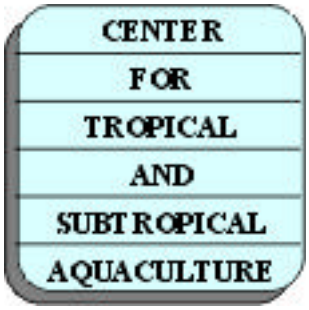

Center for Tropical and Subtropical Aquaculture

1998 Annual Accomplishment Report

(formatted for Adobe Acrobat Reader)

December 1, 1999

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Introduction

Mission

The mission of CTSA is to support aquaculture research, development, demonstration and extension education to enhance viable and profitable U.S. aquaculture.

Background

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 Center for Tropical and Subtropical Aquaculture (CTSA) is one of five regional aquaculture centers funded by the U.S. Department of Agriculture. Research projects span the American Insular Pacific, using its extensive resource base to meet the needs and concerns of the tropical aquaculture industry.

The five Regional Aquaculture Centers encourage cooperative and collaborative aquaculture research and extension education programs that have regional or national applications. Center programs complement and strengthen existing research and extension educational programs provided by the U.S. Department of Agriculture and by other public institutions. The Centers' objectives 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;
utilize the Regional Centers in a national program of cooperative and collaborative research, extension and development activities among public and private institutions having demonstrated capabilities in support of commercial aquaculture in the United States.

The Center for Tropical and Subtropical Aquaculture is jointly administered by the University of Hawaii and The Oceanic Institute. The Center offices and staff are located at The Oceanic Institute's Makapu`u Point site on windward Oahu.

Organizational Structure

CTSA funds aquaculture research, development and demonstration projects. Each year's program is the result of several groups working together for many months. A Board of Directors oversees the Center's programmatic functions, and an Executive Committee is responsible for the Center's administrative policy and functions.

In addition, CTSA has two working groups. The Industry Advisory Council (IAC) comprises members from financial institutions, aquacultural and agricultural enterprises, government agencies and other business concerns. The Technical Committee (TC) is made up of researchers, extension agents and fisheries officers.

The Board, the IAC and the TC draw their members from American Samoa, the Commonwealth of the Northern Mariana Islands, the Federated States of Micronesia, Guam, Hawaii, the Republic of Palau and the Republic of the Marshall Islands.

Administrative Center

The Center for Tropical and Subtropical Aquaculture is co-administered by the University of Hawaii and The Oceanic Institute. CTSA's Administrative Center is located at The Oceanic Institute, on the island of Oahu in Hawaii. The Administrative Center staff provides all necessary support services for the Executive Committee, the Board of Directors, the Industry Advisory Council, the Technical Committee, various project review panels and delegations and project work groups. Dr. Cheng-Sheng Lee, Center Director, supervises operation of the Center.

Executive Committee

The Executive Committee is the legal entity responsible for the Center's overall administrative policy formulation, budget and procedures. It also appoints the CTSA Director. The members of the Executive Committee are:

Dr. Gary D. Pruder, The Oceanic Institute, {Executive Committee Chairman};
Dr. Dean Smith, University of Hawaii.

Board of Directors

The Board of Directors is responsible for the development and implementation of the Center's program policy, including concurrence on total budget issues. The Board is also responsible for development of ancillary agreements with other agencies and institutions.

The members of the Board of Directors represent educational, state and non-profit private research institutions throughout the region. The Board of Directors:

establishes initial guidelines for regional aquaculture research, development and demonstration activities;
appoints and removes members of the Industry Advisory Council and the Technical Committee;
approves the proposed strategy for project selection;
approves the priority areas and goals for industry development identified by the Industry Advisory Council and Technical Committee;
approves the Annual Plan of Work, including budget allocations;
approves the Annual Accomplishment Report for consistency with the goals and objectives of CTSA and the authorizing legislation;
develops ancillary agreements with other institutions.

The members of the Board of Directors, in alphabetical order, are:

Dr. Jeff Barcinas, College of Agriculture and Life Sciences, University of Guam;
Mr. John Corbin, Hawaii State Aquaculture Development Program;
Dr. Michael Harrington, Hawaii Institute of Tropical Agriculture and Human Resources, University of Hawaii;
Dr. Charles Helsley, Sea Grant College Program, University of Hawaii;
Dr. Gary D. Pruder, The Oceanic Institute {Executive Committee Chairman};
Dr. Singeru Singeo, Land Grant Program, College of Micronesia;
Dr. Dean Smith, University of Hawaii, {Board Chairman}.

Industry Advisory Council

Members of the Industry Advisory Council include commercial aquaculture farmers, aquaculture suppliers and members of government bodies and financial institutions. Members are appointed by the Board of Directors for three-year, renewable terms. In the Industry Advisory Council's capacity as an advisory body, it provides an open information exchange forum for those involved in the aquaculture business. With the approval of the Board of Directors, contributions of the IAC can be incorporated into annual and ongoing plans for CTSA. The Industry Advisory Council:

recommends research and development needs and priorities from the perspective of the aquaculture industry;
participates as needed in the review of proposals, project progress reports, program review delegations and other functions of the Center;

recommends to the Board actions regarding new and continuing proposals, proposal modifications and terminations.

Members of the Industry Advisory Council are:

Mr. Bo Alexander, Hawaii Institute of Marine Biology, University of Hawaii;
Mr. David Barclay, Aquatic Culture and Design;
Mr. Michael Bauerlein, Guam Aquaculture Development and Training Center;
Dr. Paul Bienfang, CEA Tech USA Inc.;
Mr. Dennis Bishop, Kona Mariculture;
Ms. Rebecca Bishop-Yuen;
Ms. Mary Brooks, Pacific Aquaculture;
Mr. Steve Chaikin, Molokai Sea Farms;
Mr. Michael Crisostomo, Kurumaya Seahorse Restaurant;
Mr. Richard Croft, Pohnpei Natural Products;
Mr. Yimnang Golbuu, Palau Community College;
Mr. John Gourley, Micronesia Clam Company;
Ms. Linda Gusman, Island Aquaculture;
Mr. Steve Katase, Royal Hawaiian Sea Farms;
Mr. Robert Kern, Tropical Ponds of Hawaii;
Mr. Jeff Koch, Mokuleia Aquafarm;
Mr. Andrew Kuljis, Aquatic Farms;
Dr. Todd Lorenz, Cyanotech;
Dr. Craig MacDonald, Hawaii State Ocean Resources Development;
Mr. Richard Masse, Mangrove Tropicals
Mr. Jerry Norris, Pacific Basin Development Council;
Mr. Ramsey Reimers, Robert Reimers Enterprises;
Mr. Toshiuki Rudolph, Kukuoro Municipal Government;
Dr. Richard Spencer, Hawaiian Marine Enterprises {Industry Advisory Council Chairman and *ex officio* member of the BOD}
Mr. Ron Weidenbach, Hawaii Fish Company;
Dr. James Wyban, High Health Aquaculture;
Dr. Leonard Young, Hawaii State Aquaculture Development Program.

Technical Committee

The Technical Committee's members represent participating research institutions and state extension services, other state or territorial public agencies as appropriate, and non-profit private research institutions. The Technical Committee provides research expertise to address priorities set by the Industry Advisory Council. Members are appointed by the Board of Directors for three-year, renewable terms. The Technical Committee:

prepares Problem Statements for priority areas identified by the Industry Advisory Council;
participates as needed in project review panels, Program Review Delegations and other functions of the Center.

The members of the Technical Committee are:

Dr. Harry Ako, University of Hawaii {Technical Committee Chairman and *ex officio* member of the BOD};
Dr. Maqsudul Alam, University of Hawaii;
Ms. Kristen Anderson, Hamilton Library, University of Hawaii;
Dr. Brad Argue, The Oceanic Institute;
Mr. Richard Bailey, Sea Grant Extension Service, University of Hawaii;
Dr. James Brock, Hawaii State Aquaculture Development Program;
Dr. Christopher Brown, Hawaii Institute of Marine Biology, University of Hawaii;
Dr. John Brown, College of Agriculture and Life Sciences, University of Guam;
Mr. David Crisostomo, University of Guam Cooperative Extension Service;
Mr. Simon Ellis, Land Grant College Program, College of Micronesia;
Dr. Kevin Hopkins, University of Hawaii;
Dr. Robert D. Howerton, Sea Grant Extension Service, University of Hawaii;
Mr. Tom Iwai, Anuenue Fisheries Research Center;

Dr. Christopher Kelley, Hawaii Institute of Marine Biology;
Dr. Andrew Kuniyuki, Cooperative Research and Extension, College of the Marshall Islands;
Dr. PingSun Leung, University of Hawaii;
Dr. Shaun Moss, The Oceanic Institute;
Mr. Obichang Orak, Palau Mariculture Demonstration Center;
Dr. Anthony Ostrowski, The Oceanic Institute;
Dr. James Szyper, Hawaii Institute of Marine Biology;
Dr. Albert Tacon, The Oceanic Institute;
Mr. Howard Takata, Sea Grant Extension Service, University of Hawaii
Dr. Clyde Tamaru, Sea Grant Extension Service, University of Hawaii.

Executive Summary

Program Scope

During 1999, the Center for Tropical and Subtropical Aquaculture completed work on projects funded under its Ninth Annual Plan of Work and continued work on projects funded under its Tenth and Eleventh Annual Plans of Work. In addition, in June 1999, the Center initiated work on projects developed under its Twelfth Annual Plan of Work and began developing its Thirteenth Annual Plan of Work.

Five projects were funded under the Center's Twelfth year program, which was approved by the Center's Board of Directors on December 1, 1998. Two projects were new, and three were continuations of projects begun under the programs of previous years.

One sign of the effectiveness of the Center's program is the willingness of other agencies to provide supplemental funding for projects. Over the life of CTSA, other agencies provided $3,504,948 in additional or in-kind support to projects.

The development of the Year Thirteen program was initiated in February 1999 at the annual meeting of the Industry Advisory Council (IAC). The IAC reviewed the progress of funded projects and recommended Year Thirteen research priorities that would aid industry development. Members identified ten project areas, three of which were new or expanded areas, and five of which were continuations of projects funded under previous years. Two are continuations of projects funded under previous years with new project components added. The priority areas were:

1. Library Aquaculture Workstation -- Year Thirteen *(continuing priority)*;
2. Extension and Training Support for the U.S.-Affiliated Pacific Islands -- Year Twelve *(continuing priority with additional component);*
3. National Coordinator for Aquaculture New Animal Drug Applications -- Year Five *(continuing priority);*
4. Disease Management for Hawaiian Aquaculture -- Year Eight *(continuing priority with additional component)*;
5. Improving Hatchery and Nursery Methods for Sturgeon *(new priority)*;
6. Guam Tilapia Seed Production *(new priority)*;
7. Transitioning Hawaii's Freshwater Ornamental Aquaculture Industry *(continuing priority)*;
8. Marine Food Fish Seedstock Production *(continuing priority)*;
9. Aquaculture of Marine Ornamental Species *(new priority)*;
10. Publications *(continuing priority)*.

In February 1999, the Technical Committee (TC), acting on the IAC's recommendations, drafted problem statements for new or expanded projects. Those formed the basis for the Preliminary Plan of Work, which was approved by the Board of Directors in March 1999. The Center staff then solicited proposals for projects, and nine proposals were submitted.

In June, the Center began its four-month review process. New proposals were first subjected to external peer review by at least three experts in the project topic area. The expert peer reviewers were identified with the assistance of the directors of the other Regional Aquaculture Centers and the U.S.D.A. program administrators. Proposals for both new and continuing projects then underwent review by panels comprising members of the Industry Advisory Council and the Technical Committee. The final version of the proposals are incorporated into the Thirteenth Annual Plan of Work, which will be presented to the Center's Board of Directors for approval in December 1999. Following Board approval, the plan will be submitted to the U.S. Department of Agriculture Cooperative State Research, Education and Extension Service for final approval.

Since the inception of the Center for Tropical and Subtropical Aquaculture in 1988, it has funded 131 research, demonstration, development and extension projects. Eleven projects were active during 1999. These projects fall into six categories:

National Aquaculture Priorities;
Information Dissemination;
Extension Support to Further Industry Development;
Marketing and Economics;
Development of New Technologies;
Demonstration and Adaptation of Known Technologies.

Projects addressing national aquaculture priorities comprise:

National Coordinator for New Animal Drug Applications;
Development of Best Management Practices for Hawaiian Aquaculture.

Projects addressing information dissemination comprise:

Library Aquaculture Workstation;
Publications

Projects addressing extension support to further industry development comprise:

Aquaculture Extension and Training Support in the U.S.-Affiliated Pacific Islands;
Disease Management for Hawaiian Aquaculture;
Public Policy Impact on Aquaculture Development in Guam.

Projects addressing development of new technologies comprise:

Development of Pacific Threadfin and Milkfish Growout Technology and Production of Live Feeds and Seedstock.

Projects addressing demonstration and adaptation of known technologies comprise:

Expansion and Diversification of Freshwater Tropical Fish Culture;
Marine Ornamental Fish Culture and Preservation;
Growout Trials of the Commercially Important Opakapaka, *Pristipomoides filamentosus*

A brief listing of the principal accomplishments of the active projects in these categories during 1999 is presented below. Details on each project's funding, participants, objectives, anticipated benefits, progress and future plans are presented in individual sections on each project.

National Aquaculture Priorities

National Coordinator for Aquaculture New Animal Drug Applications

Accomplishments

As a direct result of the project, the U.S. Food and Drug Administration (FDA) announced on February 12, 1996, that it will "defer regulatory enforcement against the unapproved sales and use of an approved Human Chorionic Gonadotrophin (HCG) product as a spawning aid in fish by or on the order of a licensed veterinarian." This provides aquaculture farmers with a means of legally obtaining and using HCG until it is approved by the FDA.

In July 1998, the FDA approved Western Chemical Inc.'s supplemental New Animal Drug Application (NADA) for formalin solution to be used in the water of all finfish as an external parasiticide and all finfish eggs as a fungicide. This approval means that Western Chemical is the only manufacturer whose formalin can be labeled and sold for those uses. FDA's amendment of the animal drug regulations to reflect approval of Western Chemical's NADA became effective July 16, 1998.

In August 1999, CVM announced the NADA approval of Chorulon . On April 12, 1999, a Web site at http://ag.ansc.purdue.edu/aquanic/jsa/Aquadrugs.htm was established for the National NADA coordinator. The coordinator worked to obtain INADs, NADAs and approvals for a number of drugs that are considered high priority by the public and private aquaculture community.

On April 12, 1999, a Web site was established for the National NADA coordinator. The site, which is located at the Joint Subcommittee on Aquaculture's homepage, provides information on the goals, objectives, background, current position, international activities and progress reports of the coordinator.

Development of Best Management Practices for Hawaiian Aquaculture

Accomplishments

This project, initiated under the CTSA Eleventh Annual Plan of Work, will develop a general, standardized set of best management practices for Hawaii aquaculture. This will aid farmers in obtaining necessary permits, which has proved a major constraint to the aquaculture industry in Hawaii.

A literature review has been conducted covering best management practices for trout and channel catfish. A more thorough literature search continues to be conducted which also covers BMPs for the salmon industry in the Northwestern portion of the US.

Through the use of the Internet, a literature review has begun to detail BMPs used by the livestock and poultry industry in the US. Contact has been made with a number of both State and National agriculture industry councils and a dialogue has begun. Many state industry councils have begun to develop a proactive stance on waste management. These industry representatives have begun the development of best management practices specific to their needs.

Information Dissemination

Library Aquaculture Workstation

Accomplishments

This project, known as the Pacific Regional Aquaculture Information Service for Education or PRAISE, established remote workstations equipped with modems. From these workstations, users can connect to the information service at the University of Hawaii to perform CD-ROM database searches 24 hours a day. Six remote sites were established in Hawaii and two were established in Guam. PRAISE entered a cooperative agreement with PEACESAT, a federally funded communications satellite, whereby residents at five Pacific Island sites can directly access the Aquatic Sciences and Fisheries Abstracts (ASFA) database through an Internet connection between the local PEACESAT station and the mainland vendor. In addition, PRAISE established a home page on the WorldWide Web. Search requests can be sent to PRAISE personnel via the web page.

During 1998, the project provided PRAISE switched to Worldwide Web access of the *Aquatic Sciences and Fisheries Abstracts* database, thereby simplifying and expanding access to Hawaii users. All those with *@hawaii.edu email accounts log on to the Internet and conduct database searches via the Worldwide Web. Other users can submit search requests to PRAISE via the Worldwide Web site. In addition, PRAISE has established two remote sites, one of which is housed in the CTSA Administrative Offices at The Oceanic Institute, that can access Internet database searches.

During 1999, the PRAISE Web site, which is accessed, on average, more then 6,000 times per month, has been continually updated and enhanced. Both the Gray Literature Bibliography and the PRAISE use database are now updated regularly. A new brochure detailing services and contact information for the PRAISE services has been developed and is being distributed. This project has increased the accessibility of scientific information throughout the Pacific region.

Publications

Accomplishments

In 1999, this project produced a quarterly newsletter and continued to maintain a home page on the worldwide web. In addition, Center staff assisted with layout, editing and printing of a CTSA-funded project manual titled, *The Culture of Soft Corals for the Marine Aquarium Trade*. In addition, Center staff assisted in the publication of the extension fact sheets, *Spawning the tinfoil barb,* Barbodes schwanenfeldi *in Hawaii, Lagoon farming of giant clams (Bivalvia: Tridacnidae),* and *Farming Soft Corals for the Marine Aquarium Trade,* CTSA publication #140.

Extension Support to Further Industry Development

Aquaculture Extension and Training Support in the U.S.-Affiliated Pacific Islands

Accomplishments

At the start of this project in 1989, aquaculture was virtually non-existent in American Samoa, the Commonwealth of the Northern Mariana Islands, the Federated States of Micronesia and the Republic of the Marshall Islands. Today, the region has six giant clam hatcheries, four pearl oyster farms, six sponge farms, three coral production facilities and four tilapia farms. In addition, awareness of aquaculture and its income potential is growing rapidly.

This project provides extension and training support to aquaculturists and to government fisheries and aquaculture staff throughout the region. This support includes conducting aquaculture training courses at various locations, providing scientific advice to the FSM National Aquaculture Center and other private and public concerns, and assisting with reef surveys and reseeding programs for giant clams, sponges, pearl oysters and other species as requested by local authorities. Simon Ellis, the extension agent since January 1997, has continued the hands-on training of local individuals, which has led to substantial capacity building in the region. Native islanders operate most of the giant clam farms. As the industry grows, this knowledge will spread to other species and will achieve the ultimate goal of a self-sustaining, economically viable aquaculture industry within the region.

The project continues to produce informational publications on the culture of a variety of species. In 1998, the project produced a manual and companion video titled "Spawning and Early Larval Rearing of Giant Clams." In 1999, Ellis published *The Culture of Soft Corals for the Marine Aquarium Trade*, completed the soft coral video and published two Aquafarmer Information sheets titled: *Farming soft corals for the marine aquarium trade* and *Lagoon farming of giant clams*. A draft of a third Aquafarmer Information sheet titled *Producing pearls using the Black-lip pearl oyster* has been completed and will be published after final editing and layout work is completed.

Disease Management for Hawaiian Aquaculture

Accomplishments

This project is identifying factors that may contribute to the occurrence of bacterial disease during growout of Chinese catfish (*Clarias fuscus*) and developing strategies to control those diseases. In addition, methods of decontaminating shrimp ponds infected with the IHHN virus are being tested, groups of imported freshwater tropical fish are being surveyed to document mortality patterns, portray environmental conditions and determine the presence and prevalence of certain parasites and bacterial pathogens, and the effects of ectoparasites on cultured tilapia and mullet are being assessed.

Work in 1999 included development of a protocol for monitoring of subclinical *Amyloodinium* sp. infection of cultured moi and the use of the procedure on a commercial farm, assessment of the effect of low light conditions as an approach to control *Amyloodinium* disease in cultured moi, and investigation of the cause and control of the rickettsia-like organism (RLO) infection of cultured tilapia in Hawaii.

During year four, investigators made 172 site visits to 14 locations. During year five, investigators made 188 site visits to aquaculture sites on Oahu to assist producers address disease or production problems, or to carry-out activities related to the year five work.

Public Policy Impact on Aquaculture Development in Guam

Accomplishments

This project promoted cooperation between the Guam legislature, Guam Environmental Protection Agency (GEPA), Department of Commerce (DOC), Department of Agriculture (DOA), Bureau of Planning (BOP), Chamorro Land Trust Commission (CLTC), U.S. Army Corps of Engineers (ACOE), Department of Land Management (DLM), University of Guam, Marine Lab (UOGML) and others in the consideration and formation of sound aquaculture policy. At the Guam Aquaculture and Environmental Awareness Forum, which was held in August 1997 and videotaped, both public policies of environmental line agencies and aquaculture farmers' responses to the constraints imposed by these policies were reviewed. A total of 400 copies of the publication *Aquaculture on Guam: prospects, permits and assistance* was published and distributed under the auspices of the Agriculture Experiment Station of the University of Guam. The video "Aquaculture on Guam" was produced in-house using student help by the Agriculture Experiment Station.

Benefits were achieved through the distribution of accurate and current information affecting aquaculture development worldwide. Media coverage of the activities of the project resulted in a greater awareness and open discussion of the permitting process. Coverage included television news stories, op-ed page pieces, and letters to the editors. The project also prompted a visit by the Governor to the GADTC facility, which resulted in identification of additional local funding for the facility. During the course of the project, several new farmers began operations on-island and were given information and encouragement from the workshop.

Development of New Technologies

Development of Pacific Threadfin and Milkfish Growout Technology and Production of Live Feeds and Seedstock

Accomplishments

Results from experiments and nursery runs at commercial farms indicate that farmers can be very successful at raising threadfin, given proper guidance through extension activities. Farmers have gained confidence that survival and growth can be kept at profitable levels provided that proper facilities and techniques are employed. Feeding trials for both threadfin and milkfish have aided both in identifying the best commercial feeds and formulations for threadfin growout and in ways to minimize feed costs, which typically represent 50 percent of the production costs of any fish farming operation. Water use costs can also be lowered with the identification of threadfin loading rate requirements. Market tests have shown that threadfin is a versatile, high quality product that can be sold for $6 to $7 per pound. Markets will purchase and sell threadfin that range from 0.5 pounds to 2 pounds. The most appropriate size for farmers to grow threadfin is between 0.5 and 1 pound, which can be attained at 6 to 8 months of age at most Hawaii farms. Given estimated survival rates of fish produced under this project, the average price of $6 per pound in the round and 0.75 pound harvest weight, this CTSA Year 9 project will contribute an estimated $414,000 in net sales to Hawaii threadfin farmers. Total net sales attributed to this project during CTSA Year 8 and Year 9 is estimated at percent $508,500. The project continued to provide seedstock and technical assistance to participating farmers. This has resulted in improved on-farm survival rates averaging 98 percent.

Demonstration and Adaptation of Known Technologies

Expansion and Diversification of Freshwater Tropical Fish Culture

Accomplishments

Supporting the expansion and diversification of tropical fish culture in Hawaii is consistent with Hawaii's long-term strategy for economic diversification through fostering the development of aquafarms that can produce commercial quantities of various ornamental fish species. This project supported this strategy in a variety of ways, including successfully establishing broodstock for several popular freshwater species to distribute to Hawaii farmers; conducting a variety of feeds studies; providing extension activities in the form of technical workshops, site visitations, and verbal consultations; and producing literature regarding the culture of freshwater tropical fish.

Marine Ornamental Fish Culture and Preservation

Accomplishments

This project was designed to test the feasibility of two alternative means of providing fish for the development of a foundation for a marine ornamental fish culture industry. Both approaches involve the use, rather than the care of existing broodstock animals, one drawing upon wild stocks and the other taking advantage of animals in public display aquaria. It is anticipated that the marine ornamental fish trade will also benefit from the delivery of a species priority list and data on larval collection and growout.

Growout Trials of the Commercially Important Opakapaka, **Pristipomoides filamentosus**

Accomplishments

This project provided the first rigorous examination of the culture potential of opakapaka. It was demonstrated that opakapaka can be captured alive from deep water, can survive in captivity for at least 6 months, and can be weaned onto a pelleted diet. However, due to the poor survival of the fish during the growout experiment, the opakapaka culture does not appear to be feasible using standard techniques developed for shallow water species. Specialized techniques for deepwater species need to be developed in the future.

National Coordinator for Aquaculture New Animal Drug Applications

Dates of Work

May 1995 through September 1999

Funding Level

$35,000

Participants

Rosalie Schnick

Objectives

The overall goal of this project, which first received funding under the CTSA Eighth Annual Plan of Work, is coordination of activities for investigational new animal drug exemptions (INADs) and new animal drug applications (NADAs) to expedite approval for the use of various drugs in aquaculture. Specific objectives related to that goal are to:

serve as an information conduit between INAD and NADA applicants and the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM);
identify and encourage prospective INAD participants to become involved in specific investigational studies and NADA approval-related research;
seek the support and participation of pharmaceutical sponsors for INAD studies and NADAs and coordinate with INAD and NADA sponsors to achieve CVM approval more quickly;
guide prospective and current INAD holders on the format for INAD exemption requests and related submissions to CVM;
identify existing data and remaining data requirements for NADA approvals;
review, record, and provide information on the status of INADs and NADAs;
act as liaison and provide coordination among all the federal agencies involved in the INAD/NADA process;
provide public education related to training and guidance in obtaining INAD exemptions and pursuing NADA approval.

Principal Accomplishments

On April 12, 1999, a website at http://ag.ansc.purdue.edu/aquanic/jsa/Aquadrugs/index.htm was established for the National NADA coordinator. The site, which is located at the Joint Subcommittee on Aquaculture's homepage, was established to provide information on the goals, objectives, background, current position, international activities and progress reports of the coordinator. The coordinator worked to obtain INADs, NADAs and approvals for a number of drugs that are considered high priority by the public and private aquaculture community.

The coordinator organized, chaired and gave the keynote address at a session on worldwide cooperation toward aquaculture drug approvals at the World Aquaculture '99 conference April 26 to May 2, 1999 in Sydney, Australia.

In August 1998, comments were submitted to the Food and Drug Administration regarding the FDA Modernization Act of 1997. The FDA was urged to increase the number of aquaculture reviewers and submit to Congress the document titled "Proposals to increase the availability of approved animal drugs for minor species and minor uses."

Objective: Interest major pharmaceutical firms in developing their products, especially broad-spectrum antibacterial compounds, for aquaculture.

A major breakthrough occurred in developing a new, oral antibacterial for aquaculture. Schering-Plough Animal Health has agreed to allow the development of florfenicol as a broad spectrum antibacterial for public and private aquaculture and as the model oral drug for crop grouping research. A ballot was sent on December 31, 1997 to the stakeholders in the Federal-State Aquaculture Drug Approval Partnership program (IAFWA Project) to vote on whether to replace sarafloxacin with florfenicol as the oral antibacterial and model drug for the crop grouping research and to respond to a confidential questionnaire to determine the priority systemic bacterial diseases. The overwhelming response has been for florfenicol to replace sarafloxacin (28 votes for, none against).

New sponsors have also recently been obtained for Aqui-S™ (new drug), formalin (amended NADA), gonadotropin releasing hormone (new drug to replace LHRHa), 17 -methyltestosterone, MS-222, Ovaprim™ (new drug to replace LHRHa), and Pyceze™ (new drug).

Objective: Pursue new animal drug application sponsors for benzocaine, copper sulfate, Cutrine-Plus™, diquat dibromide, erythromycin, fumagillin, potassium permanganate, and LHRHa and work with them to obtain approvals.

Progress: Sponsors have been obtained for copper sulfate, fumagillin, and potassium permanganate. A sponsor has been obtained for Aqui-S™, a candidate anesthetic to replace benzocaine. A sponsor has stepped forward to develop Ovaprim™ and gonadotropin releasing hormone to replace LHRHa as spawning aids. No progress has been made on obtaining drug sponsors for the other drugs.

Objective: Coordinate approval activities for amoxicillin, chloramine-T, common carp pituitary, copper sulfate, EarthTec™, hydrogen peroxide, 17 -methyltestosterone, oxytetracycline and sarafloxacin.

Progress: No significant, reportable activities occurred during April 1, 1998 and March 31, 1999 for EarthTec ; however, significant, reportable activities did occur on amoxicillin, florfenicol (new drug), formalin, potassium permanganate, Pyceze (new drug), and trichlorfon.

Antibacterials

Recently the Centers for Disease Control and Prevention presented concerns about the use of all fluoroquinolones in animal health because of the potential for developing pathogen resistance to drugs used in humans. Because of this, it is doubtful that CVM will grant a new NADA on sarafloxacin or any fluoroquinolone for aquaculture uses.

Florfenicol

Schering-Plough Animal Health agreed to allow development of its florfenicol as a broad spectrum oral antibacterial for aquaculture and as the model oral drug for crop grouping research. Florfenicol is not in the class of compounds that is of concern for raising resistance in humans, and it has already been approved for aquaculture in other countries. In August 1998, representatives of Schering-Plough met with CVM regarding the development of florfenicol for aquaculture in light of CVM's new policy on disease resistance issues. CVM indicated that they would schedule a November meeting to develop a broad-based policy on this issue for all animal drugs.

The coordinator is working with the sponsor and CVM to identify existing data and outstanding requirements for approval of florfenicol in salmonids. A coordination meeting was held in La Crosse, Wisconsin on March 17-18, 1999 to discuss plans for implementation of a general U.S. Fish and Wildlife Service (FWS) investigational new animal drug (INAD) exemption for florfenical.

The coordinator met with a representative of Schering-Plough Animal Health during World Aquaculture ’99 to discuss the potential for drug approval of florfenical in Chile and worldwide.

Amoxicillin

The Coordinator obtained a sponsor for amoxicillin, an oral antibacterial. Vetrepharm Limited of Fordingbridge, United Kingdom (UK), submitted an INAD/NADA letter of intent to the Center for Veterinary Medicine on January 5, 1996. On January 16, 1996, the company was granted INAD #9659, which named AquaFuture as the U.S. representative. The NADA Coordinator met with representatives of Vetrepharm in May 1996 to discuss an action plan for the development of the INAD/NADA on amoxicillin. Two sponsors submitted INAD/NADA letters of intent.

During 1997, sponsors began considering studies on catfish, salmonids, hybrid striped bass, tilapia, walleye and yellow perch. The coordinator met with Gurvey and Berry Co. Inc., a potential amoxicillin NADA sponsor, and several industry members to discuss a strategic plan. Gurvey and Berry obtained an exemption on INAD #9853. The coordinator participated in a June 1997 conference call on a proposal to evaluate and gain approval for amoxicillin to control bacterial infections in aquaculture. The full proposal was submitted in July to the National Coastal Resources Research and Development Institute for funding but was not accepted. The coordinator met with Gurvey & Berry representatives to discuss their progress toward a NADA.

On October 19, 1998, the coordinator met with representatives of CVM and the amoxicillin sponsor GB Research Inc. to discuss the development of data that will lead to an approved NADA for amoxicillin trihydrate. The sponsor presented a plan for funding the needed research and CVM provided insight on the technical sections necessary for completion of a NADA submission.

The US representative sent out the funding plan to INAD holders through individual mailings, the USDA mailgroup and the National Coordinator in January 1999.

The coordinator requested and was able to establish a Public Master File on amoxicillin at CVM.

Chloramine-T

During H & S Chemical Co. Inc. was given information on obtaining an INAD/NADA for Chloramine-T, an external antibacterial, as a control for bacterial gill disease and flexibacteriosis. INADs for Chloramine-T were consolidated and coordinated, label claims were developed and pivotal study sites were identified. Akzo Nobel Chemicals Inc., the NADA sponsor of Chloramine-T, committed to provide the information necessary for the approval of their product in the United States and Europe. CVM concluded that para-toluene sulfonamide (p-TSA) is the major metabolite and that data necessary for calculating a tolerance for Chloramine-T in juvenile rainbow trout have been completed. A June 1996 letter to CVM's Office of Science requesting that the agency administer and monitor three required genotoxicity studies on p-TSA for the IAFWA Project has been withdrawn until studies identified by Akzo Nobel Chemicals Inc. can be acquired and evaluated. It may be possible that no funds will need to be expended on any genotoxicity studies on p-TSA.

In 1997, the coordinator sent a draft letter of intent to commit to development of Halamid, Akzo Nobel Chemicals' chloramine-T, to control or prevent external flavobacterial infections on freshwater fish. In July, Akzo Nobel Chemicals sent to CVM a letter of intent to pursue an INAD exemption that will lead to a new Animal Drug Application for chloramine-T under the company's existing INAD.

In May 1997, under the auspices of the IAFWA project, UMSC and the coordinator requested that the CVM Office of Research administer and monitor three genotoxicity studies for support of approval for chloramine-T. The request was withdrawn when Akzo Nobel Chemicals committed to fund the required studies on para-toluene sulfonamide, the major metabolite of chloramine-T.

Several active compassionate INADs are held by public aquaculture organizations. The International Association of Fish and Wildlife Agencies (IAFWA) project at the Upper Mississippi Science Center (UMSC) is developing target animal safety, efficacy, analytical methods in water and fish tissue, residue/metabolism data and the environmental assessment.

