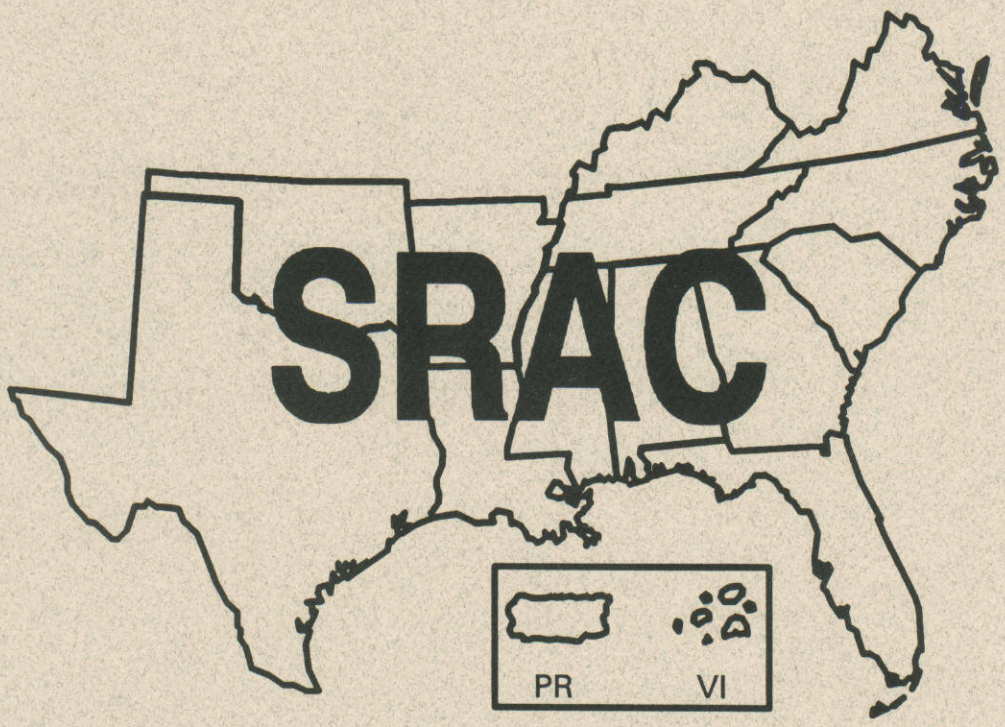
SOUTHERN REGIONAL AQUACULTURE CENTER



FOURTH ANNUAL PROGRESS REPORT

JANUARY, 1992

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FOURTH ANNUAL PROGRESS REPORT

For the Period
October 1, 1990 to September 30, 1991

January, 1992

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TABLE OF CONTENTS

I. INTRODUCTION

This Fourth Annual Progress Report of the Southern Regional Aquaculture Center (SRAC) includes progress and/or termination reports for projects supported by SRAC during the period October 1, 1990 to September 30, 1991.

Since inception of SRAC in October, 1987, eleven projects have been initiated and funded. Four projects have already been completed:

"Analysis of Regional and National Markets for Aquacultural Products Produced for Food in the Southern Region"

"Preparation of Southern Regional Aquaculture Publications"

"Performance of Aeration Systems for Channel Catfish, Crawfish, and Rainbow Trout Production"

"Develop a Statistical Data Collection System for Farm-Raised Catfish and Other Aquaculture Products in the Southern Region"

Accomplishments from these projects are included in previous Annual Progress Reports.

Projects supported by SRAC during the current reporting period were:

"Immunization of Channel Catfish" (two-year project funded at $50,000/year)

"Enhancement of the Immune Response to Edwardsiella ictaluri in Channel Catfish" (two-year project funded at $46,736 for year one and $53,264 for year two)

"Effect of Nutrition on Body Composition and Subsequent Storage Quality of Farm-Raised Channel Catfish" (three-year project funded at $275,000/year)

"Harvesting, Loading and Grading Systems for Cultured Freshwater Finfishes and Crustaceans" (three-year project funded at $125,000/year)

"Preparation of Extension Publications on Avian Predator Control in Aquaculture Facilities" (project funded for duration at $15,000)

"Educational Materials for Aquaculturists and Consumers" (three-year project funded at $39,642 for year one)

"Characterization of Finfish and Shellfish Aquacultural Effluents" (three-year project funded at $145,000 for year one)

Second and/or third year funding of the latter two of these projects will be contingent on satisfactory progress, accomplishments of the work proposed, and Congressional appropriations provided to SRAC.

A three-year project, "Food Safety and Sanitation for Aquacultural Products (Microbial)", has been prepared, reviewed, approved by the Board, and submitted to CSRS. The purpose of this project is to develop realistic criteria for consumer safety with consideration for the capability and character of the aquaculture industry and to pursue a program that will ensure the quality of aquacultural products and the growth of the aquaculture industry.

Also submitted to CSRS for approval is a project entitled, "National Aquaculture Extension Workshop", planned for March 2-8, 1992, in Little Rock, Arkansas. This workshop will involve aquaculture extension professionals throughout the United States and U.S. territories and will be funded jointly by the five Regional Aquaculture Centers.

II. ORGANIZATIONAL STRUCTURE

Title XIV of the Agriculture and Food Act of 1980 and the Food Security Act of 1985 authorized establishment of aquacultural research, development, and demonstration centers in the United States (Subtitle L, Sec. 1475[d]) in association with colleges and universities, State Departments of Agriculture, federal facilities, and non-profit private research institutions.

The Regional Aquaculture Centers encourage cooperative and collaborative research and extension educational programs in aquaculture having regional or national application. Center programs complement and strengthen existing research and extension educational programs provided by the Department of Agriculture and other public institutions.

Objectives of the Centers are to promote aquaculture research, development, and demonstration for the enhancement of viable and profitable commercial aquaculture production in the United States for the benefit of producers, consumers, and the American economy; and to utilize the Regional Centers in a national program of cooperative and collaborative research, extension, and developmental activities among public and private institutions having demonstrated capabilities in support of commercial aquaculture in the United States.

The thirteen states and two territories represented by the Southern Regional Aquaculture Center are Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, Nort h Carolina, Oklahoma, Puerto Rico, South Carolina, Tennessee, Texas, U.S. Virgin Islands, and Virginia.

Components of the Southern Regional Aquaculture Center include an Administrative Center, a Board of Directors, an Industry Advisory Council, and a Technical Committee.

A. 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 of Directors, Industry Advisory Council, Technical Committee, Steering Committees and project Work Groups are provided by the Administrative Center. Executive leadership for the Center is provided by the Center Director. Interest in aquaculture in general, and the SRAC program in particular, continues to expand as is evidenced by the number of verbal and written inquiries received by the Administrative Center. Additional SRAC Administrative Center responsibilities are detailed under Section III of this report.

B. Board of Directors

The Board of Directors is the policy-making body for SRAC. Membership of the Board of Directors for the Southern Region provides an appropriate balance among representatives from State Agricultural Experiment Stations, Cooperative Extension Services, 1890 Institutions, and the Council of Administrative Heads of Agriculture.

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 CAHA administrator from the host institution

Members of the Board are:

Dr. Harold R. Benson, Kentucky State University
Dr. William H. Brown, Louisiana State University
Dr. Gale Buchanan, University of Georgia
Dr. R. Rodney Foil, Mississippi State University (Chairman)
Dr. David E. Foster, Arkansas Cooperative Extension Service
Dr. B. G. Hicks, Tennessee Cooperative Extension Service
Dr. Hiram Palmertree, Mississippi Cooperative Extension Service
Dr. David H. Teem, Auburn University

Ex-officio Board members are:

Mr. Lester Myers, Chairman, Industry Advisory Council
Dr. James T. Davis, Co-chairman, Technical Committee
Dr. J. Larry Wilson, Co-chairman, Technical Committee
Dr. Charles G. Shepherd, 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 Technical Committee and Industry Advisory Council 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.

C. Industry Advisory Council

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

The IAC provides an open forum wherein maximum input from private and public sectors can be gained and incorporated into annual and on-going plans for SRAC. The chairman is elected by the Council members.

Members of the IAC are:

Mr. Leslie Tindal, Commissioner of Agriculture, South Carolina
Mr. William Kyser, Producer, Alabama
Mr. J. Neal Anderson, Producer, Arkansas
Mr. Harold Benoit, Producer, Louisiana
Dr. Kenneth Semmens, Producer, Georgia
Mr. Thomas R. Rhodes, Processing/ Marketing, Alabama
Mr. Huey P. Townsend, Financial Institution, Mississippi
Mr. Timothy K. Hennessy, Producer, Florida
Mr. Walter Landry, Other, Louisiana
Mr. Lester Myers, Feed Mill/Service, Mississippi (Chairman)
Mr. Jerry Williamson, Producer, Arkansas (Alternate)

IAC members serve three-year appointments having staggered terms with options for reappointment.

The IAC (1) recommends to the Board research and extension needs and priorities from an industry perspective; (2) reviews project proposals and accomplishment and termination reports; and (3) recommends to the Board, jointly with the

Technical Committee, actions regarding new and continuing proposals, proposal modifications and terminations.

D. Technical Committee

The Technical Committee (TC) is composed of representatives from participating research institutions and State Extension Services, other state or territorial public agencies as appropriate, and non-profit private institutions. Membership of the TC for the Southern Region includes 12 research scientists and 12 extension scientists representing essentially all states within the region. The TC meets as needed, but at least annually, and has a co-chairman for research and for extension. Co-chairmen serve for two years and are elected by the Committee members.

Members of the TC are:

Research:

Dr. Gary Burtle, Georgia
Dr. Ruth Francis-Floyd, Florida
Dr. J. O. Hearnsberger, Mississippi
Dr. J. A. Collier, South Carolina
Dr. J. L. Wilson, Tennessee (Co-chairman)
Dr. Delbert Gatlin, Texas
Dr. Carole Engle, Arkansas
Dr. Nick C. Parker, Texas
Dr. R. P. Romaire, Louisiana
Dr. Craig Sullivan, North Carolina
Dr. Craig S. Tucker, Mississippi
Dr. David B. Rouse, Alabama
Dr. W. R. Wolters, Mississippi (Alternate)

Extension:

Dr. M. D. Beem, Oklahoma
Dr. Martin W. Brunson, Mississippi
Dr. Charles E. Cichra, Florida
Dr. G. W. Lewis, Georgia
Dr. M. P. Masser, Alabama
Dr. Tom M. Losordo, North Carolina
Dr. Tom Hill, Tennessee
Dr. Robert Durborow, Kentucky
Dr. James T. Davis, Texas (Co-chairman)
Dr. G. J. Flick, Jr., Virginia
Dr. Wendell Lorio, Louisiana
Mr. Jack Whetstone, South Carolina
Dr. Nathan Stone, Arkansas (Alternate)

Technical Committee members serve three-year appointments having staggered terms with options for reappointment.

