PROJECT PLAN PROSPECTUS
106 Aquaculture
NP-106 (100%)
October 23 - November 19, 2001

Management Research Unit
6420-15 Aquatic Animal Health Research Unit

Old CRIS Project Number
6420-32000-011-00D

Location
Auburn, Alabama

Title
Integrated Management of Fish Diseases by Multi-Disciplinary Approaches

Scientists

Phillip H. Klesius, Lead Scientist.....	90%
Joel A.Bader.....	100%
Dehai Xu.....	100%
Richard A.Shelby.....	100%
Chhorn E. Lim.....	100%
Joyce J.Evans.....	90%
Craig A. Shoemaker.....	90%
Molecular biologist, Vacant*.....	90%
Microbiologist, Vacant*.....	100%

* Being selected and offered employment 4/16/01

Total Scientific Staff Years
8.60

Planned Duration
32 months

Signatures

/s/ Phillip H. Klesius 04/30/01
Research Leader Date approved

/s/ Not applicable
Lab, Center, or Institute Director Date approved

/s/ Edgar King 05/14/01
Area Director Date approved

/s/ Lewis Smith 05/21/01
National Program Team Leader Date approved

Key Words

Vaccine, Prevention, Diseases, Immunity, Pathogens, Virulence, Functional Genomics, Fish, Warm-water, Aquaculture, Neuropathology

Objectives

Objective 1: Development of vaccine strategies to prevent diseases of warm water species of cultured fish.

Objective 2: Identify virulence centers of bacterial, parasitic and fungal fish pathogens by functional proteomics and molecular technologies.

Objective 3: Determine interactions between nutrients, immune responses and microbes, parasites, fungi and toxins and fish to improve the understanding of susceptibility of fish to diseases.

Objective 4: Advances in understanding of neuropathology caused by microbes and toxins and relationships to abnormal aquatic animal behavior.

Need for Research

• *Description of Problem to be Solved*- Pathogenic microbes, microbial toxins and parasites are responsible for losses and injury in marine and fresh warm-water fish. The current knowledge is inadequate to develop integrated management strategies to combat fish diseases. The supply of vaccines to prevent major economically important diseases of fish is inadequate and the need to develop safe and effective vaccines are both immediate and critical. Proteomics, genomic and microbiological technologies are needed to identify the virulence centers of microbial and parasitic pathogens to provide basic understanding of their pathogenic mechanisms and the development of vaccines. The lack of basic information available on interactions between nutrients, immune responses and pathogens and fish is hampering the development of integrated management of fish diseases. Little or no information is available on understanding abnormal fish behavior and thus, basic studies on neurobiology of fish and central nerve system (CNS) pathology mediated by microbes and toxins are needed.

•*Relevance to ARS National Program Action Plan- Plant Biological and Molecular Processes*- This program falls within Component 2 (Integrated Aquatic Animal Health Management) of NS 106. The project focuses primarily on 2.7 (Conduct research and development to support approval and licensing of safe and effective new drugs, vaccines and other biologics for aquaculture). The project includes elements of 2.1 (Identify critical elements of population health management for aquatic production systems); 2.2 (Improve basic understanding of host immunity, resistance, and susceptibility to specific diseases, including genetic and nutritional factors, environmental effects and contaminants); and 2.4 (Improve understanding of the mechanisms of disease transmission, including carrier and carrier states, pathogen movements and environmental factors).

•*Potential Benefits*- Attaining these objectives will provide benefits to farmers, fish health providers, scientists and the public which will result in increased economic growth by helping to overcome the impact of disease and microbial toxins. New vaccines are expected to control diseases and reduce dependency on antibiotics and chemicals. The identification of virulence centers of pathogenic microbes and the improved understanding of interactions of nutrients,

immune responses and fish health is expected to better define integrated health management strategies in aquatic environments and production systems. The causes of fish health problems will be better understood by knowledge of the CNS of fish and CNS pathologies caused by neurotrophic microbes and toxins.

•*Anticipated Products*- New vaccines, improved fish feed and feeding practices and fish health management strategies to prevent fish diseases of warm-water fish species are the expected products.

•*Customers*- Fish farmers of cultured catfish, tilapia, hybrid striped bass and marine species in ponds, cages and water-reuse systems will benefit. Scientists will benefit from basic knowledge of the CNS of fish and the mechanisms of microbial mediated injuries that alters fish behavior. Consumers demanding safe and wholesome fish products free of antibiotics and chemicals will benefit. Trust agreement partners are involved in the vaccine development and interactions of environments, pathogenic microbes and fish health investigations.