The U.S. Fish and Wildlife Service and other INAD holders are performing pivotal chloramine-T efficacy studies and reporting good results. CVM concluded that a colorimetric method, developed by the Upper Mississippi Science Center (UMSC) under the IAFWA project, is acceptable for monitoring chloramine-T in pivotal efficacy studies.

In May 1998, the Upper Mississippi Science Center (UMSC) developed a proposed regulatory analytical method for para-toluenesulfonamide in tissues of cultured fish to support residue depletion studies and submitted it to CVM for review and comment. UMSC also issued a data call-in, organized and evaluated chloramine-T INAD efficacy data to control or prevent mortalities resulting from bacterial gill disease of cultured freshwater fish species and submitted it to CVM for review and comment.

In early 1998, data were received in response to a data call-in for all efficacy data that can support a label claim to control or prevent mortalities related to external flavobacterial infections in cultured freshwater fish. In July 1998, summary reports prepared to address efficacy technical sections to both prevent and control bacterial gill disease in selected species were submitted to CVM.

On October 24, 1998, a draft proposal was sent to CVM to review and consider for approval of chloramine-T for exclusive use on early life stage fish at public aquaculture facilities. The acceptance of this course of action is critical if the compound is to be approved for public aquaculture use under the IAFWA Project. A major impediment to approval is the lack of several acceptable mammalian toxicology studies that CVM reviewers feel are required to establish a tolerance for the marker residue of chloramine-T in rainbow trout, paratoluenesulfonamide. Such studies would cost the sponsor $700,000, which couldn't be recouped in a reasonable time based on potential use by public aquaculture. If the compound were used only on fish in early life stages, no residue should remain in fish eaten by consumers. This premise is based on setting an inherent withdrawal time well before treated fish have entered a legal public fishery. After CVM completes the preliminary review, a final proposal will be submitted to CVM in November 1998, and a December 1998 meeting will be requested to discuss implementation of the proposal.

On May 15, 1998, a regulatory analytical method for para-toluenesulfonamide in fish tissue to support residue depletion studies was submitted to CVM for review.

Akzo Nobel Chemicals, Inc. informed the NADA coordinator on March 24, 1999 that the company will not fund any additional studies to support the approval for chloramine-T, including the additional genotoxicity work that is required to establish a tolerance for chloramines-T in fish tissues. The company will continue to act as a sponsor for their product in the United States and to provide any existing materials such as protocols, environmental safety data, and product chemistry information. Because of this change, UMESC has opted to spend Akzo contributed funds being held at UMESC on the required genotoxicity studies. Formerly, UMESC was recommending that these funds be used to support target animal safety studies in salmonids. Instead, the work on salmonids will be completed by Bozeman Fish Technology Center and UMESC will conduct target animal safety studies on representative cool- and warmwater species with public funds.

The coordinator developed a fact sheet on March 29, 1999 entitled "What is needed to gain an approval of chloramines-T for salmonids?" in an attempt to answer questions concerning the drug's status.

On June 8, 1999, the coordinator met with representatives of Akzo Nobel Functional Chemicals to discuss the remaining data requirements

All submissions should be completed in 2000 for control of mortalities associated with bacterial gill disease in cool- and warmwater fish and external flavobacteriosis on salmonids, and in 2002 for control of mortalities associated with external flavobacteriosis on cool- and warmwater fish.

Erythromycin

In 1996, the coordinator met with representatives of Vetrepharm Limited to discuss the possibility of the company becoming the erythromycin NADA sponsor. In follow-up efforts, the coordinator worked with Dr. Christine Moffitt of the University of Idaho at Moscow to determine how Vetrepharm can become the NADA sponsor of erythromycin.

In 1997, the University of Idaho developed all the required NADA data with funding from the Bonneville Power Administration and submitted the data to CVM. Several pharmaceutical and chemical companies in the United States and the United Kingdom are interested in becoming sponsors of the drug for the prevention and control of bacterial kidney disease in salmonids. The coordinator met with one potential sponsor and Dr. Chris Moffitt of the University of Idaho to discuss the final stages of NADA development. Interest has been expressed in developing the drug to control streptococcal infections in tilapia and hybrid striped bass.

Sponsorship needs to be resolved; all technical sections except sponsor product chemistry submitted; risk assessment needed on potential for disease resistance; near NADA approval for bacterial disease in salmonids if gain sponsor.

Oxytetracyline

In 1996, CVM indicated that INADs for the use of oxytetracyline (OTC) as a marking agent will continue but that the agency was close to a decision about extending the NADA for that purpose to all fish. The coordinator worked with UMSC staff to coordinate the IAFWA project activities regarding OTC as an antibacterial, especially concerning the development of pivotal efficacy data. The HPLC analytical method was adapted for determining OTC levels in edible tissues of several species of fish. Bridging studies between microbiological and HPLC analytical methods were planned. INADs were consolidated under the direction of the state of Texas.

In 1997, a major effort began to obtain NADA extension and expansions under the IAFWA project and compassionate INADs. The effort is expected produce amended NADAs to:

extend OTC's use as an otolith marking agent to all cultured freshwater fish;
to include OTC as an oral antibacterial in at least one salmonid below 9 C;
to extend OTC's label as an oral antibacterial to allow use of higher dosage levels in salmonids and at least one cool or warmwater fish species;
to expand OTC's label for use as an oral antibacterial to control mortalities associate with systemic flavobacterial infections;
to extend OTC's label as an oral antibacterial to include use in at least one cool or warmwater species.

OTC is currently approved for control of certain bacterial diseases in catfish, salmonids and lobsters and as a marking agent Pacific salmon. The coordinator estimates that in the near term CVM will decide whether to approve OTC's use as a marking aid on all cultured freshwater fish and as a control for bacterial diseases on shrimp. The coordinator also estimates that CVM is one year from approving certain expansions and extensions for OTC.

A call-in of efficacy data initiated in October 1997 for any and all efficacy data on OTC that can support the extension and expansion of the NADA was evaluated beginning in May 1998, and the summary report was submitted to CVM on January 25, 1999. Assessment by UMESC scientists suggests that the data from the call-in support extending the label to include treatment of *Aeromonas hydrophila* in esocids.

An analytical method to analyze for oxytetracycline in fish feeds in support of INAD pivotal efficacy testing at participating INAD hatcheries was accepted by CVM on July 29, 1998.

An oxytetracycline residue depletion study was recently completed at low temperatures at the Quilcene National Fish Hatchery. The data should allow treatment of yearling salmonids below 9^{o}C. The report was submitted to CVM in February 1999.

The coordinator submitted a request to Pfizer, Inc. to petition CVM to recalculate the current withdrawal time for all fish treated with OTC based on a recent UMESC submission to CVM documenting the loss of OTC residues in fry-fingerling coho salmon below 9^{o}C and the new OTC tolerance in tissues of 2 μg/gm.

Oxytetracycline is currently approved for control of certain bacterial diseases in catfish, salmonids, and lobsters and as a marking agent in Pacific salmon; IAFWA Project drug; near-term approval as marking aid on all cultured freshwater fish and as a control for bacterial diseases on shrimp.

All submissions should be completed in 1999 for control of *Aeromonas* sp. in esocids, in 2000 for systemic flavobacteriosis in salmonids, and in 2001 for control of mortalities associated with systemic columnaris disease in cool- and warmwater fish.

Sarafloxacin

In 1996, the coordinator assisted the efforts of the National Research Support Program Number 7 (NRSP-7) to complete the approval process for sarafloxacin to control enteric septicemia in channel catfish.

In 1997, Abbott Laboratories, which holds the NADA, began preparing the last portion of a technical section to complete the data requirements for NADA approval. The coordinator met with company representatives to discuss completion of the data requirements and a strategy for risk assessment. The catfish industry, researchers and the U.S. Department of Agriculture agreed to consider developing a risk assessment on the use of sarafloxacin in catfish to control enteric septicemia to alleviate concerns of disease resistance developing in humans from the use of this fluoroquinolone in catfish.

Previously, most of the NADA technical sections were submitted by Abbott Laboratories and accepted by CVM for control of enteric septicemia in catfish. Recently, the Centers for Disease Control and Prevention have presented concerns about the use of all fluoroquinolones in animal health because of the potential for developing pathogen resistance to drugs in humans. It is doubtful that a new NADA on sarafloxacin or any fluoroquinolone will be allowed for aquaculture uses by CVM. Sarafloxacin was replaced by florfenical as the oral antibacterial and model drug for crop grouping research in January 1998 by an unanimous vote of the IAFWA Project stakeholders.

Microbicides

Copper sulfate

In 1996, copper sulfate INADs were consolidated under the direction of the state of Nebraska. In July 1996, CVM determined that it has no human food or environmental safety concerns over the use of copper sulfate as a microbicide, thus making approval relatively easy. The coordinator met with a potential representatives of Phelps Dodge Refining Corporation to discuss the company's interest in sponsoring an INAD/NADA on copper sulfate, data requirements for approval, and coordination activities. The company applied for an INAD/NADA on copper sulfate.

In 1997, the coordinator reviewed a draft efficacy technical section written by the Stuttgart National Aquaculture Research Center under the IAFWA project. CVM established Public Master file #5590 for those submissions on copper sulfate. The file includes reports from studies on copper sulfate residues in channel catfish, an environmental assessment on the use of copper sulfate in aquaculture, and efficacy of copper sulfate to control or prevent mortalities or the diseases associated with external aquatic parasites, bacteria and fungi of culture fresh and brackish water fish.

The Coordinator reviewed Phelps Dodge Refining Corporation's draft product chemistry technical section, which was submitted to CVM in March 1998. The sponsor received comments and responded with clarifications in July 1998. All technical sections need for copper sulfate approval have been submitted to CVM.

Efficacy and target animal safety data and the environmental assessment have been submitted to CVM by the Stuttgart National Aquaculture Research Center. All the data needed for an approval of copper sulfate have been submitted as of early 1998 and all the technical section packages are under review by CVM.

All submissions should be completed in 1999 for *Ichthyopthirius* on all fish. The claims for control or prevention of other external microbes on all fish would be based on efficacy studies that would be completed in 2001 if stakeholders are interested.

Diquat Dibromide

In July 1996 the coordinator sent a letter suggesting a potential licensee for Diquat to Zeneca Professional Products after Zeneca decided not to pursue an INAD/NADA package for Diquat because Zeneca is not in the aquaculture business. The state of Illinois generated efficacy data under INAD #8110.

The current status is that there is no commitment by potential sponsors and so is in the early development stage.

Formalin

In July 1996, CVM stated that formalin could be used safely on all fish eggs to control and prevent fungal infections if a statement is added to the label concerning the need for a preliminary bioassay on a sub-sample before the entire group is treated.

In 1997, CVM accepted data to support a NADA or supplemental NADA for formal to control certain fungi on all finfish eggs and certain external protozoa and monogenetic trematodes on all finfish. At least one NADA sponsor submitted a supplemental NADA for those purposes.

In 1997, CVM called for all formalin INAD holders to submit efficacy data that has been generated to extend the formalin NADA for the control or prevention of mortalities associated with saprolegniasis on all cultured freshwater fish. The coordinator estimates that within a year the NADA will be expanded for that purpose.

Effective July 16, 1998, CVM approved Western Chemical Inc.'s supplemental new animal drug application (NADA) for formalin solution to be used in the water of all finfish as an external parasiticide and all finfish eggs as a fungicide. The approval means that Western Chemical is the only manufacturer whose formalin can be labeled and sold for those uses. The amendment of the animal drug regulations to reflect approval of Western Chemical's NADA became effective June 18, 1998.

At the Work Planning/Coordination Meeting of the IAFWA Project held November 19-20, 1998 in La Crosse, Wisconsin, CVM reviewers indicated that a broad label of all fish to treat external fungal infections was not possible because of the lack of information for cool- and warmwater fish. IAFWA members attending the meeting voted to have UMESC proceed immediately to perform pivotal efficacy studies on cool- and warmwater fish to support an amended NADA and to delay pivotal efficacy studies at UMESC on potassium permanganate and AQUI-S™.

A study protocol was developed at UMESC to determine the efficacy of formalin for treating saprolegniasis in channel catfish and laboratory testing began in early 1999.

All submissions should be completed in 1999 for control or prevention of mortalities associated with fungal infections on salmonids and in 2000 for control or prevention of mortalities associated with fungal infections on all fish.

Fumagillin

The coordinator contacted several potential researchers about studies to determine the potential of Fumagillin to control or prevent hamburger gill disease in catfish and whirling and proliferative kidney diseases in salmonids. The compound has the potential to test the "early life stage concept" because it would be used in starter feed of very young fish. The coordinator met and corresponded with a potential NADA sponsor of Fumagillin, Sanofi Sante Nutrition Animale, about the development of an INAD/NADA in the United States.

In June 1997, Sanofi Sante Nutrition Animale submitted to CVM a letter of intent to pursue U.S. approval of fumagillin. The company is working with the coordinator to obtain a U.S. representative.

Several efforts are being made to collect efficacy data in public and private sector.

Hydrogen peroxide

The coordinator worked with Eka Nobel Inc. to submit an INAD/NADA letter of intent for hydrogen peroxide as a fungicide. In January 1996, CVM granted INAD #9671 to the company. In June, the coordinator met with Eka Nobel Inc. to discuss the procedures for the INAD/NADA for hydrogen peroxide as a fungicide and the potential for its use to control and prevent external bacteria and parasites. A Canadian environmental and safety package on hydrogen peroxide will be submitted to a veterinary master file at CVM. The coordinator reviewed a UMSC petition that CVM increase the low regulatory priority ruling on hydrogen peroxide to maximum levels of 1,000 ppm when used to control fungus on eggs.

In 1997, the IAFWA project undertook a major effort to gain approval for hydrogen peroxide. UMSC has developed efficacy and target animal safety data on the use of hydrogen peroxide to control or prevent mortalities associated with saprolegniasis on fish eggs and is developing pivotal efficacy and target animal safety data for its use to control or prevent mortalities associated with saprolegniasis on freshwater fish. UMSC is also assessing hydrogen peroxide's use to control or prevent mortalities associated with external flavobacterial infections and its use to control or prevent external parasitic infestations on cultured freshwater fish.

In April 1998, the efficacy and target animal safety technical sections for a NADA were submitted to CVM for the use of hydrogen peroxide to control or prevent mortalities associated with saprolegniasis on eggs of all fish. In June 1998, the coordinator met with CVM to discuss remaining hydrogen peroxide data requirements and an approach to obtaining them. They discussed the mechanisms for transferring the Canadian dossier to CVM and setting up a Public Master File. Enough data may exist for an early approval in the United States. CVM determined that the human food safety data requirements have been met.

UMSC submitted efficacy and target animal safety data to CVM September 18, 1998 for review and comment to support a NADA for the use of hydrogen peroxide to control and/or prevent mortalities associated with saprolegniasis on all cultured freshwater fish.

UMSC is in the process of completing experiments in collaboration with state hatcheries to determine the efficacy of hydrogen peroxide to control external parasitic infestations and to control mortalities associated with external flavobacterial infections on representative cultured freshwater fish.

FWS is modifying the protocol for hydrogen peroxide and plans to initiate an INAD in early 1999 through the National Research Support Program Number Seven (NRSP-7) for its use to control or prevent mortalities associated with external bacterial infections and control external parasitic infestations.

The sponsor is negotiating with a company to do the marketing of hydrogen peroxide for fisheries use in Canada.

All submissions should be completed in 1999 for control or prevention of mortalities associated with fungal infections on all fish eggs in the US if data from Canada is acceptable, in 2000 for control or prevention of mortalities associated with fungal infections on all fish, and in 2001 for external flavobacteriosis and bacterial gill disease on all fish and to control parasitic infestations on all fish.

Pyceze

In April 1998, the coordinator met with catfish and salmonid interests and with CVM regarding the potential development of Pyceze , a general external microbicide. In September 1998, the sponsor of the compound submitted to CVM a data package containing product chemistry, mammalian toxicology, human food safety, environmental assessment, efficacy, and target animal safety for use of the compound to control pr prevent saprolegniasis on fish eggs.

A summary data package containing product chemistry, mammalian toxicology, human food safety, environmental assessment, efficacy, and target animal safety was submitted to CVM on September 3, 1998 by the sponsor of Pyceze for its use to control and prevent saprolegniasis on fish eggs.

Meetings were held in April 1998 with salmonid and catfish interests and then with CVM regarding the potential development of Pyceze , a general external microbicide, in the United States.

Trichlorfon

In 1997, a potential sponsor, Bayer AG of Germany, expressed interest in pursuing an INAD/NADA for this drug. The coordinator sent to Bayer AG a draft letter of intent as an example of the letter Bayer can send to CVM.

In 1998, Special Local Need pesticide permits were granted by several states for use of trichlorfon on non-food fish to control predaceous insects and zooplankton.

Sea Lice Control

In September 1996, the coordinator attended a joint Canada-United States workshop on the jurisdiction of drugs and pesticides used to control and prevent sea lice on salmon. The coordinator consulted with John Pitts of Bellwether Consulting and Rob Armstrong of Sahnon Health Consortium in Canada on which chemicals to pursue as a control for sea lice on salmon. INADs for Cutrine-Plus, a parasiticide, fungicide and control for columnaris in cool and warm water fishes, were consolidated under the direction of the state of Iowa. The coordinator communicated with Applied Biochemists about its interest in sponsoring Cutrine-Plus.

In May 1997, the coordinator interacted with Cutrine-Plus INAD holders and sent a draft letter of intent to potential sponsor Applied Biochemists for its consideration.

Various drugs and pesticides (azamethiphos or Salmosan , cypermethrin or Excis) are being pursued by the US and Canada and are at various stages of registration and approval.

Pet Fish Therapeutants

In 1996, the coordinator met with the American Pet Products Manufacturers Association to develop strategies and discuss progress toward approval of drugs of interest to the pet fish industry.

There is a major effort to resolve non-food fish issues for these drugs by private consultant.

Anesthetics

Aqui-S

Aqui-S, which is approved in New Zealand, has a zero withdrawal time and offers a potential alternative to benzocaine. Because of the potential for gaining approval of an anesthetic with a zero withdrawal time in the United States, the coordinator and UMSC staff decided in 1996 to evaluate the efficacy and overall performance of Aqui-S before committing additional funds under the IAFWA Project to gain approval of benzocaine. In June 1996, the U.S. representative of Aqui-S New Zealand Ltd. met the coordinator and UMSC staff to discuss the potential for development of Aqui-S in the United States under the IAFWA Project.

In June 1997, the coordinator provided the U.S. representative of Aqui-S New Zealand with information on contracting residue chemistry studies.

UMSC, under the IAFWA project, is completing an efficacy and safety evaluation of Aqui-S in two size ranges of six representative freshwater fish species. The results will be submitted to all federal and state partnership stakeholders and cooperators of the IAFWA project for assessment. In July 1997, the coordinator surveyed all federal and state partnership stakeholders and cooperators of the IAFWA project about whether Aqui-S or benzocaine should be the candidate anesthetic for public aquaculture.

Aqui-S contains an ingredient that is similar to a major component in clove oil that also has anesthetic qualities in fish. CVM has no intention of granting low regulatory priority to clove oil, although it is designated as “generally

recognized as safe" as a food additive. Approval of clove oil would require efficacy and target animal safety data; however, it does not have a sponsor.

On November 13, 1998, the sponsor submitted an environmental assessment of Aqui-S to the CVM and has recent information from a National Toxicology Program study at the University of Arizona that the major ingredient in Aqui-S is not a carcinogen. They are close to submitting the product chemistry technical section for the drug and will provide funding for a residue chemistry study in Aqui-S at UMESC.

The FWS is initiating an INAD on Aqui-S to cover all public and private facilities in early 1999. A study protocol was developed to determine the target animal safety of Aqui-S treatments to various cultured freshwater fishes and laboratory testing has begun at UMESC.

A cooperative research and development agreement is being developed between UMESC and the sponsor to cover cooperative efforts on Aqui-S as a short withdrawal time anesthetic.

The sponsor is proceeding with worldwide drug approval and the drug is being assessed by the IAFWA Project. All submissions should be completed by 2000 for zero or low withdrawal time anesthetic for Atlantic Salmon and in 2002 for all fish.

Spawning and Gender Manipulation Aids

Common Carp Pituitary

In May 1996, as a follow-up to the April 1996 Common Carp Pituitary (CCP) meeting at CVM headquarters, CVM coordinated a conference call that covered (1) identification of researchers and design of target animal safety studies; (2) writing of the environmental assessment through the NRSP-7; and (3) potential funding sources for the target animal safety studies. A target animal safety study protocol on CCP using channel catfish written by Auburn University was reviewed. Efforts were made to find funding for the target animal safety studies needed to obtain approval of CCP.

In 1997, the sponsor and interested parties are proceeding toward obtaining NADA approval for CCP, which the coordinator estimates is at least one year away. The coordinator urged the sponsor Stoller Fisheries to submit the data package on the CCP product chemistry. The National Aquaculture Association provided funding for target animal safety studies at Auburn University. The coordinator determined the status of National Aquaculture Association funding for CCP target animal safety studies at Auburn University, the status of the studies and the status of the efficacy and target animal safety technical sections.

In addition, the U.S. Fish and Wildlife Service is completing efficacy, target animal safety and environmental assessment portions from the literature and compassionate INAD data for a NADA submission.

In August 1998, the literature review was presented at an FWS-INAD Coordination Workshop. Plans call for the document to be prepared for submission to CVM.

A researcher was identified from Mississippi State University who will complete the target animal safety studies for CCP.

Human Chorionic Gonadotropin (HCG)

In February 1996, CVM announced it will defer regulatory action on HCG as a spawning aid by or on the order of a veterinarian and strongly encouraged use of Intervet's product, Chorulon . Intervet submitted a complete NADA package for final review by CVM. In 1997, the coordinator contacted CVM to determine the status of HCG in the review process. In August 1999, CVM announced the NADA approval of Chorulon .

17 -methyltestosterone

In 1996, the coordinator helped the INAD holder for the use of 17 -methyltestosterone (MT) on yellow perch in implementing that portion of the MT INAD under the authorization of Auburn University. The coordinator reviewed a protocol written by Southern Illinois University for a target animal safety study on MT using walleye as a surrogate percid. The study has begun and is funded by the North Central Regional Aquaculture Center (NCRAC). The coordinator reviewed a proposal by Auburn University to write an environmental assessment of MT for a NADA submission to CVM. This project will be funded by the NCRAC.

In 1997, the INAD sponsors are actively pursuing a NADA approval, which is at least one year away, according to the coordinator's estimates. In June 1998, CVM responded to the environmental assessment submitted in November 1997. A response to CVM was submitted in October 1998.

The sponsor (Rangen, Inc.) is working on the chemistry section of the NADA.

Work Planned

During the fourth year of this project, the Coordinator will continue facilitating activities for investigational new animal drug exemptions and new animal drug applications to expedite approval of the use of various compounds in aquaculture.

Impacts

Establishment of the National NADA Coordinator position in May 1995 has resulted in coordination, consolidation, and increased involvement in the INAD/NADA process on 18 of the 19 high priority aquaculture drugs and activities on thirteen new drugs of interest to aquaculture. Twenty INAD/NADA sponsors have initiated INADs or confirmed their commitment to gaining approvals of their products for the aquaculture industry. Progress has been made toward unified efforts on existing and new INADs/NADAs for a variety of priority drugs. Data packages have recently been submitted to CVM for the following drugs: chloramine-T, copper sulfate, formalin (extension), hydrogen peroxide, 17 -methyltestosterone, oxytetracycline, potassium permanganate, and Pyceze™.

This enhanced coordination will help gain extensions and expansions of approved NADAs and gain approvals for new NADAs. Data on formalin were accepted by CVM and an amended NADA was granted to Western Chemical Inc., one of the current NADA sponsors of formalin. In addition, Western Chemical Inc. obtained a new NADA on MS-222, an anesthetic. An approved NADA is anticipated soon for human chorionic gonadotropin, a spawning aid.

The approval of the candidate drugs will aid the aquaculture industry to reduce mortalities associated with infectious and handling diseases and to increase their efficiency by using spawning aids and gender manipulation aids. The domestic aquaculture industry will be better able to compete with foreign producers because there will be more legal drugs to use.

Support

A number of public and private entities contribute funding for this project. In addition to the Center for Tropical and Subtropical Aquaculture (CTSA), they include Abbott Laboratories, American Pet Products Manufacturers Association (APPMA), American Veterinary Medical Association (AVMA), Catfish Farmers of American (CFA), the Center for Veterinary Medicine (CVM), Florida Tropical Fish Farms Association (FTFFA), IAFWA Project (IAWFA), Northeastern Regional Aquaculture Center (NRAC), North Central Regional Aquaculture Center (NCRAC), Western Regional Aquaculture Center (WRAC), Simaron Freshwater Fish Inc. (SFFI), Hybrid Striped Bass Producers Association (HSBPA), and the National Aquaculture Council (NAC), Fish Health section of the American Fisheries Society, Natchez Animal Supply, American Tilapia Association, U.S. Dept. of Interior NBS, Gurvey and Berry Inc., Sanofi Sante Nutrition Animale.

Year	CTSA	Industry	Other Federal	Total Other	Total Support
One	$5,000.00	$23,750.00	$80,000.00	$103,750.00	*$108,750.00*
Two	$10,000.00	$30,000.00	$71,920.00	$101,920.00	*$111,920.00*
Three	$10,000.00	$43,500.00	$76,631.00	$120,131.00	*$130,131.00*
Four	$10,000.00	*	*	$122,600.00	*$132,600.00*
Total	**$35,000.00**	**$97,250.00**	**$228,551.00**	**$325,801.00**	***$350,801.00***

* Detailed numbers for other support unavailable.

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Library Aquaculture Workstation
Pacific Regional Aquaculture Information Service for Education

Dates of Work
March 1988 through September 1999

Funding Level
$244,600

Participants
David E. Coleman (project coordinator through June 1997), Randall Buettner, Kristen Anderson (project coordinator as of July 1997), Rachel Hu, Jue Wang,Catherine Stewart Edington, Alex Stroup and Lois Kiehl-Cain, Hamilton Library, University of Hawaii;
Bin Zhang, Kapiolani Community College.

Objectives
The overall goal of this project, which was initiated under the CTSA First Annual Plan of Work and is now in its twelfth year, is to make scientific information more accessible to the aquaculture community. Specific year twelve objectives related to that goal are to:

continue to provide established services;
develop programs for user education;
canvas aquaculturists to determine other ways in which the PRAISE Web page can be used to promote Pacific aquaculture;
transfer the technology to users.

Principal Accomplishments
In 1988-1989, the Center for Tropical and Subtropical Aquaculture provided funding to establish an aquaculture workstation operated and managed by the staff of Hamilton Library, University of Hawaii. That program is known as the Pacific Regional Aquaculture Information Service for Education, or PRAISE. The workstation is a computer equipped with a multi-disk CD-ROM player, fax and modem. The service subscribes to a number of CD-ROM databases, including Aquatic Sciences and Fisheries Abstracts (ASFA), CINAHL Nursing Index, AGRICOLA, and Biological Abstracts. These databases list articles on thousands of aquaculture topics from hundreds of scientific journals.

During the first two years of the project, those interested in conducting a search could either travel to Hamilton Library at the University of Hawaii Manoa campus or call David Coleman, who would then conduct the search and fax the results to the PRAISE patron. The patron then selected the desired articles, which Coleman photocopied and faxed or mailed to the patron. Initially, a limit of 10 articles was set, but that proved too restrictive and was eliminated. An average of 15 articles are sent to patrons who can't otherwise obtain them.

During the third and fourth years of the project, investigators compiled and published *A Union List of Aquaculture Journals in Hawaii*. The catalog listed science journals held at seven key libraries that have a large collection of aquaculture literature. The catalog assisted the aquaculture community with locating journal literature. In addition, PRAISE exchanged journal holdings data with the Scripps Institute of Oceanography, the California Academy of Sciences and the Pacific Island Marine Resources Information Service of the University of the South Pacific.

During the fifth year of the project, remote workstations were established at the CTSA office, the Hawaii Institute of Marine Biology, the Aquaculture Development Program office, the Sea Grant office, the Pacific Island Network office and the University of Hawaii at Hilo. From these remote workstations equipped with modems, users can dial into PRAISE to perform database searches 24 hours a day, 365 days a year. The remote sites increased the efficiency of the service, which was demonstrated by the vastly increased numbers of searches that have been performed since their establishment.

During the sixth year of the project, additional remote workstations were established at The Oceanic Institute's site at Keahuolu on the island of Hawaii, at Anuenue Fisheries Research Center, and at the Hawaii Institute of Marine Biology computer lab. A breakthrough in the Pacific Islands' ability to access scientific information came in August 1993, when two remote workstations were established on Guam. Users at the site at the offices of the University of Guam's Cooperative Extension Service and at the Guam Department of Commerce gained access to PRAISE through a toll-free telephone line. The investigator conducted training sessions at both locations.

The establishment of two remote workstations, from which users dial into PRAISE via a toll-free telephone number, marked the first time a toll-free line was established from the Pacific Islands for CD-ROM data transmission. The vendor, MCI, experienced a number of problems before instituting reliable service. The cost of establishing the line and monthly charges totaled $2,000. A total of 54 calls--an average of 3.9 calls per month--were made. The average call lasted 20 minutes and cost $1.85 per minute or $37 per call. Providing ready access to aquaculture information has proven to be useful to the aquaculturists of Guam. However, the service was quite costly, and the system continued to experience problems.

During the seventh year of the project, PRAISE submitted data to the U.S. Department of Agriculture Science and Evaluation Study Working Committee on Aquaculture. Results of the study showed that aquaculturists were particularly interested in sources of aquaculture information from various government agencies and educational facilities. Based on this information, the Joint Subcommittee on Aquaculture approved publication of the Resource Guide to Aquaculture Information. PRAISE participated in the creation of this publication.

PRAISE entered a cooperative agreement with the Pacific Education and Communications Experiment by Satellite, or PEACESAT, to improve information access for five Pacific Island sites. Under the agreement, residents of Guam, Saipan, Pohnpei, Palau and Majuro can directly access the Aquatic Sciences and Fisheries Abstracts (ASFA) database through an Internet connection between the local PEACESAT station and the mainland vendor. This system cost $1,000 per year per locale.

A wealth of reports containing valuable, unique information are produced throughout the Pacific but never integrated into journals and conference proceedings. The inaccessibility of gray literature is a particularly serious problem in the Pacific, where libraries and other organizations that collect and disseminate information are few. Also, important work done in the region is not shared with the rest of the scientific community, which means regional work does not get the recognition it deserves. The Pacific Islands Gray Literature project was established to address this impediment to information. To date, nearly 170 Pacific Islands publications have been gathered for inclusion in the Aquatic Sciences and Fisheries Abstract (ASFA) database. In addition, *Pacific Islands Gray Literature Project: A Bibliography* was published.

PRAISE hosted the 20th Annual Conference of the International Association of Marine Science Libraries and Information Centers in Waikiki from October 9-13, 1994. Participants from more than 12 countries, including Iceland, Malaysia and Russia, attended.

Objective: Increase and ensure the continued usefulness of the PRAISE program through the use of CD-ROM database searching, telecommunications and new technologies as they develop, and disseminate information products as needed by the industry.

Use of the workstations at both Hamilton Library and remote sites continued to increase. Since establishing the electronic network, the total number of system uses increased from about 400 per year to more than 8,000 per year. The 2,000 percent rise in use of the service was accomplished with no increase in staff.

The primary focus of the Year Eight project was preparing PRAISE for integration into the Worldwide Web. To do this, the existing CD-ROM system was upgraded to effectively handle the tremendous increase in the use of the service. Hamilton Library adapted eight in-house workstations to Pentium computers. This was done in anticipation of allowing these machines to access both the Kapiolani Community College CD-ROM Local Area Network (LAN), which has better capability than the Hamilton Library LAN and the Internet. This provided access to the PRAISE WorldWide Web page as well as the aquaculture database. Test results of this system allowed progress toward other objectives. Tests were conducted at the CTSA office and Hamilton Library to determine whether it was most efficient to allow access to the aquaculture CD-ROM database either via direct telnet to Kapiolani Community College Library or via the PRAISE home page on the WorldWide Web.

In conjunction with the Joint Subcommittee on Aquaculture and the Aquaculture National Information Center, the project developed programming to allow Internet access to PRAISE. The project instituted methods to allow the Pacific Islands to have cost-effective access to PRAISE, including making PRAISE Internet-compatible. A PRAISE home page on the WorldWide Web was established and is available to Guam, Saipan and other Pacific Island sites with Internet connectivity.

In addition, the PRAISE program was positioned to take advantage of the upcoming national integration of aquaculture information, specifically by interacting with the U.S.D.A. Aquaculture Extension Service.

A new program was installed to record usage of the PRAISE Web page. In the 11-month period from February 1996 to January 1997, the Web site was accessed 61,868 times. In addition, the vendors page was accessed 454 times. In addition, the project responded to 135 requests for literature from the Pacific Islands and 95 requests for information from areas as diverse as the U.S. mainland, South America, Asia and the Middle East.

