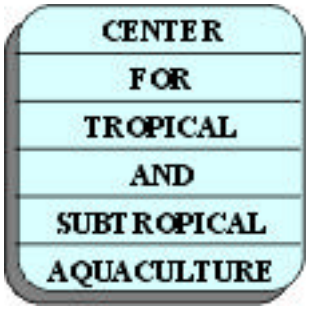

Center for Tropical and Subtropical Aquaculture

1998 Annual Accomplishment Report

(formatted for Adobe Acrobat Reader)

December 1, 1998

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Executive Summary

Mission

The Center for Tropical and Subtropical Aquaculture, or CTSA, is one of five regional aquaculture centers funded by the U.S. Department of Agriculture. The mission of CTSA is to support aquaculture research, development, demonstration and extension education to enhance viable and profitable U.S. aquaculture. Research projects span the American Insular Pacific, using its extensive resource base to meet the needs and concerns of the tropical aquaculture industry.

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.

Organization

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.

Program Scope

In April 1998, projects funded under the Center's Eleventh Year Plan of Work were initiated. The Center has funded 126 projects in its 11 years of operation. 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.

A brief listing of the principal accomplishments of the active projects in these categories during 1998 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.

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.

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.

Publications

Accomplishments

This project produced a quarterly newsletter, and an annual technical bulletin on each of the Center's active funded projects. In addition, the project provided assistance to "RAC Results: Aquaculture Extension and Training in the U.S.-Affiliated Pacific Islands." The publications project also provided production assistance to publish manuals from two projects. The manuals were titled "Spawning and Early Larval Rearing of Giant Clams" and "Pacific

Threadfin *Polydactylus sexfilis* (Moi) Hatchery Manual." The project also established and maintains a home page on the worldwide web from which Internet browsers can download publications and learn about the Center.

Extension Support to Further Industry Development

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

Accomplishments

This project provides extension and training support to aquaculturists and to government fisheries and aquaculture staff throughout the region. This support included 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. The project produced a manual and companion video titled "Spawning and Early Larval Rearing of Giant Clams."

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.

Public Policy Impact on Aquaculture Development in Guam

Accomplishments

This project is promoting 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. The benefit will be the direct participation of relevant agencies in the consideration and formation of sound aquaculture policy.

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.

Diversification of Species for Aquaculture in Guam

Accomplishments

This project developed a technique for spawning and culturing hard corals for the aquarium trade. It has also identified an artificial substrate that can be used for settling corals, which will help to identify them as cultured in the marketplace.

Demonstration and Adaptation of Known Technologies

Differential Growth Rate Studies in Cultured Commercial Sponges

Accomplishments

This project compared growth of various sponges to determine which would be best used to replant a farm. The study determined that high growth rate sponges were best to replant a farm because the sponges maintained high growth rates after being divided into cuttings, whereas slow-growing sponges maintain their slow growth rates when divided into cuttings.

Development of Improved Growout Culture for Chinese Catfish Through Ploidy and Feed Applications

Accomplishments

Significantly greater growth of triploid fish at higher temperatures was observed. These higher water temperatures are perhaps representations of typical water temperature at most Hawaii aquaculture sites. However, triploid growth was only about 10 percent greater on average than that of diploids. This is less than expected based on earlier results with *Clarias macrocephalus* but still could prove to be an advantage to farmers because triploids would reach marketable size about one month earlier than diploids. Triploid fish also exhibited significantly greater fat content when cultured at both high and low temperatures and on different feeds. Those fat profiles are considered “healthful” and could add to their market appeal. The project produced an extension fact sheet that will be published at the end of 1998.

Introduction

During 1998, the Center for Tropical and Subtropical Aquaculture completed work on projects funded under its Eighth Annual Plan of Work and continued work on projects funded under its Ninth and Tenth Annual Plans of Work. In addition, the Center initiated work on projects developed under its Eleventh Annual Plan of Work and began developing its Twelfth Annual Plan of Work.

Ten projects were funded under the Center's eleventh year program, which was approved by the Center's Board of Directors on January 27, 1998. Three projects were new, and seven 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 $3,337,348 in additional or in-kind support to projects.

The development of the Year Twelve program was initiated in March 1998 at the annual meeting of the Industry Advisory Council (IAC). The IAC reviewed the progress of funded projects and recommended Year Twelve research priorities that would aid industry development. Members identified nine project areas, four of which were new areas, and five of which were continuations of projects funded under previous years. The priority areas were:

1. Library Aquaculture Workstation -- Year Twelve;
2. Extension and Training Support for the U.S.-Affiliated Pacific Islands -- Year Eleven;
3. Disease Management for Hawaiian Aquaculture -- Year Seven;
4. National Coordinator for Aquaculture New Animal Drug Applications -- Year Four;
5. Marine Ornamental Aquaculture *(new priority);*
6. Freshwater Ornamental Aquaculture *(new priority);*
7. Marine Food Fish Seedstock Production *(new priority)*;
8. Red Tilapia Seed and Tilapia Aquaponics in Guam *(new priority)*;
9. Publications.

In April 1998, 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 May. The Center staff then solicited proposals for projects, and eight proposals were submitted.

In July, 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 will be incorporated into the Twelfth Annual Plan of Work, which will be sent to the Center's Board of Directors for approval. 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 126 research, demonstration, development and extension projects. Thirteen projects were active during 1998. 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;
Understanding *Gracilaria* Gall Syndrome;
Disease Management for Hawaiian Aquaculture;
Public Policy Impact on Aquaculture Development in Guam.

Projects addressing development of new technologies comprise:

Development of Threadfin (*Polydactylus sexfilis*) Fry Production Technology;
Development of Pacific Threadfin and Milkfish Growout Technology and Production of Live Feeds and Seedstock.

Projects addressing demonstration and adaptation of known technologies comprise:

Differential Growth Rate Studies in Cultured Commercial Sponges;
Expansion and Diversification of Freshwater Tropical Fish Culture;
Development of Improved Growout Culture for Chinese Catfish through Ploidy and Feed Applications.

Organizational Structure

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

The 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.

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. Dennis Bishop, Kona Mariculture;

Ms. Mary Brooks, Pacific Aquaculture;
Mr. Steve Chaikin, Molokai Sea Farms;
Mr. Shinji Chibana, Palau Biotech Marine Tropicals;
Mr. Michael Crisostomo, Kurumaya SeaHorse Restaurant;
Mr. Richard Croft, Pohnpei Natural Products;
Mr. John Gourley, Micronesia Clam Company;
Ms. Linda Gusman, Island Aquaculture;
Mr. Steve Katase, Royal Hawaiian Sea Farms;
Mr. Jeff Koch, Mokuleia Aquafarm;
Mr. Ray Kosaka, Discus of Hawaii;
Mr. Andrew Kuljis, Aquatic Farms;
Dr. Craig MacDonald, Hawaii State Ocean Resources Development;
Mr. Jerry B. Norris, Pacific Basin Development Council;
Mr. Ramsey Reimers, Robert Reimers Enterprises;
Dr. Richard Spencer, Hawaiian Marine Enterprises {Industry Advisory Council Chairman and *ex officio* member of the BOD}
Mr. John Taitano, Guam Aquafarms;
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;
Mr. Richard Bailey, Sea Grant Extension Service, University of Hawaii;
Dr. James Brock, Hawaii State Aquaculture Development Program;
Mr. Mark Brotman, Land Grant Cooperative Extension, Northern Marianas College;
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;
Mr. Howard Takata, Sea Grant Extension Service, University of Hawaii
Dr. Clyde Tamaru, Sea Grant Extension Service, University of Hawaii.

National Coordinator for Aquaculture New Animal Drug Applications

Dates of Work

May 1995 through May 1998

Funding Level

$15,000

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; and
provide public education related to training and guidance in obtaining INAD exemptions and pursuing NADA approval.