The TC (1) recommends to the Board research and extension needs and priorities from a scientific perspective; (2) develops problem statements for research and extension areas under consideration; (3) plans, develops, and implements regional proposals; (4) reviews proposals, accomplishment and termination reports; and (5) recommends to the Board, jointly with the IAC, actions regarding new and continuing proposals and proposal modifications and terminations.

E. Project Development Procedures

Subcommittees, Steering Committees, or Work Groups for research and extension may be appointed and will be responsible for specific planning, development and evaluation of selected regional proposals. These groups will: (1) identify specific problems for regional proposals; (2) classify and rank proposals by common factors and relationships and by adaptability for cooperative proposals; and (3) work with participating scientists to develop regional proposals for high priority areas identified by the Board, IAC and TC as appropriate for the Southern Region.

The Board-approved SRAC Operations Manual, January, 1989, is used for development of cooperative regional aquaculture research and extension activities. Guidelines used to establish regional projects include the following: (1) institutions receiving program dollars must have

a demonstrated capacity to perform the work; (2) a problem concerns two or more states or territories; (3) a project addresses programmatic issues that could not be addressed by a single institution; (4) a project requires more manpower, equipment, and facilities than are available in one state or territory; and (5) a project can be effectively and efficiently organized and conducted on a regional level.

Separate funding allocations will be made for research and for extension to ensure strong programs in each of these areas. All funds allocated for extension activities will be administered through the respective State Cooperative Extension Services.

An Administrative Advisor is appointed for each active project area, and serves as the coordinator for activities related to the project. The responsibilities of the Administrative Advisors are outlined in the SRAC Operations Manual.

III. ADMINISTRATIVE ACTIVITIES

The administrative staff of the Southern Regional Aquaculture Center provides a wide variety of support functions for the various SRAC components, including the Board of Directors, Technical Committee, Industry Advisory Council, Steering Committees and project Work Groups. These responsibilities include the following:

-- Provide documentation for, attend and assist with meetings of the Board of Directors, Technical Committee and Industry Advisory Council; prepare minutes of meetings of the Board of Directors.

-- Solicit and receive nominations for memberships on the Technical Committee and the Industry Advisory Council.

-- Center Director serves as an ex-officio member of the Board, TC, and IAC.

-- Monitor research and extension activities sponsored by SRAC.

-- Attend and participate in meetings of producers, industry representatives, scientists, and others involved in the aquaculture industry in the Southern Region and nationally.

-- Coordinate and participate in testimony before the House Agriculture, Rural Development, and Related Agencies Subcommittee on Appropriations in support of the Regional Aquaculture Centers.

-- Work with members of the House and Senate Appropriations Committees, as well as other members of Congress in the Southern Region, regarding support for the Regional Aquaculture Centers.

-- Center Director serves on the National Coordinating Council for Aquaculture.

-- Prepare Grant Application entering into funding agreement with USDA/CSRS for each fiscal year.

-- Develop and execute appropriate Letters of Agreement with participating institutions in each currently funded proposal for purposes of transferring funds and for coordinating and implementing projects approved under each grant.

-- Prepare and submit Annual Plans of Work to USDA/CSRS.

-- Serve as fiscal agent in distributing funds

as approved under each of the grants and as set forth in the Letters of Agreement with participating institutions.

-- Approve and process invoices received from participating institutions for reimbursement of expenditures.

-- Track status of reimbursement of expenditures to participating each institution for all funded proposals.

-- Monitor budgetary status and progress of each participating institutions for all funded proposals.

-- Prepare budgets for the Administrative Center, track administrative expenditures, and obtain USDA/CSRS approval for project and budget changes.

-- Prepare budget reports for the SRAC Board of Directors, tracking expenditures and status of all funded proposals and the Administrative Center.

-- Assist personnel from Grant Offices of participating institutions in establishing procedures for invoicing for expenditures and obtaining reimbursements.

-- Assist Steering Committees and Work Groups with preparation and revisions of proposals for technical and scientific merit, feasibility and applicability to priority problem areas.

-- Solicit and coordinate national reviews of project proposals.

-- Review project progress reports, publications and videos.

-- Distribute progress reports, publications and videos to Extension contacts throughout the Southern Region and to other Regional Aquaculture Centers.

-- Maintain and distribute listings of research and extension publications developed through SRAC projects.

-- Assist Administrative Advisors and Work Group chairmen.

-- Establish and maintain mailing lists for solicitation of proposals and announcements of Ad Hoc Work Group meetings; prepare and distribute Requests for Proposals and Work Group announcements.

-- Prepare and distribute SRAC Annual Progress Reports.

-- Prepare and distribute interim reports on SRAC activities to provide information regarding on-going projects.

-- Respond to requests from the public for copies of SRAC and other aquaculture publications and for general aquaculture-related information.

IV. PROJECT PROGRESS REPORTS

A. Immunization of Channel Catfish

Termination Report
For the Period
May 2, 1989 to April 30, 1991

COOPERATING INSTITUTIONS:

Auburn University, Alabama Agricultural Experiment Station (Lead Institution) - John A. Plumb, Department of Fisheries and Allied Aquaculture

Louisiana State University - Ronald L. Thune, Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine

University of Georgia - Vicki S. Blazer, Fisheries Research Unit, School of Forestry

ADMINISTRATIVE ADVISOR:

Lowell T. Frobish, Director
Alabama Agricultural Experiment Station
Auburn University, Alabama

REASON FOR TERMINATION:

Project completed.

PRINCIPAL ACCOMPLISHMENTS:

Auburn University

Objective 1A: Isolation and purification of immunocompetent antigen.

Cell extract and crude membrane protein from Edwardsiella ictaluri were used to immunize channel catfish. The antibody titer of immunized fish to cell extract and crude membrane gradually increased the first week and then more rapidly thereafter until reaching a maximum titer at four weeks post intraperitoneal injection. An anamnestic response immediately followed a booster vaccination in which the antibody titer increased in a short period of time. The antibody titers gradually declined after this point, but were still detectable 11 weeks post immunization. A whole cell formalin-killed preparation injected at 2.0×10^4 cells/fish produced lower antibody titers than 2.0×10^6 or 2.0×10^8 cells/fish, but there was no difference in antibody titers from 2.0×10^6 and 2.0×10^8 cells/injected fish. A crude membrane preparation produced similar results; antibody production resulting from 0.20 and 1.50 mg protein/fish were not different, but were higher than that stimulated by 0.02 mg protein/fish. In general, the higher the antigen concentration the higher the antibody production, but an antigen saturation point was attained. The antibody response at 25 and 30°C were similar in rate of increase and did not differ. However, antibody titer of fish at 20°C was lower than those produced at 25 and 30°C. Channel catfish exposed to E. ictaluri by immersion for two and five minutes had similar antibody response, but fish exposed for 30 minutes and eight hours had higher antibody response.

Objective 1B: Protection of channel catfish to E. ictaluri from immunization.

Cells transferred from fish immunized with E. ictaluri were used to determine the cell-mediated immune response of channel catfish, Ictalurus punctatus, and compare this response to the humoral immune response. Channel catfish (average size of 28.7 g) were immunized intraperitoneally with cell extract of E. ictaluri, and 21 days later head kidney cells from immunized and non-immunized fish were removed and transferred to other immunized and non-immunized

fish. Total white blood cell count from control fish was 2.5 x 10^6 cell/ml and 3.1 x 10^6 cells/ml in immunized fish at 21 days post immunization. The mortality was 20% in non-immunized-non-cell transferred fish, 32% in immunized-non-cell transferred fish, and 8% in both immunized-cell transferred and non-immunized-cell transferred. Head kidney cells transferred from immunized and non-immunized fish to other immunized and non-immunized fish resulted in only 8% mortality, which indicated that cell transfer played a more important role in protection than immunization alone.

Channel catfish were vaccinated intraperitoneally with cell extract, crude membrane, and a 36 kDa purified outer membrane protein from E. ictaluri. Fish were boosted 14 days later, and then 28 days after initial vaccination they were challenged with E. ictaluri. When fish were vaccinated with the 36 kDa outer membrane protein and boosted, mortality was reduced from 54.5% in the control group to 24.0% in vaccinated fish. Fish vaccinated with cell extract, crude membrane (both boosted and non-boosted), and fish not boosted with the 36 kDa protein demonstrated no degree of protection compared to control. Survivors from a natural infection of E. ictaluri demonstrated a strong relationship between degree of protection. Intraperitoneal injection of 2.0 x 10^7 cells killed 100% of fish with antibody titers of 0 to 128; 77.8% of fish with titers from 256 to 512 (medium); and 57.7% of the fish with titers of >1024 (high). The second trial using 5.1 x 10^5 cells/fish gave 72.2% mortality in fish with no detectable antibody titers, 51.3% in low antibody titer fish, 25.0% in medium antibody titer fish, and 6.5% in high antibody titer fish. These results demonstrate that channel catfish had protective antibody after they were exposed to the pathogen, but if fish are challenged with large numbers of pathogens, this protective immunity can be overwhelmed.

Application of cell extract of E. ictaluri impregnated feed showed that vaccinated fish receiving the antigen-impregnated feed every 10 days maintained their antibody titer.

USEFULNESS OF FINDINGS:

Results of the research at Auburn provide some basic knowledge to the immunity of channel catfish to E. ictaluri. It is important to know that catfish respond immunogenically to bacterial cell protein extract, and crude membrane material and that they do have an anamnestic response. Also >0.2 mg of protein was required to produce a significant immune response. Water temperature and length of exposure time to antigen are critical points to be determined. The role of cell-mediated immunity to the immune response was hinted but not proven. Protection of immunized catfish from infection of E. ictaluri was also an important key to antigen preparation (vaccine) and application. Fish exposed for two and five minutes had similar antibody response, but fish exposed for 30 minutes and eight hours had higher antibody response.

Louisiana State University

Objective 2A: Cloning of the channel catfish herpesvirus (CCV) thymidine kinase gene.