During Years 10 and 11, service for PRAISE users has been greatly enhanced by advances in electronic technology. Three major changes have taken place:

1. PRAISE switched to Worldwide Web access of the *Aquatic Sciences and Fisheries Abstracts* database, thereby simplifying and expanding access to Hawaii users. All those with *@hawaii.edu email accounts log on to the Internet and conduct database searches via the Worldwide Web. In addition, PRAISE has established two remote sites, one of which is housed in the CTSA Administrative Offices at The Oceanic Institute, that can access Internet database searches.
2. An increasing number of people are accessing the PRAISE Web site and submitting database search requests via the online forms found at the site.
3. The Pacific Islands within the CTSA region have greatly advanced in their ability to electronically access the Internet.

During Year 10, the PRAISE Web site was accessed more than 5,700 times per month, and an average of 28 research or journal search requests were submitted monthly. The Pacific vendors page was accessed 1,200 times.

During the six month period from April 1 through September 30, 1998, PRAISE users logged onto the ASFA database 2,520 times and submitted 11,300 queries. During the first half of Year 11 (April 1 through September 30, 1998), the PRAISE Web site was accessed an average of 5,550 times month. An average of 25 research or journal requests were submitted via the PRAISE web site during this period. PRAISE delivered 255 documents to users during this period. The turn-around time on research and article requests has been reduced from 4 weeks to 1 week or less due to the increased availability of stable electronic mail on many Pacific Islands.

In the subsequent 12 month period from October 1, 1998 through September 31, 1999, PRAISE users logged onto the ASFA database 5,300 times and submitted 14,259 queries. During this period, the PRAISE Web site was accessed an average of 12,788 times month. An average of 24 research or journal requests were submitted per month via the PRAISE web site during this period. PRAISE delivered 658 documents to users during this period.

During Year 11 and 12, the PRAISE Web site has been continually updated and enhanced. Both the Gray Literature Bibliography and the PRAISE use database are now updated regularly. A new brochure detailing services and contact information for the PRAISE services has been developed and is being distributed. It had been hoped that

PRAISE would become a center for videoconferencing as a method to increase communication and expand educational opportunities, however, extensive research revealed that the cost for reliable equipment and technical knowledge to maintain it was beyond the scope of this program. In addition, PEACESAT is currently developing videoconferencing for the Pacific Islands.

Objective: Increase the efficiency of PRAISE through interaction with other information agencies.

The Joint Subcommittee on Aquaculture (JSA) decided that legislative materials on the development and support of aquaculture should be included on AquaNIC, an Internet gateway to the world's electronic resources in aquaculture that is supported by the U.S. Department of Agriculture. David Coleman, a member of the JSA's Aquaculture Information and Technology Transfer Task Force, is attempting to gather relevant legislative materials from Hawaii and the Pacific Islands for AquaNIC. He met with AquaNIC coordinators in October 1995 to establish a procedure for downloading the information. Coleman gave a presentation on the cooperative project between PRAISE and PEACESAT and participated in discussions of Internet uses and procedures at the 1995 CYAMUS Marine Librarians Meeting.

In 1997, a Pacific Aquaculture Legislation section was established on the PRAISE home page on the WorldWide Web. Legislation information has been provided by the Hawaii Legislative Reference Bureau. Information from other Pacific islands is being sought.

During Year 11, Kristen Anderson, PRAISE principal investigator, presented a poster session at the annual conference of the International Association of Marine Science Libraries and Information Centers (IAMSLIC). The poster explained the benefits of an established information dissemination service for a geographically diverse region. In addition, she participated in discussions about broadening *Ariel* document delivery services, which could be an important improvement to the PRAISE service.

Objective: Increase the support base for the project through cooperative agreements with other agencies and information facilities.

The cost to establish the remote workstations was shared by all the hosting institutions. The work on the gray literature bibliography received additional funding from the Pacific Island Network, USDA's National Agricultural Library and the vendor for the ASFA database, into which the bibliography materials will be incorporated. The National Agricultural Library also provided co-funding to publish the bibliography. Additional base project funding was secured from the University of Hawaii, the Pacific Island Network and the University of Hawaii Sea Grant Extension Service.

The Pacific Aquaculture Association purchased a photocopy machine for PRAISE use. The machine has significantly sped the process of providing documents for document delivery. It will also be used to provide clean copy for scanning of Pacific Islands documents that will then be indexed in the Aquatic Sciences and Fisheries Abstracts database.

PRAISE continued to gather and provide access to Pacific Islands-specific literature for the bibliography of Pacific Islands gray literature known as the PRAISE "gray literature project." The bibliography was published in November 1995 and is available through PRAISE. An electronic version was made available on the PRAISE Web site. The National Agricultural Library, NOAA, the Pacific Aquaculture Association, and Cambridge Scientific Abstracts, the commercial vendor of the Aquatic Sciences and Fisheries Abstracts database.

In the ninth year of the project, the PRAISE Web site was expanded to allow users to download a version of the Hawaii Aquaculture Module Expert System, a software program that helps farmers to diagnose and treat disease problems in cultured tilapia. The program was developed for DOS, Windows and Macintosh computers under another CTSA-funded project. In addition, PRAISE began a "Pacific Islands Aquaculture Vendors" section of its Web site in cooperation with UH Sea Grant Extension Service. The page allows aquaculture producers a free place to advertise their products and services.

Objective: Transfer the technology to users and develop programs for user education.

User education workshops were held in Palau, Pohnpei, and the Marshall Islands. The workshops were held to train librarians, extension agents, government officials and aquaculturists in use of the PRAISE system. The workshops were conducted with the cooperation of the Sea Grant College Program and the Pacific Islands Library Association.

During Year 10, Kristen Anderson, PRAISE principal investigator, visited Palau, Saipan and Pohnpei in July 1997 to promote the PRAISE program and educate residents on the services available. In each locale, she met with Sea Grant extension agents, Fish and Wildlife personnel, college faculty, librarians and students, aquafarmers and those interested in aquaculture

During Year 10, PRAISE joined Ariel, a nationwide network of research libraries that provide electronic access to journal articles, and was listed in the IAMSLIC directory.

During Year 10 and the first part of Year 11, the principal investigator consulted at length with Dr. Leonard Young, aquaculture specialist with the Hawaii State Aquaculture Development Program, regarding ongoing user education programs. Dr. Young advocated an increase in available research education for local Hawaii aquaculture workers and producers. As a result of this, the PRAISE coordinator has taken a course in writing Web tutorials, which will provide ongoing educational opportunities, plans to incorporate increased interisland education opportunities in the Year 12 program and given individual and small group instruction to 37 individuals at UH-Manoa on how to access ASFA and Web resources for aquaculture.

Objective: Canvas aquaculturists to determine other ways in which the PRAISE Web page can be used to promote Pacific aquaculture.

During Year 10, an online electronic form was created so that Pacific vendors could submit their company and product information directly via the web site.

During Year 11, a brief questionnaire was prepared for distribution to PRAISE users via email, postal mail and the Web site. In addition, the investigator has analyzed the queries received over the past year in order to determine what additions might be beneficial. One addition currently being prepared will provide resources for those interested in aquaculture education opportunities.

The major suggestions from the questionnaire were for an aquaculture education page, a marketing page, and more concise organization. In response to these suggestions:

> educational resources are being gathered to develop an education page that will be available later in 1999;
> a marketing page has been established with the assistance of the business librarian at Hamilton Library;
> several of the longer pages are being re-categorized to make them more accessible.

Work Planned

PRAISE will continue to provide established services of database searching and document delivery. The Web site and the Gray Literature Bibliography will be continually updated and improved. Methods to improve database access for Pacific Island residents will continue to be explored. Educational workshops for farmers, students, and researchers will be held on the island of Oahu and distribution of the information brochure will continue.

Impacts

This project has increased the accessibility of scientific information throughout the Pacific region. Based on rates charged by the information industry's major commercial suppliers, (Dialog Information Service Inc. for access to Aquatic Sciences and Fisheries Abstracts, and UnCover Inc. for document delivery charges), the dollar value for PRAISE's primary services for the period April 1 through September 30, 1999, would be as follows:

2,701 log-ins at $1.20 each =	$ 3,241.20
6,265 queries averaging 3 minutes each or 313.25 hours online @ $60/hour) =	$18,795.00
367 articles at $13.50 each =	$4,954.50
TOTAL	**$26,990.50**

The PRAISE Web site is a bonus. It allows users to make requests online, publicizes research being done in the Pacific via the Gray Literature Bibliography, and gives local vendors a venue to advertise themselves to the world.

Support

This project received funding from CTSA, the University of Hawaii (UH), Sea Grant Extension Service (SGES), the National Agricultural Library (NAL), the U.S. Department of Agriculture (directly), the Center for Applied Aquaculture (CAA), the National Oceanic and Atmospheric Administration (NOAA) and jointly from the Pacific Island Network (PIN) and the Pacific Aquaculture Development Program (PADP).

Year	CTSA	Other Support							
		UH	SGES	PIN / PADP	NAL	USDA	CAA	NOAA	*Total Other Support*
1	$7,000.00	$13,400.00	$1,500.00	$0.00	$0.00	$0.00	$0.00	$0.00	*$14,900.00*
2	$6,700.00	$12,600.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	*$12,600.00*
3	$6,000.00	$12,600.00	$3,300.00	$0.00	$0.00	$0.00	$0.00	$0.00	*$15,900.00*
4	$7,000.00	$14,100.00	$4,000.00	$0.00	$2,500.00	$0.00	$0.00	$2,500.00	*$23,100.00*
5	$20,000.00	$44,175.00	$3,500.00	$10,800.00	$0.00	$0.00	$15,000.00	$0.00	*$73,475.00*
6	$17,900.00	$24,000.00	$0.00	$5,800.00	$0.00	$0.00	$0.00	$0.00	*$29,800.00*
7	$28,000.00	$12,600.00	$0.00	$5,500.00	$0.00	$0.00	$0.00	$0.00	*$18,100.00*
8	$49,000.00	$11,400.00	$0.00	$5,500.00	$0.00	$0.00	$0.00	$0.00	*$16,900.00*
9	$25,000.00	$10,500.00	$0.00	$5,000.00	$7,500.00	$0.00	$0.00	$0.00	*$23,000.00*
10	$24,000.00	$19,200.00	$0.00	$0.00	$0.00	$6,700.00	$0.00	$0.00	*$25,900.00*
11	$30,000.00	$10,500.00	$2,500.00	$0.00	$6,500.00	$0.00	$0.00	$0.00	*$19,500.00*
12	$24,000.00	$10,500.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	*$10,500.00*
Total	**$244,600.00**	**$195,575.00**	**$14,800.00**	**$32,600.00**	**$16,500.00**	**$6,700.00**	**$15,000.00**	**$2,500.00**	***$283,675.00***

Publications, Manuscripts or Papers Presented

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Aquaculture Extension and Training Support in the U.S.-Affiliated Pacific Islands

Dates of Work

August 1989 through September 1999

Funding Level

$902,470

Participants

Dr. Christine Crawford (August 1989 through July 1991),
Stephen Lindsay (July 1991 through January 1996),
Dr. Maria Haws (May through July 1996), and
Simon Ellis (January 1997 to present), College of Micronesia, Pohnpei, Federated States of Micronesia.

Objectives

The overall goal of this project, which was initiated under the CTSA Second Annual Plan of Work and is now in its eleventh year, is to provide extension and training support to private aquaculturists and to government fisheries and aquaculture staff to develop commercial and subsistence aquaculture crops within American Samoa, the Commonwealth of the Northern Mariana Islands (CNMI), the Federated States of Micronesia (FSM), the Republic of Palau and the Republic of the Marshall Islands (RMI). Specific objectives related to that goal are to:

conduct training courses in culture techniques and general biology of aquaculture species;
provide extension support to private aquaculturists and government fisheries and aquaculture staff to develop commercial and subsistence aquaculture crops within the region;
help develop and support hatcheries and growout farms for giant clams and other aquatic plant and animal species, including sponges, pearl oysters, seaweed, trochus and green snails;
assist in reef reseeding programs and surveys for giant clams, sponges and other species as requested by local authorities;
continue to act as the scientific and aquaculture advisor to the FSM National Aquaculture Center in Kosrae.

Principal Accomplishments

This project began in 1989, when the Center for Tropical and Subtropical Aquaculture funded an aquaculture extension specialist for the region. Additional funding has been provided by Sea Grant Extension Service, the Pacific Island Network, the Pacific Aquaculture Development Program, the College of Micronesia and the Federated States of Micronesia government.

Dr. Christine Crawford served as the extension specialist from 1989 to 1991, when Mr. Stephen Lindsay assumed the position. He resigned in February 1996; Dr. Maria Haws assumed the post from May through July 1996. Simon Ellis served as the extension specialist starting in January 1997.

The extension specialist provided technical advice and assistance to establish the FSM National Aquaculture Center in Kosrae and established a demonstration ocean growout farm on the reef outside the National Aquaculture Center. The specialist assisted with development of the major field site, including building and deploying enough off-bottom culture racks to hold all the clams. A training course in giant clam culture was conducted, after which trainees induced spawning in *T. gigas* and in 6-year-old *Hippopus hippopus.* A project was conducted to encourage the women of Kosrae to grow clams on the reef outside their homes. The specialist continues to act as the scientific and technical advisor to the facility and makes site visits to all the giant clam culture facilities in the region upon request.

Training courses in all aspects of giant clam spawning and culture were conducted in various locations throughout the region. Assistance was provided with giant clam reef reseeding programs, and surveys for giant clams, sponges and other species were completed as requested by local authorities. During the project's seventh year, the following training courses were held:

A training course in the culture of the marine gastropods trochus and turbot was conducted in the Marshall Islands;
More than 40 people attended an aquaculture workshop held in the Marshall Islands. The workshop included presentations on planning aquaculture projects, conducting feasibility studies and pearl oyster culture;
Presentations on various aquaculture topics were given for schools and the general public throughout the region. Presentations included "Aquaculture in the Pacific," "Reproduction in Tropical Bivalves and Gastropods," "Giant Clam Farming," "Marine Gastropod Farming," "Pearl Oyster Culture," "General Aquaculture" and "Aquaculture in the U.S.-Affiliated Pacific Islands."

A black-lip pearl oyster stock assessment was conducted in Arno Atoll, RMI, and three people were taught survey techniques.

A series of lectures on aquaculture topics were presented to science classes at the American Samoa Community College and at the FSM Community College and to groups of Peace Corps volunteers in the FSM. In addition, lectures on pearl farming and giant clam culture were given to staff of the American Samoa Department of Marine and Wildlife Resources and to staff of the Marshall Islands Development Authority.

A giant clam demonstration farm was established in Pohnpei, FSM. Three sponge demonstration farms were established in various states of the FSM, and assistance was provided in marketing a colonial tunicate that grows on sponge farming lines. All giant clam culture facilities in the region received information on aquarium markets, local and international food markets and reef reseeding. A private giant clam wholesaler in Saipan was assisted with obtaining the necessary permits and provided with information on clam availability and pricing from hatcheries in the region.

The agent provided information on aquaculture of various species in response to requests from parties throughout the region. The information covered mangrove crab, marine and freshwater shrimp, sponges, soft coral, giant clams, pearl oysters, aquacultured live rock, freshwater aquarium fish and marine ornamental fish.

Subscriptions to a relevant magazine were provided to operators of giant clam facilities, including the Marshall Islands Marine Resources Authority (MIMRA), Robert Reimers Enterprises (RRE), FSM National Aquaculture Center (NAC) in Kosrae and the American Samoa Department of Marine and Wildlife Resources (DMWR).

Slide presentations and accompanying written materials on general aquaculture were developed. In addition, the project provided funding to produce a manual and accompanying video titled *Clams to Cash: How to Make and Sell Giant Clam Shell Products*. The manual and video provide details on how to produce value-added products from giant clam shells.

Literature and advice on aquaculture of various species were provided to private concerns and government agencies in the CTSA region

The species covered included mangrove crabs, pearl oysters, marine shrimp, freshwater prawns, marine sponges, live rock, soft coral, giant clams, mullet, grouper, trochus, eels, sea cucumbers, tilapia and ornamental species. Field site evaluations were done in several cases.
Approximately 3 percent of those who requested information and advice initiated aquaculture projects.

All locations were provided with information on the documentation required under the Convention on International Trade in Endangered Species (CITES) and by U.S. Fish and Wildlife Service to allow the export of giant clam products.

The Republic of Palau, the Republic of the Marshall Islands and the Federated States of Micronesia have completed the necessary steps and obtained permits to export giant clam products for non-food uses.

Regional aquaculture businesses were assisted with developing markets for their products. Assistance with marketing and business matters remains a major concern of regional aquaculturists, most of whom have had marketing difficulties and have extensive questions regarding the topic.

Objective: Provide extension support to private aquaculturists and government fisheries and aquaculture staff to develop commercial and subsistence aquaculture crops within the region.

The extension specialist worked to help a private farm in the Marshall Islands to diversify its product line and improve soft coral culture techniques.

During the lapse between extension specialists, American Samoa requested assistance in developing a product line that included live rock, corals and invertebrates. In 1997, Ellis visited American Samoa to meet Department of Marine and Wildlife Resources personnel and to determine the status and needs of aquaculture in that locale. He also visited tilapia and eel farms there to determine if they required technical assistance.

In 1997, the extension specialist met several times with the CNMI aquaculture extension agent to coordinate a site visit to established private aquaculture facilities on Guam for six farmers from CNMI. This will be funded from the extension agent's budget.

In 1997, the extension specialist evaluated three Pohnpei sites for baitfish culture. He also participated in planning meetings for the aquaculture division of the Marine and Environmental Research Institute of Pohnpei, a facility planned for the campus of the Pohnpei Agriculture and Trade School. The Institute will initially focus on sponges, pearl oysters and baitfish.

In 1998, he designed a saltwater intake and filter system for MERIP. He also trained the staff of these schools in sponge culture and soft coral culture and helped them to establish a small soft coral farm. Further, he held a soft coral culture workshop at the MERIP facility.

The extension specialist spent eight days helping to install a flow-through holding tank system for giant clams and pearl oysters for the Marshall Islands Marine Resources Authority (MIMRA).

During 1998, Ellis arranged for Mark Inouye of the Waianae Backyard Aquaculture Program in Hawaii to conduct workshops in the CNMI on developing a rotating biological contact filter and on the pros and cons of backyard recirculating systems. In addition, he supported training in hatchery culture techniques for red tilapia in the CNMI by designing a system for which quotes were obtained. When the system is completed, training sessions will be conducted.

During 1998, he also coordinated an aquaculture planning session in Pohnpei involving interested parties from Sea Grant, COM-FSM, the FSM government and the private sector. In addition, Ellis provided technical assistance and materials for a new marine science teacher at COM-FSM. He also conducted a three-day workshop in backyard aquaculture in American Samoa.

During 1999, Ellis provided site assessment assistance to a prospective milkfish farmer in Kitti, Pohnpei, to B + K Farms in Laura, Majuro, RMI, and to a local business man in Pohnpei for a small scale prawn farm. He provided assistance to Marine and Environmental Research Institute of Pohnpei (MERIP) in design of their aquaculture facilities and seawater system, the design of their experiments on optimum coral farming practices, and continued training activities at the MERIP soft corals farm for PATS/MERIP staff and students. He conducted training for residents of Jaluit, RMI in manufacture and deployment of pearl oyster spat collectors, assisted Yap MRD with a plan for conducting pearl oyster surveys in the outer islands of YAP, advised staff and managers of the Nukuoru pearl farm on harvest timing, grant sources and future direction for their farm, and continued assisting staff of RRE with problems associated with the pearl oyster spat project in Jaluit. He assisted Pacific Water Resources in husbandry techniques for their milkfish bait farm in Pohnpei, assisted Pacific Water Resources in husbandry techniques for their milkfish bait farm in Pohnpei. Ellis initiated and continued extension and marketing assistance with the Pohnpei State MRD Lenger Island clam hatchery, continued the planning and ordering process for the tilapia hatchery in CNMI, and provided planning and design assistance to the Aquaculture Cooperative of American Samoa (AQCAS).

Objective: Conduct training courses in culture techniques and general biology of giant clams, sponges, black pearl oysters, soft corals, aquarium fishes and baitfish. Training courses will be tailored to meet the needs of individuals involved.

The extension specialist provided training and technical assistance, including demonstrations of spawning methods and husbandry techniques, to a private company with giant clam farm sites in Majuro and Mili, Marshall Islands. He also conducted a staff training course in how to cut and plant soft corals. He coordinated a pearl oyster culture training session in Majuro for staff of both a government agency and a private company. In 1998, the company began selling cultured *Sarcophytons.*

In 1997, the extension specialist twice visited the giant clam farm in Pohnpei, FSM, and once visited the FSM National Aquaculture Center (NAC) in Kosrae. He conducted a training course for the NAC technical staff to improve hatchery techniques and husbandry for giant clams, and he conducted a soft coral culture workshop at the NAC. In 1998, he provided technical assistance to the NAC in planning its new lab and seawater system.

In 1998, Ellis spent a week in Palau providing technical assistance and advice to two new private coral farms, Western Pacific Mariculture and Palau Aquaculture.

During 1998, Ellis trained staff of the Mariculture Education and Research Institute of Pohnpei in soft coral culture techniques and assisted in setting up a soft coral farm off the island of Nah Pohli. He also trained RRE personnel on Jaluit Atoll in pearl oyster spat collection techniques.

During 1998, Ellis assisted in establishing a collaborative research project between Robert Reimers Enterprises, Dr. Maria Haws and the regional extension specialist to determine the potential for black-lipped pearl oyster spat collection in Jaluit Lagoon, RMI. He coordinated equipment orders and travel arrangements for Dr. Haws. During July 1998, he and Haws visited Jaluit for two weeks to training residents in spat collection techniques, identify four sites for spat collection and deployed long lines to house the spat collectors, which will be deployed at six-week intervals.

During 1999, Ellis made a number of site visits to various clam and pearl farms throughout the region to assess their need for technical assistance and training. In response to MERIP's strong interest in coral farming, Ellis made five visits to the MERIP soft coral farm and has continued to provide training and extension support to staff on coral culture techniques.

Two followup visits were made to the black lipped pearl oyster project now operating in Jaluit, RMI and scheduled to run through February 2000. Visits were made to all collector sites, training was given in manufacturing techniques for collectors and spat identification and care.

In 1999, Ellis also surveyed the Nam lagoon to determine if oyster populations had improved since the last visit in 1997. He also assisted Yap MRD in a plan to conduct pearl oyster surveys in the outer islands of Yap.

Objective: Develop extension fact sheets, manuals and videos to educate existing and potential aquaculture producers in the region.

During 1997, the extension specialist collected footage for a video on giant clam spawning and has began writing the script. He reviewed literature for brochures or manuals or soft coral culture, giant clam spawning and handling and transport of aquarium species.

In the summer of 1998, Ellis published *Spawning and Early Larval Rearing of Giant Clams (Bivalvia: Tridacnidae)*, which is being distributed throughout the region. The companion video of the same title was also completed. He began work on a soft coral culture manual and began reviewing literature for a manual on transporting aquarium species.

In 1999, Ellis published *The Culture of Soft Corals for the Marine Aquarium Trade*, completed the soft coral video and published two Aquafarmer Information sheets titled: *Farming soft corals for the marine aquarium trade* and

Lagoon farming of giant clams. A draft of a third Aquafarmer Information sheet titled *Producing pearls using the Black-lip pearl oyster* has been completed and will be published after final editing and layout work is completed.

Objective: Conduct or organize general aquaculture information sessions and workshops on established and potential aquaculture species.

In 1997, the extension specialist prepared a slide presentation on basic aquaculture for presentations at community colleges throughout the region. In 1998, presentations were given in Pohnpei three times, and in Yap, Palau and Majuro twice.

In October 1997, Ellis coordinated workshops in Pohnpei and Majuro at which Joan Rolls of the Cook Islands taught production of handcrafts from the shells of pearl oysters and giant clams. Coordination efforts included travel arrangements, consultant agreements, workshop location and participants and materials. The agent also trained Peace Corps volunteers in various aquaculture techniques.

In 1999, Ellis continued a series of educational presentations covering basic aspects of aquaculture and potential culture species. Ellis gave a presentation at the April 1999 World Aquaculture Society (WAS) meeting in Sydney, Australia on the status of aquaculture in the region.

Ellis spent considerable time and effort in 1999 providing assistance in designing and constructing tilapia hatchery system at the local Department of Land and Natural Resources (DLNR). Followup visits will be made to provide training in tilapia spawning and hatchery techniques.

Objective: Continue to search for island residents to participate in an aquaculture internship program. Assist with development of the Pacific Regional Aquaculture Extension Program, which will be filled with local residents and eventually assume all the responsibilities of the regional extension specialist.

During 1997, the extension specialist met with the director of the Hawaii Sea Grant Micronesia and American Samoa Student Internship Program (MASSIP) program to try to identify suitable candidates for training. The program trains students to conduct research on environmental issues, which they then do in their home islands, using the results to educate local residents. He continues to seek candidates while in Micronesia.

During 1998, Ellis reached an agreement with Sharon Zeigler of MASSIP that an aquaculture internship would be an excellent placement for a MASSIP student and a job description was submitted. An intern was hired June, July and August of 1998.

The program hired Mr. Richard Croft as a part-time sponge extension specialist. During 1998, Croft accompanied Ellis on a visit to Palau to set up a sponge farm for a prospective farmer. They trained the farmer in sponge collection, cutting and stringing and assisted him in establishing a small farm of 150 sponge cuttings.

Objective: Provide aquaculture information in the form of papers, manuals and videos for producers, government representatives and interested parties throughout the region upon request.

In 1997, the extension specialist reorganized and catalogued the office collection of aquaculture literature and videos, expanded the literature collection and renewed lapsed subscriptions.

He provided books and posters on corals to MIMRA and RRE, a private aquaculture company in the Marshall Islands. He has filled request for videos and literature on giant clams, pearl oysters, sponges, mangrove crabs, corals, baitfish, shellcraft, sea cucumbers, trochus and fish transportation.

In 1999, Ellis continued to expand the literature collection at the office in Pohnpei by making literature searches and working with other information centers around the region. He continues to fill requests on an ongoing basis for a wide variety of manuals and videos.

Work Planned

The project will continue to provide extension support and training throughout the region. In addition, the pearl oyster information sheet will be published.

Impacts

At the start of this project in 1989, aquaculture was virtually non-existent in American Samoa, the Commonwealth of the Northern Mariana Islands, the Federated States of Micronesia, and the Republic of the Marshall Islands. Today the region has six giant clam hatcheries, four pearl oyster farms, six sponge farms, four coral production facilities and four tilapia farms. In addition, awareness of aquaculture and its income potential is growing rapidly. This project has provided vital technical assistance in all phases of aquaculture to farmers and government employees. The extension agent's hands-on training of local individuals has led to substantial capacity building in the region. Most of the giant clam farms are operated by native islanders. As the industry grows, this knowledge will spread to other species and will achieve the ultimate goal of a self-sustaining, economically viable aquaculture industry within the region.

Support

This project received funding from CTSA, the Federated States of Micronesia government (FSM), Sea Grant Extension Service (SGES), the Pacific Island Network (PIN), the College of Micronesia (COM), the Pacific Aquaculture Development Program (PADP), and the United Nations Food and Agriculture Organization (FAO).

Extension and Training Support in the U.S.-Affiliated Pacific Islands									
Project	CTSA	Other Support						*Total*	Total
Year	Support	FSM	COM	SGES	PIN	PADP	FAO	*Other*	Support
1	$100,000.00	$24,000.00	$7,000.00	$5,000.00	$4,000.00	$10,000.00	$0.00	*$50,000.00*	**$150,000.00**
2	$85,870.00	$26,700.00	$7,000.00	$4,500.00	$4,500.00	$4,500.00	$2,500.00	*$49,700.00*	**$135,570.00**
3	$83,600.00	$27,754.00	$7,000.00	$10,800.00	$10,800.00	$21,000.00	$6,000.00	*$83,354.00*	**$166,954.00**
4	$70,000.00	$15,000.00	$2,000.00	$0.00	$6,000.00	$4,000.00	$0.00	*$27,000.00*	**$97,000.00**
5	$75,000.00	$0.00	$3,000.00	$0.00	$2,000.00	$10,000.00	$0.00	*$15,000.00*	**$90,000.00**
6	$98,000.00	$0.00	$3,000.00	$2,000.00	$2,000.00	$16,000.00	$0.00	*$23,000.00*	**$121,000.00**
7	$70,000.00	$0.00	$3,000.00	$2,000.00	$2,000.00	$12,000.00	$0.00	*$19,000.00*	**$89,000.00**
8	$75,000.00	$0.00	$3,000.00	$2,000.00	$2,000.00	$19,000.00	$0.00	*$26,000.00*	**$101,000.00**
9	$85,000.00	$0.00	$3,000.00	$0.00	$2,000.00	$29,500.00	$0.00	*$34,500.00*	**$119,500.00**
10	$85,000.00	$0.00	$3,000.00	$0.00	$0.00	$31,500.00	$0.00	*$34,500.00*	**$119,500.00**
11	$75,000.00	$0.00	$3,000.00	$0.00	$0.00	$31,500.00	$0.00	*$34,500.00*	**$109,500.00**
Total	**$902,470.00**	**$93,454.00**	**$44,000.00**	**$26,300.00**	**$35,300.00**	**$189,000.00**	**$8,500.00**	*$396,554.00*	***$1,299,024.00***

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Disease Management for Hawaiian Aquaculture

Dates of Work

April 1993 through May 1999

Funding Level

$351,072

Participants

Dr. James Brock and Diana Montgomery, Hawaii State Aquaculture Development Program;
Dr. Brad LeaMaster, Department of Animal Sciences, University of Hawaii;
Dr. Clyde Tamaru, Sea Grant Extension Service, University of Hawaii.

Objectives

The overall goal of this project, which was initiated under the CTSA Sixth Annual Plan of Work and is now in its sixth year, is to develop management strategies to minimize losses from diseases at aquaculture farms in Hawaii. Specific objectives related to that goal are to:

provide aquaculture health management extension support to commercial farms;
identify contributing factors that may be important to the occurrence of bacterial disease during growout of Chinese catfish (*Clarias fuscus*);
field test a preventive strategy to mitigate losses of cultured Chinese catfish (*Clarias fuscus*) during growout in Hawaii due to two bacterial diseases, *Aeromonas hydrophila* and *Edwardsiella tarda* septicemia;
screen juvenile and adult cultured Chinese catfish and tilapia (*Oreochromis mossambicus*) for potential pathogenic fish viruses;
assess the infectivity of IHHN virus in feces after passage through the digestive tract of a species of water bird;
provide diagnostic and health management support to the CTSA-funded project titled "Ornamental Aquaculture Technology Transfer" and implement management practices and standard disease treatment strategies to improve fish survival and reduce the abundance of pathogenic parasites in imported groups of freshwater tropical aquarium fish;
document the principal ectoparasites and assess their effects on cultured tilapia and mullet in a traditional Hawaiian fishpond;
assess samples of *Gracilaria* spp. for the presence of *Gracilaria* Gall Syndrome (GGS), determine how the syndrome is transmitted, and identify potential chemical controls for it.
provide diagnostic support to the CTSA-funded project titled "Expansion and Diversification of Freshwater Tropical Fish Culture," and to ornamental fish hatcheries and farms;
investigate the role of contributing factors and identify control options for the rickettsia-like organism (RLO) disease of tilapia in Hawaii;
produce an operational manual for the application of the probiotic technology for shrimp hatcheries in Hawaii;
continue work on vaccination protection for control of bacterial disease of cultured Chinese catfish in Hawaii.

Anticipated Benefits

This project focuses on problems that directly affect production of crops in Hawaii's aquaculture farms and provides disease screening for other CTSA-funded projects. Examples of assistance include developing procedures for control of bacterial diseases in cultured Chinese catfish, initial investigation of a serious new disease in cultured seaweed, a study to increase the understanding of transmission of selected viruses in cultured shrimp, documentation of ectoparasites on fish cultured in Hawaiian fishponds, and work focusing on disease control in ornamental fish culture in Hawaii. These efforts will improve production of Hawaii aquaculture facilities or procure new information that will eventually lead to improved health management strategies for improved aquaculture productivity in Hawaii.

Principal Accomplishments

Objective: Provide aquaculture health management support to commercial farms.

During year one, investigators made 80 site visits to 10 farms to provide health management extension assistance under this project. During year two, investigators made 240 site visits to 10 aquaculture farms on Oahu and Hawaii to provide health management extension assistance. During year three, investigators made 158 site visits to 15 locations. During year four, investigators made 172 site visits to 14 locations. During year five, investigators made 188 site visits to aquaculture sites on Oahu to assist producers address disease or production problems, or to carry-out activities related to the year five work.

Objective: Work on the development of practical, on-farm measures to mitigate the impact of Taura syndrome on penaeid shrimp farming.

In May 1994, the second year of this project, a serious disease outbreak occurred in farmed *Penaeus vannamei* in Kahuku, Hawaii. It caused mortality rates higher than 95 percent within 14 to 30 days of stocking. Studies initiated to determine the cause and a means of controlling the disease led to the discovery of a new shrimp virus in Hawaii. The virus was thought to be the Taura Syndrome agent, which was confirmed in subsequent studies by other laboratories. Additionally, this study found that *P. stylirostris* is largely resistant to Taura Syndrome. The Hawaii farm that was affected by Taura Syndrome then began culturing *P. stylirostris* and achieved production levels equal to or greater than those achieved with *P. vannamei*.