Principal Accomplishments

As a result of a successful funding initiative, the coordinator's position has been increased from three-quarters to full time during the second year, which began in May 1996. 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.

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.

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 will schedule a November meeting to develop a broad-based policy on this issue for all animal drug.

The coordinator is working with the sponsor and CVM to identify existing data and outstanding requirements for approval of florfenicol in salmonids.

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.

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 methods 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 methods for para-toluenesulfonamide in fish tissue to support residue depletion studies was submitted to CVM for review.

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.

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.

In May 1998, efficacy data (received and organized in response to a November 1997 data call-in) were evaluated for support of the extension and expansion of the NADA. The summary is being prepared for submission to CVM.

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.

In 1998, the CDC raised concerns about the use of fluoroquinolones in animal health having a potential for developing pathogen resistance in humans, so it is doubtful that a new NADA on sarafloxacin will be granted.

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. Preliminary indications are that the label will only cover the control of *Ichthyopthirius.*

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.

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 July 16, 1998.

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.

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.

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.

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.

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.

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.

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.

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. It continues to be under review and is a high priority.

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 being prepared for submission to CVM will include revision to the calculations and assumptions in the original submission.

Work Planned

During the fifth 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*
Total	**$25,000.00**	**$97,250.00**	**$228,551.00**	**$325,801.00**	***$350,801.00***

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Understanding *Gracilaria* Gall Syndrome
termination report

Dates of Work

May 1, 1995, through September 30, 1998

Funding Level

$120,000

Participants

Dr. James Brock, Aquaculture Disease Specialist, Hawaii Department of Land and Natural Resources;
Dr. Lynda Goff, Professor of Biology, University of California at Santa Cruz;
Dr. Roger Lallone, Scientific Director, Brookwood Biomedical Laboratory;
Dr. Michael Shintaku, Assistant Professor of Plant Pathology, University of Hawaii at Hilo.

Reason for Termination

This project was terminated because no further progress could be achieved.

Objectives

The objectives of this two-year project, initiated under the CTSA Eighth Annual Plan of Work, were to:

demonstrate under controlled laboratory conditions the transmission and infectivity of the *Gracilaria* gall syndrome (GGS) agent to various *Gracilaria* strains;
describe in detail by light and electron microscopy the external and internal characteristics of *Gracilaria tikvahiae* affected by *Gracilaria* gall syndrome (GGS);
characterize the bacterial communities associated with *Gracilaria tikvahiae* unaffected and affected by GGS, including an assessment of antibiotic sensitivities, and to survey *Gracilaria* tissues for the presence of viruses;
develop a probe for identification of biochemical markers of GGS and/or the GGS agent;
develop an assay for the presence of the GGS pathogen. Once a pathogen is identified and even partially characterized, such an assay will be useful in identifying primary sources of inoculum and in evaluating the potential efficacy of certain measures designed for pathogen exclusion.

Principal Accomplishments:

Objective: Demonstrate under controlled laboratory conditions the transmission and infectivity of the Gracilaria *gall syndrome (GGS) agent to various* Gracilaria *strains, and to characterize the bacterial communities associated with* Gracilaria tikvahiae *unaffected and affected by GGS, including an assessment of antibiotic sensitivities, and to survey* Gracilaria *tissues for the presence of viruses.*

Originally these were separate objectives. However, investigators are assaying bacteria originally isolated from GGS-affected tissue for infection potential and have merged the progress of these objectives.

Dr. Shintaku's laboratory collected normal and GGS-affected seaweed samples three times from an Oahu farm and eleven times from a Hawaii farm between November 1994 and May 1996. Small (2 to 5 mg) samples of galls and of the tips of healthy tissue were crushed and spread on a culture medium. Extracts from healthy tissue showed between 5

and 50 bacterial colonies, while extracts from diseased tissue showed between 100 and 1,000 bacterial colonies. Galled tissue is more heavily colonized with epiphytic bacteria.

Dr. Shintaku archived 110 bacterial isolates from diseased seaweed, attempting to gather a collection as diverse as possible in terms of colony morphology and growth habits. Bacterial isolates that were abundant on or specific to GGS-affected tissue were selected for identification and further characterization.

Identification of the isolated bacteria was attempted using the Biolog bacterial identification system. Unfortunately, the Biolog bacterial identification database is rather limited regarding marine bacteria and was able to identify only nine of the 30 isolates to species and five to genus (Table 1).

Table 1.

Isolate	Biolog identification	similarity to database isolate (if no fit, closest sp. in database)
1Tb	*Vibrio alginolyticus*	0.824
3Tb	*V. carchariae*	0.956
141b	*V. tubiashii*	0.873
4b	*V. alginolyticus*	0.734
13a	*Vibrio sp.*	0.495 *(V. meditarreanei)*
13c	*Vibrio sp.*	0.429 *(V. anguillarum)*
407	*Vibrio sp.*	0.631 *(V. cholerae)*
23C	*Vibrio sp.*	0.429 *(V. anguillarum)*
401	*Vibrio sp.*	0.529 *(V. alginolyticus)*
402	*V. alginolyticus*	0.734
403	no i.d.	0.386 *(Acinetobacter radioresistens)*
404	no i.d.	0.616 *(Hydrogenophaga pseudoflava)*
405	no i.d.	0.212 *(Halomonas elongata)*
406	no i.d.	no pattern
407	no i.d.	0.153 *(Xanthomonas oryzae)*
409	no i.d.	0.110 *(Gilardi pink gram -)*
142b	*Kingella denitrificans*	0.793
1434	*V. alginolyticus*	0.794
1437	*V. alginolyticus*	0.718
1439	*no i.d.*	0.232 *(CDC group EF-4)*
1440	*no i.d.*	0.618 (*K. denitrificans*)
LRO1	*V. alginolyticus*	0.732
ORO1	no i.d.	0.477 (*V. carchariae*)
ORO2	no i.d.	0.460 (*V. parahaemolyticus*)
1201	no i.d.	0.126 (*Burkholderia picketii*)
1202	no i.d.	0.452 (*B. picketii*)
1203	no i.d.	0.397 (*B. picketii*)
1204	no i.d.	0.048 (*Pseudomonas syringae pv. phaseolicola*)
1302	no i.d.	no pattern

Forty-eight of the bacterial cultures isolated from GGS-affected material have been inoculated on *Gracilaria* in small flask cultures. Four bacteria induced GGS in red but not green *Gracilaria* six to seven days after inoculation. No galls were observed in the uninoculated control groups.

All four of the bacteria that produced GGS in this trial were gram-negative, and two were identified using the Biolog system as *Vibrio alginolyticus*. Unfortunately, repeated experiments using these strains have not induced GGS, which may indicate an environmental factor that predisposes *Gracilaria* for GGS susceptibility.

Dr. Goff's laboratory conducted three 3-month-long inoculation trials with the same bacteria. These inoculation experiments failed to identify a bacterial isolate capable of inducing GGS on cultured algae, despite the demonstrated ability of these isolates to induce GGS symptoms in cultured algae immediately after isolation. Algal specimens from

both sites failed to develop GGS symptoms during the 3-month incubation period. However, within a week of inoculation with cultured bacteria, several algal cultures revealed a minor degree of apical bleaching. This response, which was not universal and was not correlated with a particular bacterial culture, suggested nutrient depletion.

Dr. Shintaku has attempted to detect the presence of viruses by repeatedly isolating total nucleic acids from healthy and diseased seaweeds and assaying for the presence of double-stranded RNA using CF-11 chromatography. These attempts have not revealed double-stranded RNA. In addition, analysis of the nucleic acid extracts revealed no subgenomic species of DNA. Although this does not rule out the presence of viruses in these tissues, viruses are not abundant if they are present. In light of this, investigators are continuing their efforts to identify a bacterial causal agent.