The gene encoding the previously identified unique channel catfish herpesvirus (CCV) thymidine kinase (Tk) was preliminarily located on the CCV genome. CCV genomic DNA libraries were constructed into plasmid pUC 19 and cosmid pHC 79. Analysis of $CaCl_2$, DEAE and cationic liposome mediated transfection techniques using beta-galactoside expressing plasmid pON 105 on the channel catfish ovary cell line (CCO), a Tk deficient mutant of CCO (CCOBr), and the brown bullhead cell line (BB) revealed cationic liposome transfection of CCO cells to be the most effective combination. More importantly, cationic liposome mediated transfection of whole CCV-DNA onto CCO or

CCOBr cells was the only method that effectively produced infectious viral progeny. This is the first account showing purified CCV-DNA to be infectious. Subsequently, cationic liposome mediated co-transfection of cloned wild type CCV-DNA with the Tk deficient mutant of CCV (CCVAr) in marker rescue assays mapped the mutation within the 18 Kb direct repeat ends of the genome.

In addition, the polymerase chain reaction (PCR) was used (Jack Numberg, Cetus Corporation) to amplify regions flanked by sequences with homology to degenerate primers corresponding to conserved amino acid sequences among herpesvirus Tk's. Three PCR generated fragments were isolated, cloned into pUC 19 and sequenced. The corresponding amino acid sequence of the presumptive coding strand of one sequence (405) showed limited homology to the mammalian cytoplasmic and poxvirus Tk. A weak specific hybridization signal was located on the Eco RI L-fragment, which is located on the direct repeat ends of the CCV genome, when DNA-DNA hybridization analysis was performed using the purified ^{32}P nick-translation labeled 405 fragment. The combined marker rescue and PCR data mapped the Tk gene within the direct repeat region of the CCV genome. With subsequent subcloning of restriction digested cosmid clones, the Tk gene was mapped to within a 3.1 Kb fragment of the 18 Kb direct repeat ends of the genome. This location is unique among herpesviruses, indicating significant divergence from previously identified herpesvirus gene arrangements.

Objective 2B: Cloning and expression of the S-layer protein gene of Aeromonas Hydrophila.

The S-layer protein gene of A. hydrophila was cloned in the phagemid expression vector Lambda ZAP II (Stratagene, La Jolla, California). A. hydrophila genomic DNA was partially digested with Eco RI to yield fragments ranging in size from 2 to 10 kilobase pairs (Kb) and shotgun cloned into the phagemid vector Lambda ZAP II. Under isopropylthio-B-D-galactosidase (IPTG) induction of the Lac Z promoter, approximately 25,000 plaque forming units (PFU) were screened on nitrocellulose membranes using an enzyme-linked immunosorbant assay (ELISA). Thirty positive clones were identified and purified. The p Bluescript SK-plasmid was excised from the phagemid by co-infection with VCSM13 helper phage (Stratagene) and transformed into fresh host cells to produce double-stranded plasmid DNA. The transformed cells were then used for analysis of the inserts by agarose gel electrophoresis and determination of protein expression by Western blot analysis.

Two of the original 30 clones, which contained approximately a 9.4 Kb insert, expressed two proteins with molecular weights of 85 and 81 Kb. Both proteins were expressed with or without IPTG induction, indicating that the S-layer protein gene is contained within the Eco RI fragment and is under the control of its own promoter rather than the Lac Z promoter of the vector. Restriction enzyme digests of the 9.4 Kb Eco RI fragment were subsequently subcloned into p Bluescript.

USEFULNESS OF FINDINGS:

The first steps of engineering CCV as a vaccine vector were accomplished. The thymidine kinase was cloned and mapped to a 3.1 kh DNA fragment in the CCV genome. Also the immunodominant S-layer protein of A. hydrophila was cloned for insertion into the CCV-TK gene. This non-reverting mutant of CCV that expresses the bacterial antigen can provide protection against the bacterium and CCV. The research also establishes a foundation for developing a CCV based vaccine vector system that could be used with other catfish pathogens.

University of Georgia

Objective 3A: Determine if specific immune resistance to E. ictaluri can be enhanced through dietary manipulation in non-immunized, bath and orally immunized, and oral only immunized fish from each experimental diet.

Four laboratory prepared feeds were compared. These feeds were identical except for the lipid source which was beef tallow, soybean oil, menhaden oil or an equal combination of all three lipid sources. Within each diet group, macrophage function and antibody production were compared in non-immunized, bath-immunized, orally-immunized and bath followed by an oral boost.

There were significant differences among the groups in macrophage function. In general, macrophages from fish fed menhaden oil and the combination feed had an enhanced ability to kill engulfed, live E. ictaluri. Bath immunization further enhanced this killing, however, oral immunization, with our oral preparation, did not. Although the menhaden group had the highest macrophage killing activity, it had the lowest growth.

Objective 3B: Compare the above groups for survival after challenge with virulent E. ictaluri.

An attempt was made to challenge fish from each group with a live, virulent strain of bacteria. Unfortunately, it was unsuccessful in that there were very few mortalities even in the non-immunized group.

Objective 3C: Evaluate fatty acid profiles and vitamin E content of the diets and tissues of fish from each group.

Liver and muscle were removed from representative fish from each group. Fatty acid profiles of the tissues reflected the dietary fatty acid profiles. Fish fed menhaden oil had the highest percentages of the long-chained polyunsaturated n-3 fatty acids. Tissue samples to be used for vitamin E analysis were unfortunately lost during a power outage.

Objective 3D: If dietary enhancement is seen, determine least amount of time enhancing diets would have to be fed in order to balance optimal protection and cost-effectiveness.

Because of the above results, three diets (commercial, beef tallow and combination) were tested instead of the two diets proposed. Swim-up fry were received from LSU and divided into 24 groups, which included non-immunized and bath immunized (which were later orally boosted). All groups were fed commercial feed for varying times before switching to the lab diets in order to determine if time on the lab diets had any effect on protection. Groups were maintained on lab feeds four, two or one week prior to the oral boost and on these feeds until challenged three weeks later.

Using a sub-sample of fish from each group, fish were bath-challenged with a strain of E. ictaluri which killed fish within three days of injection. Using 10^{12} bacteria and increasing the time of exposure to the bacteria to two hours, there were still very few deaths. A second challenge after a rapid temperature change was attempted. Again this was not successful in killing fish. It is believed water quality parameters, fish strain and/or stress must play an important role in the bacteria's ability to enter the fish and overcome the defense mechanism. Some of these are currently being investigated.

So as to obtain some useful information from this experiment, it was decided to examine some of the actual macrophage killing mechanisms and the effects of both diet and vaccination on them. Phagocytes produce various oxygen radicals as a bactericidal mechanism against engulfed

organisms. The production of intracellular superoxide anion as well as the extracellular secretion of superoxide anion and hydrogen peroxide can be measured.

It was found that vaccination enhanced the ability of macrophages to produce intracellular superoxide anion after phagocytosis of live E. ictaluri. The production was increased 8.5x in the fish fed combo feed, 6.5x in fish fed the commercial feed and only 2.3x in fish fed the beef tallow feed. The extracellular secretion of superoxide anion and hydrogen peroxide was increased in vaccinated fish from the combo and commercial groups but not in the fish fed beef tallow.

The conclusion is that nutritional manipulation can be used to potentiate the immune response. Certain lipids appear to be very useful in the immune response and macrophage function. A combination of menhaden oil, soybean oil and beef tallow is probably the best regarding both disease resistance and growth. If lipid is used as a dietary enhancement prior to vaccination, these feeds would have to be fed for 3-4 weeks (at 24°C or above) prior to vaccination.

USEFULNESS OF FINDINGS:

This work indicates that a combination of dietary immunopotentiators and vaccination programs could significantly reduce losses due to E. ictaluri.

PUBLICATIONS:

Vinitnantharat, S. 1991. Humoral and cell-mediated immune response of channel catfish, Ictalurus punctatus, to Edwardsiella ictaluri. Ph.D. Dissertation, Auburn University, AL. 155 pp.

Plumb, J. A. and S. Vinitnantharat. 1991. Kinetics of the immune response in channel catfish to Edwardsiella ictaluri. 16th Annual Eastern Fish Health Workshop. Martinsburg, WV. June, 1991.

Hanson, L. A. 1990. Biochemical characterization and gene mapping of the channel catfish herpesvirus (CCV) encoded thymidine kinase, a selectable site for homologous recombination. Ph.D. dissertation. Louisiana State University, Baton Rouge, LA. 70803.

Awad, M. and R. L. Thune. 1991. Cloning and expression of the S-layer protein gene of Aeromonas hydrophila. Proceedings of Annual Meeting of Fish Health Section of American Fisheries Society, Portland, OR. p. 29.

Lingenfelser, J. T., V. S. Blazer and R. E. Klinger. 1991. Metabolic activation of channel catfish macrophages. 16th Annual Eastern Fish Health Workshop. Martinsburg, WV. June, 1991.

B. Enhancement of the Immune Response to Edwardsiella ictaluri in Channel Catfish

Termination Report
For the Period
May 2, 1989 - September 30, 1991

COOPERATING INSTITUTIONS:

Clemson University (Lead Institution) - J. R. Tomasso and T. E. Schwedler, Department of Aquaculture, Fisheries and Wildlife

Texas A&M University - D. M. Gatlin and W. H. Neill, Department of Wildlife & Fisheries Sciences; D. H. Lewis, School of Veterinary Medicine

University of Georgia - Vicki S. Blazer, Georgia Cooperative Fish & Wildlife Research Unit

ADMINISTRATIVE ADVISOR:

J. R. Fischer, Director
South Carolina Agric. Experiment Station
Clemson University
Clemson, South Carolina

REASON FOR TERMINATION:

Project completed.

PRINCIPAL ACCOMPLISHMENTS:

Effect of Selenium on the Immune Response

Purified diets containing adequate vitamin E (60 IU/kg) were supplemented with 0, 0.25 and 10 mg/kg Se and fed to immunized and non-immunized fingerling catfish in aquaria to evaluate the effects of dietary selenium on immunocompetence and disease resistance to E. ictaluri. At the end of the 15-week feeding trial, selenium status of fish fed the various diets was confirmed by analysis of selenium-dependent glutathione peroxidase (SeGSH-Px) activity in liver. Fish fed the basal diet were selenium deficient as evidenced by significantly ($P < 0.05$) reduced SeGSH-Px activity as compared to fish fed diets supplemented with 0.25 and 10 mg/kg Se. However, supplemental selenium in the diet did not improve immunocompetence of catfish based on assessment of antibody titers, phage neutralization, peritoneal macrophage activity and resistance to a challenge by live E. ictaluri. In fact, selenium deficiency actually improved the resistance of catfish to bacterial challenge. Similar responses have been observed in some selenium-deficient mammalian species.

Subsequent experiments to evaluate the combined effects of dietary selenium and vitamin E on immunocompetence of channel catfish are proposed since these nutrients have complementary biochemical functions which may interact synergistically.