Two bioassay studies were conducted to assess the effect of vaccination with a commercial bacterial lipopolysaccharide (LPS) preparation on acute, lethal exposure to TSV in juvenile SPF *Penaeus vannamei*. High mortality was observed after TSV exposure in both the vaccine and the control groups of shrimp.

Kahuku Shrimp Company's reproduction and hatchery operations have had limited production due to technical, feed, and disease problems. This project provided advisory and diagnostic assistance to the farm. Nauplii production improved, but hatchery output continued to be unstable.

Diagnostic testing as well as assessment of the population impact from IHHNV infection are being done at a commercial shrimp farm. Shrimp populations in all areas of the farm are being monitored. During year three, evaluation of 116 specimens by dot-blot gene probe indicated that IHHNV infection was in the growout area of the farm, but not in the broodstock, reproduction or hatchery facility. Because the farm was working with *P. stylirostris*, the initial strategy for control in grow-out was to switch to *P. vannamei*. This strategy appears to be working; Taura Syndrome has not recurred on that farm.

Taura syndrome disappeared in Spring 1995. The project investigators continued to monitor shrimp farms in Kahuku, Hawaii, examining 248 shrimp taken in 25 case submissions from two farms. Histopathology examinations of the shrimp revealed no lesions diagnostic for Taura syndrome, which supports the conclusion that TSV is no longer present in shrimp populations in Hawaii.

Objective: Field test an approach for IHHNV decontamination of shrimp ponds.

During year one, six ponds at the site of the former Amorient Aquafarm in Kahuku, Hawaii, were selected for the study. All six ponds were drained, and the soil's moisture content and pH balance were measured. The three control ponds were refilled with water within three days of draining. The surfaces of the three remaining ponds were spread with a layer of lime at a rate of 2,500 pounds per acre and were left undisturbed for two weeks.

Investigators sampled soil from the limed ponds weekly to determine the moisture content and pH level. The lime distribution was uneven, so the pH rose to 11 in some areas while remaining unchanged in other areas. After two weeks, the limed ponds were refilled and stocked at a per-acre rate of 60,000 *Penaeus vannamei* and 10,000 *P. stylirostris*; the latter are highly susceptible to IHHNV. Results were very disappointing. *P. stylirostris* survival and shrimp production levels were no better in the limed ponds than in the control ponds.

Investigators also conducted several trials to measure the effect of solar heating on the surface temperature of soil. Soil surface temperatures were recorded at 64 to 66 C for one to two hours in the afternoon in soils exposed to direct sunlight. Because these temperatures are high enough to inactivate the known penaeid shrimp viruses, solar heating should be considered as a practical means to disinfect shrimp pond bottoms.

Objective: Assess the infectivity of IHHN virus in feces after passage through the digestive tract of a species of water bird.

During year one, two cages were constructed to hold the two juvenile night herons obtained for the study. The birds adapted well to captivity and readily ate fish and fresh shrimp.

During year two, three trials were conducted in which IHHNV-infected frozen shrimp carcasses (100 grams) were fed to an avian vector host and the feces from the bird were collected over five to six days following ingestion of the infected shrimp. The feces collected in the trial have been stored frozen and will be analyzed for IHHNV and infectivity to juvenile *P. stylirostris.* The studies to identify IHHNV in the feces from the bird vector have been postponed because of a backlog of work in our bioassay facility. The bird fecal samples for IHHNV evaluation by PCR-plus dot blot will be examined by the Aquaculture Pathology Group at the University of Arizona.

In year three, six aquaria were each stocked with juvenile SPF *P. vannamei* from a population shown to be susceptible to TSV infection and disease. The resident avian vector was not fed for one day (Day Zero) because prior work showed that this bird clears the gut of contents within 12 to 18 hours. On Day One, the avian vector was fed 100 grams of previously frozen shrimp carcasses known to harbor infective TSV. The following morning, feces were collected from the plastic sheet on the bottom of the cage, and the majority of the feces were inoculated into the first shrimp tank in the bioassay room. The avian vector was not fed on Day One, and a small amount of droppings (0.5 gram) were collected and inoculated into the water of shrimp tank #2. On Day Two, the bird was fed its normal ration of fish, and the plastic sheet was cleaned, disinfected, dried and replaced on the bottom of the cage. On Day Three, the bird feces were collected and inoculated into the water of shrimp tank #3. The process was repeated on Days Four and Five so that five tank groups of indicator shrimp were exposed to feces of the bird. The sixth tank group of indicator shrimp served as the negative control for the trial. Feces collected after ingestion of shrimp were inoculated into aquaria with susceptible indicator shrimp. The bioassay was run through Day 21 and terminated. Shrimp survival in the six tank groups was 90 percent or higher, and no clinical indication of Taura Syndrome (TS) appeared in any animals in the study. Histologically, none of the 60 shrimp examined had tissue changes suggestive of TS. These results indicated the absence of transmission of the Taura Syndrome virus from avian feces to the susceptible juvenile *P. vannamei* indicator shrimp. However, infection of the bioassay shrimp did not occur in the two trials, and the findings do not support the hypothesis that TSV survived through the digestive tract of the shrimp-eating bird.

Three trials were conducted in which IHHNV-infected shrimp carcasses were fed to the avian vector. Two of three feces samples collected within 24 hours after the birds ingested the shrimp were positive for IHHNV by the PCR method. Fecal samples collected on subsequent days were negative for IHHNV by the PCR method.

In addition, a trial was conducted to assess whether the tilapia RLO organism could be transmitted in bird feces to susceptible tilapia. Known RLO-infected dying tilapia were fed to the avian vector over five consecutive days. Each day the feces on the plastic sheet covering the bottom of the bird cage were collected and added to the water in a tank containing juvenile, RLO-free tilapia. Thirty days following addition of bird feces neither clinical, gross pathological or histological signs of RLO disease were found in the exposed or the unexposed control groups of tilapia. Survival was 95 to 100 percent in both groups of fish. These findings indicate that the Hawaiian tilapia RLO does not appear to survive in an infectious form in feces after passage through an avian digestive tract.

Objective: Test the susceptibility of the endemic caridean shrimp, Halocaridina rubra*, to infection and disease by the penaeid shrimp viruses IHHN and the newly discovered Taura Syndrome virus (TSV).*

During year three, work was conducted on two fronts. The first continued studies on birds as vectors of shrimp viruses. The second front tested *H. rubra* as a possible reservoir host for IHHN and TSV. A colony of about 100 *H. rubra* was established in a 300-gallon holding tank in the isolation area at AFRC. The population of *H. rubra* were

provided by Dr. Richard Brock, who collected the animals from a coastal pond in Kona, Hawaii. Virus challenge bioassay trials with *H. rubra* began in December 1996. The results indicate that *H. rubra* is not susceptible to disease from either IHHNV or TSV and does not serve as a reservoir host for IHHNV.

Objective: Produce an operation manual for application of the probiotic technology for shrimp hatcheries in Hawaii.

During year four, information was gathered on procedures used for probiotic application at two commercial shrimp hatcheries in Central and South America.

Objective: Test the efficacy of hydrogen peroxide to control common ectoparasite infestation of culture fishes.

Work continued to test hydrogen peroxide, which the Food and Drug Administration (FDA) has classified as a low regulatory priority compound for aquaculture, as a chemical control agent for treatment of common ectoparasite infestation of cultured freshwater and marine fish. During year five of the project, juvenile moi (*Polydactylus sexfilis*) with *Amyloodinium* sp. infection of the gills were treated with hydrogen peroxide to determine the fish's tolerance to hydrogen peroxide (e.g. concentration and duration) and to test the compound's efficacy as a chemical control of *Amyloodinium.*

Juvenile moi were found to tolerate exposure to hydrogen peroxide at 150 ppm for 30 to 60 minutes. Twenty-six fish were used in the study; 13 were hydrogen-peroxide treated and 13 were untreated control fish. Gill wet-mounts of treated juvenile moi showed significantly reduced numbers of *Amyloodinium* sp. This indicates that hydrogen peroxide may be an effective chemical for the treatment of ectoparasite disease caused by *Amyloodinium* species.

Hydrogen peroxide was also tested as a treatment to control two Chinese catfish ectoparasites, *Gyrodactylus* sp. and *Trichodina* sp. Juvenile Chinese catfish were found to tolerate 30 minute treatment with 250 ppm hydrogen peroxide. Twenty-four hours after treatment, no *Gyrodactylus* sp. or *Trichodina* sp. were observed in wet-mounts of skin scrapings from the treated catfish, but an average of 18.4 *Gyrodactylus* sp. and 12.4 *Trichodina* sp. per fish appeared in wet mounts of skin scrapings of untreated control fish. This indicates that hydrogen peroxide may be useful as a chemical treatment for the control of ectoparasite infections in Chinese catfish.

However, some producers experienced mortalities in Chinese catfish following treatment with hydrogen peroxide. Rach *et al*. (1997) reported a reduced tolerance of fish to hydrogen peroxide exposure when fish were held in higher water temperature. During year five, a study was conducted to test whether hydrogen peroxide toxic to Chinese catfish when administered in "warm" rather than "cool" water. Forty juvenile catfish weighing between 5 and 48 grams each were divided into two groups, which were subdivided into four batches of five fish each. Each batch of five fish was released into a bucket filled with 12.5 liters of water. Four buckets were placed into a tank in which the level of water was adjusted so that buckets were immersed to about 75 percent of the depth of the bucket. In each group, two buckets of fish were unexposed controls and two buckets of fish were administered hydrogen peroxide. In the high temperature group, the four buckets of fish were acclimated overnight in heated 29 C water. The second group of four buckets of fish were held in a tank with water at the ambient temperature of 23°C. Two buckets in the each group were administered hydrogen peroxide at a rate of 150 ppm for 30 minutes, after which they were transferred to buckets with clean water at their treatment temperatures. The control groups were handled in a similar fashion. Following treatment, all bucket groups of fish were held and observed for 24 hours. None of the Chinese catfish died during the study, which did not demonstrate increased toxicity of hydrogen peroxide to Chinese catfish at higher temperatures.

Objective: Develop a protocol for monitoring of subclinical Amyloodinium sp. Infection of cultured moi and the use of the procedure on a commercial farm.

Experience has shown that once the moi have started dying from OD, the losses will be high even if the fish are treated with hydrogen peroxide and/or transferred to a clean raceway. An analysis of these problems led to the conclusion that the outcome for hydrogen peroxide treatment or management procedures practices for the control of OD would be more favorable if intervention was initiated prior to the onset of clinical disease (morbidity and

mortality) of the fish. In year five of the project, a protocol was devised, tested, modified and found to be suitable for the monitoring of subclinical infection by *Amyloodinium sp*. of moi.

The gill biopsy/microscopic trophont count method was applied weekly by project members or farm staff to check moi for *Amyloodinium* sp. infection. The moi tanks were sampled 85 times over a period of 22 weeks. In a few cases for specific problem tanks, the trophont counts were made every few days. Fifty-five (65%) of the tanks sampled had one or more fish positive for trophonts. For 30 of the tank samples (35%) the gill biopsy samples from the five fish were negative for trophonts. Even with a five fish sample size, *Amyloodinium* sp. infection was detected well in advance of the occurrence of clinical disease. When trophont counts were observed to rise, steps were taken to mitigate an outbreak of OD.

A study was undertaken to evaluate if a larger sample size of fish per tank would increase the sensitivity of detection of trophonts. The frequency of trophont positive biopsy specimens for samples of 10 fish were compared with samples from five fish. The frequency of trophont positive biopsy specimens was not statistically different (P=0.65) for the 10 or five fish groups. These findings indicated that a sample size of five fish per tank provided adequate sensitivity for the gill biopsy procedure.

The gill biopsy procedure was found to be effective as a means to monitor subclinical *Amyloodinium* sp. infection in cultured moi. The observations indicated that moi were asymptomatic and feeding well in tank populations when the average trophont count/field remained at 5 or less. There was danger of an impending outbreak of OD when the average trophont count/field score rose to 20 or higher. Average trophont count/field scores of >75 were associated with clinical signs of OD in the moi.

Objective: Assess the effect of low light conditions as an approach for the control of Amyloodinium sp. disease of moi cultured in Hawaii.

Weekly monitoring of the *Amyloodinium* sp. trophont count coupled with hydrogen peroxide treatment and transfer to a clean tank was found to help advert losses from OD. However, complications arose when there were no empty tanks available for the fish to be transferred to and the procedures were found to be time consuming and labor intensive. A simpler approach was needed for the prevention of OD. This led to studies to evaluate the maintenance of low light as a means to inhibit the life cycle of *Amyloodinium* sp. in moi culture tanks. The rationale for using darkness to combat OD was based on the belief that a significant reduction in light would slow down or stop the proliferation of macro algal on the bottom of the moi tanks and that this would reduce the favorable substrate for *Amyloodinium* sp. Investigators also speculated that the low light conditions would be unfavorable to the dinoflagellate (swimming) stage of this parasite.

The first experiment was conducted in small circular tanks at Anuenue Fisheries Research Center (AFRC). Eight moi were transported to AFRC from the commercial moi farm on Oahu. These fish were collected from a raceway where the fish were exhibiting clinical signs of *Amyloodinium* infection (flashing, not eating, discolored and mottled appearance). The eight fish were separated into two groups of four fish each and randomly assigned to two, 270 liter tanks with continuous aeration and flow through of salt water. One of the tanks was covered to almost complete darkness with the use of a cover and shade cloth. The other tank was left uncovered.

On the day of arrival at AFRC, each of the eight fish were examined separately, gill biopsies made, trophonts counted and the results recorded. Three days later all of the fish were re-examined and trophont count determined. The gill exam was repeated one month after the fish had been stocked into the tanks. During the holding period the fish were fed daily a commercial pelleted fish feed.

After one month, the covered tank was uncovered to expose the tank sides and bottom to sunlight. Likewise, the uncovered tank was covered to greatly reduce exposure to sunlight. Twenty-seven days later the fish were re-examined by gill biopsy and the trophont count determined.

A companion study was also carried out at the commercial moi farm. Moi from a tank population with high trophont count were moved into a covered tank. Gill biopsy/trophont counts were made from fish in the darkened tank on days 14 and 25 following transfer.

In the AFRC trial the bottom of the covered tank remained clean while the sides and bottom of the uncovered tank had a thick algal growth. In the covered tank, by one month the trophont count had declined below a level detectable by the gill biopsy/microscopic examination procedure. When the light conditions were switched, trophonts were detected at a low level in the now uncovered tank, but had declined below detectable in the then covered tank. Similar observations were found for the moi transferred to the covered tank at the commercial moi farm. The trophont count declined to a very low level within the 25 days of the observation period.

The results of these trials strongly suggest that maintenance of moi under limited light conditions may be an effective means for the prevention of OD.

*Objective: Identify contributing factors that may be important to the occurrence of bacterial disease during growout of Chinese catfish (*Clarias fuscus*) and develop practical strategies for the control of bacterial diseases during their growout.*

During year one, scientists in Thailand who have experience with disease management in freshwater aquaculture fishes in Asia were contacted for information. They forwarded a series of publications on the culture, environmental quality and disease problems for *Clarias* spp. The publications provided comparative information on diseases that have been a problem in Hawaii.

A study was initiated to determine the occurrence and severity of disease episodes in Chinese catfish reared in tanks. The study tracked the occurrence and severity of disease episodes in six tanks of catfish given the same feed. Fourteen dead fish were retrieved from the six tanks during the first sampling period. Those fish and, to a far lesser degree, the fish sampled for weighing showed physical changes that suggested internal bacterial infection, such as swelling of the abdomen over the anterior lobes of the kidney or small skin sores.

Investigators collected a set of water specimens from the six tanks and measured bacterial levels. The bacterium *A. hydrophila* was retrieved in samples from five of the tanks; the bacterium *E. tarda* was not identified from any of the samples.

Skin scrapings from a sample of five fish per tank were done to monitor the prevalence and the relative abundance of ectoparasites. The scrapings showed two types of ectoparasites, *Tricodina* sp. and *Gyrodactylus* sp. that are commonly associated with cultured Chinese catfish in Hawaii. The findings suggest that *Tricodina* sp. infestation had declined in the older groups of fish. *Gyrodactylus* sp. were found in fish from only one tank.

An initial database was developed on the physical and chemical water quality parameters in Chinese catfish culture tanks. Water samples were collected four times over approximately 24 hours. The samples were evaluated for temperature, dissolved oxygen, pH, carbon dioxide, hydrogen sulfide, secchi disc turbidity, hardness, alkalinity, chloride, total ammonia, nitrite, nitrate and ortho-phosphate.

Water quality and commercial diet factors were evaluated in relation to the onset of disease episodes in Chinese catfish populations under farm conditions. The initial sample findings suggested that juvenile Chinese catfish can tolerate large diurnal variations in temperature, dissolved oxygen, carbon dioxide and pH without the occurrence of high mortality episodes of bacterial or ectoparasitic disease. However, evaluation of the physical and chemical measurements during disease outbreaks suggested a positive correlation between occurrence of disease and elevated levels of ammonia or nitrate. Elevated levels of these compounds were associated with one of two factors: either the water supply is temporarily lost due to mechanical or electrical failure, or the phytoplankton/biological filtration community in the culture tank failed.

Bacterial pathogens were isolated from dead Chinese catfish during disease outbreaks. *A. hydrophila* accounted for an average of 80 percent of viable bacteria in the water samples. *E. tarda* rarely was isolated from catfish culture tank water. In addition, the bacterial pathogen *A. hydrophila* is the dominant flora in the water of these catfish culture tanks during periods of minimal losses to bacterial infection. This suggests that the animals are normally exposed to relatively stable numbers of *A. hydrophila* continuously throughout the culture period and that disease events involving this pathogen involve the contribution of more etiological variables than the bacteria and the host fish.

During year three, Vitamin-C-fortified feed was obtained for a trial to examine effect of high dietary vitamin C (>1,000 mg per kilogram) as a treatment to reduce bacterial disease losses in Chinese catfish farmed in Hawaii. Six cages were installed in the Hawaii Fish Company's reservoir in Mokuleia, which had suffered persistent crop losses due to bacterial diseases. Three hundred juvenile Chinese catfish were stocked into each of the six cages at densities of 100 fish per cubic meter and grown for 30 weeks. Catfish survival rates ranged from 77 percent to 95 percent, with an average survival rate of 86 percent. Those catfish fed a diet not fortified with Vitamin C had higher survival rates. Growth of catfish was slightly higher in the control diet group, but the difference was not statistically significant. No beneficial effect was found for growth or survival in those Chinese catfish fed the ration with a high level of Vitamin C. The findings indicate that farmers would not realize improved production by feeding a ration fortified with a high concentration of Vitamin C.

Objective: Assess the effect of low versus high stocking density on survival and final harvest production of Chinese catfish.

During year three, investigators evaluated whether holding juvenile Chinese catfish under crowded conditions would predispose the fish to fatal bouts of bacterial and/or parasitic diseases. For 14 weeks, subadult Chinese catfish were held in plastic bucket within a raceway tank. The catfish were stocked in some bucket replicates at densities of 10 to 50 catfish per bucket (800 to 4,000 fish per cubic meter). By week 14, the biomass of fish reached 2.9 kilograms per bucket (200 kg per cubic meter) in some bucket replicates. During weeks 10 through 12, an outbreak of fatal mouth and head rot occurred in some of the bucket groups of fish. Fish mortality was independent of catfish density and biomass. Catfish deaths ceased within two weeks following clearing the detritus that had accumulated around the buckets in the holding tank. Investigators suspect that one or more factors associated with the accumulation of particulate detritus in the experimental system was important to the onset of the disease. The study results did not support the hypothesis that crowding predisposes Chinese catfish to increased prevalence of fatal infectious disease from opportunistic bacterial pathogens, fungi, protozoa or a monogenetic trematode.

Objective: Assess the antibody response of Chinese catfish held in cool versus warmer water conditions.

Two trials were conducted to determine the antibody response of juvenile Chinese catfish inoculated with an *A. hydrophila* FKC bacterin and held in cool vs. warm water conditions. The null hypothesis was that the fish maintained in the warmer water would have a greater antibody response when compared to the fish maintained in the cooler water.

The mean water temperature was 22.5°C with a range of 20.5 to 24.0°C in the cool water treatment and the mean water temperature was 29.1°C with a range of 27.0 to 30.7°C in the warm water treatment. At 28 days post bacterin inoculation, the surviving catfish in the four buckets were bled and the antibody titers determined to *A. hydrophila*. There was no measurable antibody titer in any of the serum samples from the bacterin and control groups in the two temperature treatments. Investigators suspect that the lack of detectable antibody response in the bacterin treatment groups resulted because of 1) the lower titer (2.0 x 105 CFU/fish) of *A. hydrophila* in the bacterin preparation used for the trial and 2) because the fish were not given a booster inoculation.

The second trial was carried out from February through April, 1999. Two rectangular raceway troughs (46 cm wide x 222 cm long x 14 cm deep) were used in the study. In each trough the water flow was adjusted to allow for at least 100% exchange every other day. In one of the troughs, the water temperature was heated by four Visi Therm® 150 volt, 200 watt immersion heaters. Water temperature was measured and recorded twice daily in each trough with the use of a centigrade thermometer. One hundred juvenile, 10 to 20 gram, Chinese catfish were obtained from a commercial farm for the trial. The fish were randomly stocked into the two troughs and allowed to acclimate for three weeks prior to the start of the experiment. During the acclimation period and the trial the fish were fed a commercial pelleted feed to satiation two to three times daily.

At the start of the trial the fish were divided into two groups in each trough. Each group of fish was placed into a 20 liter bucket immersed in the trough. The volume of water was maintained at approximately 9.5 liters in the buckets. The sides of the buckets were modified with multiple 1 cm in diameter holes to allow the trough water to circulate in and out of the bucket. In the warm water treatment, the immunized and control bucket groups were stocked with 26

and 31 juvenile catfish, respectively. In the ambient temperature (cool) water trough, we stocked 24 and 19 juvenile catfish into the immunized and control buckets, respectively.

The fish were immunized by an IP (intraperitoneal) injection of 0.1 ml (2.0 x 106 CFU/ml) of the formalin-killed bacterin. The controls were injected IP with 0.1 ml of 0.85% sterile saline solution. Booster injections of bacterin and saline were administered three weeks after the initial dose.

The optimal water temperature conditions for Chinese catfish are 26°C - 35°C (Klein, 1996). The findings from the trial suggest that juvenile Chinese catfish have reduced capacity to mount an antibody response when held in cooler water.

Objective: Assess bath immersion exposure of Chinese catfish to live A. hydrophila

In year five of the project, investigators conducted two trials to test the possibility that immersion exposure of juvenile Chinese catfish to live *A. hydrophila* could be applied as a practical means to induce protective immunity in juvenile catfish to Aeromonas disease.

Sixty juvenile Chinese catfish were obtained from a commercial farm for the first trial. The average weight of the catfish was 14.2 grams. The fish were divided into two groups of thirty fish each and stocked into 270 liter circular tanks with constant flow through. The fish were allowed to acclimate for one week prior to starting the trial. Thirty of the fish were placed into a 4-liter beaker that contained a 2 x 106 CFU ml-1 of live *A. hydrophila* in Trypticase Soy Broth (TSB). The immersion treated fish were treated for one hour. The remaining 30 fish (controls) were held for one hour in a 4 liter beaker filled with fresh water. The exposed fish started showing signs of stress almost immediately after being placed in the bacterial broth. After a few minutes the fish settled down to the bottom of the beaker and were very still. Forty minutes into the treatment the fish were once again very active in the beaker, breathing frequently and appeared stressed. After one hour, all the exposed fish were dead, while the control fish appeared to be fine. Following the immersion treatment, the surviving fish in the control group were replaced into their holding tank.

One week later a second trial was conducted, using the control fish from the first trial. The 30 fish were divided into three groups of 10 fish per group. These fish were immersed in the same concentration of bacterial broth as used in the first trial, but for a shorter exposure time. Group 1 was immersed for ten minutes, Group 2 for twenty minutes, and Group 3 for thirty minutes. The fish tolerated the three exposure times with no apparent signs of stress. They were returned to the holding tank and four weeks later blood samples were collected to test for antibody response to *A. hydrophila*. A control group was not used in this preliminary trial. The 30 catfish survived the shorter immersion exposure times, but measurable antibody was not detected in the blood of the fish in the three groups.

Objective: Field test a preventive strategy to mitigate losses of cultured Chinese catfish during growout in Hawaii due to two bacterial diseases, Aeromonas hydrophila *and* Edwardsiella tarda *septicemia.*

During year one, an isolate of *Aeromonas hydrophila* was propagated and used to produce an autogenous bacterin for a vaccination trial that was conducted in year two. Fifty-four days following inoculation challenge with a known lethal dose of the same strain of *A. hydrophila*, survival of the bacterin-treated fish was 53 percent compared to 24 percent for the unvaccinated control group. No antibody titer to A. *hydrophila* was measured in the saline-vaccinated control fish tested in either the day 22 or day 54 samples. The results suggest that vaccination may be a practical tool to advert or reduce *A. hydrophila*-induced mortality of farmed Chinese catfish.

An autogenous formalin-killed bacterin was prepared from *Edwardsiella tarda* isolated from diseased Chinese catfish. In February and April when water temperatures were cooler, vaccination-plus-booster-plus-challenge trials were conducted with juvenile Chinese Catfish following a similar protocol to that applied in the *A. hydrophila* study. Following inoculation challenge with a known lethal dose of the same strain of *E. tarda*, survival was low in both the vaccinated and the control groups.

The two *E. tarda* vaccination trials gave different results than the trial with *A. hydrophila*: exposure of the *E. tarda* vaccinated fish did not elicit development of a serum antibody response, and no inoculation challenge was observed. Although further study is necessary before the reason is understood for the lack of an antibody response in the experimental fish, cooler water temperature is a plausible explanation. If this is the reason, then seasonal temperature changes will be an important criteria in the administration of bacterins to Chinese catfish.

During 1998, a second trial was started to assess the effect of vaccination with a killed bacterin on the survival of juvenile Chinese catfish that are subsequently given a high titer intramuscular inoculation of *E. tarda*. One hundred juvenile Chinese catfish were obtained from a commercial farm on Oahu, transported alive to AFRC, divided into two groups and released into two 300-liter tanks equipped with running freshwater and continuous aeration. The fish were fed a commercial catfish floating pellet and observed daily. A formalin-killed bacterin with Freunds adjuvant was prepared using an isolate of *E. tarda*, which was recovered from Chinese catfish with edwardsiellosis. The catfish in one group were inoculated with the bacterin on Day One, and will be given a booster on Day 30 of the trial. On the same days, fish in the control group will be inoculated with saline.

Saprolegnia sp. and, perhaps, other aquatic saprophytic fungi cause losses of eggs and post-hatched fry Chinese catfish. The Food and Drug Administration's Center for Veterinary Medicine (CVM) classified hydrogen peroxide as a low regulatory priority compound. Hydrogen peroxide was evaluated as a treatment for fungal infection of Chinese catfish eggs and fry at two farms. Chinese catfish hatching tanks dosed at 300 to 500 ppm hydrogen peroxide for 15 minutes as a single or multiple treatments had increased hatch of catfish fry and obviously lower presence of fungal mats than untreated control tank batches. These observations indicated that hydrogen peroxide is a useful chemical control for fungal infections of Chinese catfish eggs and fry.

During year three, three preliminary experiments were conducted to test the hypothesis that elevated ammonia in Chinese catfish culture water results in the fish becoming more susceptible to lethal bacterial septicemia from *Aeromonas hydrophila*.

The first trial tested the feasibility of a static bath exposure to ammonia to find an ammonia level that, with moderate duration exposure (e.g., five to 10 days), was likely to be stressful but not lethal to juvenile Chinese catfish. Water for the trials had total hardness and alkalinity of 150 to 170 mg liter and 60 to 80 mg per liter, respectively. A 100-gallon treatment tank and a 100-gallon control tank were set up in the Anuenue Fisheries Research Center (AFRC) hatchery. Each tank was stocked with 10 juvenile Chinese catfish. One day after stocking, ammonium chloride was added to the treatment tank to achieve a total ammonia level of about 5 mg per liter. Daily thereafter for six days, total ammonia, pH and temperature were measured in both tanks. Total ammonia, pH and temperature varied from 4.0 to 5.2 mg per liter, 7.4 to 7.6 and 27 to 30 C, respectively, in the treatment tank and from 0.5 to 0.6 mg per liter, 7.2 to 7.6 and 27 to 30 C, respectively, in the control tank. The catfish in both groups fed actively and showed no difference in behavior or survival.

On Day Seven, additional ammonium chloride was added to the treatment tank to achieve a calculated total ammonia level of about 20 mg per liter. This ammonia level is at least twice as high as the highest level measured in local Chinese catfish culture systems. Total ammonia, pH and temperature level varied from 22 to 24 mg per liter, 6.9 to 7.2 and 29.5 to 30 C, respectively, in the treatment tank and from 1.0 to 1.5 mg per liter, 6.9 to 7.2 and 28 to 29.5 C, respectively in the control tank. The fish were held for four days under these water conditions with no apparent difference between the control or the treatment groups.

Water in the two tanks was exchanged, and the temperature allowed to stabilize for 24 hours. Sodium carbonate was added to increase the pH in both the treatment and control tanks. The pH varied between 8.9 and 9.6 over a period of two days in the two tanks. Fish in both tanks displayed no adverse behavior and fed well. Ammonium chloride was added to the treatment tank to achieve an estimated 5 mg per liter of total ammonia. The fish remained active over the four-day test period in both the control and treatment tanks. The pH level varied from 8.5 to 8.9 during this period, but total ammonia began to decline. Nitrite and nitrate were found to be elevated in the treatment tank water.

Both tanks were drained, cleaned, filled and restocked with the juvenile catfish. After one day of acclimation, sodium carbonate was introduced to each tank, and, after several hours, ammonium chloride was added to achieve about 10 mg per liter total ammonia in the treatment tank water. The fish were held under these conditions for several days, and

then the trial was terminated. Total ammonia and pH remained elevated in the treatment tank through the exposure period with no apparent adverse effect on the fish.

The results of the trials suggested that Chinese catfish could tolerate moderate duration exposure to total ammonia of about 10 mg per liter in water with pH of 8.5 to 9.0 and temperature of 28 to 30 C. This would result in the fish being held in water with an unionized ammonia concentration in the approximate range of 2 to 4 mg per liter. The level generally regarded as safe is 0.02 mg per liter for prolonged exposure to unionized ammonia in freshwater fish. This protocol subjects the experimental fish to about 100 times the safe level for a moderate duration, which should be sufficiently high to elicit a stress-induced effect on the immune function of Chinese catfish, if ammonia has this effect on this species of fish.

Preliminary studies to test the protocol for injection exposure of Chinese catfish to a predetermined concentration of the bacterium, *Aeromonas hydrophila*, demonstrated that an inoculum of about 105 viable bacteria per fish can be delivered for the bacterial challenge aspect of the study.

In year five of the project, a survey was conducted to determine if Chinese catfish in commercial production systems had circulating antibodies to *Aeromonas hydrophila* and *Edwardsiella tarda*. The null hypothesis was that the catfish did not have circulating antibodies to *A. hydrophila* and *E. tarda* and this was an important reason why farm raised fish periodically suffered disease episodes from these bacterial pathogens. The survey showed that the antibody to the bacterial pathogens was detected in some of the Chinese catfish sampled from the three farms. The findings clearly refute the null hypothesis and demonstrated that farm-raised catfish may have antibody titer to one or both of the two bacteria. Antibody titers were observed in juvenile through the adult life stages of the fish and differences were found for the prevalence of catfish with antibody titers between the three locations.

Investigators felt that the differences reflect the variability in the exposure of groups of catfish to the two organisms due to differing farm and batch specific culture circumstances. For example, the fish sampled from Farm 1 were collected during a clinical outbreak of *edwardsiellosis* on the farm. In that case 88% of the fish sampled had measurable antibody to *E. tarda*. On the other hand, none of the 30 fish sampled and tested from farm 3 had an antibody titer to *E. tarda*. Of importance here is that we have never diagnosed *edwardsiellosis* from fish sampled from farm 3.

*Objective: Screen juvenile and adult cultured Chinese catfish and tilapia (*Oreochromis mossambicus*) for potential pathogenic fish viruses.*

During year one, 60 tilapia of various ages and sizes were collected from five Oahu locations for a virus isolation study. Evaluation of various organ tissues revealed no viruses. These results support findings from previous tilapia disease cases on Oahu. Both wild and cultured tilapia (*Sarotherodon melanotheron* and *Oreochromis mossambicus*) populations have been afflicted by a previously unrecognized syndrome that causes high mortalities and has negatively affected production at several Oahu farms. Analysis of dying tilapia from various Oahu sites suggests that the cause is an intracellular rickettsia-like organism (RLO).

Dead and dying Chinese catfish from an Oahu farm that has a history of chronic disease problems were evaluated by cell culture methods for viruses. Evaluation of various organ tissues showed no evidence of an infectious virus.

During year two, about 300 juvenile to adult tilapia collected from five locations on Oahu were tested for pathogenic viruses. The results indicate that pathogenic viruses are not obviously present in cultured populations of tilapia on Oahu.

