22,487</u>	2008-38500-19251
	Total Yr. 1	\$37,595	
	Yr. 2-01/01/12-12/31/12	\$15,434	2008-38500-19251
		<u>28,088</u>	2010-38500-21142
	Total Yr. 2	\$43,503	
Yr. 3-01/01/13-12/31/13	<u>\$43,798</u>	2010-38500-21142	
<b>Project Total</b>	<b>\$124,896</b>		
*Identifying Determinants for Development of Live-Market Grading Standards for Crawfish. Dr. Ray McClain, Louisiana State University, Principal Investigator	Yr. 1-01/01/11-12/31/11	\$34,599	2007-38500-18470
		10,017	2008-38500-19251
		<u>5,336</u>	2010-38500-21142
	<b>Project Total</b>	<b>\$49,952</b>	
Potential Marketing Structures for the Catfish Industry. Dr. Carole Engle, University of Arkansas at Pine Bluff, Principal Investigator	Yr. 1-01/01/11-12/31/11	\$21,951	2007-38500-18470
		<u>103,045</u>	2008-38500-19251
	Total Yr. 1	\$124,996	
	Yr. 2-01/01/12-12/31/12	\$106,961	2008-38500-19251
		<u>18,039</u>	2010-38500-21142
	Total Yr. 2	<u>\$125,000</u>	
<b>Project Total</b>	<b>\$249,996</b>		
*Project Completed			

Project	Duration	Funding	Grant No.
Reproduction and Larval Rearing of Freshwater Ornamental and Marine Baitfish. Dr. Cortney Ohs, University of Florida, Principal Investigator	Yr. 1-01/01/11-12/31/11	\$56,532	2007-38500-18470
		<u>\$111,246</u>	2008-38500-19251
	Total Yr. 1	\$167,778	
	Yr. 2-01/01/12-12/31/12	\$139,444	2008-38500-19251
		<u>29,985</u>	2010-38500-21142
	Total Yr.2	\$169,429	
	Yr. 3-01/01/13-12/31/13	<u>\$162,637</u>	2010-38500-21142
	<b>Project Total</b>	<b>\$499,844</b>	
*Effects of Mosquito Abatement Pesticides on Various Life Stages of Commercially Important Shellfish Aquaculture Species in the South. Dr. Craig Plante, College of Charleston, Principal Investigator	Yr. 1-03/01/11-02/29/12	\$39,973	2008-38500-19251
	<b>Project Total</b>	<b>\$39,973</b>	
*Development of Baitfish, Goldfish, and Ornamental Fish Hatchery Methods. Dr. Anita Kelly, University of Arkansas at Pine Bluff, Principal Investigator	Yr. 1-03/01/11-02/29/12	\$19,022	2007-38500-18470
		34,935	2008-38500-19251
		<u>5,991</u>	2010-38500-21142
	<b>Project Total</b>	<b>\$59,949</b>	
Improving Catfish Broodstock Management by Manipulating Diet, Stocking Densities and Sex Ratios. Dr. Rebecca Lochmann, University of Arkansas at Pine Bluff, Principal Investigator	Yr. 1-01/01/12-12/31/12	\$58,755	2008-38500-19251
		<u>57,105</u>	2010-38500-21142
	Total Yr. 1	\$115,860	
	Yr. 2-01/01/13-12/31/13	\$127,400	2010-38500-21142
	Projected-Yr. 3	<u>\$145,125</u>	
	<b>Project Total</b>	<b>\$388,385</b>	
*Project Completed			



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