Effect of Vitamin E on the Immune Response

Channel catfish fingerlings were acclimated to laboratory conditions and fed diets containing 0, 60 or 2,500 IU/kg vitamin E for 3.5 months. Half of the fish in each treatment were immersion vaccinated after 0.5 months using formalin-killed E. ictaluri. These fish also received an oral booster two months later.

After 3.5 months, the vaccinated fish had a significantly higher phagocytic index than the non-vaccinated fish (2-way ANOVA). Phagocytic index in the immunized fish also increased significantly in a diet-dependent manner in the vaccinated groups (1-way ANOVA). Bactericidal activity was significantly affected by diet in both vaccinated and non-vaccinated groups (1-way ANOVA); however, no pattern was evident. In general, bactericidal activity was higher in the non-vaccinated groups (2-way ANOVA). The groups (both vaccinated and non-vaccinated) fed the high vitamin E diet were significantly more resistant to red blood cell peroxidation than the groups fed the intermediate and low vitamin E diets. All groups responded similarly to challenge by injection of live bacteria.

Effect of Levamisole on the Immune Response

Initial studies revealed that bath immunization of catfish fingerlings and adults with formalin-killed-bacterins of E. ictaluri did not yield consistent immune responses with respect to protection and various serologic parameters. Hence, a pilot study was conducted comparing various formalin- and heat-killed preparations for efficacy in bath immunization of catfish. The bacterin which proved superior was prepared from a two broth culture, washed 2x in saline, autoclaved and the turbidity adjusted to Macfarland standard 4, and diluted 10x in a bath wherein fish were held for 20 minutes. Four groups of fish were studied, i.e., a control group maintained on

a conventional diet containing no Se, a group maintained on 1% Carrisyn (an immunopotentiator), and a group which received levamisole after having been immunized. Each of the four groups were subdivided into subgroups, one of which had been immunized and the other which had not been immunized. All the fish which were immunized had significant agglutinin titers two weeks after immunization; the agglutinin titers of those fish which had received Carrisyn were 2- to 8-fold higher than control fish; levamisole treatment and selenium deprivation enhanced serologic response approximately 2-fold. Challenge studies using 2XLD50 live organisms revealed that selenium deprivation and the incorporation of Carrisyn enhanced protectiveness of the immunization protocol.

Effect of Combinations of Vitamin E and Selenium on the Immune Response

Channel catfish were fed five diets containing combinations of selenium and vitamin E (0 IU/kg E and 0 mg/kg selenium; 60 IU/kg E and 0 mg/kg selenium; 0 IU/kg E and 0.25 mg/kg selenium; 240 IU/kg E and 1.0 mg/kg selenium). Fish were fed the experimental diets for at least 120 days. Half of the fish receiving each diet were vaccinated by immersion in 5×10^9 formalin-killed cells per ml on day 90, given oral boosters (15.7×10^{10} formalin-killed cells/kg) on days 104-106, and sampled beginning on day 120. Production of intracellular and extracellular superoxide anion by macrophages, glutathione peroxidase activity of liver, red blood cell resistance to peroxidation, and resistance to challenge by live bacteria were determined. In all treatments except the double deficient group, immunization significantly enhanced the intracellular production of superoxide anion subsequent to phagocytosis of E. ictaluri. The two selenium-deficient groups produced the lowest amounts of superoxide anion in both vaccinated and non-vaccinated groups. Extracellular superoxide anion in both vaccinated and non-vaccinated groups was lowest in the double deficient group. Glutathione peroxidase activity was lowest in the two selenium deficient treatments, highest in the high selenium treatment, and unaffected by vaccination status. Fish fed the two vitamin E-deficient diets were more susceptible to red blood cell peroxidation than fish fed the remaining three diets. No significant differences were observed in the challenge studies.

Effect of Levamisole, Cortisol and Stress on Immune Response

Experimental design of this study involved five vaccinated treatments in duplicate (total of 10 groups, 20 fish/group): (1) control; (2) levamisole for four days prior to initial sampling; (3) cortisol (1700 mg/kg) two weeks prior to initial sampling; (4) cortisol + levamisole and (5) Carrisyn. Immunologic assays were conducted five weeks after initiating the study. Mean plasma cortisol concentration of fish not receiving cortisol was 8.2 ng/ml, while that of fish receiving cortisol was 163.0 ng/m. Pronounced immunosuppression was observed in fish receiving cortisol in that none of these vaccinated fish developed agglutinins during the study. In fact, of the cortisol treated fish, 4/10 fish in one replicate group and 7/10 in the other succumbed before the study was terminated. The ameliorating effect of levamisole in counteracting the immunosuppressive effect of cortisol was not statistically significant ($p < 0.05$). The resistance enhancing effects of Carrisyn were further verified in that bactericidal and phagocytic assays were enhanced by a factor of 2-3 fold over control fish which had agglutination titers of 1:64-1:256. Eighty-two percent of the fish survived bacterial challenge. None of the fish which had received Carrisyn succumbed to challenge.

In an effort to ascertain the potential ameliorating effects of levamisole and Carrisyn upon stress-induced immunosuppression, the fish from one replicate of each treatment were stressed

by placing in nets just below the surface of the water for 24 hours. Three fish in each tank were processed for: a) phagocytic assays, b) bacterial challenge, c) phage neutralization and d) plasma cortisol assay prior to and immediately after imposing the 24-hour stress test. Across all treatments, stressed fish possessed a mean cortisol level of 93 ng/ml while unstressed fish possessed a mean cortisol level of 11 ng/ml. Phagocytic assays and other indicators of immune responsiveness were higher in levamisole treated, vaccinated stressed fish, than in those vaccinated stressed fish which had not received levamisole. However, there was no difference in resistance to challenge between stressed and non-stressed levamisole-treated fish. Approximately 40% of the stressed fish which had been immunized ultimately succumbed to bacterial challenge whether or not they had received levamisole. The immunopotentiating effects of Carrisyn were not observed in stressed fish in that there appeared to be no difference in survival to challenge or other immunoassays between fish receiving Carrisyn and those not receiving Carrisyn.

USEFULNESS OF FINDINGS:

The findings of this research project will be useful in:

(1) designing diets to help promote immunity to E. ictaluri;

(2) designing diet/vaccination regimes for promoting immunity to E. ictaluri; and

(3) designing further studies to develop techniques to more efficiently immunize channel catfish against E. ictaluri.

PUBLICATIONS:

Blazer, Vicki S. 1991. Piscine macrophage function and nutritional influences: A review. Journal of Aquatic Animal Health 3:77-86.

C. Effect of Nutrition on Body Composition and Subsequent Storage Quality of Farm-Raised Channel Catfish

Annual Progress Report
For The Period
October 1, 1990 to September 30, 1991

COOPERATING INSTITUTIONS:

Auburn University (Lead Institution)	
Fisheries	R.T. Lovell
Ag. Economics	Upton Hatch
Kentucky State University	
Aqua. Res. Center	J.H. Tidwell
	C. Webster
Louisiana State University	
Forestry, Wildlife & Fisheries	R.C. Reigh
Food Science	J. S. Godber
Mississippi State University	
Delta Research and Extension Center	E.H. Robinson
Biochemistry	R.P. Wilson
Wildlife & Fisheries	H.R. Robinette
Agric. Economics	J.E. Waldrop
Food Science & Human Nutrition	J. Hearnsberger
Texas A & M University	
Wildlife & Fish. Science	D.M. Gatlin
University of Georgia	
Food Science & Tech.	J.J. Jen
	Y.W. Huang
	D.A. Lillard
	P.E. Koehler
	R. EitenMiller
Georgia Exp. Station, Griffin, Ga.	M. Erickson
Coastal Plains Exp. Station, Tifton, Ga.	G. Burtle

ADMINISTRATIVE ADVISOR:

Gale A. Buchanan, Associate Director
Georgia Agricultural Experiment Station
Tifton, Georgia

PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1: In a study at Auburn University, diets containing five DE/protein ratios were fed at satiation rate to year-2 (first growing season) channel catfish in ponds. The DE/protein ratios (10.0, 9.4, 8.5, 7.7, and 7.0 kcal/gram) were affected by changing protein percentages from 24 to 28, 32, 36 and 40. Weight gain and feed conversion were the same for all treatments. Dressing percentage was not different among treatments (67%). Fat percentage in fillets decreased as dietary protein increased (8.6% to 6.5%).

The Food Science Department, University of Georgia, obtained fish from the feeding study at Auburn. Fat contents of fillets from catfish fed feeds containing 26, 32, and 38% protein were decreased as protein increased. Results from nine months storage showed that all fillets stored at the lower temperature (-28°C) had significantly lower 2-thiobarbituric acid (TBA) number and free fatty acid content than those stored at -18°C for vacuum and non-vacuum packaging methods. No significant differences for sensory quality, TBA number, pH, ammonia and free fatty acid content of fillets stored at the same temperature were found regardless of dietary treatment or packaging method.

New procedures for measuring lipid oxidation in frozen stored fish were evaluated at the Georgia Agricultural Experiment Station, Griffin. In general, neither hydroperoxides, conjugated dienes, nor fluorescent pigments served as sensitive measures of oxidation in minced muscle tissue during the first six months of storage. The ability to detect production of TBA and loss of tocopherol during the first six months of storage, however, suggest their use as indicators for the onset of the propagation stage of lipid oxidation.

Diets with supplemental L-carnitine were evaluated for channel catfish grown in ponds in the summer of 1991 at the Georgia Coastal Plain Experiment Station. Diets were formulated with 0, 0.05%, or 0.1% supplemental L-carnitine. The fish will be harvested the week of September 23. Analyses of whole body lipid and protein and muscle lipid and protein will be completed by December 15, 1991. Frozen storage quality will be measured over a six month period at -18°C. Changes in TBA values, free fatty acids, pH, and ammonia of the fillet portion will be measured.

Results of a 2-year study at Louisiana State University showed that supplementation of a low-fat (2.5% lipid) commercial catfish feed with either 2% or 4% lipid in the form of menhaden oil, catfish oil, and tallow, did not affect the average gain of year-3 channel catfish raised at commercial densities in ponds. Among fish fed 4% supplements, menhaden oil produced significantly greater liver weight than catfish oil, which produced significantly greater liver weight than tallow. Fish fed 2% lipid supplements showed no differences in liver weight among lipid types. Visceral fat was higher in catfish fed 4% tallow than in those fed 2% tallow. The opposite result occurred among fish fed catfish oil and no difference was found among fish fed menhaden oil. Fish fed 4% tallow contained more visceral fat than those fed equal amounts of menhaden oil or catfish oil. Feeding 2% supplemental lipid did not affect visceral fat. Dressing percentage did not differ among fish fed the three lipid supplements at the 2% level, but at 4% lipid, those fed menhaden and catfish oil had lower dressed weight than fish fed tallow.