In addition, the propagation of GGS-affected *G. tikvahiae* has not been successful under laboratory conditions. Diseased seaweeds brought in from the field and cultured under a variety of conditions seem to cure themselves within several weeks. Laboratory-cultured GGS-affected *G. tikvahiae* again manifested GGS symptoms in only one case, which could not be correlated with any obvious culture conditions and could not be repeated. In that case, the cuticle showed no obvious bacterial infestation.

The irregular occurrence of GGS on farms, which has not been related to any obvious physical or biological variables, has been an additional obstacle to the investigation. This has made a consistent source of GGS affected algae extremely difficult to obtain and precluded intensive investigation of this syndrome.

In 1997 - 1998, Dr. Shintaku established laboratory cultures of both GGS-affected *Gracilaria* and GGS-free *Gracilaria* from mariculture systems on Oahu and the island of Hawaii. The cultures were maintained under a light and temperature regime intended to mimic field conditions (29 C, 16:8, high light). Cultures were maintained in half-strength Provasoli's Enriched Seawater media with constant shaking at 50 rpm. Unialgal cultures of unaffected *Gracilaria* for use in inoculation experiments were derived from stock cultures from Oahu and Hawaii. These cultures were propagated for three weeks under the conditions described with media changes every seven days. This has promoted vigorous growth and demonstrated the absence of GGS in thalli used in subsequent manipulations. Each replicate was split into three subcultures and propagated for two more weeks with regular media changes.

In addition, investigators continued to work on cultures of four bacteria cultured from GGS-affected *Gracilaria*. Previous reports indicated that these bacteria were capable of inducing GGS symptoms in cultured *Gracilaria* after five to seven days. Individual bacterial lines, the strain or species status of which was unknown, were restreaked from individual colonies of the provided stocks at total of three times until microscopic examination determined that a single bacterials morphotype was present. That was restreaked onto Difco Marine Agar plates (media 2216), incubated at 29 C and propagated from single colonies.

Each isolated morphotype was grown in liquid culture (10 milliliters Difco Marine Borth 2216 at 29 C to an O.D. of 0.5, diluted ub PES/2 to a final concentration of 106 cells per milliliter and added to one of two replicate algal specimens in 150 milliliters of sterile PES/2. Controls were run in replicates by the addition of either E. coli, Vibrio harveyii or the bacterialsumbionts of Prionitis lanceolata (all at the same effective concentrations) or by the addition of sterile PES/2 (0.22 filtered).

Assays were performed with shaking at 50 rpm under the above cultured conditions and observed daily. After two weeks, media in all cultures were replaced with sterile PES/2 and monitoring was continued on a daily basis for an additional two weeks. Bi-monthly media replacement and weekly observations were carried out for an additional two months. The entire process was completed after three months and was repeated four times using the same isolated bacterial cultures and algal replicate specimens. New algal specimens were derived from control stocks that had been inoculated with sterile seawater.

Bacterial isolates provided by Dr. Shintaku were also identified by 16S rDNA sequence. The rDNA genes of all isolates were amplified by polymerase chain reaction (PCR) using the universal bacterial primers (p28F and p1452r), cloned into pBluescript KS+ (Stratagene, La Jolla, California), which had been prepared as a t-vector by restriction digest (EcoRV) and complete sequences determined in our laboratory using an ABI automated sequencer, dye termination chemistry, the vector primers and an internal primer (p568r) designed by our lab. The Check_Chimera and

Similarity_Rank functions of the Ribosomal Database Project were then utilized to determine preliminary relatedness of these isolates to available bacterial 16s sequences and to ensure that single sequences, not mosaics, had been amplified.

Two species specific oligonucleotide probes were designed against variable regions of the determined 16s sequences (from the provided bacterial cultures) and utilized to confirm, by whole cell fluorescence hybridization, the presence of these bacteria in association with disease *Gracilaria* species.

Phylogenetic analysis of the provided bacterial isolates was carried out by sequence comparisons of the amplified 16s rDNA sequences using Paup 3.1 under the conditions of parsimony and compared to analyses performed using both distance and maximum likelihood optimality criteria.

Inoculation experiments carried out in our lab did not identify, from the bacteria provided by Dr. Shintaku, a bacterial isolate capable of inducing GGS on cultured *Gracilaria*. No algal specimens form either Oahu or Hawaii manifest symptoms associated with GGS during any of the four three-month incubation periods. Control inoculations, with other bacteria or with sterile seawater, were similarly unaffected. Microscopic examination of assayed *Gracilaria* did not show bacterial pitting and decomposition of the cell wall material, a condition identical to that observed in all control thalli.

Observations of a small percentage of inoculated cultures (<10%:3/48 replicates inoculated with the bacteria provided by Dr. Shintaku) revealed minor apical bleaching, but this was deemed unusual, was not statistically significant and was not correlated with a particular bacterial culture. This response was similar to that observed in older algal cultures and suggested the involvement of nutrient depletion was involved. This was supported by observations of algal cultures after the first media change at two weeks in which specimen apices regained their color.

Sequence comparison of the complete 16s rDNA sequences of the bacteria obtained from Dr. Shintaku revealed two phylotypes out of four cultures provided. These bacteria were revealed by phylogenetic analysis, as members of the alpha and gamma sub divisions of the Proteobacteria, most closely related to the genera Roseobacter and Alteromonas respectively. Placement of these previously uncultivated bacterial lineages on phylogenetic trees was consistent under parsimony and using both distance and maximum likelihood methods. Bootstrap analyses provided strong support for the placement of these bacteria.

Morphologically both bacterial phylotypes were revealed as small, non-motile rods measuring ca 1 x 2 . Whole cell hybridization, *in situ* in sections of both infected and uninfected *Gracilaria* species did not reveal the presence of the provided bacteria at any time during any of the inoculation trials or from concentrated specimens of bacteria obtained from infected or uninfected algal cultures. All bacteria in all trials were assayed and were hybridized, on the same microscopic slides, by the universal bacterial probe 338r.

Even though bacterial infestation of a thickened epidermal cuticular layer is associated with GGA affected algae, there is no indication that this is the cause of abnormal growth in this alga. It is just as parsimonious to presume that the observed bacterial infestation is due to a weakening of the algal thallus associated with GGS. In this scenario, the bacterial aggregations on affected algae are an opportunistic invasion of an already sickened thallus.

The failure to reproduce the symptoms associated with GGS on cultured specimens of *Gracilaria* may be due to several factors. Although the bacterial cultures provided were derived from affected material, the demonstration of causation by a single organism requires re-isolation of pure cultures by classical microbiological technique. During this process and/or the process of maintenance on agar plates, the infective ability of GGS agent -- if indeed it is bacterial -- may have been lost. Also possible here, GGS may be due to the action of a microbial consortium that would be lost in the process of pure culture manipulation.

The propagation of GGS affected *Gracilaria* has not been 100 percent successful under laboratory conditions. Affected individuals propagated under a variety of conditions can spontaneously “cure themselves” with several weeks after transfer into new culture media. In only one instance has laboratory cultured *Gracilaria* manifesting GGS reproduced the lost symptomology. This occurrence was not correlated with obvious culture conditions and was not observed to repeat. In this case, there was no obvious bacterial infestation of the cuticle.

Whole cell hybridization results indicate that the bacteria tested as pure culture isolates are probably not the disease agents. The absence of these bacteria, at least in numbers sufficiently large enough to be detected, or in a physiological state allowing detection, from both normal and GGS-affected *Gracilaria* suggests that these isolates my be water column inhabitants or casual associates. No evidence is available that shows either of these microbial phylotypes represents a significant bacterial associate of diseased or normal *Gracilaria*. In addition, the phylogenetic position of these microorganisms suggests as numerous other laboratories have recently shown, that a large number of previously unknown and uncultivated bacterial phylotypes from the alpha and gamma subdivisions of the Proteobacteria are associated with marine sediments, macro- and microorganisms including mussels, starfish, brittle stars, squid, intertidal algae (Ulva) and phytoplankton (Dinoflagellaes especially).