However, efforts under this objective were re-focused after the outbreak of a new disease observed in both wild and cultured tilapia populations on Oahu. *S. melanotheron* and *O. mossambicus* juveniles to adults were found susceptible to the disease, which has resulted in a high rate of mortality of fish populations. The disease has negatively affected production on the majority of commercial tilapia farms on Oahu.

Pathologically, tilapia affected by this syndrome have multiple, large pyo-granulomas systemically. An intracellular bacteria-like organism was identified by special histological stains and in transmission electron microscopy

preparations of lesions from affected fish. Attempts to culture the organism on a variety of artificial bacterial media have been unsuccessful, as have attempts to grow the intracellular organism in cell culture.

Work on this new tilapia disease revealed the causative agent to be a species of rickettsia. The Hawaiian tilapia RLO is the most important disease facing tilapia farmers on Oahu because of the disease's potential to cause high mortalities of fish. Further work on the agent and the disease are detailed below.

Objective: Investigate the cause and control of the rickettsia-like organism (RLO) infection of cultured tilapia in Hawaii.

At warmer water temperature, RLO apparently occurs in tilapia as a subclinical infection. With the present diagnostic methods available for this agent, it is difficult to know if asymptomatic tilapia are carriers or free of the RLO organism. A trial was completed to assess the effect of corticosteroid administration on the expression of RLO disease in tilapia. Investigators obtained 25 tilapia from a population believed to have subclinical infection by RLO. The fish were divided into two groups of 12 and 13 fish and were stocked into two tanks. The tanks were set up with a continuous flow-through freshwater and aeration. The fish were acclimated for one week prior to the start of the experiment. Statistical relationships were determined for some of the data collected in the trial using the statistical program, StatView™ 4.0. Comparison of the means were preformed by paired T-test.

The mean±standard deviation (S.D.)was 35.0±13.31 grams and 29.7±14.26 grams and not statistically different (P=0.48) for the weight of the tilapia in the treatment and control groups, respectively, in the trial. On day one the tilapia in the treatment group were administered 20 mg/kg methylprednisolone [Depo-Medrol®] by intramuscular injection. The tilapia in the control group were administered a similar volume of sterile 0.85% saline by intramuscular injection. The trial was carried-out for 15 days during which time the groups were monitored for signs of morbidity and mortality. The temperature was measured daily in each tank in the morning. The mean±standard deviation for the water temperature measured values was 21.28±0.50°C and 21.0±0.40°C (P=0.02) for the treatment and control tanks, respectively.

On day 15 all of the fish were killed with an overdose of MS-222 anesthetic, given a necropsy examination and tissues were processed by routine histopathology methods. The results for the trial suggest an increase in the activity of RLO infection in the methylprednisolone-exposed group when compared to the control group during the 15 day period of observation. There was a 23% mortality in the depo-treated group, and no losses in the control batch of fish. The prevalence of fish with gross skin sores and/or changes in the spleen (multifocal granulomas and/or enlargement of the spleen) was approximately 30% higher in the depo-treatment group when compared to the control group. The mean microscopic count of granulomas in the spleens was about 1.8 times greater in the depo-treated group as compared to the control group.

The findings support the conclusion that immunosuppression is probably an important feature in the expression of RLO disease in tilapia. Further, prior treatment with a corticosteroid could be applied to enhance the activity of the RLO in tilapia and may be useful as an approach to improve detection of RLO in batches of tilapia in regions where RLO is endemic.

Objective: Assessment for RLO Infection and the Sensitivity to RLO Challenge of a Group of Juvenile Tilapia From the Waianae Coast Community Alternative Development Corporation (WCCADC), Waianae, Oahu

Over the past decade tilapia have been produced in tanks at the Waianae Coast Community Alternative Development Corporation (WCCADC) and distributed to participating community members on the Leeward Coast of Oahu. There has never been a reported or documented case of RLO disease in the tilapia grown in the WCCADC facility. Studies were undertaken with tilapia from WCCADC to achieve the following two objectives: 1) assess tilapia from the WCCADC for infection by the tilapia RLO, and 2) determine the susceptibility of juvenile tilapia from WCCADC to RLO challenge.

Thirty juvenile tilapia were obtained from a tank at the WCCADC and transported live to Anuenue Fisheries Research Center, Sand Island. The tilapia were anesthetized in MS-222, killed by severing the spinal cord at the base

of the skull, visually examined for skin sores or other external lesions, the abdomen was dissected, the spleen examined, removed and placed in Davidson Fixative. The spleens were processed by routine histopathology methods. The slides were stained with hematoxylin and eosin (H&E). Skin sores, gross changes in the spleen or granulomas were not found in the 30 fish.

A second group of 40 juvenile tilapia was obtained from another tank group and brought to AFRC for evaluation. The animals were divided into two groups of 20 fish and stocked into two tanks. The tanks were set up with a continuous flow-through of freshwater and aeration. The morning water temperature averaged 21.0±1.0°C during the trial. The fish were fed a commercial pelleted fish feed daily during the course of the trial. The fish were acclimated for one week prior to the exposure to RLO. Five adult tilapia from a tank at AFRC in which RLO infection was known to occur were placed into one of the tanks. This tank group was designated as the "exposed" group. The two groups of fish were maintained for 51 days of observation. Moribund and dying tilapia were removed daily and suitable specimens were preserved in Davidson Fixative. On day 51, all of the tilapia were removed from the two tanks, anesthetized in MS-222, killed, necropsied and tissues collected and preserved in Davidson Fixative. The tissues were processed and prepared as described previously. At the termination of the trial, the mean±S.D. body weight was 26.9±14.08 grams for the tilapia in both groups.

Tilapia mortality started on day 15 from exposure to the RLO carrier fish in the exposed group of tilapia. The mortality was 60% in the RLO-exposed group and 0% in the control group of tilapia. By histopathology, 100% of the moribund or dead tilapia (n=8), collected and preserved from the exposed group during the trial, were found to have numerous granulomas in the spleen and other organs. Of the eight surviving tilapia on day 51 in the exposed group, 100% of the fish had moderate to abundant granulomas in the spleen. None of the 20 tilapia in the control group had granulomas in the spleen or other organs examined microscopically. Five of five (100%) of the original, AFRC, RLO carrier tilapia survived through the course of the trial. All of these fish were found to have RLO-type granulomas in the spleen.

The findings from the study of the tilapia from the WCCADC project indicated that 1) the tilapia tested from the WCCADC tanks were free of the tilapia RLO, and 2) the strain of tilapia grown at WCCADC is susceptible to high morbidity and mortality, when exposed to the tilapia RLO, when held at cooler (<24°C) water temperature

Objective: Evaluate the effect of low temperature on expression of RLO disease.

In this trial, juvenile tilapia that had been raised in warm water were split into two sub-populations, and 250 fish were stocked in each of two aquaria placed in different locations at the Pacific Discus Hatchery in Kaimuki. One aquarium held warm water, and the other held water that was chilled by exposure to strong trade winds. The group was split into two sub-populations, and temperature data loggers were placed in both aquaria. Fish were fed a commercial pelleted ration.

Water temperatures averaged 24.45 1.01 C in the treatment cold water group and 26.96 1.01 C in the control warm water group. For the first nine days of the trial, water temperature in the control tank fluctuated between 26.5 C and 29.2 C on most days, and the water temperature in the treatment tank fluctuated between 21.5 C and 26.3 C. On day 15, the first mortalities were noticed in the treatment tank; they progressed from then, doubling almost daily. The trial was terminated on Day 24 with 4 percent survival of the tilapia stocked in the treatment cold water tank. No tilapia died in the control tank although the water temperature dropped on Day 11 of the trial. RLO disease in fish sampled from the treatment tank on Days 15 and 21 was confirmed by histopathology. Fish sampled from the control tank on Day 21 did not exhibit histopathology lesions diagnostic for RLO disease despite the tank's exposure to a low temperature period. The results support the hypothesis that exposure to low water temperatures for more than 1 week is a permissive factor for expression of RLO disease in tilapia. The group of fish used in the experiment originated from a location where RLO is believed to be endemic in tilapia populations and apparently were asymptomatic carriers of the RLO agent. RLO disease expression occurs when the fish are exposed to a stressor. Future studies to control the RLO status of asymptomatic populations of tilapia can be designed using exposure to a period of low water temperature as the permissive stressor for the expression of RLO disease.

A population of 50 juvenile tilapia were received from a Molokai farm and stocked into an outdoor, flow-through tank at Anuenue Fisheries Research Center. The fish were observed for mortality and assessed for RLO disease for 93

days. Daily water temperatures varied between 21 and 25 C, and fish were fed a commercial trout chow. On Day 50, 12 larger tilapia from Nuuanu reservoir were stocked into the tank with the Molokai fish. The tilapia in Nuuanu reservoir are known to be free of RLO and highly susceptible to RLO disease. The Nuuanu fish were stocked into the tank to give an additional means to detect the RLO agent. Mortality of tilapia was negligible in the tank over the entire observation period. On the day the fish were received, the spleens of 10 fish in both the Molokai group and the Nuuanu group were collected and examined histologically. Histological evidence of RLO disease was not found in any fish from either group. The results indicate that the RLO agent was not present in the tilapia submitted from the Molokai farm.

Objective: Provide diagnostic and health management support to the CTSA-funded project titled "Ornamental Aquaculture Technology Transfer," and implement management practices and standard disease treatment strategies to improve fish survival and reduce the abundance of pathogenic parasites in imported groups of freshwater tropical aquarium fish.

During year one, eight groups of freshwater tropical fish, imported for the CTSA-funded project titled "Ornamental Aquaculture Technology Transfer," were evaluated and found to be free of diseases.

During year one, diagnostic assistance was provided to three farmers who were losing fry and juvenile discus (*Symphysodon discus*) stock. Parasite and water quality problems were found. Parasite treatments were suggested and solutions to the water quality problems were recommended. Water quality monitoring data on temperature, dissolved oxygen, pH, carbon dioxide, alkalinity, hardness, chloride, total ammonia, nitrite and nitrate are being gathered at one of the ornamental fish farms. The data will help in determining appropriate water quality parameters for tropical fish culture in Hawaii.

During year two, 13 diagnostic case submissions were processed from the Ornamental Aquaculture Technology Transfer project. Diagnostic assistance was provided for 26 case submissions of freshwater and marine aquarium fishes. Parasites and water quality problems were found, and recommendations were provided for treatment and improving the environmental conditions in the holding tanks or aquaria. During year three, project personnel made 158 site visits to 15 farms. Forty-five of the 326 case submissions (14 percent) processed for laboratory diagnosis comprised tropical ornamental fish.

During year five, 457 case submissions were received and processed for necropsy, bacteriology or histopathology examination. Of these cases, 114 submissions (25%) made up of 1138 animals, were ornamental aquatic animals (predominantly fishes). Five were processed for electron microscopy evaluation.

A study was conducted to assess the efficacy of oral treatment with praziquentel HCL for elimination of the Asian tapeworm (*Bothriocephalus* sp.), which was found in adult live-bearers imported by a commercial farm. Thirty adult gold-and-black tuxedo swordtails from an imported group held in a tank at a commercial farm were obtained for the treatment study. The fish were divided into two groups of 15 and stocked into separate buckets, which were placed in a larger holding tank. The buckets were supplied with continuous flow-through freshwater and aeration. The water level in the holding tank was held at 2 cm below the level in the test buckets, and the water temperature ranted between 23 and 24 C. Dr. Harry Ako of the University of Hawaii prepared a test diet by grinding Feline Droncit tablets into a fine powder and adding it to Nutra diet #0 at a rate of 400 mg per 100 grams of feed. The tuxedo swordtails in on budget were fed the medicated feed once a days for 10 days, while the control group was fed the Nutra diet without medication for the same period. Each group of fish were examined daily, and dead fish were removed and necropsied. The trial was terminated 48 hours after the last application of feed on Day 10. At termination of the trial, all fish were necropsied; their entire intestinal tracts were removed and examined for tapeworms under the light microscope. Two fish in the Droncit treatment group were found dead during the study (day 0 and day 4). An Asian tapeworm was recovered from the intestine of one of these two fish. Asian tapeworms were not found in the intestine of any of the 28 fish examined at the end of the trial. Thus, the level of infection by Asian tapeworms in the study animals was insufficient to allow for a meaningful assessment of the efficacy of Droncit for tapeworm elimination. The study must be repeated using fish with a higher prevalence of tapeworm infection.

During year five of the project, a study was conducted to test the hypothesis that the *Henneguya* sp. might be capable of infecting gouramis by fish to fish horizontal transmission. *Henneguya* sp. is a genera of myxosporidian

parasite that has many species that infect fish in the wild. Juvenile gouramis (*Colisa* sp.) in two shipments imported into Honolulu from Asia were found by histopathology examination to have moderate to heavy infection by what appeared to be an intralemallar (capillary) form of *Henneguya* sp. The disturbing features of these cases were the high prevalence of the *Henneguya* sp. cysts and the heavy mortality in the two imported groups of gouramis.

Although *Henneguya* sp. is known at times to cause high mortalities of fish, there appears to be scant information on the life cycle of this parasite. It was unclear to us if the heavy infection by *Henneguya* sp. was the result of contamination before the fish were shipped or occurred during and after (direct horizontal transmission) the fish were shipped.

Ten juvenile gouramis were donated to the study by Brian Cole, Windward Community College Sea Grant College Program, the University of Hawaii. The fish were divided into two groups of five fish and stocked into two 270 liter circular tanks with continuous aeration and water exchange. Four juvenile gouramis were obtained from the population with the *Henneguya* sp. infection of the gills. These fish were placed into a screen enclosure setup in one of the two circular tanks. The fish in the trial were fed a commercial pelleted fish feed. The period of exposure was maintained for 33 days. Fish were observed daily and the observations recorded. At the termination of the trial, all of the surviving fish were preserved in Davidson Fixative and later the gills and other organs were prepared and processed by routine histopathology methods. The tissue sections were stained with hematoxylin and eosin. During the 33 days of the trial, three of the four gouramis died in the imported, *Henneguya* sp. infected group. None of the five gouramis in the exposed and non-exposed tanks died. Histologically, the single remaining imported gourami had a heavy infection of the gills by *Henneguya* sp. None of the five gouramis in the exposed group or the five gouramis in the control tank group had microscopic evidence of infection by *Henneguya* sp.

The results of the trial suggest that the imported gouramis arrived in Honolulu with the heavy and often fatal infection by *Henneguya* sp. Furthermore, there was no indication found that horizontal transmission by *Henneguya* sp. occurred in the exposed group of gouramis.

Objective: Document the principal ectoparasites and assess their effects on cultured tilapia and mullet in a traditional Hawaiian fishpond.

Two species of tilapia (*O. mossambicus* and *S. melanotheron*) and mullet (*M. cephalus*) were collected from cages or net pens in Heeia Fishpond, a traditional Hawaiian fishpond, and examined for ecto-parasites in histological preparations. A total of 250 tilapia, most of which also were examined for infestation levels of *Caligus* sp. and *Neobenedia melleni* and 46 juvenile mullet were evaluated in the study. Tilapia exhibited infestation or infection with *Caligus* sp., *Neobenedenia melleni*, *Trichodina* sp., and *Scyphidia* sp. Mullet exhibited ectoparasite infestation or pathogenic infections with digenetic trematode metacercaria, Epitheliocystis, *Trichodina* sp., *Scyphidia* sp., *Myxobolus equisquamalis*, and *Eimeria* sp.

Objective: Assess samples of Gracilaria spp. for the presence of Gracilaria Gall Syndrome, determine how the syndrome is transmitted, and identify potential chemical controls for it.

During year one, a cooperating commercial seaweed farmer constructed a greenhouse for on-site experiments with infected *Gracilaria*. A series of observations on the farm led to the suspicion that the fresh water might be a potential source of GGS. In March, a two-month experiment was undertaken to compare the onset and severity of GGS in *Gracilaria* exposed to different freshwater treatments. Two replicates were done of each of the following experiments:

seaweed held in seawater and rinsed in unsterilized freshwater every three days;
seaweed held in a mixture of 80 percent seawater and 20 percent unsterilized freshwater;
seaweed held in a mixture of 80 percent seawater and 20 percent UV-sterilized freshwater;
seaweed held in full strength seawater with no exposure to freshwater.

The effect of adding penicillin to seawater containing GGS-positive seaweed was tested. Preliminary observations suggest that penicillin reduces or eliminates GGS symptoms, which supports previous tests in flask cultures of GGS-positive *Gracilaria*. This finding implies that a bacterial agent may cause GGS.

In Year Two, further experiments at Hawaiian Marine Enterprises showed that the farm's fresh water supply was not a factor in GGS and that the trace nutrient solution used in seaweed culture was not a GGS contaminant. A series of trials were carried out to determine *Gracilaria* sp.'s tolerance level to each of seven fisheries therapeutants and the efficacy of the chemicals to control clinical GGS.

During the follow-up chemical treatment trials, the HME staff noted that GGS-afflicted seaweed in both chemically treated groups and untreated control groups in test aquaria recovered. Meanwhile, *Gracilaria* grown in the commercial culture tanks on the farm remained afflicted with GGS. This finding, confirmed repeatedly, led the investigators to think that some condition in the greenhouse had a curative effect on GGS. Further experiments led to the hypothesis that constant circulation in the aquaria seemed to have curative effect on GGS-afflicted seaweed.

The commercial seaweed culture tanks were aerated for 12 hours per day. As a result of the experiments, the air blowers in the commercial farm tanks have been run continuously with good results. This indicates that this physical factor can effectively control GGS on this farm. However, another land-based *Gracilaria* farm continues to have GGS outbreaks despite the use of constant aeration.

Impacts

This project assisted producers and research groups to understand the cause of diseases in groups of cultured ornamentals, food fish and marine crustaceans. Recommendations were provided to producers and researchers for the control of disease episodes, which resulted in more effective management of disease problems.

This work has had eight principal impacts to date. The first was the demonstration that Chinese catfish respond to vaccination with a formalin-killed preparation of *Aeromonas hydrophila*. This opens the possibility of using vaccination as a means to mitigate disease from *A. hydrophila*.

The second impact was the demonstration of the usefulness of hydrogen peroxide as a means to control fungus infections of eggs and fry and as a chemical control agent for the ectoparasites *Trichodina* sp. and *Gyrodactylus* sp.

The third impact was the discovery that the new tilapia mortality syndrome is caused by a rickettsia-like organism (RLO). The identification of the etiological agent for the disease will help in developing practical control strategies.

The fourth impact was the discovery that Taura Syndrome is caused by a virus. Since the disease was first recognized in Ecuador in 1992, Taura Syndrome was believed to be caused by exposure to banana fungicides. This project, working in cooperation with Dr. Donald Lightner at the University of Arizona, was first to demonstrate that Taura Syndrome was caused by an infectious agent, which was a small virus. Work in several laboratories has confirmed this. This discovery has fundamentally changed the way many shrimp farmers in the Western Hemisphere view Taura Syndrome.

The fifth impact was the experimental determination and later field confirmation that *Penaeus stylirostris* is relatively resistant to disease impacts from Taura Syndrome. This finding led to the use of *P. stylirostris* at a shrimp farm in Kahuku, Hawaii, and the subsequent demonstration of the alternate species approach as a practical option for controlling Taura Syndrome.

The sixth impact was the discovery that continuous aeration reversed the progression of GGS in afflicted seaweed. This discovery led to the change to continuous aeration in the commercial culture tanks on the farm and, to date, an absence of re-occurrence of GGS on the HME farm site.

A seventh impact was the determination that the horizontal transmission of *Henneguya* sp. indicated a low risk of fish to fish transmission in the captive fish held in isolation tanks.

The eighth impact was the indication of strategies for the prevention and control of *Amyloodinium* sp. disease in farmed moi. For *Amyloodinium* sp. the deliverables from the project were the development of a gill biopsy procedure

for monitoring subclinical infection by *Amyloodinium* sp. and the demonstration that low light conditions are likely to be an effective means for prevention of OD in farmed moi.

Work Planned

During year six of the project, investigators plan to:

continue providing diagnostic support for the CTSA-funded ornamental fish projects, commercial hatcheries and farms;
investigate the role of contributing factors and identify control options for the rickettsia-like organism disease of tilapia in Hawaii;
produce an operational manual for the application of the probiotic on shrimp farms in Hawaii;
assist the high health shrimp producers in Hawaii acquire additional family lines of high health shrimp;
determine the efficacy of hydrogen peroxide as a treatment for the control of columnaris disease;
continue work on vaccination protection for control of bacterial diseases in cultured Chinese catfish;
develop extension publications reporting project results.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), the University of Hawaii (UH), the Hawaii State Aquaculture Development Program (ADP) and Sea Grant Extension Service (SGES).

		Other Support				Total
Year	CTSA	UH	ADP	SGES	Total Other	Support
1	$41,638	$15,988	$5,329	$0	$21,317	$62,955
2	$68,116	$10,658	$5,329	$0	$15,987	$84,103
3	$49,916	$13,323	$5,329	$0	$18,652	$68,568
4	$49,989	$13,323	$7,600	$1,000	$21,923	$71,912
5	$66,451	$13,323	$5,329	$0	$18,652	$85,103
6	$74,962	$13,323	$5,329	$0	$18,652	$93,614
Total	**$351,072**	**$79,938**	**$34,245**	**$1,000**	**$115,183**	**$466,255**

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Expansion and Diversification of Freshwater Tropical Fish Culture

Dates of Work

October 1997 through June 1999

Funding Level

$260,000

Participants

Dr. Clyde Tamaru, Brian Cole, Richard Bailey, Sea Grant Extension Service, University of Hawaii
Dr. Christopher Brown, Hawaii Institute of Marine Biology, University of Hawaii.

Objectives

The overall goal of this three-year project, initiated under the CTSA Ninth Annual Plan of Work, is to demonstrate to Hawaii aquaculturists the feasibility of ornamental fish production as a viable alternative cash crop. Specific objectives related to that goal are to:

expand production and distribution of tropical fish species and add new farms to production in Hawaii;
operate an incubator hatchery for the production of 2-week-old larvae of selected egg-layers for distribution to Hawaii farmers;
expand technical assistance to more demonstration farm sites and include small-scale commercial breeders to increase production and diversification of species;
expand the current number of farms/individuals commercially producing freshwater ornamentals and diversify tropical fish culture by incorporating an additional five species into the inventory being supported by CTSA;
collaborate with the private sector and the state aquatic veterinarian on the importation of additional fish species by providing facilities and maintenance during the quarantine period; during this period, diagnostics to screen the fish for pathogens will also be provided;
introduce production and marketing infrastructure scenarios to tropical fish growers;
conduct extension activities in the form of three technical workshops, site visits, verbal consultations and literature to support development of additional Hawaii farms culturing freshwater tropical fish;
confirm stocking densities for at least two ornamental species that result in optimal growth and survival;
demonstrate out of season maturation and spawning of at least one freshwater ornamental fish species (i.e., rainbow or redtail sharks);
demonstrate optimal stocking densities of one ornamental fish species in a commercially available recirculating system and determine economic feasibility;
complete a paper study of aquatic ornamental plants that hold potential for culture in Hawaii.

Anticipated Benefits

Supporting the expansion and diversification of tropical fish culture in Hawaii is consistent with Hawaii's long-term strategy for economic diversification through fostering the development of aquafarms that can produce commercial quantities of various ornamental fish species. Hawaii has an ideal environment for this type of aquaculture activity with year-round warm temperatures, soft and slightly acidic water. In addition, the culture of ornamental fishes appeals to a much broader audience as opposed to the culture of food fish because of the relatively smaller sizes of fishes and consequent smaller water volumes required for ornamental species.

Production of egg-laying tropical fish species has been hampered by lack of a hatchery from which farmers can obtain seedstock. Operation of a hatchery will allow the project to produce sufficient quantities of fry for farmers to gain experience in growout, grading, sorting, transporting and marketing commercial quantities of these animals.

Providing technical assistance to farmers is the main focus of this project. Technical assistance for those involved in this fledgling endeavor will ensure that the industry gains a firm foothold. Training farmers in disease management for these animals is an important aspect of this technical assistance.

During this reporting period, work was completed on year two and begun on year three of the project.

Principal Accomplishments

Objective: Operate an incubator hatchery for the production of 2-week-old larvae of selected egg-layers for distribution to Hawaii farmers.

Project personnel successfully establish broodstock of tinfoil barbs, rainbow sharks, albino rainbow sharks, tiger barbs and blue and gold gouramis at the project facility at Windward Community College. Maturation of selected broodstock species was monitored, and mature individuals were either induced or conditioned to spawn. Three species of egg layers – rainbow sharks, red tail black sharks and tinfoil barbs – achieved gonadal maturation during the reporting period. These species require hormonal induction of final maturation and spawning of broodstock. Tiger barbs and gouramis, which are characterized as temporary paired tank spawners, can be conditioned to spawn. Maturation drops sharply in October and November and does not appear to resume until March. This indicates that these species exhibit discrete spawning seasons, which would mean that farms culturing these species would also exhibit seasonal production. Farmers would find the ability to control maturation and spawning in order to produce fish on demand advantageous. Technology to do so would provide farmers with the means to take advantage of seasonal fluctuations in the market. As a first step toward developing such technology, project personnel monitored environmental parameters, including water temperature and day length, which influence maturation and correlate them with seasonal maturation data for rainbow sharks, red tail black sharks and tinfoil barbs. The correlation clearly shows the day-length and water temperature at which the percentage of mature animals increases.

Objective: Expand technical assistance to more farm sites and include small-scale commercial breeders to increase production and diversification of species.

A series of disease management workshops was conducted in collaboration with Dr. James Brock. The workshops were held at the Cooperative Extension Service facility in Hilo and at the University of Hawaii at Hilo campus and attended by 85 individuals. The workshops, which repeated those held on Oahu during Fall 1996 under the "Ornamental Aquaculture Technology Transfer" project, were titled:

Overview of Principal Diseases, January 4, 1997;
Use of the Microscope for Disease Diagnosis, January 18, 1997;
Environmental Agents and Water Quality Factors, February 1, 1997;
Disease Control, February 15, 1997;
Use of the Microscope for Disease Diagnosis, March 1, 1997.

Interviews with producers indicated several areas in which technical assistance could be provided to improve overall productivity. The first area was that of which feeds were best for these ornamental species. Very little information is available regarding nutritional requirements of ornamental fishes, so project personnel collaborated with small-scale commercial producers to determine which commercial feed for food fishes would be suitable for use with ornamental fishes. All tests were completed during the reporting period and the results were printed in a series of Honolulu Aquarium Society monthly newsletters.

A series of experiments compared a mahimahi feed, a salmon feed and a standard flaked feed. Results demonstrated that both the mahimahi feed and the salmon feed, which had feed conversion ratios of 0.8 each, were superior for growing marble angelfish and golden angelfish and are 10 times cheaper than the flake food tested, which had a feed conversion ratio of 1.3. In addition, a palatability test, designed by Dr. Harry Ako, was conducted to determine whether the aquaculture feeds obtained superior results because fish preferred their taste. The five-day test carefully records the amount of one feed eaten by a group of fish in comparison to other feeds. Results indicated that the fish preferred the mahimahi and salmon feeds to the flake feed.

Tests were also conducted with the ornamental variety of carp, known as *koi*. These tests indicated that the koi preferred Laguna Supreme Formula and Hikari Gold feeds, which produced about three times the increase in fish length and in weight gain over the other feeds tested. This supports the hypothesis that more palatable feeds should yield faster growth than less palatable feeds.

Tests were conducted to assess the strengths and weaknesses of three flake feeds, Tetramin, Tri-Flake and Black Gold. The study used emperor tetra, guppies and tinfoil barbs to test the feeds. Results indicated that the flake feed preferred by guppies (Tetramin) does not result in superior growth. Tri-Flake was found to support the greatest gain in body weight, but interestingly, only for female guppies. The results on palatability and resulting growth differs from that reported for koi, but the nutritional quality of the feeds used in that study were similar. Once again we see a familiar pattern emerging with a more palatable feed not necessarily being the most appropriate feed for supporting growth. The results are consistent with the overall goals of a major feed manufacturer for hobbyists as the delight in watching fish feed with gusto is one of the main highlights of raising fish. From an aquaculture standpoint, the most palatable feed may not necessarily mean that it is the best feed for commercial production.

Additional tests examined growth and survival of guppies fed a commercial salmon fry feed, a commercial trout chow, a grower's mix consisting of spirulina, trout chow, Lansy, mahimahi feed and egg yolk, and a pureed beef heart, beef liver and garlic custom mixed feed. Those fish given the beef-heart-and-liver mix had a significantly lower survival rate than those given the other three feeds. The lower survival rate resulted in large part from the poor water quality resulting from use of this feed mix. Feed conversion ratios for the first three feeds ranged from 0.6 to 0.7; an estimated feed conversion ratio for the beef-heart-and-liver mix was not obtained. The two commercial feeds were much lower in price. The results suggest that use of commercial feeds would yield good results at the lowest cost to the producer.

Feeds used in maturation and spawning of discus, angelfish, guppies, goldfish, gouramis and barbs were examined for total and essential fatty acids. Investigators were looking for similarities in the feeds' nutritional profiles. The feeds examined were live earthworms, mosquito larvae, *Moina*, a beef heart preparation, black tubifex worms and red tubifex worms.

The results strongly suggest that a particular fatty acid plays an integral role in the reproductive mechanism of a large number of ornamental fishes; interestingly, this fatty acid is different from that required by marine fishes. Several workshops and short articles on the findings about feeds were prepared. Workshops titled "Ornamental Feeds," were presented to the Goldfish Club of Hawaii in Honolulu on September 15, 1996; to the Hawaii Tropical Fish Farmers Association in Hilo on October 5, 1996; to the Backyard Aquaculture Hui on Maui on November 23, 1996; and to the Honolulu Aquarium Society on April 4, 1997.

Another feeds study investigated the growth rate of *Moina* using "green water" as the source of the food. *Moina* is a frequently used food source in freshwater ornamental fish culture popular due to its size (males 0.6-0.9 mm, females 1.0-1.5 mm) and ability to be produced in relatively large quantities. Results indicate that the population in culture doubles within a 24-hour period, which makes this a suitable organism for use as a live feed. The study also showed that nutritional content of *Moina* was significantly improved by the addition of yeast and Algamac-2000. What remains to be determined is whether boosting the fatty acid profiles offers an advantage (growth, survival, stress resistance, reproduction) in freshwater ornamental fish culture.

An experiment was conducted to examine whether *Moina* can substitute for *Artemia* nauplii as an initial live food organism. The experimental design provided emperor tetra larvae (2-3 days post-hatching) with screened (250 µm nytext) *Moina* and examined if any fish larvae survive. In all cases, larval survival was above 70% and the overall average survival for all three trials was above 80%, which is no different than that obtained using *Artemia* nauplii, demonstrating that *Moina* can substitute for brine shrimp nauplii when rearing emperor tetra larvae. It remains to be determined how many other species would show similar results. One clear advantage of the use of *Moina* over that of brine shrimp is that the *Moina* do not die after you stock them with the larvae. If they are not eaten they will remain in the tank and filter the water and apparently can survive and grow along with the larvae. When the larvae get large enough however, they are quickly eliminated from the rearing tank and serve as a cue that the fish would like to eat something bigger.

On June 5, 1997, investigators presented a workshop on ornamental fish culture at the University of Guam PEACESAT station. The workshop, which reached 51 PEACESAT stations in 22 countries, was a two-way, interactive audio broadcast. In addition, investigators conducted site visits and evaluations in Guam, Pohnpei, FSM, and Majuro, RMI. Only Majuro proved unsuitable for ornamental fish culture because freshwater resources and the transportation infrastructure are limited.

Investigators made two oral presentations at the World Aquaculture Society (WAS) meeting in February 1998 in Las Vegas.

Project personnel collaborated with the state aquatic veterinarian to obtain 17 -methyltestosterone that will be used in feed to masculinize fish. Requests for the masculinizing feed were received during the reporting period and is a service to be continued.

Objective: Conduct extension activities in the form of technical workshops, site visitation, verbal consultations and providing literature in support of developments of additional farms geared towards the culture of freshwater tropical fish in Hawaii.

Providing technical assistance is a major activity of the project and from feedback throughout the aquaculture community this is one of the most valued services being provided by the project.

Between the reporting period of November 1997 thru December 1998 a total of 807 requests for technical assistance were responded to by the CTSA funded ornamental fish specialist. Request for technical assistance came in the form of verbal consultations (456), written material (274) and site visits (77). A total of 429,382 fry and broodstock were distributed to participating farmers.

In addition, a manual covering packing and shipping practices was completed and printed using funds from a previously supported project. The manual was then used as a focal point to conduct an additional three workshops held in collaboration with the Sea Grant Extension Service, Aquaculture Extension Network. Project personnel also gave presentations at the Honolulu Aquarium Society on rotifer culture and new feeds and how to evaluate them.

Species obtained under the auspices of previous projects were used for demonstration activities in another series of workshops. These workshops were a collaborative effort between CTSA, SGES, and ADP, and focused on providing hands on training on induction of spawning techniques that were also implemented during the reporting period. Resulting fry (225,000 tinfoil barbs and 200,000 rainbow shark fry) were distributed to workshop participants.