The Food Science Department at Louisiana State University determined that lipid oxidation

in fresh fillets from catfish fed 4% menhaden oil or catfish oil was higher than in fillets from catfish fed 2% of oils. Oxidation was lower in fillets from catfish fed 4% tallow than in those from fish fed 2% tallow, perhaps because of reduced unsaturated fat levels. No apparent differences in sensory (taste) perception among treatments were determined by a trained taste panel. Lipid oxidation in frozen fillets increased over time. Lipid in fillets from tallow-fed fish was less oxidized over time than that from fish fed menhaden oil, however, sensory scores were not significantly different among treatments.

Objective 2: Second-year data from a study at the Delta Research and Extension Center, Stoneville, Mississippi, designed to evaluate high-protein finisher feeds on yield and fattiness in catfish have been analyzed. Fish were reared to a final weight of 0.9 to 1.1 kg. There were no differences in weight gain or proximate composition of edible tissue of fish regardless of dietary protein level (38 or 32%) or length of time the high-protein feed was fed. Visceral fat was higher in females as compared to males. Fish from the third year study will be harvested in October, 1991.

The Department of Wildlife and Fisheries at Mississippi State University has analyzed second year data from a study to evaluate effects of dietary protein level (32 vs 38%) and feeding regime (satiation or restricted). Catfish fed 32% protein feed to satiation gained less weight than fish fed 38% protein feed under a restricted feeding regime. Fish fed under restricted feeding had lower feed conversions than fish fed to satiation. Fish on the 32% restricted feeding regime had more visceral fat than fish on the 32% protein feed to satiation or the 38% restricted feeding regime. Fish from the third year study will be harvested in October, 1991.

The Food Science and Human Nutrition Department at Mississippi State University is currently evaluating second-year fish from the two studies described above. TBA, tissue phospholipids, and flavor are being evaluated. Available data indicate no differences in the keeping quality of frozen fillets regardless of the dietary treatment.

Three studies were completed at Kentucky State University in 1991. Study one was an evaluation of the effects of feeding frequency (once or twice daily) at two protein levels (32 and 38%) for fish grown to harvestable sizes in ponds. Study two was a similar protocol for fish raised in cages. Study three was an investigation of the effects of food deprivation and re-feeding on fat level and fatty acid profiles of different tissues.

In the pond study, there were no significant differences in growth or body composition of channel catfish due to protein level, feeding frequency, or their interaction. In the cage study, fish fed a diet containing 38% protein had significantly higher weight gain than fish fed the diet containing 32% protein. Feeding frequency did not affect growth or body composition, but the fish fed twice daily had a higher dressing percentage than those fed once daily. Depriving channel catfish of feed for 28 days caused an increase in n3 highly unsaturated fattyacids (C-20, C-22) in muscle and liver, but a reverse trend in the brain. Except palmitic acid (C-16), no other fatty acid changes were observed. Body composition was not affected. The results suggest that channel catfish are able to maintain stability in body composition and fatty acid profiles in tissue for at least 28 days.

Objective 3: An experiment was conducted at Texas A & M to determine the effects of the natural antioxidants, vitamin C and rutin (a bioflavonoid), on channel catfish. Supplemental vitamin C improved growth and health of the fish as well as increased resistance of fillet samples to oxidation as measured by TBA.

However, rutin had only limited effects on the measured parameters. In another feeding trial, the effects of dietary glutathione (0, 0.1, and 1%) and lipid (5 and 10%) were investigated in a 10-week feeding trial. The diets containing 10% lipid generally produced higher weight gain and feed efficiency values as well as higher levels of abdominal, whole-body and muscle lipid. The higher level of dietary lipid also reduced the stability of fillets to oxidation as measured by TBA. Dietary glutathione did not significantly affect any of the measured parameters.

Studies are continuing by personnel in the Biochemistry Department at Mississippi State University to determine effects of feeding tristearin, catfish oil, menhaden oil, and a 50:50 mixture of catfish and menhaden oil on the fatty acid composition of membrane polar lipids. Tissue fatty acid analysis is underway.

USEFULNESS OF FINDINGS:

This year's data with different sizes of catfish agree with previous years' data (year-2 and year-3 fish) that increasing the dietary ratio of protein to energy reduces body fat in the fish but does not significantly enhance fresh or frozen keeping quality of processed fish. Also, there appears to be no benefit in finishing year-2 and year-3 fish on a high protein diet to reduce body fat. Feeding frequency or feed deprivation (4 weeks) also has limited affect on body fat in fish. Type of fat in the diet (beef, soybean, menhaden) at levels of 2 and 4%, does not seem to affect growth rate, muscle fat or keeping quality of year-2 or year-3 channel catfish.

These results suggest that feed composition and feeding strategy are less responsible for quality changes than post-harvest processing and storage conditions. Various dietary supplements such as vitamins E and C and carnitine may be useful in enhancing frozen keeping quality of processed catfish products.

PUBLICATIONS:

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Webster, C. D., J. H. Tidwell, D. H. Yancey. Effect of protein level and feeding frequency on growth and body composition in cage-reared channel catfish. Progressive Fish-Culturist. Accepted.

Webster, C. D., J. H. Tidwell, J. A. Clark, and D. H. Yancey. Effects of feeding diets containing 34 or 38% protein at two feeding frequencies on growth and body composition of channel catfish. Journal Applied Aquaculture. Accepted.

Erickson, Marilyn C. 1991. Extraction and quantitation of tocopherol in raw and cooked channel catfish. Journal of Food Science 56: 1113-4.

Erickson, Marilyn C. 1991. Susceptibility of striped bass and hybrid striped bass to oxidation during frozen storage. 88th Annual Meeting Southern Association of Agricultural Scientists, Food Science and Human Nutrition Section, February 3-6, Fort Worth, Texas. Abstract. pp. 14-15.

Erickson, Marilyn C. 1991. Frozen storage stability of two channel catfish strains. Annual Meeting of Institute of Food Technologists. June 2-5, Dallas, Texas. Abstract. p. 145.

Erickson, Marilyn C. 1991. Acceleration of lipid oxidation during cooking of refrigerated minced channel catfish muscle. Fourth Chemical Congress of North America. August 25-30, New York City, New York. Abstract. Paper 124.

Huang, Yao-Wen, R. R. EitenMiller, D. A. Lillard, and P. E. Koehler. 1991. Storage quality of iced channel catfish fed different protein levels. Journal of Food Quality. 14:345-354.

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Huang, Yao-Wen and Menghe Zheng. 1991. Chemical, microbiological and sensory quali-ties of vacuum skin packaged channel catfish stored at 4°C. Abstract. Annual Meeting of Institute of Food Technologists. June 1-5, Dallas, Texas.

Huang, Yao-Wen, R. R. EitenMiller, D. A. Lillard, and P. E. Koehler. 1991. Chemical microbial quality of packaged fresh and frozen farm-raised catfish. Abstract. 8th World Congress of Food Science and Technology. September 29 - October 4, Toronto, Canada.

Liu, Q. Interaction of supplementary carnitine and lysine on growth, tissue lipid and protein content of fingerling channel catfish. Master's Thesis. University of Georgia.

Li, Menghe and R. T. Lovell. 1991. Growth, feed efficiency and body composition of second and third-year channel catfish fed various concentrations of protein to satiety in production ponds. Aquaculture. Accepted.

Li, Menghe and R. T. Lovell. 1991. Comparison of satiate and restricted feeding of channel catfish with various concentrations of dietary protein in production ponds. Aquaculture. Accepted.

D. Harvesting, Loading, and Grading Systems for Cultured Freshwater Finfishes and Crustaceans

Annual Progress Report
For The Period
October 1, 1990 through September 30, 1991

COOPERATING INSTITUTIONS:

Louisiana State University (Lead Institution) - Robert P. Romaire, Tom B. Lawson, James L. Avery

Auburn University - J. W. Jensen, J. M. Grizzle, L. L. Lovshin, R. K. Goodman

Clemson University - John A. Collier, Thomas E. Schwedler

Memphis State University - Kenneth B. Davis, James F. Payne, Bill A. Simco

Mississippi State University - M. J. Fuller, James G. Dillard, M. W. Brunson

University of Georgia - George W. Lewis, James Shelton

University of Southwestern Louisiana - Jay V. Huner

ADMINISTRATIVE ADVISOR:

W. H. Brown, Associate Director
Louisiana Agricultural Experiment Station
La. State University Agricultural Center
Baton Rouge, Louisiana

PROGRESS OF THE WORK AND PRINCIPAL ACCOMPLISHMENTS:

Auburn University

Evaluation of a turbine pump, vacuum pump, and lift-net (boom-and-basket) to load market-size channel catfish into transport tanks was

completed. Channel catfish were harvested from 1-acre (0.4 ha) ponds using the three loading devices and stocked into 0.1 acre (0.04 ha) ponds for three weeks. Survival and individual weight gain of catfish after three weeks did not differ among the three loading devices and were as follows: turbine pump, 85.3% and 0.07 lb (34 g) gain; vacuum pump, 89.0% and 0.07 lb (32 g); and lift net, 94.0%, and 0.15 lb (68 g). Three species of pathogenic bacteria were isolated from internal organs. Two days post-harvest, bacterial disease was most common in fish harvested with the turbine pump, and the turbine pump caused more abrasions, lacerations, and broken fin spines, and higher serum AST and LDH activities (enzymes released from injured tissue) in catfish than the vacuum pump or lift net. The turbine pump causes more traumatic injury to market-size catfish than the lift net or vacuum pump.

Channel catfish fingerlings were loaded into transport tanks with the turbine pump and lift-net in March and May. After a three-week holding period in ponds, no difference was observed in survival (96%) or individual weight gain between the two loading devices. External, gross injuries to the fingerling catfish were evaluated and AST and LDH enzyme levels measured. Analysis of blood samples and analysis of data were not yet completed as of September 1991.

The catchability of seines with a gathered-netting mud line (GNML) or rubber-roller mud line (RRML) was evaluated on market-size catfish in ten experimental earthen ponds. The mean total weight of catfish removed by the GNML seine on the 1st, 2nd, and 3rd haul was 57%, 18%, and 8%, respectively, and for the RRML seine, 77%, 12%, and 4% on the 1st, 2nd, and 3rd seine haul. More fish were removed with the RRML seine for first seine haul and the first and second seine haul combined compared to the GNML seine, but the total fish removed was the same for both gears for the three seine hauls combined.