It is quite possible that the causative agent of GGS remains a microbial pathogen but that is not indicated from the analyses of the bacterial cultures provided. Clearly more bacteria should be isolated and screened and shown to be associates of both infected and uninfected *Gracilaria* as there is no indication, at present, that the bacteria provided to date are the responsible organisms.

Objective: Describe in detail by light and electron microscopy the external and internal characteristics of Gracilaria tikvahiae *affected by* Gracilaria *gall syndrome.*

Dr. Goff's laboratory conducted this work. More than 1,000 sections of GGS-afflicted and healthy seaweed have been extensively examined using glycol methacrylate resin (JB-4) and various cytochemical staining techniques. Healthy seaweed shows *Gracilaria*'s normal morphology: three cell layers--epidermal, cortical and medullary-- are present, and the relative sizes of the cells within each layer are normal. The cuticle of healthy seaweed 1 to 25 microns wide and is covered with a thin, extensive layer of assorted epiphytic bacteria.

In contrast, GGS-afflicted seaweed show extensively indented scalloped and extremely thickened (up to 105 m) cuticle areas. When stained with cytochemical dyes, these indentations appear tightly packed with small rods (1 x 25 m) indistinguishable from bacteria. Despite extensive survey of afflicted tissues, these symptoms are the only indication of a pathology that may be afflicting these obviously abnormal algae. There appears to be no internal (inter- or intracellular) symbionts or pathogens (bacterial, fungal, algal or animal), although several instances of bacteria-containing "scallops" within the cuticle of the GGS-afflicted thalli appeared to have penetrated the algal epidermis. Areas of GGS-afflicted thallus which demonstrate these symptoms also seem to be correlated with hyperplasia of the algal epidermis. This induction of epidermal cell division and thickening of the algal cuticle may be a host response to bacterial infection. Current work is focusing on serial sectioning and reconstruction of a GGS-afflicted algal gall.

In summary, these investigations have revealed extensive pitting of a thickened epithallial layer of GGS-affected cell wall material. This layer of material is clear in preparations for light microscopy and electron transparent in preparations for TEM. Within this thickened layer of putative polysaccharide there appears to be localized proliferation of densely packed bacterial aggregations. These bacteria are uniform in size, implying that they are of a single type.

Objective: Identify biochemical markers of GGS and/or the GGS agent.

Dr. Lallone found that some preliminary experiments documented differences in protein and carbohydrate content of detergent extracts of normal and affected cultures of *G. tikvahiae*. Aqueous extracts from GGS positive and negative plants were made and concentrated 10X by ultrafiltration using 10K molecular weight cut-off membranes and analyzed by SDS-PAGE under reducing and non-reducing conditions. The slab gels were stained using Coomassie Blue or PAS to reveal either proteins or carbohydrates. Investigators detected a group of proteins, of low to intermediate molecular weight and sensitive to reduction by 2-ME in extracts of GGS negative plants, which are either absent or degraded to smaller molecular weight fragments in GGS-positive material. In addition, investigators observed increased amounts of low (but not high) molecular weight, PAS positive carbohydrate material in affected plant extracts compared to non-affected plant extracts.

Investigators made several non-detergent extracts of infected plant material. These extracts were also analyzed by SDS-PAGE and were found to contain a variety of high and low molecular weight proteins. Diseased plants were

ground in a blender, soluble material was extracted by aqueous incubation at 4C, insoluble residue was removed by filtration, and soluble material was fractionated by sequential ultrafiltration. Soluble material was fractionated using a series of decreasing molecular weight cutoff membranes (300D, 100D, 30K, and 10K). Banding patterns reveal both similarities and differences between each fraction. In addition, similar extracts and fractionations were performed on apparently healthy plants taken from two distant culture sites (one of which has to date had no record of similar disease symptoms). When examined in parallel clear differences could be found between each of the various fractions taken from plants collected from each of the various sites.

Based on these observations, investigators immunized four groups of animals. Each group of three rabbits received injections of extracted material taken from stocks of ultrafiltration-fractionated extracts of diseased plants. Antibodies were also raised to healthy *Gracilaria*.

Antisera collected from these animals was cross-reacted with healthy *Gracilaria* tissue, labeled, and used to probe a Western blot containing extracts (fractionated as described above) from diseased tissue. These probes react strongly with a compound approximately 30 kD in mass in GGS-affected tissue. Further, the reactive antigen does not appear as a prominent band when total protein is visualized. We do not know if this compound is produced by the diseased plant, perhaps as a stress response, or is a component of the pathogenic agent.

The antibodies were formatted into a sensitive immunoassay and used to test water samples taken at regular time periods from production tanks. Records were kept during the sampling period so that correlations could later be made between a positive immunoassay test and the appearance of disease. Surprisingly, the antibodies raised to diseased plants showed no immunoreactivity with any of the water samples despite appearance and disappearance of disease whereas the antibodies raised to healthy plants did. Furthermore the concentration of immunoreactive material in the water samples was highest days before the visual appearance of disease. In light of this surprising result plants showing early signs of disease were collected, crudely dissected, and separate detergent extracts were made of plant stems, healthy growing tips, and galled growing tips. The extracts were probed with the same antibodies used in the immunoassay. The highest concentration of immunoreactive material was found in healthy tips and the lowest concentration was found in diseased tips. This indicates that components of healthy plant tissue may be released into the water prior to appearance of disease. This needs to be verified by comparing the material found in water samples with material extracted from healthy growing tips. The destruction of normal plant tissue and release of critical components is likely to be due to proteolytic enzymes, either from a bacterial or plant source. Dr. Lallone is exposing normal plant tissue to various commercially available bacterial, plant, and animal derived enzymes and monitoring the release of immunoreactive material from the plants. In addition, Dr. Lallone's group is screening the bacterial cultures obtained from Dr. Shintaku for reactivity with the above-mentioned antisera.

In summary, several lines of evidence implicate a bacterial causal agent in this disease interaction. These include a therapeutic response to ampicillin and the *in vitro* induction of GGS with several bacteria. However, the apparent induction of GGS by several different bacteria and the inability to consistently reproduce these results confounds our findings.

Furthermore, there appears to be an intimate association between aggregations of surface bacteria found in tissue indentations and GGS-affected thalli, but very little internal tissue invasion. The outer layers of GGS-affected tissue become greatly thickened in addition to acquiring those bacteria-filled indentations. However, investigators have not yet elucidated the temporal relationship between heavy bacterial colonization, wall thickening, and GGS. An axenic culture system for *Gracilaria* culture is in place in Dr. Goff's laboratory, and work continues towards maintaining tissue in the galled state. The failure to reproduce the symptoms associated with GGS on cultured specimens of *G. tikvahiae* may be due to several factors. Although the bacterial cultures provided were derived from affected material, re-isolation of pure cultures by classical microbiological technique was necessary. During this process, the GGS agent may have been lost. Another possibility is GGS may be due to the action of a microbial consortium which would be eliminated in single-culture inoculation of axenic algae. Dr. Lallone has had success in generating GGS-related antibodies, and these are being used to determine the mechanism of GGS induction, and to help ascertain which bacteria are unique to GGS-affected material.

Objective: develop an assay for the presence of the GGS pathogen. Once a pathogen is identified and even partially characterized, such an assay will be useful in identifying primary sources of inoculum and in evaluating the potential efficacy of certain measures designed for pathogen exclusion.

Results regarding the sequence analysis of the 16S ribosomal RNA genes from several isolates showed promise. This analysis supported the MIDI-based identification of these strains as *Cytophaga* species and provided a means to design a PCR-based diagnostic assay. Such an assay was used and found to be extremely sensitive for the detection of the bacterial wilt pathogen, *Pseudomonas solanacearum*, in ginger.