In addition to the shipping manual, two other technical manuals were printed and distributed during the reporting period covering the topics of *Artemia* enrichment and the commercial production of the Gourami. Two additional manuals, *Spawning and production of the Serpae tetra*, and *Spawning the tinfoil barb,* Barbodes schwanenfeldi *in Hawaii* were completed and will be used for a series of workshops being planned for the upcoming reporting period.

In response to a request by the editor of *International Aquafeed*, project work group members produced a summary article about commercial feeds for use in the aquarium trade.

A complete listing of articles published and presentations made can be found in the section *titled Publications, Manuscripts and Papers Presented* in this report.

Objective: Expand the number of farms commercially producing freshwater ornamentals and diversify tropical fish culture by incorporating five additional species into the inventory supported by the project.

During the period from October 1, 1997, through March 31, 1998, investigators surveyed 75 industry members to determine what additional species should be considered. The suggested species were

1. *Serpae tetra* (long fin variety)
2. Lemon tetra

3. High fin red or red wag platy
4. Ram Cichlid
5. Lyretail swordtail

The species selected represent either a new category of life history/production scenario not covered during previous projects or an elevation in the value of a particular species (e.g., hi-fin platy or variatus). With regard to the higher value livebearers, the culture scenario also changes as added attention will need to be given to selection of broodstock as well as their propagation. A major service to the community will be the establishment of a reliable broodstock of hi-fin varieties of either livebearer as they are difficult to obtain. This also applies for the dwarf cichlid and European sources tapped in order to achieve this objective. All species are in various stages of being amplified and at the time of the preparation of this report, F1 individuals for each species had already been generated. A growth curve for the *Serpae tetra* was determined during the reporting period and has been summarized in a production handout and a workshop series is being planned for the upcoming period. The broodstock obtained for the hi-fin redwag platy appears to be a good one as greater than 90% of the progeny have the hi-fin trait. All other species are under various stages of amplification.

Objective: Collaborate with the private sector and the state aquatic veterinarian with the importation of additional species by providing facilities and maintenance during quarantine period where diagnostics to screen incoming fish for pathogens will also be provided.

All of the new fish being amplified were successfully screened during the reporting period in collaboration with Dr. James Brock. The facilities used were at the Windward Community College aquaculture production facility and represents a contribution in kind to the project. Likewise, the screening of the fish also represents a contribution in kind to the project.

Objective: Introduce production and marketing infrastructure scenarios to tropical fish growers in Hawaii.

During June and July 1998, a series of six workshops was held in collaboration with the Pacific Business Center. The workshops were telecasted to Hawaii Interactive Television sites on the Neighbor Islands to maximize participation. The results of attendees' critiques of the workshops reflect the industry's desire for a continued flow of information and technical assistance.

Objective: Demonstrate out of season maturation and spawning of at least one freshwater ornamental fish species (i.e., rainbow or redtail sharks).

In October 1998, a group of 216 young adult rainbow sharks were moved into an indoor facility at the Hawaii Institute of Marine Biology. They were distributed into twelve glass aquaria (29 gallons each) at a density of 18 fish per tank. Water was provided through a recirculating system that was flushed for one hour on a weekly basis. Feeding was done ad libitum 3-5 times per day using a pelleted commercial diet (Biokyawa 2000) and a frozen paste, which was made from homogenized beef heart, liver and spinach. Overhead lighting was regulated with a timer at 14 light: 10 dark and the room temperature was maintained between 80-84 F with a thermostatically controlled space heater. The control group consisted of fish for the same group that was held under ambient conditions in an outdoor fiberglass tank.

Changes in maturation were at first assessed visually, examining for individuals that were showing physical signs of becoming gravid (plump sides). From February, fish were examined by gonadal biopsy by first anesthetizing fish with 200 ppm 2-phenoxyethanol and using a 1 mm plastic cannula. Oocytes that were retrieved were examined under a dissecting microscope for size and position of the germinal vesicle, both indices used for staging ovarian maturation. Experience in the past five years revealed that the rainbow shark population held at Windward Community College reach the state of maturity at which they can be induced to spawn in August-September and the state of maturation of the control group was consistent with previous observations. No mature individuals were found in fish being held under ambient conditions in February. In contrast, almost all of the fish (male and female) under the extended day length and elevated temperature were at the state of maturation at which induction of spawning could be achieved.

The results clearly demonstrate that the altered environmental conditions have resulted in an advancement of maturation of broodstock by at least six months. Induction of spawning trials will be reported on during the upcoming reporting period.

Objective: Demonstrate optimal stocking densities of one ornamental fish species in a commercially available recirculating system and determine economic feasibility.

It has been reported that the island of Oahu's underground freshwater resources will be fully accounted for by the year 2010 and additional means of supplying potable water for the populace will have to be considered. These indications highlight the increased demand for the limited resource of freshwater. As a result, water reuse becomes an important issue in the development of the freshwater ornamental fish industry in Hawaii.

The initial investigation of the study conducted in response to this objective began with characterizing the "new tank syndrome" by stocking 90 juvenile rainbow sharks into 10-gallon glass aquaria equipped with virgin biofilters. Daily feeding rates consisted of two meals starting out at about 220 mg of feed and ended up at approximately 280 mg per day. Water quality parameters that were monitored included temperature, pH, total ammonia nitrogen, nitrate and nitrite. The study found that the nitrogenous waste products peaked (ammonia by day 18, nitrite by day 30) followed by a decrease to undetectable levels. The changes were explained by a growth of the detoxifying bacteria and clarifying bacteria in the biofilter as well as on the tank walls.

The second phase of the work used the same tanks and "pushed" the system by increasing density and the feeding rate. In addition to the control tanks, two tanks were seeded with the commercial bacteria flock "Cycle" and the remaining tanks were seeded with "Cycle" and the additional commercial bacterial flock "Waste Control". Each tank was stocked with 160 fish and feedings began at 280 mg per day and increased to over one gram a day. Ammonia remained undetectable until day 54 of the trial when fish were eating about 640 mg of feed a day, which meant that during the early phase of the trial, the Nitrosomonas bacterial already present were able to handle the conversion of all of the ammonia produced to nitrite and the Nitrobacter converted all of the nitrite to non-toxic nitrate. Although the bacteria colonies were apparently overwhelmed beginning on the 54th day of the trial, eventually all tanks were able to acquire sufficient amounts of bacteria to handle the amounts of nitrogenous waste being produced by the fish.

Turbidity was another factor that followed a similar trend in that the addition of the commercial preparations of bacteria significantly improved the clarity of the treated tanks. However, all tanks became equally turbid by the end of the growout period suggesting that even with the addition of exogenous bacterial flora to seed the tanks, the limits of the recirculating system were exceeded.

The water quality parameter that exhibited temporal changes that reached toxic levels was pH, which was observed to continuously fall during the course of the trial in all tanks. Although the addition of exogenous bacteria resulted in significant differences in the observed ranges in pH, all tanks exhibited a decreasing ability to maintain pH during the growout trials. Mortalities were observed only during periods when pH dropped to a level of 4.1, which indicates that the amount of fish and bacteria at this stage are producing so much carbon dioxide that the pH of the entire system is being forced towards acidic levels and can overwhelm a system.

The results from these activities provided an indication of the threshold of a recirculating system and some working parameters. From these small-scale trials, parameters were established as follows. For 38 liters of water, 0.4 liters of biofilter space to account for 700-800 mg of feed per day with no water exchange. If there is a daily water exchange the amount of feed can be increased to approximately 1,100 mg/day. For aquarium fishes, this would be essentially equivalent to 13 inches (body length) of fish per gallon of aquarium water used. The results of the experiment were summarized in an article submitted to the Honolulu Aquarium Society and printed in their monthly newsletter.

Objective: Complete a paper study of aquatic ornamental plants that hold potential for culture in Hawaii.

In addition to teleost, aquatic plants are also a mainstay of the industry. Until recently, aquatic plants were exclusively collected from the wild. However, culture technologies of freshwater aquatic plants have evolved to the

point where they also have become a viable industry. Technologies such as tissue culture techniques have been employed to amplify certain species of plants that are slow in reproducing and growout is being conducted in hot-houses at commercial scale.

In spite of these positive aspects, it is important to first assess whether such an industry is viable in Hawaii. Price lists were obtained for six species of aquatic plants (*Egeria densa, Cabomba caroliniana, Echinodurus bleheri, Vallisneria torta, Anubias barteri and Crytocoryne balanse*) from three different distributors. Analysis of the information concluded that the competition for breaking into the market with the plants surveyed would be difficult considering their very low estimated farm-gate price. However, Hawaii may still be able to compete as a niche market (which is typical of Hawaii's aquaculture commodity list) by providing species that are not readily available. Another option would be to take advantage of the lack of restrictions for the importation of any species of aquatic plants and establish a transshipping business where large numbers of plants are imported and then marketed. Prices would have to be competitive with U.S. mainland distributors. Although technologies are available to mass culture aquatic plants, there are apparently no "large" commercial tissue culture laboratories for plants in general in Hawaii, which may indicate that a laboratory of the size and type needed may not be commercially viable in Hawaii.

Work Planned

The amplification process will continue for the five species identified as desirable by producers. A workshop will be conducted in conjunction with the distribution of the anticipated first species, *Serpae tetra*, and pamphlets describing the culture of each of the species will be produced for distribution. The possibility of conducting the stocking densities activity on a farm site will be explored. If this is not possible, a tank field will be set up to complete this work. Now that out of season maturation has been achieved for rainbow sharks, spawning trials will be conducted to demonstrate the successful production of larvae. A manuscript summarizing this activity will be produced. Three identical tanks, each equipped with a different recirculating system, will be constructed and stocked to compare the effectiveness of the different units and validate the findings observed in the earlier small scale experiments. An extension handout will summarize the findings. A draft of the project paper will summarize the information being collected regarding the potential for the culture of aquatic ornamental plants in Hawaii. A series of workshops will be held covering a variety of topics including the commercial production of the *Serpae tetra* and the popular spawning workshops. Pending the completion of the live feeds work, a workshop is also tentatively planned on this topic. Investigators will continue to provide technical assistance to farmers as needed.

Impacts

Whether the project is successful in achieving its goal to foster development of the freshwater ornamental fish industry in Hawaii can be measured by a review of the number of farms that report freshwater ornamentals as a commodity. The State Department of Aquaculture provides a list every year of the number of farms and what they produced. Based on the lists obtained for the years 1992 through 1998, there is a significant increasing trend in the number of farms that produce freshwater ornamentals since the start of CTSA support in 1994. The 1998 data reflect an increase of nearly four times the number of farms listed in 1994. Another method to measure impact is by comparing the amount invested in the program with the amount produced by the industry. The Aquaculture Development Program reported approximately $450,000 in gross sales of freshwater fish in Hawaii in 1997. This amount already exceeds the amount invested in freshwater ornamental projects since their inception. The freshwater ornamental growers have reached a sufficient mass that they can now be considered a small industry in Hawaii's aquaculture scene and shows every indication of continued growth. The industry is already taking steps to diversify on their own as reflected in the number of species being marketed by local wholesalers well beyond the ones focused on by this project. As investigators look ahead for possible constraints that face this growing industry, the work focusing on recirculating systems will continue to be important as freshwater is a limited resource in an island environment.

Support

This project received financial support from CTSA and Sea Grant Extension Service, University of Hawaii and in-kind support from 12 farms and three state agencies.

		Other Support				Total
Year	CTSA	SGES	Industry	State of Hawaii	Total Other Support	Support
One	$50,000.00	$20,000.00	in kind	3 agencies	$20,000.00	$70,000.00
Two	$100,000.00	$20,000.00	in kind	in kind	$20,000.00	$120,000.00
Three	$110,000.00	$12,272.00	in kind	in kind	$12,272.00	$122,272.00
Total	**$260,000.00**	**$52,272.00**	**--**	**--**	**$52,272.00**	***$312,272.00***

Publications, Manuscripts and Papers Presented

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Growout Trials of the Commercially Important Opakapaka, *Pristipomoides filamentosus* *termination report*

Dates of Work

April 1998 through March 1999

Funding Level

$30,000

Participants

Dr. Christopher Kelley, Hawaii Institute of Marine Biology
Dr. Anthony Ostrowski, The Oceanic Institute
Dr. E. Gordon Grau, Hawaii Institute of Marine Biology

Reason for Termination

This project was terminated because all the objectives were completed.

Objectives

The overall goal of this project, which was initiated under the CTSA Eleventh Annual Plan of Work, was to establish commercial production of cultured opakapaka, *Pristipomoides filamentosus.* To achieve this goal, an economically feasible culture technology for this species must be developed and subsequently transferred to commercial farmers. This project focused on the growout aspect of culture technology (seedstock production was already being investigated.) Specific objectives related to that goal were to:

collect 200 live opakapaka juveniles from nursery grounds located outside of Kaneohe Bay;
transport the fish to The Oceanic Institute (OI) and maintain the fish in an isolated tank for a quarantine period of one week;
conduct a growout experiment to determine the survival and growth rate of juveniles fed either a raw or pelleted diet while being held under either low or ambient lighting conditions;
provide farmers with our results as well as updates on our progress via CTSA reports, the submission of at least one publication in a refereed journal, and at least one newspaper article.

Principal Accomplishments

Objective: Collect 200 live opakapaka juveniles from nursery grounds located outside of Kaneohe Bay.

The Hawaii Institute of Marine Biology (HIMB) was responsible for accomplishing this objective. HIMB participants subcontracted Mr. Randy Cates, the owner of the 32 ft Raden, *Ho'okupu*, to perform the collections. Mr. Cates had already been working with HIMB on its existing deepwater snapper project, and he and his staff were trained by HIMB participants in techniques for collecting live fish from deep water. Ten trips were carried out on the *Ho'okupu* between June 1 and July 11, 1998. Table 1 provides the results of these trips.

A total of 440 juvenile opakapaka were caught by hook and line from depths of 73-110 meters and brought up to the surface using hydraulic reels. Once on board, fish with over-inflated air bladders were "deflated" with the use of a hypodermic needle and placed inside the *Ho'okupu*'s live bait well where they remained until the boat returned to

HIMB. One hundred and thirty-five of these fish did not survive by the time the boat docked and therefore the overall survival rate at capture was 69%. This objective was achieved on schedule.

Table 1: Summary of opakapaka collection trips made by the Ho'okupu.

Trip #	Date	Location	Depth (m)	# Caught	# Survived	Survival Rate
1	6/1	Kaneohe Bay	73-101	24	19	79 %
2	6/3	Kaneohe Bay	73-101	59	43	73 %
3	6/9	Kaneohe Bay	73-82	46	27	59 %
4	6/11	Kaneohe Bay	73-91	15	11	73 %
5	6/12	Kahana Bay	73-91	19	13	68 %
6	6/13	Kaneohe Bay	79	52	33	63 %
7	6/15	Kaneohe Bay	73	64	40	63 %
8	6/16	Kaneohe Bay	73-110	51	35	69 %
9	6/17	Kaneohe Bay	73-91	68	52	76 %
10	7/11	Kaneohe Bay	79-101	42	32	76 %
Total			**73-110**	**440**	**305**	**69 %**

Objective: Transport the fish to The Oceanic Institute (OI) and maintain the fish in an isolated tank for a quarantine period of one week.

HIMB was responsible for the transportation of the fish to The Oceanic Institute (OI), and OI was responsible for maintaining the fish under quarantine. All 305 fish that survived capture were placed into an aerated transport tank on the back of a pickup truck and taken to OI. Twenty-one fish captured on trip 2 were brought back to HIMB as a result of a handling problem that was subsequently corrected. It was determined that opakapaka juveniles could not be transferred from tank to tank by conventional nylon hand nets. All fish were subsequently captured and transferred from the live well and transport tank with only the use of water-filled plastic bags. This method reduced the amount of abrasion suffered by the fish during transport. A small percentage of the fish taken to OI already showed symptoms of exophthalmos or "pop-eye" during the transport that presumably resulted from barotrauma experienced during capture. After arriving at OI, the fish in the first three transports were immediately dipped for 3-4 minutes in temperature-acclimated freshwater before placement into five m^3 circular, black quarantine tanks (three m^3 working volume). However, the freshwater dip was discontinued because of high stress-related deaths, and subsequent groups were simply placed into tanks. All groups were treated with 10 ppm nitrofurazone *in situ* for 2 hours each day for a period of ten days. After treatment, water flow in the tanks was adjusted to 30 liters/minute. Temperature and salinity in the tanks were maintained at 26-27 C and 33 ppt, respectively.

During quarantine, the health status of the fish was evaluated and remedial actions taken to correct problems. Numerous additional cases of pop-eye appeared as well as recurrences of distended air bladders that were a result of capture from deep waters. Fish that developed pop-eye in both eyes were considered blind and immediately culled from the population. Fish that developed distended air bladders were held submerged in a 15-liter bucket and their air bladders deflated again using a needle inserted through the anal pore at a 45 angle. It was not uncommon for the same fish to re-inflate several times during this period. Dead and moribund fish were quickly examined for the presence of gill and skin ectoparasites, dissected, and autopsied. By the end of the quarantine period on July 15, OI had a total of 184 completely healthy specimens, which was 92% of our target number. Total fish culled or that had died during this phase, due largely to pop-eye and complications from air bladder deflation, was 35%. This objective was achieved on schedule.

Objective: Conduct a growout experiment to determine the survival and growth rate of juveniles fed either a raw or pelleted diet while being held under either low or ambient lighting conditions.

OI was responsible for accomplishing this objective. Following the quarantine period, the 184 healthy fish were weighed (g), measured (cm fork length), and divided equally (23 fish/tank) into eight, 10 m^3 circular, black experimental tanks (7 m^3 working volume) located outdoors. Since the last group of fish arrived from HIMB on July 11, each tank

was initially stocked with 20 fish on July 15. On July 30, three additional fish were added to each tank from the last group after they had completed quarantine. Fish were first anesthetized *in situ* with 80 ppm MS-222 before handling. Initially, each tank was covered with two pieces of 80% shade cloth to limit algal growth. A third piece of cloth was added 56 days into the trial to further limit incident and stray light source infiltration.

Four tanks of fish were fed a raw diet consisting of squid, smelt, and krill. The other four tanks of fish were fed a moist, dough-like mash consisting primarily of the Moore-Clarke Marine Grower diet. The fish were weaned onto the mash by initially feeding them dry pellets mixed with raw smelt (50:50 w/w mixture). After a short period, the smelt was discontinued and the pellets were subsequently moistened by soaking them in water for 5 minutes. The fish accepted these "hydrated" pellets only after they were molded into a dough-like mass. All fish were fed twice daily (0800 and 1500 hrs) to satiation.

The original design of this trial included monthly measurements of fish performance. This schedule was changed after it became clear that handling increased deaths and the incidence of pop-eye. After the initial check in July (week 0), the fish were weighed and measured on only two other dates, October 29, 1998 (week 15) and January 28, 1999 (week 28). During these checks, the water level in the tanks was reduced to one m^3. Fish were captured and placed into a holding tank containing 80 ppm MS 222 where they lost equilibrium after 30-45 seconds. After measurement, each fish was placed into a plastic bag filled with ambient seawater held inside a 15-liter bucket. Once recovery was imminent, the bag with the fish was placed back into a cleaned experimental tank, and the fish was released.

Table 2 (on the following page) provides the survival rates, mean forklengths, and mean weights from each tank for weeks 0, 15, and 28. At week 0, mean forklengths and weights ranged between 18.2-19.0 cm and 104.6-115.5 g, respectively. By week 15, these values had increased to 22.5-26.5 cm and 216.4-439.8 g, while survival rates ranged between 39-74%. By week 28, the survival rates had dropped to between 0-48%. All of the fish in two of the tanks (i.e., tanks 4 and 5) died between weeks 15 and 28. The mean forklengths and weights in tanks that had surviving fish ranged between 26.1-31.1 cm and 343.9-689.1 g, respectively. In both treatment groups, there was clearly a tank effect with respect to both survival and growth. This was most likely due to disease problems observed during the experiment. Deaths occurred frequently (about one fish every day) and included fish that died due to starvation, pop-eye, and unknown causes. One group of fish fed the raw diet exhibited heavy mortality associated with unusual kidney histopathology. The cause of the condition is unknown.

Table 2: Summary of the data by tank

Treatment	Tank	Week	N	Survival	Mean FL (cm)	Mean Wt (g)
Pelleted Diet	**1**	**0**	23	100 %	18.6 1.5	108.7 30.0
		15	13	57 %	22.8 2.9	236.2 116.6
		28	11	48 %	26.1 2.8	343.9 121.5
	4	**0**	23	100 %	19.0 1.4	115.5 27.9
		15	17	74 %	22.5 2.5	216.4 88.6
		28	0	0 %	-	-
	5	**0**	23	100 %	18.7 1.5	114.8 31.9
		15	12	52 %	23.5 3.1	260.0 132.5
		28	0	0 %	-	-
	6	**0**	23	100 %	18.6 2.0	112.7 50.3
		15	11	48 %	23.0 4.1	251.5 161.2
		28	8	35 %	26.5 2.9	366.1 142.9
Raw Diet	**2**	**0**	23	100 %	18.3 1.7	108.8 25.8
		15	15	65 %	25.9 2.1	388.5 128.6
		28	11	48 %	28.8 3.5	520.5 225.7
	3	**0**	23	100 %	18.2 2.0	104.6 38.2
		15	17	74 %	26.5 3.3	439.8 188.0
		28	10	43 %	31.1 2.8	689.1 188.7
	7	**0**	23	100 %	18.4 1.5	109.8 28.6
		15	9	39 %	24.4 2.6	319.3 121.4
		28	4	17 %	26.8 2.4	398.3 107.8
	8	**0**	23	100 %	18.4 1.5	106.0 28.8
		15	16	70 %	26.3 3.0	409.6 171.4
		28	3	13 %	27.2 2.0	394 112.7

Table 3 summarizes the same data by treatment. Mean survival rate, forklength, and weight were higher in the raw diet group in comparison with the pelleted diet group. By week 28, 30% of the fish in the raw diet group had survived versus 21% in the pelleted diet group while the mean weights differed by more than 150 g. These differences, however, were not significantly different.

Specific growth rates (SGR, i.e., % body weight gain/day) and % weight increase were not shown, but were likewise, not significantly different (0.763 vs. 0.763 and 466.9 vs. 483.3 respectively). The food conversion rate (FCR) however, was significantly higher in the raw diet group in comparison to the pelleted group. The raw diet was apparently a better converter of fish weight at 1:1.4 than the pellet feed which converted at 1:6.1. This was probably due to a 5-week acclimation period of converting the fish to pellets. This was finally accomplished after moistening the pellets in water prior to feeding. During this period, most of the feed was flushed down the drain before the fish could eat it.

Table 3: Summary of the data by treatment

Treatment	Week	N	Mean Survival	Mean FL (cm)	Mean Wt (g)
Pelleted Diet	**0**	23.0 0.0	100 0 %	18.7 0.2	113.0 2.8
	15	13.3 1.3	58 6 %	23.0 0.4	209.3 53.0
	28	4.8 4.8	21 21 %	26.3 0.2	355.0 11.1
Raw Diet	**0**	23.0 0.0	100 0 %	18.3 0.1	107.5 2.4
	15	14.3 1.8	62 8 %	25.8 1.0	389.3 51.5
	28	7.0 3.5	30 15 %	28.5 1.5	500.5 104.3

A problem with interpreting the results of the growout experiment is the high mortality rate observed in both treatment groups. Of the original 184 fish used in this experiment, only 47 had survived to week 28. Of these, 21 had either unilateral or bilateral pop-eyes. Only 26 fish completed the experiment in reasonably good health.

These fish were moved to a larger and brighter square cement tank where pop-eye still continues to develop. Other physical problems observed during the experiment were parasites in the viscera and gill plates, a thinning of the epidermis dorsally behind the head, and necrosis of the swim bladder. These problems need to be resolved before this species can be considered as an aquaculture candidate. This task was completed on schedule.

Objective: Provide farmers with our results as well as updates on our progress via CTSA reports, the submission of at least one publication in a refereed journal, and at least one newspaper article.

Both HIMB and OI were responsible for accomplishing this objective. Required progress reports have been submitted to CTSA with this being the final report for year 1. Due to the poor survival rate of the fish during the growout experiment, workgroup members from both HIMB and OI are still deciding whether an attempt should be made to submit the results for publication. No newspaper articles are planned. This objective, therefore, has been only partially achieved.

Impacts

This project provided the first rigorous examination of the culture potential of opakapaka. It was demonstrated that opakapaka can be captured alive from deep water, can survive in captivity for at least 6 months, and can be weaned onto a pelleted diet. However, due the poor survival of the fish during the growout experiment, the opakapaka culture does not appear to be feasible using standard techniques developed for shallow water species. Specialized techniques for deepwater species need to be developed in the future.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), the University of Guam (UOG), the National Science Foundation and the Department of the Interior, Office of Insular Affairs.

		Other Support			
Year	**CTSA**	**University**	**Industry**	**Total Other**	**Total**
One	$30,000.00	$0.00	$0.00	$0.00	$30,000.00
TOTAL	**$30,000.00**	$0.00	$0.00	*$0.00*	**$30,000.00**

Publications, Manuscripts or Papers Presented

No publications were produced as a result of this project.

Development of Pacific Threadfin and Milkfish Growout Technology and Production of Live Feeds and Seedstock

Dates of Work

April 1993 through March 1999

Funding Level

$331,934

Participants

Dr. Anthony Ostrowski, The Oceanic Institute
Dr. James Szyper, Hawaii Institute of Marine Biology
Dr. Clyde Tamaru, Sea Grant Extension Service
Michael Fujimoto, Anuenue Fisheries Research Center

Objectives

The overall goal of this three-year project, which was initiated under the CTSA Ninth Annual Plan of Work, is to develop growout technology for Pacific threadfin (*Polydactyulus sexfilis*) and milkfish (*Chanos chanos*) and transfer that technology to the aquaculture industry. Specific objectives related to that goal are to

- determine Pacific threadfin growout requirements by:
  - evaluating suitable growout facilities,
  - identifying appropriate stocking densities and loading rates,
  - identifying appropriate commercial feeds,
  - and identifying market potential;
- determine milkfish growout requirements by:
  - identifying suitable commercial feeds and assessing the animal's potential as baitfish;
- provide commercial farmers with live feed cultures and threadfin and milkfish seedstock, which includes
  - maintaining broad-based threadfin and milkfish broodstock capabilities to supply eggs or juveniles to cooperating farmers,
  - supplying live feed starter kits to cooperating farmers,
  - maintaining cooperative efforts and contacts between farmers and researchers;
- Provide farmers with a spreadsheet template to calculate costs of production and identify specific areas to improve productivity.

Principal Accomplishments

Progress over the last six months of the project concentrated primarily on production and distribution of remaining fingerling threadfin and milkfish to participating farmers and on field testing of the economic spreadsheet. Marketing efforts were delayed because of cancellation of events or changes in dates, and budget cuts within the State of Hawaii's Aquaculture Development Program (ADP). Development of a promotional brochure for threadfin was to be cost-shared between the ADP and the CTSA. A no-cost extension of the project through October 1999 was approved. This extension was requested because the start date of the project, which was delayed until July 15, 1998.

The production and distribution of fingerlings to local farmers has been completed. A total 354,998 threadfin and 137,500 milkfish were distributed. These numbers represented 41.6% and 37.5% more, respectively, than what was targeted for the project. In addition, two feeding trials were conducted that indicated the potential for polyculture of milkfish and shrimp (*Penaus vannamei*) in ponds. Results indicated that total biomass in a pond can be increased by

an average 22.4% with the combination, although total crop value was not affected. Some farmers in Hawaii currently culture milkfish and shrimp together in ponds. Development of optimum polyculture methods would allow these farmers to maximize their profit potential.

The threadfin production and financial model (TPFM) was taken to several farmers to estimate on-site production costs. Inputs from farmers are currently being used to revise the model and make it more user friendly. We anticipate to have the model finalized by the extension date deadline.

The outlook for completion of the project objectives is very good. Technical pamphlets on threadfin growout and milkfish semi-intensive culture are currently in progress. The TPFM will be finalized and a version will be available for publication on the web. The additional fish supplied by the project should enhance efforts to promote threadfin and milkfish culture in the state.

Objective: Identify suitable commercial facilities in Hawaii for growout of threadfin through on-site growout trials.

The primary facilities available to raise threadfin on commercial farms in Hawaii are tanks, ponds, Hawaiian fishponds and net pens. Several commercial farms agreed to participate in growout trials designed to evaluate the suitability of potential facilities and identify production constraints and advantages in each. To replicate results, two runs were scheduled for each farm site at the beginning and end of the natural spawning season. In each trials, farmers were to grow fish from the same spawn feeding the same commercial feed, Moore-Clark mahimahi diet.

A total of 110,000 juveniles were distributed to six cooperating farms on Oahu and Hawaii. In addition, 4,560 fish were stocked in growout facilities at The Oceanic Institute (OI) and Hawaii Institute of Marine Biology (HIMB) for designed experiments. At commercial farm sites, fish were stocked into either large production tanks or pen facilities in Hawaiian fishponds; target harvest densities were 15 kilograms per cubic meter in growout tanks and 8,000 pounds per acre in pens. At OI, fish were stocked into each of four replicate 10-foot-diameter and 20-foot-diameter tanks at densities of 50 fish per cubic meter and at initial loading rates of 0.1 kilogram per liter per minute water flow. The water flow increased to 1.0 kilograms per liter per minute at the end of the trial to determine the effect of tank size on fish growth and to serve as a growth reference for the commercial facilities. At HIMB, fish were stocked into eight 1.2-cubic-meter pens at either 30 or 60 fish per cubic meter to determine the effect of stocking rate on growth and survival in these systems. Graduate students living on the island fed the fish in this experiment. Threadfin were sampled at 30-day intervals, and growth and survival rates compared with those raised at OI. Three farms on the island of Hawaii and one on Oahu received 30-day-old fish that were raised through Nursery I on standardized feeding and management methods developed under the previous CTSA-funded three-year project titled "Development of Fry Production Technology." OI staff provided hatchery facility owners with technical assistance regarding setting up the nursery facilities.

Results from farm sites have been encouraging; successful commercial harvests from both the first and second runs are appearing in the market place. Overall survival rates were 78 percent; about 34,000 fish ranging from 0.75 pounds to 2 pounds remain to be sold. Threadfin have grown well in both brackish water and saltwater conditions. Anecdotal evidence suggests that fish cannot survive for extended periods at salinity below 20 parts per thousand. Nursery survival at the farm sites ranged between 50 and 90 percent, while survival in the OI nursery averaged 85 percent. At Day 102, the average weight of fish ranged from 45 to 60 grams at cooperating facilities; the average weight of fish at OI was 64.2 grams at Day 95. The first harvest from this group began in January, 1997, when fish were 7 months old. The second rearing was conducted in early November and targeted 50,000 juveniles for distribution. All the farm facilities agreed to replicate the first run.

Results of the HIMB cage experiments indicate no difference in weight gain and specific growth rate between the two stocking densities; however, results should be viewed with caution. Feed conversion ratios and survival rates were poor for both densities. Problems and indicators for the poorer biological performance include:

> the personnel maintaining the experiment changed numerous times, which may have led to improper feeding; feed not immediately consumed by fish in the cages would fall through the bottom mesh of the cage and be consumed by ornamental fish living in the canal, which may have lead to poor feed conversion;

few threadfin were observed to have died during the experiment, but when fish were harvested, overall survival rates were low. Extraction of threadfin from the cages may have occurred, thus accounting for the poor survival rates;
Cages were built with a PVC frame and covered with a one-eighth-inch nylon mesh net. The netting became weak from sunlight and bio-fouling after the third month and needed repair and cleaning.

Despite these problems, the fish grew and survived, so this type of environment should be considered in future investigations involving growout in cages. Perhaps better results can be achieved with increased supervision and cages built from stronger materials.

Objective: Identify suitable, locally available commercial feeds for threadfin growout.

Based on recommendations from the previous CTSA-funded three-year project titled "Development of Fry Production Technology," farmers are using a Moore-Clark mahimahi diet for threadfin growout. An experiment was conducted to determine if other commercial feeds would be suitable for threadfin growout at lower feeding costs. Four feeds were tested, Moore-Clark (MC) Threadfin (45 percent protein and 14% lipid), MC New Age Salmon (45 percent protein and 18 percent lipid), Rangen Salmon Grower (45 percent protein and 15 percent lipid) and Rangen EXTR 450 (45 percent protein and 15percent lipid). Each diet was fed to satiation twice a day to 40 Day-60 fish stocked four replicate tanks, for a total of 16 1.5-ton tanks. Fish were stocked at a mean weight of 9.8 grams and grown to Day 116. Results indicated significant differences in growth and survival of threadfin given the four diets.

All aspects of biological performance were best in fish given the MC Threadfin diet. Weight gain was 34 percent greater for these fish those for those given the New Age Salmon diet.
Fish given the MC New Age Salmon and Rangen EXTR 450 had comparable performance but not as good as the Threadfin diet.
Fish given the Rangen Salmon Grower performed less favorably than the fish given the other diets. Weight gain was 29 percent less than weight gain with Rangen EXTR 450.

The Moore-Clark threadfin or mahimahi diets provide optimum biological performance for threadfin growout.