A commercial mechanical grader and counter (Fischtechnik Fredelsloh, D-3413 Moringen, West Germany) was evaluated for grading speed, fish size variation after grading, and counting accuracy with channel catfish fingerlings. About 1,540 lb (700 kg) of fingerlings were graded and counted into four size groups in 56 minutes, a rate of 26.6 lb (12.1 kg) of fingerlings/minute. Variation in each group was low; inch group 4-5, 5-6, 6-7, and > 7 had size variations of 0.29, 0.25, 0.26, and 0.42 inches, respectively. Use of a microprocessor to count fingerlings over-counted the true number by 5%. Large quantities of fingerlings can be accurately counted and graded quickly with this system.

Clemson University

Laboratory evaluation of electrical currents to enhance channel catfish harvest were completed. Electrodes placed in the mud line of an experimental seine and parallel to the mud line about 16 inches (40.6 cm) above the bottom of the seine gave the best response in water with conductivity of 390 μmhos. Continuous exposure of catfish to voltages to above 10 V would stun the fish, but pulsing the voltage on for 0.1 second and then off for 1.0 second allowed the fish to recover and move away from the seine. Voltages up to 30 V did not harm the fish. Electrical current prevented the accumulation of fish against the seine but did not prevent them from bumping into the seine.

Harvesting experiments were conducted in 0.1-acre (0.04 ha) earthen ponds with an electrical seine. Forty-eight seine hauls were made, 12 with no voltage (control) and 36 with different voltages varying from 7.5 V to 30 V. Initial indications reveal no differences in catfish catch with the electrified seine and the control. Poor results of the electrical seine in ponds may have been caused by water of low conductivity (140 μmhos). Water conductivity in ponds will be increased to levels common in the Mississippi

Delta (500 μmhos) with salt and the experiment repeated in Year 3.

Louisiana State University

Five, 4- to 5-acre (1.8-2.2 ha) crawfish ponds were filled with water in mid-October and crawfish harvest began in February and ended in May. Pyramid traps were used at a density of 24 traps/acre (60 per ha). Crawfish were trapped in each of 2 ponds, 5 consecutive days per week, 3 consecutive days per week, and 3 consecutive days every other week (biweekly). Crawfish yield increased 44% with an increase in trapping days from 3 days biweekly to 3 days/week, but catch increased only 4% when trapping effort was increased from 3 days/week to 5 days/week. Crawfish yield was as follows: 5 trapping days/week--1,971 lb/acre (2,208 kg/ha); 3 days/week--1,895 lb/acre (2,122 kg/ha); and 3 days biweekly--1,318 lb/acre (1,476 kg/ha). No decrease in crawfish yield resulted from a decrease in trapping frequency to 3 days/week from the present commercial recommendation of 5 days/week. Data is being analyzed for the effects of the three trapping strategies on size of crawfish harvested.

A spiral crawfish grader (Crawfish Combines, Inc., New Iberia, Louisiana) was modified to fit in a harvesting boat. The grader uses a pair of horizontal, diverging rollers to separate the crawfish into four size grades. Results from laboratory trials were used to establish roller spacings for field trials. Results of field trials produced mean group sizes of 26 crawfish/lb (17 g/crawfish), 19/lb (24 g), 17/lb (27 g), and 14/lb (33 g), which closely conforms to the size grades requested by crawfish buyers. The speed of the grader was not good, and frequent stopping of the grader was required to clear the machine of debris. A grader that uses vibrating bars will evaluated in Year 3.

Production of the video "Warmwater Fish: Harvesting, Handling and Transportation" continued. Additional film was taken on harvesting of catfish and other finfish species. The film script was edited to clarify government regulations with regards to use of approved chemicals. Seventy-five percent of the video footage was complete as of September, 1991.

University of Georgia

The University of Georgia was a non-scheduled participant in the second year of the Harvesting, Loading, and Grading project.

Memphis State University

Physiological responses of channel catfish fingerlings harvested and loaded from ponds at Auburn by lift net and turbine pump were evaluated. Plasma concentrations of cortisol and chloride were used to determine the degree of stress imposed on catfish by the two devices. Chloride concentrations were stable during harvest and loading with both devices. Cortisol concentrations increased during harvest and loading and it returned to pre-harvest levels within a few days. These data indicate that the turbine pump causes no more physiological stress in loading catfish fingerlings than the lift net.

The ionoregulatory ability of red swamp and white river crawfish in different salinities is similar and is temperature dependent. The lowest concentration of hemolymph chloride and osmotic pressure in the animals held in freshwater occurs at 75°F (24°C). Both species hyporegulate their hemolymph up to 20 parts per thousand (ppt) salinity at which point the hemolymph becomes isosmotic and remains isosmotic at salinities up to 35 ppt.

Mississippi State University

Mississippi State University economists collected data for harvesting and loading studies with

channel catfish (Auburn University) and crawfish (Louisiana State University). LSU researchers have provided data on cost of harvesting crawfish using traditional trapping methods. Data was received from Auburn on experiments that compared the vacuum pump and turbine pump with the traditional lift net for loading market-size catfish. The alternative loading technique with greatest potential is an 8-inch (20 cm) hydraulic-powered turbine pump. A larger, 10-inch (25 cm) turbine pump may be more effective in harvesting and loading pond-run, market-size catfish, and it is scheduled to be tested in commercial catfish ponds in Mississippi in Year 3. Efforts to update a 1983 report on the cost of harvesting and loading catfish in the Mississippi Delta using traditional methods were begun and most of the data required has been obtained.

University of Southwestern Louisiana

University of Southwestern Louisiana (USL) designed and fabricated a capture net (trawl), now referred to as a "crawfish skimmer", to harvest crawfish. The net, which is made from 1.5-inch (38 mm) stretched and square mesh polyvinyl netting and is mounted to a conventional crawfish harvesting boat, is fished in tandem and suspended from the bow of the boat. Net width is seven feet (2.15 m). The net was evaluated in two crawfish ponds at the USL experimental farm from April through mid-June. Test hauls were made in mid-morning, and each haul, about 1,300 feet (400 m), was about five minutes. Tests were made with and without attractant (bait). A 25% protein crawfish feed was used as the attractant. Use of an attractant increased the effectiveness of the net. Crawfish catch in the net hauls over baited lanes (mean = 38.4 lb/haul, 17.5 kg; range 14-88 lb, 6.4-40 kg) was 3.4 times greater than in net hauls over non-baited areas (mean = 13.4 lb/haul, 6.1 kg; range 5-39 lb, 2.3-17.7 kg). Only 1.8% (range 0-6.3%) of the crawfish caught over bait lines were soft compared to 4.4% (range 1.1-13.4%) caught in trawls in non-baited lanes, indicating that the attractant concentrated on hard crawfish. Average size of the hard crawfish caught in trawls was 29 crawfish/lb (15.5 g/crawfish). Crawfish caught in baited traps were larger than those caught in trawls. Plant debris had a tendency to clog the net.

USEFULNESS OF FINDINGS:

The research efforts in Year 2 provided potential useful benefits to catfish and crawfish producers. Vacuum pumps do not appear to have much promise for harvesting and loading market-size or fingerling catfish. The turbine pump appears to be an effective device for loading market-size catfish that will be sent to processing plants. A seine with a modified mud-line with rubber rollers appears to be more efficient in harvesting catfish in ponds with irregular soft mud bottoms than a seine with a traditional mud-line. A German manufactured finfish grader and counter was able to accurately grade and count large quantities of catfish fingerlings quickly, and the device may have potential for commercial use in the catfish industry.

Crawfish harvesting research has determined that a reduction in trapping effort from three consecutive trapping days/week results in no significant reduction in catch compared to the current recommendation of five days/week. Personnel with the Louisiana Cooperative Extension Service that recommended three day/week trapping to selected crawfish producers in the 1990-1991 season reported that most of the producers observed no reduction in crawfish catch and significant savings in bait and labor cost by trapping fewer days/week. The potential of a trawl or net system for harvesting crawfish has been demonstrated but it requires further evaluation. Commercially available in-boat crawfish graders have mechanical limitations in grading capacity that must be corrected before they can be recommended to crawfish producers.

WORK PLANNED FOR NEXT YEAR:

Harvesting, loading, and grading research with channel catfish at Auburn University will continue to evaluate catfish injury caused by lift nets and turbine pumps; graders will be evaluated for sorting market-size and fingerling catfish; and market-size catfish harvested and loaded with lift nets and turbine pumps will be re-stocked and their susceptibility to angling investigated. Clemson University will field test the electrical seine in ponds in which conductivity of the water has been increased to levels common in the Mississippi Delta. Louisiana State University will evaluate seines and trawls to harvest crawfish; a crawfish harvesting and grading workshop with be conducted in southwest Louisiana in 1992; an in-boat grader that uses vibrating bars to segregate crawfish will evaluated; and the video "Warmwater Fish: Harvesting, Handling and Transportation" will be completed. The University of Georgia will host a "Southern Regional Fish Farming Field Day" at Pineland Plantation Fish Farm in Newton, Georgia, on October 24, 1991. Memphis State University will continue research on the physiological stress imposed on catfish and crawfish by the various harvesting and loading devices tested at Auburn University and Louisiana State University. Mississippi State University will analyze data from catfish harvesting and loading studies and crawfish harvesting studies to determine the comparative economic benefits of alternate harvest methods compared to conventional methods. Mississippi State University economists and extension personnel will develop and prepare extension publications on the economics and techniques of harvesting, loading, and grading catfish.

PUBLICATIONS:

Lawson, T.B. and R. Romaire. In press. Evaluation of two new trap types and aerator-induced water currents for harvesting procambarid crawfish in ponds. J. Shellfish Research.

Rode, R., L. Lovshin, and R. Goodman. In press. Comparison of three fish-loading systems to harvest food-size channel catfish (Ictalurus punctatus). Aquacultural Engineering.

E. Preparation of Extension Publications on Avian Predator Control in Aquaculture Facilities

Annual Progress Report
For The Period
October 1, 1990 to September 30, 1991

COOPERATING INSTITUTIONS:

Texas Agricultural Extension Service (Lead Institution) - James T. Davis

Mississippi Cooperative Extension Service - Martin Brunson

Arkansas APHIS/ADC/USDA - M. Hoy

Georgia Cooperative Extension Service - George Lewis

Louisiana APHIS/ADC/USDA - W. F. Stevens

Mississippi APHIS/ADC/USDA - Frank Boyd

Texas APHIS/ADC/USDA - Gary Littauer

Mississippi S & T Field Station APHIS/ADC/USDA - Alvin Stickley, Jr.