Scientific Background

The project is closely coordinated with the Unit's CRIS 6420-32000-010-00D, "Provide the Fish Farmers Improved Tools of Management of Fish". Basic research findings made on early and rapid detection of pathogens, development of vaccine delivery systems and immunostimulant strategies of the project "Provide the Fish Farmers Improved Tools of Management of Fish" will be applied in this integrated aquatic animal health management project. The project is also coordinated by CRADA with Intervet, Inc. on "Characterization and Testing of Flexibacter and Streptococcus Vaccines", Trust Agreement with Kent Seatech on "Prevention of Infectious Diseases in Hybrid Striped Bass", Hydromentia on "Health Management of Hybrid-striped Bass in Re-circulating Water Systems", and MinAqua Fisheries on "Fish Health Related to Streptococcus Vaccination"' for field testing of vaccines against fish pathogens that cause losses in catfish, tilapia and hybrid striped bass. By specific agreement, the project is coordinated with Auburn University, Department of Fisheries and Allied Aquacultures on "Fish Health: Bacterial Genomics Research for Vaccine Development" on functional genomics of bacterial pathogens and vaccine development, and State of Delaware, Department of Natural Resources and Environmental Control Division of Water Resources on "Delaware Inland Bay Fish Health" to determine the effects of microbes and toxins on fish behavior. Similar work is being conducted at National Warm Water Aquaculture Center on applied health management and nutrition by specific agreement "Catfish Health and Nutrition Management". Fish health and genetic research at the ARS National Cold and Cool Water Aquaculture Research Center and Catfish Genetic Unit will be coordinated with proposal objectives.

Approach and Research Procedures

Objective 1

• *Experimental design*-

Existing and new microbiological, functional proteomics, genomics and vaccine technologies will be applied to fish pathogens and toxins. Bacterin, modified live, recombinant and DNA vaccines will be formulated and tested for safety and efficacy in wet laboratories and production systems to prevent diseases caused by bacterial, fungal and parasitic pathogens of fish. The vaccine induced immune responses will be characterized to determine the nature of the

protective immunities by humoral and cellular immunological assays.
• *Contingencies*- Adapt newly developed proteomic, genomic and immunological technologies to develop safe and efficacious vaccines to prevent fish diseases.

Objective 2
• *Experimental design*-
Existing and new functional proteomics and genomics technologies will be used to identify virulence centers of microbial and parasitic pathogens. Existing and new challenge models for fish pathogens will be used or developed to characterize the role of the virulence centers in infectivity of fish pathogens. Assays will be developed to define virulent isolates as opposed to non-virulent isolates of species of bacteria by microbiological and proteomics technologies.
• *Contingencies*- Adapt newly developed proteomic, genomic and immunological technologies to identify virulence centers of fish pathogens.

Objective 3
• *Experimental design*-
Conduct studies on nutrients, immune responses and pathogens to establish interactions that promote disease resistance against fish pathogens. Correlate the effect of nutrients and anti-nutritional factors on the fish immune system using antibody and cellular assays. Evaluate feed, feed additives and feeding practices that promote enhanced natural and vaccine mediated disease resistance in wet laboratory and field trials.
• *Contingencies*- Modify nutrients, determine optimal concentrations, improve digestion and availability of nutrient for the formulation of feed and feeding strategies that enhances protective immune responses after vaccination.

Objective 4
• *Experimental design*-
Conduct *in vitro* studies employing primary and established cultures of cells from the brain and CNS to identify and characterize cellular injuries mediated by microbial toxins using cytotoxicity assays, flow cytometry, electron, laser and light microscopy analysis and histological and immunohistological analysis. *In vivo* toxicity and microbial challenge models will also be employed to determine the effects of microbes and toxins on fish behavior.
• *Contingencies*- Modify techniques or employ new proteomics and immunological technologies to characterize and define CNS damage caused by microbes and toxins.

Collaborators
ARS- Bill Wolters, Stoneville, MS and Don Freeman, Stutgart, AR will be required for expertise and access. Outside ARS- Kent Seatech, Meca, CA; Hydromentia, Okeechobee, FL, Intervet, Millsboro, DE and MinAqua Fisheries, Renville, MN by existing trust and cooperative research and development agreements for expertise and access. Edythe Humphries, Delaware Fish Health Program, Dover, DE by specific agreement will be required for expertise and access. John Grizzle, John Liu and other faculty of Auburn University by specific agreement for expertise and access. Lester Khoo, David Wise, Ming Li, Ed Robinson of the National Warmwater Aquaculture Center, Stoneville, MS by specific agreement for expertise and access. Carl Webster of the Aquaculture Center, Kentucky State University, Frankfort, KY by specific agreement for expertise and access.

Conflicts of interest- See attached list which covers all scientists included on this project.