Impacts

A management strategy aimed at GGS is clearly needed. The rapid progression of GGS outbreaks coupled with the severity of GGS symptomology combine for high yield losses and the expenditure of much effort in tank dumping. Unfortunately, the GGS causal agent was not identified.

Support

This project received support from CTSA, the University of Hawaii at Hilo, University of California at Santa Cruz and Brookwood Biomedical Laboratory.

		Other Support				
Year	**CTSA**	**UH**	**UCSC**	**Brookwood**	**Total Other**	**Total Support**
One	$60,000	$8,400	$12,000	$10,000	$30,400	$90,400
Two	$60,000	$8,400	$12,000	$10,000	$30,400	$90,400
Total	$120,000	$16,800	$24,000	$20,000	$60,800	$180,800

Publications, Manuscripts or Papers Presented

No publications or manuscripts were prepared during the reporting period.

Library Aquaculture Workstation
Pacific Regional Aquaculture Information Service for Education

Dates of Work
March 1988 through September 1998

Funding Level
$166,600

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 eleventh year, is to make scientific information more accessible to the aquaculture community. Specific year eleven 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, more than 100 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 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.

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.

During Years 10 and 11, the PRAISE Web site has been continually updated and enhanced. This included an upgraded version of the Pacific Gray Literature Bibliography and the PRAISE user database.

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.

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 be explored, as will the viability of video conferencing.

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, 1998, would be as follows

2,520 log-ins at $1.20 each =	$ 3,424.00
11,300 queries averaging 3 minutes each (565 hours online at $60 per hour) =	$33,900.00
255 articles at $13.50 each =	$3,442.50
TOTAL	**$40,766.50**

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (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*	**Total Support**
1	$7,000.00	$13,400.00	$1,500.00	$0.00	$0.00	$0.00	$0.00	$0.00	*$14,900.00*	**$21,901.00**
2	$6,700.00	$12,600.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	*$12,600.00*	**$19,302.00**
3	$6,000.00	$12,600.00	$3,300.00	$0.00	$0.00	$0.00	$0.00	$0.00	*$15,900.00*	**$21,903.00**
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*	**$27,604.00**
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*	**$93,480.00**
6	$17,900.00	$24,000.00	$0.00	$5,800.00	$0.00	$0.00	$0.00	$0.00	*$29,800.00*	**$47,706.00**
7	$28,000.00	$12,600.00	$0.00	$5,500.00	$0.00	$0.00	$0.00	$0.00	*$18,100.00*	**$46,107.00**
8	$49,000.00	$11,400.00	$0.00	$5,500.00	$0.00	$0.00	$0.00	$0.00	*$16,900.00*	**$65,908.00**
9	$25,000.00	$10,500.00	$0.00	$5,000.00	$7,500.00	$0.00	$0.00	$0.00	*$23,000.00*	**$48,009.00**
10	$24,000.00	$19,200.00	$0.00	$0.00	$0.00	$6,700.00	$0.00	$0.00	*$0.00*	**$49,910.00**
11	$30,000.00	$10,500.00	$2,500.00		$6,500.00	$0.00				**$49,511.00**
Total	**$190,600.00**	**$174,575.00**	**$12,300.00**	**$32,600.00**	**$10,000.00**	**$6,700.00**	**$15,000.00**	**$2,500.00**	***$227,775.00***	**$441,775.00**

Publications, Manuscripts or Papers Presented

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Guenther, K. and D. E Coleman. 1994. *Pacific Islands Gray Literature Project: A Bibliography*. Center for Tropical and Subtropical Aquaculture Publication #115. Waimanalo, Hawaii.

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

Dates of Work

August 1989 through September 30, 1998

Funding Level

$827,470

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 tenth 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 1988, 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;
In June, an aquaculture workshop held in the Marshall Islands was attended by more than 40 people. 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.

Because no extension specialist was on the job for eight months in 1996 and Mr. Ellis is new, he spent considerable time during the first six months familiarizing himself with his region, the status of aquaculture, government representatives and private individuals involved in aquaculture.

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.

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.

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 begun writing the script. He is also working on a giant clam spawning manual. 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. He began work on a soft coral culture manual and began reviewing literature for a manual on transporting aquarium species.

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 trained Peach Corps volunteers in various aquaculture techniques, including

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. A job description was submitted and a suitable candidate is being sought in the program.

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.

In May 1998, the program hired Mr. John Sprague as a business development extension agent. Ellis coordinated Sprague's visit to Pohnpei and provided input for a five-year development plan Sprague is writing.

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.

Work Planned

The project will continue to provide extension support and training throughout the region. In addition, the manual and video on giant clam culture will be published and one workshop on soft coral culture will be held in the Marshall Islands.

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, 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 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**
Total	**$827,470.00**	**$93,454.00**	**$41,000.00**	**$26,300.00**	**$35,300.00**	**$157,500.00**	**$8,500.00**	*$362,054.00*	***$1,189,524.00***

Publications, Manuscripts or Papers Presented

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Haws, Maria. 1996. *Gems from the Sea: A Pearl Oyster Culture Manual*. Center for Tropical and Subtropical Aquaculture Publication Number 127. Waimanalo, Hawaii.

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Disease Management for Hawaiian Aquaculture

Dates of Work

April 1993 through May 1998

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 were 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 to provide health management extension assistance under this project.

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 this reporting period, 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: 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: 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 which, 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.

*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. Further work on the agent and the disease are ongoing. 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.

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 the first six months of 1998, 36 case submissions comprising 390 ornamental fish were received and processed for necropsy, bacteriology and histopathology examination. Two cases were processed for electron microscopy evaluation.

A study was conducted to asses 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.

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 (GGS), 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 work had six principal impacts. 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.

Work Planned

During year six of the project, investigators plan to:

continue providing diagnostic support for the CTSA-funded ornamental fish projects and for ornamental fish producers;
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;
continue monitoring for IHHNV on shrimp farms;
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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Differential Growth Rate Studies in Cultured Commercial Sponges
termination report

Dates of Work

April 1993 through April 1998

Funding Level

$156,087

Participants

Richard Croft, College of Micronesia;
Dr. Michelle Kelly-Borges, Natural History Museum, London, UK.

Reason for Termination

This project was terminated because all objectives were completed.

Objectives

The objectives of this five-year project, which was initiated under the CTSA Sixth Annual Plan of Work, are to:

improve the efficiency and production of commercial sponge farms by determining the factors responsible for variable growth rates in cultured sponges;
obtain additional biological data; and
maintain the existing demonstration nursery.

Principal Accomplishments

Objective: Improve the efficiency of commercial sponge farms.

During the first year of the project, sponges were selected, cut, tagged and planted for use in several investigations that were undertaken at the same time. The first study examined the cause of the large differences in growth rates of sponges growing under the same conditions.

The hypothesis was that certain areas of the parent sponge actively grow while other areas do not, which may explain why some sponges planted in the demonstration farm grew only 3 percent per month while others planted three feet away on the same line grew up to 15 percent per month. If this hypothesis proved true, the faster growing parts of the wild sponge would be used as cutting material.

Ball-shaped and bowl-shaped sponges were selected and cut into three layers—a top layer, a middle layer, and a bottom layer. The center of each layer was then cut out, resulting in a donut-shaped outside section and a disk-shaped inside section. (The top layer from a bowl-shape sponge did not have an inside section.) The six groups from the ball-shaped sponges and five groups from the bowl-shape sponges resulted in 1,100 cuttings, which were segregated by groups, tagged and planted.

Within four to five weeks after planting the cut areas had healed, and cuttings were weighed for the first time. Each tagged section was weighed every six months. For some unknown reason, portions of some cuttings died and fell off just after planting. Thereafter, the cutting grew normally. If the lower growth rates were due to this “die back,” growth

rates would rise and the range of the average monthly growth rates would narrow. During the second and third years of the project, measurements showed this to be true.