United States Fish and Wildlife Service

ADMINISTRATIVE ADVISOR:

Milo J. Shult, Associate Director
Texas Cooperative Extension Service
Texas A&M University System
College Station, Texas

PROGRESS OF THE WORK AND PRINCIPAL ACCOMPLISHMENTS:

In response to the demand for more factual information about control of depredation by avian predators, this project has undertaken to produce a 20-minute educational video that will provide identification guidelines for the common predatory birds that affect aquaculture operations. Information on their economic importance, effectiveness of control measures, and how to use these control devices or techniques will be provided.

This avian predator management video will be useful to catfish, baitfish, crawfish and other producers, as well as creating awareness within the general public of the extent of the problem. All field film footage has been taken and scripting has been initiated. As soon as the script has been reviewed and approved, it will be voiced and sent out for final review. The video should be completed within the next six months.

Five, two to four page, fact sheets will also be developed through this project. These fact sheets will discuss identification of avian predators of major importance, their damage to aquaculture products, consumption rates, seasons of occurrence, recommended control measures and sources of technical assistance. Two of these fact sheets, "Frightening Techniques for Reducing Bird Damage at Aquaculture Facilities" and "Control of Bird Predation at Aquaculture Facilities - Strategies and Cost Estimates" by Gary Littauer have been completed. Alvin Stickley, Jr., has completed a fact sheet entitled "Avian Predators on Southern Aquaculture". These three fact sheets were peer reviewed and edited, and have been distributed throughout United States. Copies were requested and furnished to a number of Fish and Game agencies in addition to Extension cooperators. The two remaining fact sheets are being processed at this time. They delineate passive measures for bird control and regulations affecting the methods allowed for bird control in the United States. Completion of all of these items is planned within the next six months.

WORK PLANNED FOR NEXT YEAR:

No changes are contemplated in the project format or details.

F. Educational Materials for Aquaculturists and Consumers

Annual Progress Report
For The Period
May 1, 1991 to September 30, 1991

COOPERATING INSTITUTIONS:

Texas Agricultural Extension Service (Lead Institution) - James T. Davis, Katheleen Ladewig, Thomas Valco, Billy Higginbotham

Alabama Cooperative Extension Service - Michael Masser, John Jensen

Ekk Will Tropical Fish Farm - Timothy K. Hennessy

Florida Cooperative Extension Service - Charles Cichra, Ruth Francis-Floyd, Jerome V. Shireman, Roger Rottmann

Georgia Cooperative Extension Service - George W. Lewis, Ronnie Gilbert, George Schuler

Kentucky Cooperative Extension Service - Robert Durborow

Louisiana Cooperative Extension Service - Wendell Lorio, Fred Baker, Michael Moody

Mississippi Cooperative Extension Service - Martin W. Brunson

Mississippi Agricultural and Forestry Experiment Station - Craig S. Tucker, Martine van der Ploeg

North Carolina Agricultural Extension Service - Thomas Losordo

Tennessee Cooperative Extension Service - Thomas Hill

ADMINISTRATIVE ADVISOR:

Milo J. Shult, Associate Director
Texas Agricultural Extension Service
Texas A&M University System
College Station, Texas

PROGRESS OF THE WORK AND PRINCIPAL ACCOMPLISHMENTS:

For aquaculturists to take advantage of the increase in fish and shellfish consumption in the world, the latest research, development, marketing and consumer education materials must be available. This project will prepare and distribute information needed to assist aquaculturists in making the best informed decisions associated with production and marketing practices. In addition, information for retailers and consumers will be made available to insure that handling of produce in supermarkets, restaurants and the home meets food safety standards.

A cooperative regional project such as this allows producers and consumers throughout the region to utilize educational materials without each state having to produce their own. This is estimated to save the Extension Services in the Southern Region approximately $7,000 per fact sheet and $20,000 per video. This translates to a total savings to the 15 states and territories in the Southern Region of nearly $6 million for a SRAC investment of less than $150,000. As other regions use the materials, the savings will be even higher. Judging by the requests received, the usefulness to the industry exceeds most Extension publications as aquaculture is the sole commodity that has shown expansion throughout the past 20 years.

The Steering Committee and Work Group outlined the schedule for submission of the fact sheets and videos. Progress in preparation of these materials is excellent. Within the short time the project has been active, six fact sheets have been peer reviewed and are currently in the editing process. At least five others are in the peer review process and should be sent to the editor within a month. The other 17 fact sheets scheduled for year one are in various stages of preparation.

Some of the areas to be covered by fact sheets are off-flavor, water quality, water reuse systems, fish health, induced spawning, processing procedures, fee fishing, alligator production, basic food safety, pumping and power systems, and oyster and clam production.

Progress on the videos is somewhat slower since they are scheduled for completion in years two and three. Scripting has been started on one video and shooting areas selected for two others. Areas to be covered by vidoes are handling of fish and shellfish by retailers and consumers; processing procedures to meet quality standards; and production of channel catfish fingerlings.

USEFULNESS OF FINDINGS:

Distribution to user groups has not started at this time.

WORK PLANNED FOR NEXT YEAR:

No changes are anticipated in the project plans at this time.

G. Characterization of Finfish and Shellfish Aquacultural Effluents

Annual Progress Report
For The Period
May 1, 1991 to September 30, 1991

COOPERATING INSTITUTIONS:

University of Florida (Lead Institution) - Jerome Shireman

Auburn University - Claude E. Boyd

Clemson University - David E. Brune

Louisiana State University - Donald C. Huffman, Robert P. Romaire

Mississippi State University - John E. Waldrop, Craig S. Tucker

North Carolina State University - Jeffrey M. Hinshaw, Thomas M. Losordo

Texas A & M University - James T. Davis

University of Arkansas - Carole R. Engle

University of Georgia - Gary J. Burtle

Waddell Mariculture Center - J. Stephen Hopkins

ADMINISTRATIVE ADVISOR:

Dr. John T. Woeste, Dean
Florida Cooperative Extension Service
University of Florida
Gainesville, Florida

PROGRESS OF THE WORK AND PRINCIPAL ACCOMPLISHMENTS:

The aquacultural industry has expanded considerably in the southern United States and is now a major agricultural endeavor. The purpose of this study is to characterize effluents from finfish and shellfish operations. Best management practices will be identified that are sound technically and economically. Results will be evaluated and extension publications developed that describe problems and best management practices to solve real and potential problems.

Effluent characterization -- catfish: Twenty-five commercial channel catfish ponds were selected in central and west-central Alabama to collect water samples. Samples were collected from near the surface and bottom in spring and summer of 1991. Samples were analyzed for water quality variables by procedures decided upon by the SRAC Work Group.

Water samples were also collected during the summer growing season from 20 commercial channel catfish ponds on three farms in west-central Mississippi. Samples were collected from the surface and bottom of each pond adjacent to the drain pipe. Single analyses were conducted for each variable listed in the project proposal.

Effluent characterization -- striped bass: Virginia State University withdrew from the project as they were unable to establish a water quality laboratory. The work to be done by Virginia State University will be done by Waddell Mariculture Center.

The striped bass hybrid (SBH) sampling schedule was interrupted by the withdrawal of Virginia State University. Waddell Mariculture Center (WMC) expanded its sample collection because of this change. WMC has made several equipment and procedural changes in its water quality analysis laboratory to conform to SRAC effluent study guidelines and to facilitate simultaneous handling of larger numbers of samples. The first of eight quarterly samples were taken in August to early September. A total

of 24 ponds were sampled with either two or three samples from the water column of each pond. In addition, the inlet water was analyzed at each site for a total of 63 samples. For each pond and each level of the water column (surface, mid-water, bottom), the following were determined and/or recorded:

farm name
pond number
sample depth
aeration rate
water exchange rate
fish size
fish biomass
feed rate
temperature
dissolved oxygen
pH
ammonia nitrogen
nitrite nitrogen
nitrate nitrogen
soluble reactive phosphate
suspended solids
percent organic matter
settleable solids
total nitrogen
total phosphorous
BOD (3 dilutions)
salinity
coliform bacteria

Twenty-one of the ponds are located at four commercial farms. Three of the ponds are at the Research Center -- two are used for refining intensive production techniques, and one for holding broodstock. Two of the commercial farms utilize ground water, one utilizes surface freshwater and the fourth utilizes brackish surface water. The data have been entered into spreadsheet format and will be made available to those responsible for modeling upon receipt of the coliform bacteria analysis results from the commercial laboratory. However, due to the cyclic nature of pond dynamics, WMC feels that the quarterly sampling may not be frequent enough to determine the absolute range of several important parameters. Therefore, WMC is expanding its efforts in SBH effluent characterization by measuring all of the parameters mentioned above on a weekly, and in some cases, daily basis. This more intensive sampling program will be conducted in research ponds at WMC. SRAC will not incur any additional expenses over that already allocated for SBH effluent characterization.

Effluent characterization -- crawfish: The period from May 1 through September 30, 1991, was used to purchase equipment and supplies, select commercial ponds for study, and to employ personnel for the project. At least 16 commercial crawfish ponds have been selected for characterization of effluent quality and others may be included. The ponds represent cultivation systems that include the following pond types and forage plantings: rice-crawfish ponds (rice-crawfish rotation, rice set-aside); permanent ponds (planted with either rice, sorghum-sudan grasses or colonized by native terrestrial/native aquatic vegetation); and wooded ponds (native terrestrial/native aquatic vegetation and leaf litter). The ponds, which are located in eight parishes (counties) of south-central and southwest Louisiana, include those that replace water ("pump and flush") for maintenance of water quality and those that use paddlewheels to recirculate and maintain water quality.

Estimates of vegetative biomass (crawfish forage) began in some ponds in September and will continue into October. Collection and analysis of effluent samples will begin in October or approximately three weeks after ponds are filled.

Trout literature review: The purpose of this portion of the project is to review and summarize existing information on effluent characteristics of trout production facilities, and to determine the need for any additional monitoring or characterization of effluents from trout facilities in the

South. Working toward this goal, we have obtained copies or summaries of approximately 63 articles published since 1970, which are pertinent to the characterization of effluent from trout production facilities. The vast majority of efforts made by investigators and culturists to date have focused upon removal of suspended solids from effluent from salmonid production facilities. Very little effort has apparently been directed at the treatment or removal of dissolved nutrients from salmonid production effluents.