The Moore-Clark mahimahi diet was specifically formulated to meet the protein and energy requirements of the mahimahi, a fast-growing, open ocean predator. Short-term research has shown that Pacific threadfin require less protein and more carbohydrates in their diet. Confirmation of the suitability of the lower protein diet should be obtained in long term commercial growout. An experiment was conducted at OI to determine the effect of both tank size and a mahimahi diet and a diet specifically formulated to for threadfin.

Moore-Clark was contracted to produce the mahimahi diet and a threadfin diet made with the same dietary ingredients but formulated to match threadfin macro nutrient needs. Four 10-foot- and four 20-foot-diameter tanks were stocked with Day 65 threadfin at a density of 50 fish per cubic meter of working volume. Fish had been fed a 2.5 mm mahimahi diet containing 55 percent protein from Day 45 through Day 65. At Day 65, duplicate 10-foot- and 20-foot-diameter tanks were switched to the respective experimental diets. Fish were fed twice daily to satiation. Fish were weighed and measured every 30 days until harvest at Day 180. Results of the protein experiment indicate that tank size and percentage of protein significantly affect growth and performance of threadfin.

Threadfin given a diet containing 55 percent protein gained 9 percent more weight than threadfin given a 45 percent protein diet.
Threadfin grown in 20-foot-diameter tanks gained 12 percent more weight than fish grown in 10-foot diameter tanks.
Survival was similar in the two sizes of tanks but was 3.4 percent better in threadfin given the 55 percent protein diet.
Specific growth rates were similar between the two protein levels but were 5.7 percent better in the 20-foot-diameter tanks.

The differences in growth and tank size should be considered when planning a growout facility. Based on these results, a tank size of at least 20 feet and a diet consisting of either 45 percent or 55 percent protein should be utilized to optimize biological performance for threadfin growout.

Objective: Determine the loading rate that promotes optimum growth and feed utilization in threadfin growout.

The most expensive operating costs for an onshore growout facility are those associated with pumping seawater. The amount of water required to maintain fish at high density is largely related to tolerance limits to un-ionized ammonia. These tolerance limits determine the biomass of fish that can be raised with a given flow of water. This is termed as the loading rate, often measured in kilograms of fish per liter of water flow per minute. Determination of optimum loading rates for threadfin is crucial in the assessment of economic feasibility of threadfin culture in Hawaii because electrical rates are among the highest in the nation.

Four 10-cubic-meter tanks were each stocked with 450 Day 60 threadfin. Duplicate tanks were used for each loading rate treatment. Water flow rates were set to reach target harvest load of 2.0 and 1.0 kilograms per liter per minute at Day 180. Fish were fed twice a day to satiation with a Moore-Clark Marine Grower diet of 55 percent protein and measured and weighed monthly. Water samples were taken to determine total ammonia and pH during peak periods of the day prior to each monthly sample. A 24-hour study of how ammonia cycles through the different flow rates was conducted prior to harvest. Results indicate that threadfin can be raised with water flow rates than originally believed. Survival and growth of fish raised to harvest size showed no difference at either loading rate, despite the fact that total and un-ionized ammonia levels in the high loading rate treatment tanks (2kg/l/m) were always twice that in the standard (1 kg/l/m) loading rate treatment tanks. In fact, those fish raised at 2kg/l/m had a 1.49 feed conversion ratio, which was slightly lower than the 1.60 feed conversion ratio of those fish raised at 1kg/l/m. This information will greatly reduce water use costs at flow-through facilities and provide a starting point for determination of carrying capacities at facilities that may plan to recirculate water. Threadfin appear to tolerate un-ionized ammonia levels as high as 0.08 ppm quite well for short period of time and 0.04 ppm for longer periods without adverse effects.

Objective: Generate socio-economic and market characteristics of harvested threadfin through sales to distributors and other outlets.

Economic success of threadfin culture in Hawaii will depend upon proper identification of market outlets, volume and proper characteristics as well as appropriate off-the-farm price. Approximately 5,400 pounds of threadfin were sold to supermarkets and distribution outlets willing to test market and promote the product. KTA Superstores in Hilo, Hawaii, participated in the test marketing. Weekly harvests of 400 total pounds of 0.75-pound threadfin began in January and continued through February. An additional 700 fish were raised to 6 months of age in two 20-foot-diameter tanks. The threadfin were given the Marine fish diet fed to satiation twice daily and sampled every month for weight gain and length. Markets had suggested that a 2-pound threadfin would fetch a higher price, which stimulated the research to answer the production costs and profitability of raising a fish to that size. Growth and feed conversion were compared between the two periods of growth.

Results indicated that growout to an average 1.5 pounds is feasible within a year, but growth and feed conversion are adversely affected. During the first 126 days of growout, which spanned Day 65 through Day 191, fish gained an average of 371 grams, for a specific growth rate of 6 percent of body weight per day, and had a feed conversion rate of 1.1 and survival of 98 percent. During the last 138 days of growout, which spanned Day 205 through Day 343, fish gained an average of 266 grams, for a specific growth rate of only 0.4 percent of body weight per day, and had a feed conversion rate of 2.6; however, survival remained at 98 percent. The slowed growth and higher feed conversion were attributed to the development of sexual products and sex reversal in some fish beginning at approximately six months of age. Overall feed conversion from Day 65 to Day 343 was 1.7. The results indicated that growout of threadfin to the larger 1.5- to 2-pound market size increases feed costs by 55 percent and may not be economically feasible for farmers unless premium prices can be obtained.

During the months of January and February, KTA test marketed a total of 2,600 pounds of threadfin. Another 1,350 pounds were test marketed during April and May. The fish were well received and had a shelf life of 8 to 11 days. Threadfin obtained from the wild did not last as long on the shelf. The longer shelf life is due to the immediate preservation of tissue when a cultured threadfin goes straight from a culture tank into a briny ice bath. The manager of KTA noted that the cultured threadfin was the best tasting fish he had eaten.

Fish distributed last year to farmers have already appeared in the market place. Off-the-farm price is $6.50 per pound for 0.75- to 1-pound fish and $2 per pound for a 2- to 3-ounce fish. Product quality has been high, and demand growing. Cultured threadfin have been sold to Times Supermarket, which has advertised them at $10.99 per pound, to Safeway and Daiei, which have advertised the threadfin for $8.99 per pound and to Rick's Restaurant in San Francisco. One restaurant end-user estimated that he could sell threadfin daily if his purchase price was between $5.50 and $5.75 per pound. End-users have noted that shortened operculum and other gill anomalies present in 15 to 30 percent of cultured threadfin make no difference in their acceptance of the product or its price.

Objective: Identify commercially available feeds suitable for milkfish growout and assess its potential as baitfish.

Juvenile milkfish readily consume and grow well on a wide variety of commercial feeds available in Hawaii. However, the feeds that produce the best growth have not been determined, nor have the costs effectiveness of the feeds currently in use. In addition, although evidence indicates that milkfish juveniles may prove useful as bait for commercial and sport fishermen, their effectiveness has not been clearly established nor has the extent of the market been identified. Experiments to determine the feeding cost (price of feed x feed conversion) of raising juvenile milkfish on a cross-section of commercial feeds and to identify potential baitfish outlets for milkfish juveniles by supplying fish to commercial and sport-fishing vessels.

A commercial feeding trial was conducted to determine the suitability of four local fish diets. Sixteen 1.5-ton round fiberglass tanks were stocked with 100 Day 60 milkfish with an average weight of 3 grams. Four replicate tanks of fish each were fed one of four commercial feeds: Sunfish Pellet, which cost $0.30 per pound; Catfish pellet, which cost $0.46 per pound; trout pellet, which cost $0.64 per pound, and mahimahi pellet, which cost $0.55 per pound. Fish were fed to satiation twice daily and weighed at the end of the experiment on Day 116. The results indicated the following:

The mahimahi, trout and catfish diets produced similar weight gain, survival and specific growth rates of fish.
The mahimahi diet yielded the best feed conversion ratio and overall feeding cost.
Overall performance was poorest in fish given the sunfish diet, although survival was unaffected.

The mahimahi diet produced the best growth and lowest feeding costs and should be considered when raising milkfish for profit.

Milkfish fingerlings from these trials were used to conduct a swordfish longline trial from March 20 through April 1, 1997, aboard the NOAA research vessel "Townsend Cromwell" in cooperation with Dr. Chris Boggs of the Honolulu lab. Dr. Boggs agreed to compare the use of live milkfish and dead bait in alternating baskets, which are a series of hooks differentiated by the distance between buoys that keep the mainline afloat. Data on effectiveness, types of fish caught and preference of bait was collected.

Approximately 3,000 7-month-old milkfish were transferred to a live bait well on the ship. Eight longline sets, each averaging 150 hooks, were baited with live milkfish versus frozen squid. The milkfish were arranged in groups of approximately 50 on either the beginning, middle or end of the longline set. The longlines were set at 8 p.m. and 3 am and hauled in at 11 p.m. and 3 am. Thirteen sets were cast using frozen squid only. A total of 1,854 hooks were deployed. The catch rate per 100 hooks for live milkfish was 0.69 and for frozen squid was 0.42. The catch rate on the squid only lines was 0.32. Because the total number of trials and fish caught was small, the data is inconclusive but looks favorable for further study.

Live milkfish was a hardy bait at 18 to 25 C, but significant mortalities were observed when temperatures dropped below 15 C. Improvements in the holding conditions such as water flow and oxygen will facilitate better overall health of live milkfish on board a vessel.

Objective: Supply threadfin and milkfish seedstock to commercial farms.

A total of 115,000 threadfin were distributed to five cooperating farms, OI and HIMB. A total of 97,000 day 40 milkfish were distributed to 15 farms on Oahu, Molokai, Maui and Hawaii. Growout resulted in successful commercial harvests of approximately 58,000 of the fish; another 34,000 are nearing harvest. Market sizes ranged from 0.5 pounds to 2 pounds. Threadfin have grown well in either brackish or saltwater conditions in flow-through tanks or pens in

Hawaiian fish ponds. Anecdotal evidence suggests that fish cannot survive below 20 ppt salinity for extended periods. Fish grow best at dissolved oxygen levels of 5.0 ppm or higher but reportedly can tolerate oxygen levels as low as 2.0 ppm provided they have had prior acclimation to low oxygen levels. Nursery survival at farms ranged between 50 and 90 percent, while survival in the OI nursery averaged 85 percent. Growth rates at the farm sites have not been comparable to those attained at OI for a variety of reasons; however, most farms can produce 0.5-pound fish by 6 months of age and 0.75-pound fish by 8 months of age.

Growout trials of milkfish at farms exhibit an overall survival rate of 63 percent. Approximately 45,000 milkfish, each averaging about 0.75 pounds, remain in growout. Markets for milkfish range from 5 to 6 inches for pan frying (entire fish) to 1 or 2 pounds whole, de-boned for restaurants. Prices average between $2.50 to $3.50 per pound for a whole fish.

1998 Report

During the period from April through September 1998, 126,200 threadfin were distributed to eight of the 13 participating commercial farms. A hatchery run in April produced 98,000 fish with an average survival rate of 23.4 7.6%. Another 20,500 fish were made available to farmers from terminated experiments at OI. An additional 200,000 eggs were provided to Anuenue Fisheries Research Center, which, in turn, produced 7,700 fish that were distributed to a participating farm. Survival rates on farms range from 48 percent to 99 percent and average 84 percent.

A total of 116,400 milkfish were distributed in August to 22 different farmers on Oahu, Molokai, Maui and Hawaii. Fish were shipped at 38 days old and a much larger size (0.050 grams) than in previous years, when Day 32 fish weighing 0.020 grams were shipped. This probably contributed to the survival rates of 98 percent overall. In addition, 200,000 milkfish eggs were distributed to two farmers. One farmer obtained 0 percent survival, while the other obtained 40 to 50 percent survival.

Additional semi-intensive runs are scheduled before the end of the milkfish spawning season. Investigators anticipate distributing close to 140,000 fry, which represents an increase of 40 percent over the original target.

1999 Report

During the period from October 1998 through March 1999, 354,998 pacific threadfin were supplied to farmers on Oahu, Hawaii, and Maui for CTSA Year 11, more than the 100,000 animals originally targeted. The threadfin were distributed to 11 out of the 13 farms between April and December 1998. The hatchery run conducted in April produced 98,000 fish while the hatchery run in November produced 128,844 fish. Another 128,154 fish were made available to farmers from terminated experiments at OI. The outer island fry were packed at between D25 (0.035g)-D30 (0.06g) at 500-1000 fry/bag. Survival from fish shipments ranged from 55-99%. Mortality during shipments were mostly due to delays with airline carriers. Consequentially, changes were made in pack out protocol to compensate for these delays. In addition, threadfin eggs were supplied to farmers on Oahu and Hawaii. OI provided Anuenue Fisheries Research Center (AFRC) a total of 600,000 eggs, resulting in 18,200 fry that were distributed to Bruce Smith's farm. Ryan Murashige's farm received 800,000 eggs of which no fry were successfully grown.

Higher mortality is generally experienced at outer island farms due to shipping deaths and the need to conduct Nursery phase I. Ryan Murashige averaged between 75-85% survival while Ben Krause averaged between 60-70% survival, a typical survival rate for a new farmer. More experienced farmers are averaging 85%, the standard set at OI. The key problem encountered at Bruce Smith's farm on Oahu is continued outbreaks of Oodinium, which account for the vast majority of deaths.

Prices for the threadfin still range from $5.00 - $7.00/lbs. Concerns from farmers regarding the moi industry include potential satiation of local markets, production costs, and price variation.

OI maintains a concerted effort in trying to supply farm demands by providing farmers with additional fish from other ongoing research projects and by providing the state's AFRC with eggs to conduct rearings targeted at supplementing OI's efforts.

Objective: refine semi-intensive and extensive culture techniques for production of milkfish fry from saltwater ponds.

1998 report

Four semi-intensive outdoor larval rearing trials were conducted between July and September, yielding a total of only 13,900 milkfish fry. Problems with inconsistent spawning and algae contamination from the ponds compromised egg quality in several cases. Additional trials are targeted for late fall.

A 12-week milkfish-and-shrimp polyculture feeding trial was initiated in mid-July. Sixteen, 1.5-ton outdoor rearing tanks were stocked with 1-gram Pacific white shrimp at a density of 40 shrimp per square meter. Four replicate tanks each were then stocked with zero, five, 10 and 20 milkfish weighing about 1 gram each. All tanks were inoculated with OI shrimp round pond water to establish a diatom bloom. The bloom is being maintained at a Secchi disk reading of 40 cm. Animals in all tanks were fed the same amount of a $^{3}/_{32}$-inch pelleted feed based on only shrimp biomass. This ensured that, initially, the milkfish would not eat pellets directly but young shrimp would. After six weeks, shrimp were weighed. Treatments with only shrimp exhibited a mean growth rate of 1.53 0.17 grams per week. Shrimp growth declined proportionately with increasing milkfish density. Milkfish have grown well, but an interim weight has not been taken to avoid excessive stress on the animals. It is suspected that total biomass produced and total feed conversion will be the key factors that will provide the most interesting information from this trial, which will terminate in mid-November 1998.

1999 Report

A new strategy was implemented in 1999 for extensive larval rearing runs. A new product called Aquamats was utilized to produce a supplemental organic food source consisting of primary phytoplankton, zooplankton, and detrital bacteria. Aquamats are made from a synthetic substrate that resembles sea grass in appearance. The Aquamat provides aquatic structure to support phytoplankton growth. The phytoplankton lies at the base of the food chain and provides ideal support for a wide ranging community of zooplankton in the 40-200 micron range. These zooplankton provide the preferred diet for larval fish development. The Aquamats will be examined along with current extensive rearing techniques and compared for growth and survival.

Objective: Identify cooperating farms and provide ongoing technical assistance in threadfin and milkfish nursery and growout.

The assistance provided to farmers by OI staff and Sea Grant extension agents had been the key to the success of farms raising these fishes. OI staff and Sea Grant extension agents coordinated activities to visit cooperating farms, identify qualified participants, and assist them in rearing strategy, facility design and, in some cases, pickup and stocking of seedstock. On-site visits helped farmers to identify needs and rectify deficiencies to ensure high survival of seedstock. Under the previous year of the threadfin fry project and the milkfish project, no technical assistance was provided and only an estimated 21,000 of the 150,000 fish distributed reached harvest size. During this project year, technical support has helped farmers to achieve threadfin survival rates of 80 percent and milkfish survival rates of 63 percent. Those rates could be improved with more education and extension activities for farmers. Threadfin feeding guides were supplied to farmers and helped them to maintain rapid fish growth and survival, particularly in the nursery stage. Commercial feed shipments for threadfin were lowered by coordinating with a Hawaii company to share a shipping container. This has reduced the cost of the mahimahi diet from $0.75 per pound to $0.55 per pound to Oahu. In addition, OI supplied farmers with <2.0-mm nursery feed and 2.5-mm feed on a reimbursement basis.

1998 Report

Technical assistance to the industry has been primarily through on-site visits and correspondence on the internet and telephone. Activities have been coordinated with participating farmers regarding system design, feeding strategies, disease, stocking densities, ordering feed supplies, fish transport and handling. During the first six months of the third year of the project, site visits averaged 1.5 per month, including several trips to the Big Island. Dialog with farmers through phone, fax and the Internet averages about 15 hours per week. These activities resulted in the start of a new threadfin farm, which has been producing good survival rates and may well become a major threadfin producer.

Particular efforts were directed at remedies for outbreaks of *oodinium* on an Oahu farm. A recently initiated program saved a large number of fish. Fish are monitored daily for unusual mortality. The farmers noted that mortality

increased from 100 to 200 to 300 fish daily over a three-day period in his raceway systems. These fish were diagnosed with *Oodinium* infestation. The remaining fish were corralled to one end of the raceway and water flow increased by 400 percent to flush the parasite from the gills and away from fish to prevent re-infestation. Mortality declined the next day. The fish will be transferred to a new tank after a 3- to 5-minute freshwater dip.

1999 Report

In addition to the continued information exchange through phone calls, the Internet, and site visits, the transfer of larval rearing technologies will be a major focus in the coming year. This will initiate a weaning process so farmers become more self-sufficient and do not have to rely on OI for seedstock.

Objective: Provide farmers with a spreadsheet template to calculate costs of production and identify specific areas to improve productivity.

Investigators have been working on the design and programming of a threadfin production and financial model (TPFM). TPFM is a MS Windows-based program designed to take detailed production and financial information from farmers for the first three years of operation and assumes that production will stabilize from the third year on for 20 years. The modules for data entry, analysis and reports, and sensitivity analysis are as follows:

Data Entry

- Overall information: to enter fish price, fingerling cost, energy cost, and lease rent.
- Production Schedule: to enter projected monthly stocking, mortality, average fish weight, production, feed use, and water temperature for the first three years;
- Tank Utilization: to enter size, utilization, and water exchange of tanks;
- Labor: to enter labor requirement and cost;
- Energy: to enter energy requirements and cost;
- Capital: to enter capital investment cost;
- Other Costs: to enter shipping, chemical and other operating costs;
- General Financial Information: to enter tax rates, interest rate and length of loan.

Analysis and Reports

- Depreciation: to allow users to choose from one of three depreciation methods (straight line, double declining balance, and sum-of-year's digits) for depreciation calculations;
- Interest: to calculate the interest and principal payments for a loan;
- Profit and Loss: to calculate annual profit and loss statements for 20 years;
- Cash Flow: to project the annual cash flows for 20 years and calculate the internal rate of return, net present value and break-even prices.

Sensitivity Analysis

- To analyze the sensitivity of profitability with respect to changes in fish price, fingerling cost, feed cost, water use, energy use, labor cost and production.

1999 Report

Initial work was devoted to the design and programming of a threadfin production and financial model (TPFM). TPFM is a Microsoft Windows-based program designed to assist threadfin farmers in analyzing their production economics and financial returns for their operations. The program is designed to take detailed production and financial inputs from the farmers for the first three years of operation and assumes that production will stabilize from the third year on for 20 years.

The TPFM program has been field tested and modified. The program and its documentation are ready for distribution. Cost of production information has been gathered from two farmers and their production economics are being analyzed at this time. In addition, the program has been applied to evaluate the economics of moi production using the production parameters from the OI experimental production trials as well as the economics of culturing moi in offshore cages. The results of these economic evaluations are being scrutinized at this time and will be available during the next reporting period.

Objective: Produce a promotional brochure and value-added gill tags for threadfin, and provide market size fish to media events and distribution outlets for evaluation and exposure to expand local and export markets.

The project participated in three Hawaii promotions and one export promotion. At each event, market sized threadfin were provided for cooking and sampling. Recipes were developed and distributed along with a survey form to measure consumer attitudes and preferences. Where appropriate, media kits, which contained information about threadfin, a press release and recipes, were distributed.

The Hawaii Hotel and Restaurant Expo, the largest restaurant trade show in Hawaii, attracted more than 1,500 chefs, hotel food and beverage buyers and supermarket buyers from around the state. Threadfin was presented on ice via a large exhibit space set up by the Aquaculture Development Program in the entrance of the exhibition hall. Steamed threadfin samples were distributed to attendees, market data was collected via a survey form, and a list of threadfin farms was distributed to interested chefs and buyers. Media kits were distributed to local news media.

The Hawaii State Farm Fair, the state's largest public event promoting agriculture, attracts more than 125,000 people over 10 days. According to Fair organizers, more than 13,000 people were at the Fair on August 1, when celebrity chef Sam Choy spoke about threadfin and prepared fish provided by the project before a huge crowd. While he was doing his demonstration, his staff prepared and distributed more than 300 large servings of threadfin for the public to sample. Consumer data was collected via a survey form and copies of Mr. Choy's recipe were distributed.

Hawaii Cooks with Roy Yamaguchi is a popular local cooking show featuring celebrity chef Roy Yamaguchi. The Fifth Anniversary Festival highlighted some unique Hawaii products, including threadfin, which was supplied by the project. Chef Yamaguchi conducted a threadfin cooking demonstration before 500 people.

The project also supplied threadfin for the Kapalua Wine and Seafood Symposium July 18-19, 1998. This is one of the most prestigious food events in the country. Some of the best Hawaii, mainland and international chefs are invited along with Hawaii and national food media. The project provided moi for a vertical fish tasting seminar conducted by Howard Deese of the Ocean Resources Branch of the Hawaii Department of Business, Economic Development and Tourism. Threadfin was sampled and compared with high value food fish from around the world, including opakapaka, tai snapper and sole flounder. Threadfin was found to be better or equal to these premium fish.

Since the start of the project year, the gill tag manufacturer discontinued making the metal tags because of health concerns about rust. Therefore, the project will purchase a plastic gill tag. However, the plastic tag is more costly, so the order will be reduced to 10,000 tags. The gill tag wording and design was developed with help from the aquaculture industry. It will bear the words "Hawaiian Alii Moi" and "Fish of Hawaiian Royalty" to emphasize the Hawaii mystique and its historical reservation for the Hawaiian Alii. Tags and a tagging gun will be distributed to two export wholesalers for trial.

In addition, ADP staff is doing the design and pre-production work on the promotional brochure. It will contain information on the history of threadfin, modern production, product attributes and recipes. Five thousand copies will be printed and distributed to seafood wholesalers.

Work Planned

During the remaining months of this project, investigators will:

Finalize the technical pamphlet for threadfin growout.
Finalize the technical pamphlet for milkfish semi-intensive rearing.
Complete enterprise budget and software application.
Finalize threadfin promotional pamphlet.

Impacts

Results from experiments and nursery runs at commercial farms indicate that farmers can be very successful at raising threadfin, given proper guidance through extension activities. Farmers have gained confidence that survival and growth can be kept at profitable levels provided that proper facilities and techniques are employed. Feeding trials for both threadfin and milkfish have aided both in identifying the best commercial feeds and formulations for threadfin growout and in ways to minimize feed costs, which typically represent 50 percent of the production costs of any fish farming operation. Water use costs can also be lowered with the identification of threadfin loading rate requirements. Market tests have shown that threadfin is a versatile, high quality product that can be sold for $6 to $7 per pound. Markets will purchase and sell threadfin that range from 0.5 pounds to 2 pounds. The most appropriate size for farmers to grow threadfin is between 0.5 and 1 pound, which can be attained at 6 to 8 months of age at most Hawaii farms. Given estimated survival rates of fish produced under this project, the average price of $6 per pound in the round and 0.75 pound harvest weight, this CTSA Year 9 project will contribute an estimated $414,000 in net sales to Hawaii threadfin farmers. Total net sales attributed to this project during CTSA Year 8 and Year 9 is estimated at percent $508,500.

End-users will provide farmers and potential investors with valuable information to meet market demands and the economic rationale for increased production for both threadfin and milkfish. Because gill deformities do not affect growth or survival of fish, the issue should not be a critical concern to threadfin farmers. Demand for milkfish as a food product is very apparent, especially in the Filipino community. Some farmers have sold milkfish as small as 5 to 6 inches for drying or frying. Most of the milkfish distributed by this project are still being grown out and will be sold to markets for $2.50 to $3.50 per pound when they reach approximately 1 pound. Project sales of milkfish from CTSA activities are estimated to be $135,000. Commercial fishing trials with live milkfish need further investigation as to whether it is a more economical and better bait. The milkfish appears to be viable bait in Hawaii especially for backyard producers. At this point, a market for small, 2- to 3-inch milkfish appears to be available for the baitfish producer. Continued investigation and demonstration will be required before fishermen will commit to purchasing milkfish for bait.

1998 Report

Continued emphasis on providing technical advice to farmers has improved on-farm survival rates of both threadfin and milkfish from the previous year. Commercial farmers currently have an estimated 72,638 threadfin, and 113,974 milkfish have been distributed to farmers. Based on current survival rates, this should result in farm-gate sales of nearly $272,390 for threadfin (54,478 pounds produced at an average 0.75 pounds sold at $5 per pound) and $85,480 for milkfish (28,494 pounds produced at an average 0.25 pounds sold at $3 per pound).

Although direct impacts from marketing efforts are difficult to measure, both threadfin farmers and distributors have confirmed increased sales through several channels including new restaurant customers and increased volume demands from existing restaurant and supermarket customers, and export distributors over the summer. Threadfin is now being served in upscale Mainland restaurants. Joan Clarke, Honolulu Advertiser Food Editor, confirmed that she saw threadfin on the menu in prestigious restaurants in Chicago and in Napa, California.

Threadfin has been featured in the local press and mentioned by food editors of both the Honolulu Advertiser and the Honolulu Star-Bulletin. The November issue of *Food Arts Magazine,* which is one of the leading chef trade magazines in the country, will carry an article on threadfin as a result of the Kapalua Wine and Seafood Symposium. Results from surveys show that threadfin has been well received by consumers.

Development of the computerized enterprise budget should prove to be a significant tool for farmers both for a predictive tool and to track current stocks. It is a user-friendly design, which does not require knowledge of Excel or other data processing packages. The budget is also easily adapted to other species of fish and will be available to farmers who do not raise threadfin.

1999 Report

As the fish production increases in Hawaii, so does the value of the aquaculture industry. Commercial farmers

currently have an estimated 354,988 threadfin and 137,500 milkfish that have been distributed. Based on current survival rates, this should result in off the farm sales of nearly $931,867 for threadfin (186,373lbs. produced at an average ¾lb. at $5/lb.), and $134,061 for milkfish (44,687lbs. produced at an average1/2lb. at $3/lb.). A million dollar a year industry is verification enough to the success of the CTSA seedstock provision program.

Development of the synthetic enterprise budget should prove to be a significant tool for farmers both for a predictive tool and to track current stocks. It is a user-friendly design, which does not require knowledge of Excel or other data processing packages. The budget is also easily adapted to other species of finfish, and also will be available for use by farmers who do not raise threadfin.

Support

This project received support from the Center for Tropical and Subtropical Aquaculture (CTSA), the National Oceanic and Atmospheric Administration and the National Marine Fisheries Service (NOAA/NMFS), the University of Hawaii (UH) and the UH Sea Grant Extension Service (SGES).

		Other Support						
Year	**CTSA**	**UH**	**NOAA/NMFS**	**SGES**	**Sales of Fish**	**OI**	**Total Other**	**Total Support**
One	$112,000.00	$0.00	$0.00	$0.00	$0.00	$86,681.00	$86,681.00	$198,681.00
Two	$109,934.00	$0.00	$15,500.00	$12,000.00	$25,639.00	$85,138.00	$138,277.00	$248,211.00
Three	$110,000.00	$4,246.00	$0.00	$0.00	$0.00	$63,361.00	$67,607.00	$177,607.00
Total	$331,934.00	$4,246.00	$15,500.00	$12,000.00	$25,639.00	$235,180.00	$292,565.00	**$624,499.00**

Publications, Manuscripts or Papers Presented

Ostrowski, A. C. and A. Molnar. 1998. Pacific Threadfin *Polydactylus sexfilis* (Moi) Hatchery Manual. Center for Tropical and Subtropical Aquaculture Publication Number 132. 96 pp. Waimanalo, Hawaii.

Public Policy Impact on Aquaculture Development in Guam
termination report

Dates of Work
October 1996 through April 1999

Funding Level
$29,950

Participants
Jeffrey Tellock, Carl Kittle and Richard Carandang, Guam Department of Commerce
Jocelyn Bamba, U. S. Department of Agriculture
Dr. Ilse Silva-Krott, Dr. John Brown and John Turner, College of Agriculture and Life Sciences, University of Guam
Richard DeVoe, South Carolina Sea Grant Consortium
Dr. Gary Pruder, The Oceanic Institute

Reason for termination
This project was termination because all the objectives were completed.

Objectives
The overall goal of this one-year project, initiated under the CTSA Ninth Annual Plan of Work, was to lower the entry barrier for new aquaculture farms through establishment of a unified policy regarding aquaculture development and to streamline the regulatory process. This includes identifying Guam government policies that hinder the expansion of the aquaculture industry; developing changes in government policies and regulatory processes to promote aquaculture development in Guam; educating Guam government administrators and elected officials about the problem of inhibitory regulations and policies and using a strategic planning session to help them develop a plan for improving the permit process and regulatory environment; educating existing and potential aquaculture farmers about the existing permit requirements and about proposed changes in the permit process. Specific objectives related to that goal were to:

prepare a case study of permitting problems experienced by Guam aquaculture producers;
identify and document Guam government policies that hinder the expansion of the aquaculture industry;
educate existing and prospective aquaculture farmers about permitting and the regulatory process and proposed changes in the process so they can monitor and support changes that are in the interest of the industry.

Principal Accomplishments

Objective: Prepare a case study of permitting problems experienced by Guam aquaculture producers.

This objective was regarded as providing the best illustration of Guam's perceived aquaculture permit and regulatory problems. Just prior to the start of the project, a new, 5-acre milkfish farm opened within a four-month time frame, which refocused this project objective. However, in this particular case, the milkfish farm was well financed and its discharge location was determined to have a low impact potential. The University of Guam agreed to provide use of its video equipment to complete the project objectives.

During the 12 years prior to the inception of this project, no new aquaculture ponds were built on Guam. However during the period from October 1996 to January 1998, four moderate to large farms received full or partial permits. One new intensive tilapia farm opened on Guam; a second farm opened without a full set of permits; a third farm rehabilitated existing ponds and reopened; and a fourth farm received most of the necessary permits. These activities caused this objective to be refocused.

The need for publication of a case study detailing the frustrations of the permitting process on Guam changed during the course of the project. Investigators had planned to select one or more farms for the case study, but none of the farmers agreed to the extensive interviews necessary for a proper case study. Although investigators had hoped to gain their cooperation, the permitting process is a sensitive issue for most farmers and none of those who were going through the process were willing to be interviewed for attribution in a published report.

Objective: Identify and document Guam government policies that hinder the expansion of the aquaculture industry.

Requests for background information and invitations to the August forum were sent to Dr. Gary Pruder and Mr. Richard DeVoe in November 1996. Investigators reviewed the Florida Aquaculture Bill (FAB) and other materials from the GADTC library, from Guam Cooperative Extension Service and from the six-year, CTSA-funded "Aquaculture Effluent Discharge Program." Investigators also held community-wide informal interviews to develop background information. Those materials served as the basis of discussions with the lead agencies affecting aquaculture development on Guam. A more informed and coordinated regulatory environment appeared to be developing on Guam. Significantly, the Department of Commerce (DOC) committed to the renewed development of the industry on Guam. In 1994, the DOC promoted a "One-Stop Aquaculture Permit" program, designed to streamline the confusing and time-consuming aquaculture permit process. The "One-Stop" program would provide for automatically approved permits for backyard and small aquaculture facilities and those medium-sized farms that have fulfilled environmental design and impact considerations. However, one segment of the program, the Master Aquaculture Permit Application (MAPA), could be a problem in the legislative, regulatory agency and federal approval processes.