As noted, the majority of the inside cuttings from most of the layers showed smaller overall increases in size. Cuttings from the center of each disk had none of the black outer skin that covers parts of cuttings from the outside edge. This appeared to be the only physical difference between these two groups. The longer time needed for the inside cuttings to become totally covered again by the black skin may slow their overall growth rates. All 11 groups of sponge cuttings showed a wide range of growth rates—from a low of 1 percent to a high of 17 percent—similar to those exhibited during the first growth rate study.

A second component to this experiment examined the differences in growth rates between cuttings taken from various sections of bowl-shaped and ball-shaped parent sponges. Ball-shaped and bowl-shaped parent stock were each cut into a top layer, a middle layer and a bottom layer. The center of each layer was cut out, resulting in a donut-shaped outside section and a disk-shaped inside section (although the top layer of a bowl-shaped sponge does not have an inside section.) The resulting 1,100 cuttings were then segregated into 11 groups, tagged and planted together.

> Tagged sponges from both the bowl-shaped and ball-shaped parent stock grew an average of 4 to 7 percent monthly. The growth rates of all 11 groups of sponge cuttings range widely: from a low of -2 percent to a high of 18 percent. This may be attributed to the die-back of portions of some of the cuttings. If so, the lower growth rates should climb during the next 12 months, and the range of growth rates should narrow. At least twice as many cuttings from the inside section of the top, middle and bottom sponge layers showed negative growth as cuttings from the outside layer. This may be because the inside cuttings do not have any of the black "skin" that covers the outside of sponges and probably protects the cuttings.

Another study examined the hypothesis that sponges exhibiting higher growth rates retain these high rates even after being cut into smaller pieces. If so, these should be the sponges used to replant or expand a farm, thus improving farm efficiency. Only those sponges with a growth rate of ten percent or more per month were selected for this experiment. High growth-rate sponges were divided into 100 cuttings, tagged and planted. None of the cuttings exhibited any "die-back." After only 12 months, many of these cuttings had reached the minimum commercial size of more than 600 grams, live, wet weight. After 24 months of growth, every sponge weighed at least 600 grams, and some weighed more than 1,200 grams. Larger sponges have greater commercial value. During the first growth study sponge cuttings took 24 months or more to reach commercial size, and 36 months to reach the size of the cuttings in this study. This clearly indicates that these sponges should be used to replant or expand a farm.

The third study examined whether cutting sponges with low growth rates stimulates their growth. The principal investigator selected 80 cultured sponges that weighed 400 grams or more and had exhibited growth rates of less than 5 percent per month. These sponges were divided into two or more cuttings of at least 200 grams each. The resulting 197 cuttings were tagged, weighed and planted. These cuttings grew at monthly rates ranging from 3 to 9 percent and averaging 5 percent. Some of these cuttings exhibited a partial "die back." Overall, the cuttings taken from slow-growing parent stock continued to grow slowly, with very few reaching commercial size after 24 months of growth. This indicated that slow-growing sponges in a commercial farm should be harvested and sold off as soon as they reach the minimum commercial size.

Objective: Obtain additional biological data.

Dr. Michelle Kelly-Borges, a sponge systematist and ecologist who was hired as a project consultant, identified the Pohnpei sponge as *Coscinoderma mathewsi.* She designed several experiments for the project. These experiments will examine whether:

> the final morphology of cultured sponges is determined by the morphotype of the wild donor sponge, or by the environmental conditions during the culture period.
> the final morphology of cultured sponges is determined by the position in the donor sponge from which the explant was cut, or by the environmental conditions during the culture period.
> the growth rate of cultured sponges is determined by the position in the donor sponge from which the explant was cut or by the environmental conditions during the culture period.

The wild donor sponges in the Pohnpei lagoon have three distinct morphologies: spherical or ball-shaped, vasiform or bowl-shaped, ring-shaped and digitate, which have small, finger-like extensions from the surface. Cuttings were taken six zones—the outside top, the inside top, the outside middle, the inside middle, the outside bottom and the inside bottom of the sponge—within each sponge morphotype. Vasiform sponges have no inside top, and ring-shaped sponges have only the outside-top and outside bottom. A minimum of three parent sponges of each morphotype and a minimum of 10 cuttings from each zone were required for statistical purposes.

Each of three parent sponges of the four morphotypes was selected, divided into the six zones, cut. Approximately 900 cuttings were then tagged and planted by zone group. After four to five weeks, 10 or more healthy cuttings from each zone group were selected and placed into a basket. Cuttings were randomly selected from this basket and replanted for 12 to 18 months.

In January 1997, 24-month growth measurements were taken from these sponges. The sponges continued to exhibit a wide range of growth rates, ranging from a low of 2 percent to 3 percent to a high of more than 12 percent. The average monthly growth rate for all groups of sponge cuttings was from 5 percent to 7 percent. The data gathered is being analyzed by Dr. Michelle Kelly-Borges. However, a number of preliminary conclusions can be drawn from the raw data.

First, in every case, cuttings taken from the outside of the parent sponges grow faster than cuttings taken from the inside of the parent sponges. One reason is that cuttings taken from the outside sections of the parent sponge have more black skin covering them than cuttings taken from the inside sections. The cuttings from the inside sections must therefore expend energy in developing a new black skin over their entire surface before they can start growing. However, this does not explain the large difference between the maximum sizes achieved by cuttings from different areas of the parent sponge. In almost every case, the difference between the maximum size of cuttings from the inside and the maximum size of cuttings from the outside is around 100 grams and in several cases ranges as high as 200 grams.

Second, the ball-shaped sponges appear to exhibit higher growth rates. However, average growth rates for all four groups of sponges is close to the same, and the apparent difference may be resolved by a more detailed analysis of the data.

Third, the survival rates for all four groups of sponges was 100 percent.

Fourth, the final morphology of the cultured sponges is still unclear. When the 24-month measurements were taken, the majority of the cuttings appeared ball-shaped regardless of the shape of the parent sponge. Most but not all of the cuttings that appeared to be digitate-shaped were taken from digitate-shaped parent stock, but some cuttings taken from ball-, vase- and ring-shaped parent sponges appear to be developing digitate shape. No cuttings appeared to have developed a vase shape nor a ring shape. The latter was expected because ring-shaped sponges are thought to be either the remainder of older vase-shaped sponges or sponges that grew around rocks or coral heads. Table 1 summarizes average growth data over 24 months for cuttings taken from each section of the four shapes of parent sponges.

Table 2 summarizes 24 months of maximum growth data for cuttings taken from each section of the four shapes of parent sponges. Table 3 summarizes 24 months of minimum growth data for cuttings taken from each section of the four shapes of parent sponges. The reader should bear in mind that ring-shaped and vase-shaped sponges do not have inside sections.

Table 1. Average Growth for Cuttings From Each Section of the Parent Sponges						
Shape of Parent Stock	Top Section		Middle Section		Bottom Section	
	Outside	Inside	Outside	Inside	Outside	Inside
	Size / Monthly Change	Size / Monthly Change	Size / Monthly Change	Size / Monthly Change	Size / Monthly Change	Size / Monthly Change
Ball	7%	480 g / 5%	549 g / 7%	494 g / 5%	541 g / 7%	479 g / 5%
Digitate	564 g / 7%	488 g / 5%	536 g / 6%	485 g / 5%	536 g / 6%	488 g / 5%
Vase	543 g / 6%	- -	554 g / 7%	511 g / 6%	564 g / 7%	510 g / 6%
Ring	552 g / 7%	- -	-	-	541 g / 6%	--