Crawfish economics: Progress to date includes assembling investment and operating cost data for facilities and equipment associated with alternative crawfish production systems. Performance rates and other parameters have been established that will permit defining differences in both fixed and operating costs associated with alternative effluent management systems. This data base will permit making detailed cost assessments of the various pond management systems being evaluated for effluents at both Experiment Station demonstration units and participating farm producer units.

Best Management practices: Six 1.0 acre ponds on the Delta Research & Extension Center, Mississippi State University, have been monitored throughout the summer. Three ponds will be harvested this fall without draining the ponds and three ponds will be drained after harvest. Fish will be restocked in the winter and grown until the fall of 1992. Water quality in all six ponds will be monitored until final fish harvest.

Texas A & M biologists have collected background water quality data from the experimental ponds to be used in the best management practice study. The ponds will be stocked after this analysis with phytophagous fish.

Agency survey: Extension personnel have been contacted in each state to conduct this survey to determine effluent regulations that apply in each state. Some information has been received and all information will be compiled during the remainder of the year.

USEFULNESS OF FINDINGS:

The project has not proceeded to a point where enough data has been collected to report findings.

WORK PLANNED FOR NEXT YEAR:

Work begun in May will proceed next year. This will include continuation of sampling for effluent characterization. This data will be used by Clemson University to develop effluent models. These models will help aquaculturists to predict the carrying capacity of local water bodies that might receive effluents.

The best management practice studies started by Auburn University and Mississippi State University will continue. In addition, researchers from The University of Arkansas at Pine Bluff and Mississippi State University will begin economic studies of best management practices and treatment technologies for those systems that produce effluents. Studies at the University of Georgia will look at ways and means to utilize catfish effluents for irrigation of crops. Modeling efforts by Clemson will continue and modeling of best management practices will begin at North Carolina State University. During year two of the project, extension specialists will begin to prepare extension publications.

V. SUMMARY

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

The Board of Directors, the policy-making body for SRAC, utilizes recommendations from an Industry Advisory Council and a Technical Committee to determine priorities for new and continuing aquaculture research and extension projects for the Southern Region. The Industry Advisory Council membership represents different segments of the aquaculture industry throughout the Region and provides valuable inputs for identifying priorities from an industry perspective. The Technical Committee is composed of research and extension scientists from essentially all states within the region and identifies priorities from a technical perspective.

Four SRAC projects were completed during 1990:

"Analysis of Regional and National Markets for Aquacultural Products Produced for Food in the Southern Region"

"Preparation of Southern Regional Aquaculture Publications"

"Performance of Aeration Systems for Channel Catfish, Crawfish, and Rainbow Trout Production"

"Develop a Statistical Data Collection System for Farm-Raised Catfish and Other Aquaculture Products in the Southern Region"

Final reports for each of these projects were included in the SRAC Third Annual Progress Report, January, 1991.

Two projects which were initiated in 1989 and completed in 1991 worked on control of enteric septicemia of catfish (ESC) caused by the bacterium Edwardsiella ictaluri. ESC is considered to be one of the most significant disease problems in channel catfish production.

The project **"Immunization of Channel Catfish"** explored immunization of channel catfish for disease protection. Channel catfish can be immunized with extracts from the bacterium E. ictaluri and these fish show some level of protection. The degree of immune response is dependent upon antigen concentration, exposure time and water temperature. Fish that survive an E. ictaluri epidemic and have high antibody levels are protected. Vaccinated fish will retain a high antibody level when fed a vaccine incorporated into the feed.

Fish fed diets with menhaden oil had an enhanced ability to intracellularly destroy E. ictaluri, when compared to fish fed diets with soybean oil or beef tallow. A diet containing all three lipid sources gave the best results regarding both disease resistance and growth. Feeding diets known to potentiate the immune response for two to four weeks prior to vaccination may be the most effective way of decreasing mortalities due to enteric septicemia.

The first steps in engineering the channel catfish virus (CCV) as a vaccine vector were accomplished. The thymidine kinase gene was cloned and mapped to the direct repeat ends of the CCV genome. In addition, a surface layer

protein of Aeromonas hydrophila was cloned and is available for insertion into the CCV- TK gene. In future research, the thymidine kinase gene will be deleted from the CCV genome and replaced with the gene encoding the A. hydrophila S-layer. This gene will be under the control of the TK promoter and the resultant recombinant will be a stable, attenuated, non-reverting mutant of CCV that expresses the bacterial antigen, thus providing protection against both the bacterium and CCV.

The project **"Enhancement of the Immune Response to E. ictaluri in Channel Catfish"** was also completed in 1991. The purpose of this study was to determine if dietary vitamin E, selenium and levamisole (an immuno-enhancing agent) affected the ability of channel catfish to develop immunity to E. ictaluri, the causative agent of enteric septicemia, following vaccination. In general, fish fed diets deficient (based on generally accepted dietary requirements) in either vitamin E or selenium did not develop immunity after vaccination as well as fish fed "normal" or above normal diets. Feeding diets containing above normal levels of vitamin E and selenium generally did not improve the development of immunity. Levamisole did not enhance the immune response as it does in other vertebrates. However, a second immunoenhancer, Carrisyn, did show some potentiating effect on the development of immunity.

The findings of this research project will be useful in (1) designing diets to help promote immunity to E. ictaluri, (2) designing diet/vaccination regimes for promoting immunity to E. ictaluri, and (3) designing further studies to develop techniques to more effectively immunize channel catfish against E. ictaluri.

Work is continuing on the three-year project **"Effect of Nutrition on Body Composition and Subsequent Storage Quality of Farm-Raised Channel Catfish"**.

Studies with year two and year three channel catfish showed that increasing the protein percentage, or increasing the protein-energy ratio, in the feed reduced fat in the muscle of the fish, but this change in fat content did not affect the fresh and frozen keeping quality of the processed fish. Increasing protein percentage of the feed from 24 to 38% did not increase weight gain when the fish were fed to satiety. Phase feeding, or finishing the fish on a high protein feed, did not influence growth or fat content of the fish; neither did frequency of feeding (once versus twice daily feeding).

Type of fat added to catfish feed, at 2 or 4% levels, did not significantly affect growth rate, body fat content or lipid oxidation of the frozen flesh of the fish. Including the antioxidants vitamin E and vitamin C in catfish diets reduced lipid oxidation in the flesh of the fish. An improved method for measuring lipid oxidation in frozen catfish flesh, may be to determine the peroxidation ability index/mole of vitamin E.

Work done by scientists on the project **"Harvesting, Loading, and Grading Systems for Cultured Freshwater Finfishes and Crustaceans"** has produced valuable information. Harvest methods which reduce costs, minimize stress, improve product quality, and facilitate grading by size are major needs of channel catfish and crawfish producers in the Southern Region. New and modified equipment and techniques for harvesting, loading and grading catfish and crawfish have been evaluated by research and extension scientists at seven universities.

Studies are being conducted under conditions similar to those practiced by commercial producers using commercially available equipment. The economic benefits of the new or modified technologies are being evaluated by agricultural economists. Information derived from the research has been and is currently being disseminated by extension educators through on-farm

demonstrations and regional workshops. One video and seven publications are in press or in preparation.

A turbine pump has shown good potential as an alternative to the standard lift net for loading catfish. The pump loads catfish much faster and it may be an effective device for loading market-size catfish that will be sent to processing plants. A catfish harvesting seine (net) has been improved by modifying the mud-line (seine bottom) to more effectively harvest catfish in ponds with irregular, soft mud bottoms. A commercial finfish grader/counter developed for other species of finfishes was found to accurately grade and count catfish fingerlings quickly, and the device may have potential for commercial use in the catfish industry.

Crawfish harvesting research has demonstrated that a reduction in trapping effort to three days/week from the current five or six days/week results in no significant reduction in catch over the harvest season, but a significant reduction in bait and labor costs is obtained. Commercially available in-boat crawfish graders have engineering limitations, and researchers are modifying these units to increase their efficiency and utility for crawfish producers.

In 1990, a project entitled **"Preparation of Extension Publications on Avian Predator Control In Aquaculture Facilities"** was initiated. Bird predation has become an increasingly important problem on aquaculture facilities during the past decade. Estimates of a 300 percent increase in avian predators on fish have been promulgated.

One fact sheet that has been completed is an attempt to provide information on identification of birds which are problem predators and those which are only a nuisance. This has been well received by aquaculture producers as well as environmental groups.

A second publication provides information on devices that frighten birds away from the production facilities. Both positive aspects and limitations of these types of gear are presented. The third publication is an attempt to provide some insight into the cost of avian predator control as well as some strategies that have proven effective.

During the coming year fact sheets on passive management techniques and federal regulations on control techniques will be distributed. In addition a video on bird habits and possible control measures is in its final stages of production and this will be distributed also.

The project **"Educational Materials for Aquaculturists and Consumers"** was initiated on May 1, 1991. It provides for the preparation of over 50 fact sheets and four videos. Some of the areas to be covered by fact sheets are off-flavor, water quality, water reuse systems, fish health, induced spawning, processing procedures, fee fishing, alligator production, basic food safety, pumping and power systems, and oyster and clam production. Areas to be covered by vidoes are handling of fish and shellfish by retailers and consumers, processing procedures to meet quality standards, and production of channel catfish fingerlings.

During the first year, it is anticipated that 28 of the fact sheets will be completed and distributed throughout the Southern Region and informational copies sent to all states and territories. The best decisions are made by informed people. This project is intended to provide the best information available to producers and consumers. With such preparation, aquaculture will continue to expand and serve the people in the United States.

Also begun in 1991 was the project **"Characterization of Finfish and Shellfish Aquacultural Effluent"**. The purpose of this study is to

characterize effluents from finfish and shellfish operations and to identify best management practices that are sound technically and economically. The information gathered in this study will be used to develop extension publications and other educational materials that will help fish farmers and regulatory agency personnel to better understand the problems both groups face.

The first year of the study was designed primarily to collect data and review pertinent trout effluent literature, begin to evaluate best management practices, conduct a survey of state aquaculture regulations, and begin a modeling effort to identify effluent carrying capacities of receiving waters. The study is on schedule as effluent samples have been collected, a review of the trout literature is well underway, extension personnel in each state have begun to collect information pertaining to effluent standards, and best management practice studies have been started. Data collection will continue during the remainder of the first contract period.

During the second year, data collection will continue and new portions of the study will begin. The agency survey will be completed at the end of the first contract year and extension education materials will be prepared describing and explaining the regulations for each state.

The interest among aquaculture scientists to work cooperatively on these regional projects has been exceptionally good. There is broad-based representation from throughout the Southern Region for both research and extension inputs into these projects. The potential benefits from these efforts appear to be quite significant.