The MAPA was designed to allow most of the required aquaculture permits to be compiled into one "master" application. The acting director of the Department of Land Management wrote a memorandum stating that MAPA creates a potential impediment because it would still have to fulfill the required "Zoning Development Plan Review." Further, once an executive order or legislative approval is sought for "One-Stop," a number of compliance issues will be raised by other regulatory agencies. This could potentially prevent acceptance of the "One-Stop" program and the streamlined permit processes for backyard to medium-sized farmers and the advocacy and implementation of best management practices (BMPs). Because of that, investigators planned to suggest that regulatory agencies approve the "One-Stop" measures regarding automatic permit approval for non-controversial applications and BMPs. As an alternative to the MAPA segment of "One-Stop," an interim solution will be suggested that provides for establishment of a position to assist aquaculturists through the existing zoning, environmental, and building permitting process. This position would collect all necessary permits to commence and complete an aquaculture development. The DLM director's memorandum advised against creating "another process in addition to the one existing." In light of this point, it will be further suggested that each regulatory agency develop specific policy standards concerning the aquaculture industry, coordinate with other lead agencies to define the specific area of jurisdiction of each agency, and standardize enforcement and monitoring procedures. Additionally the "One-Stop Aquaculture Permit" program addresses the size requirements for qualifying as a backyard operation.

Project investigators met with the Guam EPA (GEPA) Water Pollution Control Director prior to reviewing the Guam Water Quality Standards to determine their effect on aquaculture development. In January 1997, the Guam EPA, which administers Guam Water Quality Standards, requested that all interested parties submit written recommendations concerning the "Proposed Revised Guam Water Quality Standards" (PRGWQS). Investigators reviewed the PRGWQS and noted that the "Statement of Policy" does not mention the aquaculture industry. Investigators called attention to this oversight in light of the fact that five commercial aquaculture farms larger than 4 acres and 10 to 15 smaller farms are operating on Guam.

Project investigators convened an informal panel discussion to increase involvement in the PRGWQS by regulatory agencies and the Guam scientific community. During this meeting, alternatives and options were developed to offer GEPA for consideration involving policy recognition of aquaculture, the application of ambient standards to aquaculture effluents and the possibility of using aquaculture discharges to create wetlands. At a second meeting held in May 1997, the aquaculture discharge as a wetland "creator" option was favorably discussed by the GEPA, ACOE and DOC. The PRGWQS is undergoing a field application in Umatac, Guam, where secondary waste water is being used as a wetland "creator."

As a result of the project work, GEPA and other agencies realized that the PRGWQS was important to the revitalized development of the industry on Guam. This is significant because the Guam Water Quality Standards are normally reviewed only once every three years. In the spirit of cooperation engendered by the project and participating regulatory agencies, GEPA extended the review period to accommodate the project's "Aquaculture Permit Application Work Group."

In 1990, Public Law 20147 charged the Territorial Planning Council (TPC) with developing an alternate to the Guam Land Use Plan. The new, comprehensive development plan, known as the *I Tano'ta* Land Use Plan (pronounced "E-Tan-o-ta"), is being formulated. Project personnel is providing information to facilitate the inclusion of aquaculture parks and define a minimum size for backyard aquaculture facilities that could be established regardless of zoning restrictions.

The director of the Department of Land Management (DLM) issued a memo providing details on five of the eleven "Intensity Districts" as permitting "aquaculture activities/facilities." The project requested clarification of the exact aquaculture zoning designations and copies of the maps defining the zoning limitations of the "aquaculture activities/facilities" of the proposed Intensity Districts.

Investigators reviewed the Aquaculture Effluent Discharge Program's case studies, particularly that of the Natural Energy Laboratory of Hawaii Authority (NELHA) facility for its description of the formation of an aquaculture park and resultant regulatory success. The case study will be submitted to DLM for consideration in developing the aquaculture segments of the comprehensive land use plan.

Investigators requested position statement regarding the discharge of aquaculture effluent to wetlands from ACOE, Department of Agriculture (DOA) and GEPA.

An Environmental Impact Assessment (EIA) was beyond the scope of this project, but use of an EIA could provide information needed to facilitate the potential development of an aquaculture park in a specific location. Investigators requested GEPA and DOA to provide position statements regarding the use of an EIA for blanket permit clearance purposes. This will require additional cooperation from the CLTC, BOP and the DLM.

The Chamorro Land Trust Commission (CLTC) is formulating policy regarding use of excess and inactive military lands that reverted to the Guam government, which will be leased to those people recognized as "Chamorros." In June 1997, the CLTC began a 99-year agricultural lease program. The CLTC has been asked to consider an aquaculture park as part of its agriculture lease program. In addition, other government agencies were asked to comment on this idea.

The DOA Division of Aquatic and Wildlife Resources (DAWR) controls all introductions of non-indigenous life forms into Guam. The accidental introduction of the brown tree snake has significantly reduced bird life, garnered negative international publicity, damaged electrical power generation, and directly threatened humans. Because of this, the DOA is wary of introducing any non-indigenous species to Guam and will allow non indigenous species introduction only on a strict, "case by case" basis, according to a personal communication with Mr. Gerry Davis, DAWR. DOA has reiterated that new species introduction is not impossible, only very carefully scrutinized. Any party interested in importing exotic species to Guam must provide exhaustive biological backgrounds of those species. Milkfish and tilapia fry are currently imported to Guam by several producers.

At the Guam Aquaculture and Environmental Awareness Forum, which was held in August 1997 and videotaped, both public policies of environmental line agencies and aquaculture farmers' responses to the constraints imposed

by these policies were reviewed. More than 150 individuals attended the forum, including potential and current aquaculture producers, representatives of 13 government agencies and five members of the Guam Senate. Presentations were given by:

Dr. Jeff T. Barcinas, Dean, College of Agriculture and Life Sciences, University of Guam;
Mr. Frank Dayton, U.S. Army Corps of Engineers
Mr. Randy Sablan and Mr. Michael Gawel, Guam Environmental Protection Agency
Mr. Joseph Borja, Administrative Director, Chamorro Land Trust Commission;
Mr. Jeffrey Tellock, GADTC, Guam Dept. of Commerce;
Senator John C. Salas;
Mr. Marvin Aguilar, Guam Dept. of Agriculture;
Dr. Robert Richmond, University of Guam Marine Laboratory;
Dr. Gary Pruder, The Oceanic Institute, Hawaii;
Mr. Richard DeVoe, Director, South Carolina Sea Grant Consortium;
Mr. John Anderson, Director, Dept of Land Management;
Mr. Gerry Davis, Aquatic and Marine Resources, Guam Dept of Agriculture;
Senator Alberto Lamorena.

Interviews with two farmers were videotaped, and other farmers and environmental agency representatives were interviewed informally. Several constraints were identified; among them were Guam water discharge and NPDES permits, federal wetlands permits, local grading, building and land use permits and various permits associated with moving seedstock. A primary frustration for farmers is the lack of a single point of contact to deal with the permitting process and the cost in time and money of dealing with the process.

Several meetings were held with local environmental agency personnel regarding the development of a one-stop permitting process for new aquaculture operations using a Master Aquaculture Permit Application. The Department of Commerce seems to have shelved this initiative. The local environmental agencies strongly resisted the concept of a general permit for small, backyard intensive systems. Their position was that such a permit is unnecessary because they don't intend to monitor such systems, which they anticipate will have little environmental impact. Guam EPA promulgated its proposed revised Guam Water Quality Standards, public hearings on which were postponed because of the effects of Typhoon Paka. The governor vetoed the I Tano`ta land use plan, which is being revised by the Senate. Passage of this plan could have significant impact on the aquaculture regulatory environment, its progress is being closely monitored. Finally, the Chamorro Land Trust Commission is interested in the possibility of developing some of its property as an aquaculture industrial park with a single master permit and environmental impact assessment. However, development of such a park would be a long-term effort far beyond the scope of this project.

Objective: Educate existing and prospective aquaculture farmers about permitting and the regulatory process and proposed changes in the process so they can monitor and support changes that are in the interest of the industry.

A total of 400 copies of the publication *Aquaculture on Guam: prospects, permits and assistance* was published and distributed under the auspices of the Agriculture Experiment Station of the University of Guam. The video "Aquaculture on Guam" was produced in-house using student help by the Agriculture Experiment Station. Copies were made for lending purposes for prospective aquaculturists who visit the Cooperative Extension Service or the Guam Aquaculture Development and Training Center (GADTC) seeking assistance in starting a farm.

An "Aquaculture Point Paper," intended to provide a basis for developing policy considerations, was prepared and distributed to relevant agencies, UOG and local aquaculturists. Investigators anticipated adding other policy considerations to this document, which will be used as one of the Forum round table discussion topics. It may ultimately serve as the basis for development of needed actions by regulatory agencies, legislators and the governor.

The general consensus of the regulatory agency speakers was that aquaculture could and should be a viable industry on Guam, but that regulatory agencies would not consent to public actions that diminish their regulatory authority or freedom. Farmers vented their frustrations with the line agencies and paperwork involved in obtaining permits required to operate their farms. The governor and senators supported aquaculture as long as they could be

given assurance of environmental protection. No concrete process to lift the regulatory burden from farmers was proposed.

Impacts

This project benefited the aquaculture industry by garnering participation of relevant agencies in the consideration and formation of sound aquaculture policy. Benefits were achieved through the distribution of accurate and current information affecting aquaculture development worldwide. Media coverage of the activities of the project resulted in a greater awareness and open discussion of the permitting process. Coverage included television news stories, op-ed page pieces, and letters to the editors. The project also prompted a visit by the Governor to the GADTC facility, which resulted in identification of additional local funding for the facility. During the course of the project, several new farmers began operations on-island and were given information and encouragement from the workshop.

Support

This project received support from the Center for Tropical and Subtropical Aquaculture, the Guam Department of Commerce (DOC) and the University of Guam (UOG).

		Other Support			
Year	CTSA	DOC	UOG	Total Other	TOTAL
one	$29,950.00	$14,947.00	$8,000.00	$22,947.00	$52,897.00
Total	**$29,950.00**	$14,947.00	$8,000.00	**$22,947.00**	**$52,897.00**

Publications, Manuscripts or Papers Presented

Fitzgerald, William J., Jr. 1999. *Aquaculture on Guam: prospects, permits and assistance.* (Revised by John W. Brown and Anthony Benavente). Agricultural Experiment Station, College of Agriculture and Life Sciences, University of Guam. 11 pp.

Brown, John W. 1999. *Aquaculture on Guam* (video). Agricultural Experiment Station, College of Agriculture and Life Sciences, University of Guam. 26 minutes.

Marine Ornamental Fish Culture and Conservation

Dates of Work

May 1998 through April 1999

Funding Level

$49,200

Participants

Dr. Christopher Brown, Hawaii Institute of Marine Biology
Dr. Jeri Fox, Hawaii Institute of Marine Biology

Objectives

The objectives of this project, initiated under the CTSA Year 11 Plan of Work, are to:

Collect larvae from the wild, return them to the laboratory and rear them to market size.
Collect culture material from the Waikiki Aquarium.
Test the suitability of a green-water culture system primed with actively photosynthesizing monocellular algae and nutrient-enriched trocophores as a larviculture system.
Develop a prioritized list of marine ornamental species for aquaculture development.
Transfer the technology to industry.

Anticipated Benefits

This project was designed to test the feasibility of two alternative means of providing fish for the development of a foundation for a marine ornamental fish culture industry. Both approaches involve the use, rather than the care of existing broodstock animals, one drawing upon wild stocks and the other taking advantage of animals in public display aquaria. It is anticipated that the marine ornamental fish trade will also benefit from the delivery of a species priority list and data on larval collection and growout.

Principal Accomplishments

Objectives: Collect larvae from the wild, return them to the laboratory and rear them to market size using a green-water culture system.

Investigators obtained and tested a large, fine-mesh seine net to capture larvae. Extensive testing proved it unsuitable because it takes four strong people to haul it out by hand. The hauling process is slow and allows a large number of larvae and juveniles to escape. In addition, the process requires two 17-foot Boston whalers, which are costly to rent. The wet net is extremely heavy and difficult to handle. Moving it onshore requires a forklift and a large space for hanging and drying the net. The net is also affected by tidal currents. Night trials proved most encouraging, especially in calmer waters. Light attractants draw large numbers of appropriate-sized larvae (<1 cm).

Investigators constructed two types of light attractants to use when gathering larvae at night. The first is a waterproof floating light attractant that can be deployed and anchored if desired, for use in conjunction with nets. It uses a 12-volt battery, with three submersible light fixtures, switches and an anchoring device. The attractant apparatus appears to be effective in gathering larval and juvenile fishes. Preliminary attempts at encircling it with the purse-seine net were made, but yields were relatively small. However, investigators feel that resulted from the design of the net.

The second approach to attract larvae was pursued with shoreline-based lighting. Three fixtures have been deployed around the Coconut Island Marine Laboratory, in locations that have nearby electrical outlets, nearby deep water and will not interfere with HIMB research nor annoy Kaneohe Bay residents. The fixtures were placed using concrete reinforcement bars driven into the coral rubble. Investigators are experimenting with traps and netting devices to collect the larvae that are attracted.

Objective: Collect culture material from the Waikiki Aquarium.

A set of culture tanks, supplies and materials were obtained and prepared for use. Algae and zooplankton cultures were begun using starter cultures from The Oceanic Institute, the University of Hawaii and the Waikiki Aquarium. Evaluations showed *Nanochloropsis* sp., *Tetraselmis* sp., *and Isochrysis galbana* to be the best monocellular algae for the project purposes. A working relationship, including paperwork and interviews of project staff, was established with the Waikiki Aquarium. Project personnel then visited the Aquarium during night hours and successfully collected convict tangs, yellow coral gobies and cardinalfish. Frogfish eggs were also collected, although after they were brought to HIMB, it became apparent that they were not fertilized. Eggs and larvae were transported to the HIMB experimental hatchery, where they were offered a variety of starter feeds, including both strained and unstrained rotifers, copepods and algae. None of the preliminary culture trials proved successful, although some larvae survived well beyond the first feeding stage.

In addition, six cohorts of the endemic Hawaiian seahorse, which has been tentatively identified as *Hippocampus kuda*, were obtained. The larval seahorses were exposed to a variety of diets, including the three species of algae previously mentioned, both size-sorted and unsorted rotifers, copepods, *Artemia* nauplii and pelleted feed. In each case, survival was only to Day Five, at which time mass mortality resulted. Day Five was identified as a critical period for ongoing studies, including the use of hormones to attempt to accelerate development and improve larval survival, as investigators have done successfully with other marine species.

The retrieval of larvae from Waikiki Aquarium display tanks is not as straightforward as was previously thought. Large numbers of larvae are needed for culture trials at standard mariculture densities, and efficiency of collection is challenging. The presence of fish that readily consume edible eggs and larvae complicates the problem. Consequently, investigators concluded that the most viable approach is to collect fertilized eggs shortly after they are produced. They designed and refined a variety of egg collectors for that purpose. The first is a trap affixed to the display tank's outflow pipe. Conventional traps of this sort do not handle a high flow volume, so several generations of collectors were tested before a suitable one was found. It is basically a large floating chamber with a nytex panel that can screen out fertilized eggs.

A second type of trap exploits air-lift energy to direct water flow through nytex screen bags by positioning them in the back of display tanks. The drawback inherent in this type of collector is that they detract from the appearance of the display and are considered unsuitable for leaving in place during times when the Aquarium is open to the public. A third method of retrieving eggs of demersal spawners has been to place ceramic tiles in various tanks for species that are showing this sort of nesting behavior. As with the airlift collectors, these present some concerns about suitability for displays.

Objective: Develop a prioritized list of marine ornamental species for aquaculture development.

A Prioritized Species List (Table 1) was compiled based on input solicited from numerous representatives of the Hawaii aquaculture community. The fishes are ranked in their relative order, with the higher priority fish appearing at the top of the list. In general, the species endemic to Hawaii appear at the top of the list; those species are considered a higher priority for culture for reasons involving both market competition and conservation considerations. The feasibility of cultivation and market demand were also considered. The clownfishes and a few other species already being cultivated in Florida offer some potential for culturists seeking to begin production sooner rather than later. Although some appropriate technology is available for this purpose, further development is required for efficient mass-culture to become profitable.

Special considerations are indicated by asterisks (*). **Damselfishes** can be cultivated and constitute a significant segment of the marine ornamental market, but unrestricted imports of wild specimens undermine the profitability of captive rearing. It remains possible (some say even likely) that restrictions on such importations will be imposed, so the development of suitable technology for mass culture of the numerous species of damselfishes favored by the marine ornamental trade is strongly recommended. The **seahorses** include a range of species (in this context, also other pipefishes), at least one of which is endemic to Hawaii. Some are already being cultivated. Their nurturing of young contributes to the prospects of successful cultivation of a wider range of species, and continued technical development is considered advisable. A second asterisk also appears adjacent to "Market" for seahorses, because additional markets exist besides the aquarium trade. Dried seahorses are a traditional dietary supplement in China, although supplying product to this market is almost certain to have unwanted image consequences for the business profiting from this sort of trade. **Triggerfishes** construct nests and consequently eggs can be collected efficiently; also they include numerous highly valued species, some of which are endemic to Hawaii. Cultivation appears to be a difficult task, however. The **Bangai cardinalfish** is a mouthbreeding species, and can be bred in captivity with only moderate difficulty. The cohort size is small, however, so the cost of producing fry is relatively high.

TABLE 1: Prioritized Species List

Species or Group	Florida Culture	Benthic Eggs	Hawaii Export	Expected Difficulty	Market
Yellow Tang	No	No	#1	High	Vast
Pygmy angelfishes	No	No	Yes	High	Solid
Sargassum fishes	No	*	Yes	Moderate/high	High price
Hawkfishes (longnose, etc.)	No		Yes	High	Small but popular family, carnivorous
3-spot damselfish	No	Yes	Yes	Easy-moderate	Low price, high volume*
Misc. damselfishes	No	Yes	Some	Moderate	Varies*
Neon goby	Yes	Yes	No	Moderate	Accepts tank-reared fish
Seahorses	? (Australia)	*	Few	Moderate	Solid*
True angelfishes	Few	No	Some	High	76 spp.
Butterflyfishes	No	No	Yes	High	Good, but requires coral in diet
Surgeonfishes	No	No	Yes	High	Numerous spp.
Wrasses	No	No	Yes	High	Numerous spp.
Triggerfishes	No	Yes*	Yes	High	Good
Clownfishes Tomato Clarke's Pink Skunk Orange Maroon Wide-banded Cinnamon Red saddleback	Yes	Yes	No	Moderate	Established, competitive
Clownfishes Percula False percula	Yes	Yes	No	Easy-moderate	High volume/low price
Bangai cardinalfish	Yes	*	No	Moderate	High value
Neon dottyback	Yes	Yes	No	Moderate-high	Good
Striped dottyback	Yes	Yes	No	Moderate-high	good

Support

This project received support from the Center for Tropical and Subtropical Aquaculture.

Year	CTSA	Total
One	$49,200	$49,200
Total	**$49,200**	**$49,200**

Publications, Manuscripts and Papers Presented

No publications, manuscripts or papers were presented during the reporting period.

Development of Best Management Practices for Hawaiian Aquaculture

Dates of Work

April 1998 through January 1999

Funding Level

$10,000

Participants

Dr. Robert Howerton, Sea Grant Extension Service, University of Hawaii; Dr. David Ziemann, The Oceanic Institute; Kristen Anderson, Pacific Regional Aquaculture Information Service for Education, Hamilton Library, University of Hawaii; Farm Service Agency, U.S. Department of Agriculture; Department of Health, Clean Water Branch, State of Hawaii.

Objectives

The overall goal of this project, initiated under the CTSA Eleventh Annual Plan of Work, is to develop a practical Best Management Practices Manual that will assist aquaculture farmers in managing facilities more efficiently and allowing them to comply with discharge regulations. Specific objectives related to that goal are to:

Conduct a comprehensive literature review of current and proposed Best Management Practices for aquaculture systems in the United States (e.g., trout, channel catfish, salmon);
Review BMPs developed for other industries nationwide (beef, poultry, dairy, silviculture) and in Hawaii (sugar, pineapple) to determine how BMPs support and facilitate compliance with effluent discharge regulations;
Evaluate documents generated at other Regional Aquaculture Centers concerning effluent discharge and best management practices for aquaculture;
Examine international aquaculture BMPs to determine how these may apply to Hawaii aquaculture;
Interact with USDA Farm Service Bureau to outline BMP criteria for aquaculture farmers to follow, allowing them to be eligible for federal crop disaster assistance;
Develop a BMP for Hawaiian Aquaculture manual that outlines practical guidelines, recommendations and defining principles that Hawaii aquaculture farmers can use to comply with permit regulations and increase farm efficiency.

Anticipated Benefits

The Best Management Practices Manual will have three-fold benefits. Aquaculturists, by following recommendations outlined in the manual, will

Potentially be more profitable;
Eligible for federal farm disaster assistance;
Conform with effluent discharge regulations that govern aquaculture operations in Hawaii.

Principal Accomplishments

Objective: Conduct a comprehensive literature review of current and proposed Best Management Practices for Aquaculture systems in the United States.

A literature review has been conducted covering best management practices for trout and channel catfish. A more thorough literature search continues to be conducted which also covers BMPs for the salmon industry in the Northwestern portion of the US.

Objective: Review BMPs developed for other industries nationwide and in Hawaii to determine how BMPs support and facilitate compliance with effluent discharge regulations

Through the use of the Internet, a literature review has begun to detail BMPs used by the livestock and poultry industry in the US. Contact has been made with a number of both State and National agriculture industry councils and a dialogue has begun. Many state industry councils have begun to develop a proactive stance on waste management. These industry representatives have begun the development of best management practices specific to their needs.

Objective: Evaluate documents generated through other Regional Aquaculture Centers concerning effluent discharge and best management practices for aquaculture.

A project manual, "Characterization and Management of Effluents from Aquaculture Ponds in the Southeastern United States," published by the Southern Regional Aquaculture Center, was obtained and reviewed. A number of references were obtained from this manual. The NRAC and WRAC have also been contacted concerning possible references for BMPs in their region.

Objective: Examine international aquaculture best management practices and determine how these may apply to Hawaii aquaculture.

During a USAID-sponsored trip to the Philippines, the principal investigator obtained information about BMPs and effluent discharge during meetings with Philippine government officials in the Bureau of Fisheries and Central Luzon State University aquaculture researchers.

Objective: Interact with USDA Farm Service Agency to outline BMP criteria for aquaculture farmers to follow, allowing them to be eligible for federal crop disaster assistance.

The principal investigator contacted the USDA Farm Service Agency, which agreed to meet to develop BMP criteria for Hawaii aquaculture farmers.

Develop a BMP for Hawaiian Aquaculture manual that outlines practical guidelines, recommendations and defining principles that Hawaii aquaculture farmers can use to comply with permit regulations and increase farm efficiency.

A general outline of the proposed manual has been written.

Work Planned

Use PRAISE to thoroughly review BMPs developed for agriculture industries. This review will be written and submitted to CTSA Management practices used in traditional agriculture that facilitate compliance with effluent discharge regulations will be incorporated into the aquaculture BMP where relevant;
Compile documents relating to aquaculture effluent discharge, current and proposed BMPs and research efforts relating to the National Pollution Discharge Elimination System. A review of these reports will be written and submitted to CTSA;

Use PRAISE to examine international aquaculture BMPs and determine how they may apply to Hawaii aquaculture. The literature will be thoroughly searched to examine how aquaculture industries in other countries manages aquaculture effluents. A review of international BMPs will be written with an emphasis on what practices may be applicable to Hawaii aquaculture;
Submit a draft of the Hawaii BMP manual to farmers, appropriate regulatory agencies and members of the CTSA Technical Committee for review. Comments will be incorporated into a final manual for distribution to end users, the Hawaii aquaculture community.

Support

This project received support from CTSA, the University of Hawaii and Maui county government.

Year	CTSA	Other Support			Total Support
		University of Hawaii	Maui County	*Total Other Support*	
One	$ 10,000.00	$ 1,948.00	$ 1,200.00	$ 3,148.00	$ 13,148.00
Total	$ 10,000.00	$ 1,948.00	$ 1,200.00	$ 3,148.00	**$ 13,148.00**

Publications, Manuscripts and Papers Presented

No publications, manuscripts or papers were presented during the reporting period.

Publications

Dates of Work

March 1990 through October 1999

Funding Level

$175,000

Participants

Dr. Kevan L. Main (through March 1997), Cheng-Sheng Lee (starting February 1997) Patti Killelea-Almonte (through February 1999), Jean McAuliffe (starting March 1999), Center for Tropical and Subtropical Aquaculture, The Oceanic Institute.

Objectives

The overall goal of this project is to disseminate information on aquaculture. Specific objectives related to that goal are to:

publish a quarterly newsletter to communicate information about the activities of the Center for Tropical and Subtropical Aquaculture and its funded projects and the latest information about aquaculture from the nation and the region;
develop and publish a technical bulletin to communicate the status and progress of current activities to the CTSA Board of Directors, Industry Advisory Council and Technical Committee. The bulletin will also be sent to aquaculturists in the Pacific region and upon request to other interested parties;
produce and publish progress reports of selected CTSA-funded projects. These publications will be distributed free of charge to commercial producers, aquaculture researchers, extension agents and other interested parties throughout the Pacific region, with limited distribution in the United States;
duplicate and distribute the other Regional Aquaculture Centers' videos and publications to information networks throughout the Pacific region
develop and maintain a home page on the Worldwide Web through which information about CTSA activities can be disseminated.

Anticipated Benefits

In many locations in the Center for Tropical and Subtropical Aquaculture region, access to information is extremely limited, which handicaps the development of aquaculture. This project helps to overcome that obstacle by disseminating research results and other information that bears directly on commercial aquaculture production.

Principal Accomplishments

Objective: Publish a quarterly newsletter to communicate information about the activities of the Center for Tropical and Subtropical Aquaculture and its funded projects and the latest information about aquaculture from the nation and the region.

In August 1989, the Center developed and published the inaugural issue of its quarterly newsletter, *CTSA Regional Notes*. The staff handles all aspects of production for the Center's newsletter, including interviewing, researching and writing articles, and shooting or obtaining photos. *Regional Notes* provides the latest information on Center activities and aquaculture throughout the Pacific region. Published four times per year, it is distributed to

approximately 1,000 individuals, organizations and universities worldwide. In 1990, the newsletter was expanded by one-third and began carrying two regular columns:

"PRAISE Pages" is a bibliography of journal articles; the column is prepared by David E. Coleman, coordinator of the CTSA-funded *Pacific Regional Aquaculture Information Service for Education*. In each newsletter issue, Coleman compiles a bibliography on a specific topic of interest to *Regional Notes* readers;
"Aquatips" provides recommendations and suggestions on specific aquaculture topics and problems from researchers and extension agents.

The newsletter also features news on CTSA-funded projects, government assistance programs for aquaculture, publications and various information services that are available. In addition, it provides profiles of individuals who provide services to aquaculturists, job openings in the region, and announcements about training courses.

Objective: Develop and publish a technical bulletin to communicate the status and progress of current activities to the CTSA Board of Directors, Industry Advisory Council and Technical Committee. The bulletin will also be sent to aquaculturists in the Pacific region and upon request to other interested parties.

In February 1990, the Center staff developed and published its first set of *Project Updates*, technical bulletins that are distributed to the CTSA Board of Directors, Industry Advisory Council and Technical Committee and to extension agents and other interested parties upon request. Each set of *Project Updates* contains separate bulletins from one to six pages long on each active, funded project. Each bulletin provides details on the principal accomplishments for each objective and the principal investigators. In addition to writing and editing the bulletins, the staff does the artwork, layout and design and works with printers to produce the final publication.

The Publications project produced a 70-minute movie titled *CTSA Video Project Update*. Center staff assisted with writing the script and shooting the background footage for the video. The staff worked closely with the Sea Grant Communications director on the editing and final production of the video. The *CTSA Video Project Update* was prepared to provide the CTSA Board of Directors, Industry Advisory Council and Technical Committee with the latest results from 12 Center-funded projects. The video was shown at the Industry Advisory Council meeting in March 1995 and at the Technical Committee meeting in April 1995 and was distributed throughout the region. A similar video, which featured different projects, was produced and shown in 1996.

Objective: Produce and publish progress reports of selected CTSA-funded projects. These publications will be distributed free of charge to commercial producers, aquaculture researchers, extension agents and other interested parties throughout the Pacific region, with limited distribution in the United States.

The Center staff assists with publication of selected project progress reports. Staff assistance includes editing the grammar and style of the reports, proofreading and designing them and working with printers to produce the final documents.

During 1995, the Center staff assisted with publication of a bibliography developed under the two-year, Center-funded project titled "Exploratory Study of Hawaii and Guam as High Health Aquaculture Stock Centers." The bibliography, titled *A Bibliography of Specific Pathogen-Free Organisms*, was published as CTSA publication number 116 in April 1995. The Center staff also assisted with publication of an extension fact sheet developed under the Center-funded project titled "Ornamental Aquaculture Technology Transfer." The fact sheet, titled *Raising the Silver Arowana* (Osteoglossum bicirrhosum), was published as CTSA publication number 117 in May 1995.

During 1996, the Center staff assisted with publication of a manual on making value-added products from giant clam shells. Titled *Clams to Cash: How to Make and Sell Giant Clam Shell Products,* the manual was produced as part of the Center-funded project titled "Extension and Training Support in the U.S.-Affiliated Pacific Islands" and was published as CTSA publication number 125 in August 1996.

Center staff also assisted in editing a sponge manual and four extension fact sheets that were produced under the Center-funded project titled "Production of CTSA Educational Extension Materials."

During 1997, the publications assisted with publication of an extension fact sheet titled *Aquafarmer Information Sheet: Prevention of Black Gill Disease in Marine Shrimp*. The fact sheet, produced by the Center-funded as part of the project titled "Gill Discoloration in *Penaeus stylirostris*," was published as CTSA Publication Number 126 in October 1997. Center staff edited the information sheet and did the layout and design.

Center staff also provided the text and photos for the brochure titled *Regional Aquaculture Centers Results: Research and Extension Solutions for Aquaculture.* USDA requested that each of the five regional aquaculture centers provide information for one segment of the brochure.

In 1998, Center staff assisted with layout and printing of two manuals produced by CTSA-funded projects. The manuals were *Spawning and Early Larval Rearing of Giant Clams (Bivalvia: Tridacnidae),* which was CTSA publication number 130, and *Pacific Threadfin,* Polydactylus sexfilis *(Moi), Hatchery Manual*, which was CTSA publication number 132. The latter was printed with Publications budget funds. In addition, Center staff edited, designed , layer out and oversaw printing of *Triploid Chinese Catfish*, an extension fact sheet resulting from a CTSA-funded project.

In 1999, Center staff assisted with layout, editing and printing of a CTSA-funded project manual titled, *The Culture of Soft Corals for the Marine Aquarium Trade*, CTSA publication #137. In addition, Center staff edited, layed out and oversaw printing of the extension fact sheet , *Spawning the tinfoil barb,* Barbodes schwanenfeldi *in Hawaii*, CTSA Publication #136. Editing and coordination of printing was provided by Center staff for two other extension fact sheets titled, *Lagoon farming of giant clams (Bivalvia: Tridacnidae),* CTSA publication #139 and *Farming Soft Corals for the Marine Aquarium Trade*, CTSA publication #140. Editing work was also begun in late 1999 on *Producing Pearls Using the Black-lip Pearl Oyster,* (Pinctada magaritifera), CTSA Publication #141, which should be published before the end of the year.

Objective: Duplicate and distribute the other Regional Aquaculture Centers' videos and publications to information networks throughout the Pacific region.

The Center staff has duplicated 16 videos produced by the other Regional Aquaculture Centers and distributed them to extension agents, libraries and aquaculture concerns throughout the region. The Center staff also maintained a library of all videos produced by the Regional Aquaculture Centers and loaned them to interested parties upon request. In addition, the Center staff distributed publications produced by the other Regional Aquaculture Centers to extension agents and libraries throughout the region.

Objective: Develop and maintain a home page on the Worldwide Web through which information about CTSA activities can be disseminated.

The Center staff also assisted in development of a CTSA home page on the Worldwide Web. The web site is maintained by the Center staff and contains news and CTSA publications as well as links to AquaNIC, the PRAISE web site and sites maintained by other Regional Aquaculture Centers. During 1998, artwork was commissioned for the site and the site was redesigned and reformatted, resulting in a more attractive, user-friendly environment.

Work Planned

The Center staff will continue to produce the quarterly newsletter, assist as needed in editing, layout and printing of publications of selected CTSA-funded projects and maintain the Web site. A new CTSA information brochure will also be designed and produced. In addition, plans to update the design of the quarterly newsletter and extension fact sheets will also be implemented in the coming year.

Impacts

This project has helped to disseminate aquaculture research results and information throughout the region in order to enhance viable and profitable U.S. aquaculture production that will benefit consumers, producers, service industries and the American economy.

Support

This project was funded by the Center for Tropical and Subtropical Aquaculture (CTSA) through grants from the U.S. Department of Agriculture.

One	$ 10,000.00	$ 10,000.00
Two	$ 10,000.00	$ 10,000.00
Three	$ 12,000.00	$ 12,000.00
Four	$ 15,000.00	$ 15,000.00
Five	$ 38,000.00	$ 38,000.00
Six	$ 18,000.00	$ 18,000.00
Seven	$ 18,000.00	$ 18,000.00
Eight	$ 18,000.00	$ 18,000.00
Nine	$ 18,000.00	$ 18,000.00
Ten	$ 18,000.00	$ 18,000.00
TOTAL	**$ 175,000.00**	**$ 175,000.00**