Table 2. Maximum Growth Rates for Cuttings Taken from Each Section of the Parent Sponges						
Shape of Parent Stock	**Top Section**		**Middle Section**		**Bottom Section**	
	Outside	Inside	Outside	Inside	Outside	Inside
	Size / Monthly Change	Size/ Monthly Change	Size / Monthly Change	Size / Monthly Change	Size / Monthly Change	Size / Monthly Change
Ball	825 g / 13%	650 g / 10%	835 g / 13%	620 g / 8%	620 g / 13%	625 g / 9%
Digitate	760 g / 12%	620 g / 9%	720 g / 11%	590 g / 8%	785 g / 12%	650 g / 8%
Vase	780 g / 12%	-	755 g / 12%	680 g / 10%	765 g / 12%	680 g / 9%
Ring	755 g / 11%	-	-	-	765 g / 11%	-

Table 3. Minimum Growth Rates for Cuttings Taken from Each Section of the Parent Sponges						
Shape of Parent Stock	**Top Section**		**Middle Section**		**Bottom Section**	
	Outside Size / Monthly Change	Inside Size / Monthly Change	Outside Size / Monthly Change	Inside Size / Monthly Change	Outside Size / Monthly Change	Inside Size / Monthly Change
Ball	400 g / 3%	355 g / 3%	395 g / 3%	380 g / 3%	360 g / 3%	380 g / 3%
Digitate	385 g / 3%	350 g / 2%	380 g / 3%	365 g / 3%	380 g / 3%	370 g / 2%
Vase	370 g / 3%	-	390 g / 3%	355 g / 2%	425 g / 3%	385 g / 3%
Ring	405 g / 3%	-	-	-	410 g / 4%	

An investigation was conducted to determine how environmental conditions affect sponge growth rates and the final morphology of cultured sponges. A minimum of 10 cuttings taken from the six areas (top inside, top outside, middle inside, middle outside, bottom inside and bottom outside) of both vase-shaped and ball-shaped parent stock were planted 15 meters deep at three sites.

Site #1 is off the shore of the main island close to a mangrove stand that extends 150 meters from the shore. Beyond the mangrove is a 200-meter-wide reef flat. The site is sheltered with little wave action and almost no current.

Site #2 is near a patch reef midway between the main island and the barrier reef and midway between two lagoon islands. This site is subject to the strongest currents of the three sites and some wave action during high tide and strong winds.

Site #3 is near the inside edge of the barrier reef. The site is subject to mild currents and some wave action.

Each site was planted with 110 cuttings comprising 10 cuttings from each section of each of the two parent morphologies. Each of the total of 330 cuttings was tagged with identification regarding the parent shape and the section from which it was taken. The start of this experiment was delayed because of difficulty in finding a ball-shaped parent sponge large enough to supply the necessary cuttings. All the planting was completed in November 1996. The first growth measurements will be taken under the fifth and final year of this project. Investigators measure oxygen levels, carbon dioxide levels and salinity levels once a week at all three sites. In addition, temp erature, tide flow and water clarity are measured once a week at all three sites.

The first measurements were taken in January 1997. The measurements given in Tables 4 through 6 were taken in January 1998 and represent 12 months of growth. The measurements seem to indicate that sponges grow better in areas of high current flow. The PI, on his own, will continue to measure the sponges for another year and will provide the data to CTSA.

Table 4. Relationship Between Sponge Growing Environment and Morphology and Growth Rates
Average Growth Rates for Each Section

	Top Section				Middle Section				Bottom Section			
	Outside		Inside		Outside		Inside		Outside		Inside	
	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change
Site #1 Near the Mangrove / Shore												
Ball Shape	355 g	6%	350 g	6%	360 g	7%	350 g	6%	375 g	7%	350 g	6%
Vase Shape	365 g	7%	N/a	N/a	375 g	7%	365 g	7%	375 g	7%	360 g	6%
Site #2 Near a Patch Reef in the Center of the Lagoon												
Ball Shape	420 g	9%	380 g	7%	420 g	9%	395 g	8%	440 g	9%	390 g	8%
Vase Shape	420 g	9%	N/a	N/a	420 g	9%	395 g	8%	430 g	9%	390 g	8%
Site #3 Near the Inside Edge of the Barrier Reef												
Ball Shape	360 g	6%	350 g	6%	375g	7%	360 g	6%	365 g	6%	345 g	6%
Vase Shape	385 g	7%	N/a	N/a	N/a	N/a	365 g	7%	380 g	7%	365 g	6%

Table 5. Relationship Between Sponge Growing Environment and Morphology and Growth Rates: Maximum Growth Rates for Each Section												
	Top Section				Middle Section				Bottom Section			
	Outside		Inside		Outside		Inside		Outside		Inside	
	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change
Site #1 Near the Mangrove / Shore												
Ball Shape	395 g	8%	360 g	7%	415 g	10%	370 g	7%	415 g	10%	390 g	9%
Vase Shape	410 g	9%	N/a	N/a	425 g	9%	395 g	8%	405 g	9%	405 g	8%
Site #2 Near a Patch Reef in the Center of the Lagoon												
Ball Shape	490 g	12%	430 g	9%	450 g	11%	440 g	10%	475 g	12%	440 g	10%
Vase Shape	460 g	11%	N/a	N/a	475 g	12%	450 g	11%	485 g	12%	440 g	10%
Site #3 Near the Inside Edge of the Barrier Reef												
Ball Shape	395 g	8%	370 g	7%	420 g	7%	360 g	6%	365 g	6%	345 g	6%
Vase Shape	410 g	8%	N/a	N/a	425 g	10%	395 g	8%	415 g	9%	395 g	8%

Table 5. Relationship Between Sponge Growing Environment and Morphology and Growth Rates: Minimum Growth Rates for Each Section												
	Top Section				Middle Section				Bottom Section			
	Outside		Inside		Outside		Inside		Outside		Inside	
	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change	End Size	Monthly Change
Site #1 Near the Mangrove / Shore												
Ball Shape	325 g	5%	330 g	4%	310 g	5%	325 g	5%	345 g	6%	325 g	4%
Vase Shape	330 g	5%	N/a	N/a	330 g	4%	345 g	5%	340 g	6%	320 g	4%
Site #2 Near a Patch Reef in the Center of the Lagoon												
Ball Shape	340 g	6%	340 g	5%	400 g	8%	355 g	5%	395 g	7 %	355 g	7%
Vase Shape	390 g	7%	N/a	N/a	360 g	5%	340 g	6%	365 g	6%	340 g	6 %
Site #3 Near the Inside Edge of the Barrier Reef												
Ball Shape	330 g	4%	330 g	4%	350 g	6%	320 g	4%	330 g	5%	310 g	4%
Vase Shape	355 g	6%	N/a	N/a	350 g	6%	340 g	5%	340 g	5%	330 g	4%

Several environmental conditions were measured at each site once a week. The same measurements were taken for each site on the same day. For example, the oxygen measurements were taken at all three sites on the same day, as were the carbon dioxide and salinity measurements. However, all measurements were not taken on the same day. When the first few measurements were taken, three were taken at each of the test sites: one from the center of each test side and one from about 15 meters to each side (perpendicular to the normal current flow. This was to see if water chemistry was different inside and outside the farm. These three measurements were taken six times over a six-week period. Because no difference appeared in any of the samples taken from the three locations at each site, measurements were taken from only one site after the first six weeks. For the first nine weeks, samples were also taken at the water surface and at the sponge growing depth (about 15 meters). No surprisingly, the greatest differences between sites appeared at the surface; few differences appeared at the sponge growing depth. All chemical measurements were taken using a standard water test kit, a pH meter and a refractometer. The current strength was measured by releasing a white plastic bag, which had zero buoyancy, and measuring how far it moved in a five-minute period. A line was stretched from where the bag ended up to its release point and then taken to the surface and measured. Water clarity was measured by how far a white plastic bag could be seen horizontally just over each test site. Water temperature was measured by a high-low thermometer that was permanently situated at each site.

Site #3 had some current and the best water clarity. Site #1 had virtually no current and the worst water clarity. Site #2 had the strongest current by far and water clarity between the other two sites. Following is a summary of the measurements itaken at each site. Please note that the nitrite measurements are not included because the test kit could measure nitrite levels only down to 0.05 and all measurements taken were less than that.

Table 6a. Measurements taken at Test Site #1 (Near the Mangrove/Shore)

	Temperature Range		Current Strength (in feet)	Water Clarity (in feet)	pH	Salt	Ammonia	Oxygen	Carbon Dioxide
	High	Low							
Ave	83	87	2	11	7.6	32	0.21	6.5	6.5
Max	96	90	3	14	7.9	35	0.40	6.9	7.2
Min	81	85	1	8	7.3	27	0.10	6.1	6.4

Table 6b. Measurements taken at Test Site #2 (Near a reef patch in the center of the lagoon)

	Temperature Range		Current Strength (in feet)	Water Clarity (in feet)	pH	Salt	Ammonia	Oxygen	Carbon Dioxide
	High	Low							
Ave	82	87	36	31	7.6	34	0.30	6.7	6.8
Max	84	89	68	39	7.9	39	0.60	7.1	7.3
Min	81	84	14	21	7.4	30	0.10	6.1	6.4

Table 6a. Measurements taken at Test Site #3 (Near the inside edge of the barrier reef)									
	Temperature Range		Current Strength (in feet)	Water Clarity (in feet)	pH	Salt	Ammonia	Oxygen	Carbon Dioxide
	High	Low							
Ave	83	87	30	51	7.4	34	0.30	6.6	6.7
Max	85	89	49	58	7.9	36	0.50	7.1	7.1
Min	80	85	9	42	7.2	30	0.10	6.0	6.2

Objective: Maintain the existing demonstration farm.

Under the CTSA-funded Sponge Aquaculture Demonstration Project, five nursery areas were planted with more than 10,000 sponges. Two of these sites have been used for the growth experiments, and the other three sites provided cutting material to help local residents establish private sponge farms. Approximately 4,600 sponges from the nursery areas were provided to sponge farmers.

The nursery sites have also been used as training sites to train local people in sponge farming techniques. From late 1993 through 1996, 10 individuals started the training courses offered at these sites. Six of them started private sponge farms in Pohnpei's lagoon.

Although a variety of fouling organisms normally grow on sponge planting lines, in two areas of the nursery a species of colonial tunicate found growing on the surface of the cultured sponges caused the sponges to be misshapen. The nursery site was monitored monthly, and the tunicates were cleaned off the sponges and lines. Project personnel continued to monitor the tunicate and cleaned the sponges and growing lines monthly.

A large number of the hanging lines used to suspend the sponges from the growing lines had broken and were replaced with heavier test lines. In addition, a number of the main growing lines had broken and were replaced. Project personnel continue to replace the growing lines and the main lines.

The project investigator gave two presentations at the 1997 meetings of the World Aquaculture Society. One presentation covered sponge farming in general and progress toward establishing a new aquaculture industry in Micronesia and the second talk reported growth data generated in this project. In addition, project personnel presented a poster session on sponge aquaculture techniques used in Micronesia from the 1930s to the present.

Impacts

This project has shown that sponges with high growth rates retain those high growth rates when cut into smaller pieces and replanted and should be used to replant and expand commercial farms to increase their efficiency and profitability. By the same token, slow-growing sponges retain their slow growth rates after being cut and replanted, so they should be harvested and sold as soon as they reach minimum size.

All 11 groups of sponge cuttings showed growth rates ranging from a low of 1 percent to a high of 17 percent. The lower rates appeared to result from portions of some of the cuttings dying back. The wide range in growth rates appeared not to depend upon the area of the parent sponge from which the cutting came and appeared to be the same for cuttings from both the ball-shaped and the bowl-shaped parent stock. In addition, sponges grow faster in

areas of high current, so farmers should select sites with good current flow. This information will help farmers to improve their productivity.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), the College of Micronesia (COM), Pohnpei Marine Resources Division (PMRD) and Pohnpei Natural Products (PNP).

		Other Support				Total
Year	**CTSA**	**COM**	**PMRD**	**PNP**	**Total Other**	**Support**
One	$30,380	$75	$625	$7,565	$8,265	$38,645
Two	$39,746	$75	$475	$7,735	$8,285	$48,031
Three	$30,000	$75	$550	$6,830	$7,455	$37,455
Four	$40,000	$50	$715	$8,015	$8,780	$48,780
Five	$16,000	$400	$0	$3,465	$3,865	$19,865
Total	**$156,126**	**$675**	**$2,365**	**$33,610**	*$36,650*	***$192,776***

Publications, Manuscripts or Papers Presented

Kelly-Borges, M. 1996. Research report on aquaculture of the sponge *Coscinoderma mathewsi* in the Pohnpei Lagoon, Micronesia. Natural History Museum. London, United Kingdom.

Expansion and Diversification of Freshwater Tropical Fish Culture

Dates of Work

May 1996 through August 1998

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;
introduction 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.

Anticipated Benefits

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.

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, that 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 total of 267,432 fry were produced and distributed to farmers during the reporting period. In addition, 5,592 broodstock of 10 species were distributed to farmers during the reporting period.

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.

The workshop series will be repeated on Molokai with support from the Maui County Aquaculture Extension project.

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. 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.

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.

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.

Between November 1997 and February 1998, the freshwater ornamental specialist responded to 180 requests for technical assistance. He provided 138 verbal consultations, filled 28 requests for written materials and made 24 site visits.

In addition, a draft manual on packaging and transporting ornamentals was written and sent for review. Three workshops on the topic were held; the first was in December on Oahu, the second was in January in Hilo and the third was in March in Maui. In February, project personnel also gave presentations at the Honolulu Aquarium Society on rotifer culture and new feeds and how to evaluate them.

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

Project personnel collaborated with the state aquatic veterinarian to obtain 17 -methyltestosterone that will be used in feed to masculinize fish.

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. Lemmon tetra
3. High fin red or red wag platy
4. High fin marigold variatus
5. Ram cichlid.

Farmers also suggested other species, including lyretail swordtails for artificial insemination work, that represent either a new category of life history/production or an elevation in the value of a particular species, such as the

highfin platy or variatus. Investigators are identifying sources of stock and working with the state aquatic veterinarian to establish guidelines for importing these new species.

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.

Work Planned

Investigators will acquire broodstock of the additional five species identified as desirable by producers and begin the quarantine and amplification process at the project site. In addition, they will produce a fact sheet for each species and distribute the fact sheets with the fish. They will also produce a set of guidelines for quarantine procedures and a video or manuscript -- or possibly both -- of the workshops. They will continue to provide technical assistance to farmers and will conduct another set of workshops on hormonal induction of spawning.

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

Tamaru, C. S. and H. Ako. 1997. Growth of the angelfish, *Pterophyllum scalare*, using various commercial feeds. *I`ao O Hawai`i*. Volume 1997, Issue 4.

Tamaru, C. S., H. Ako, R. Pagurigan and C. Chun. 1997. Growth of guppies, *Poecilia reticulata*, using various commercial feeds. *I`ao O Hawai`i*. Volume 1997, Issue 5.

Ako, H. and C. S. Tamaru. 1997. Palatability and growth supporting characteristics of koi feeds. *I`ao O Hawai`i*. Volume 1997, Issue 6.

Tamaru, C. S. and H. Ako. 1997. Essential fatty acid profiles of maturation feeds used in freshwater ornamental fish culture. *I`ao O Hawai`i*. Volume 1997, Issues 8 and 9.

C. S. Tamaru, H. Ako and R. Paguirigan. (in press) Essential fatty acid profiles of maturation feeds used in freshwater ornamental fish culture. *Hydrobiologica*.

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

Dates of Work

April 1993 through September 1998

Funding Level

$221,934

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

Determine Pacific threadfin growout requirements.

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 form 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.

Determine milkfish growout requirements.

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 salt water 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.

1998 report

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

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.

Objective: Identify cooperating farms and provide ongoing 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 form 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.

1998 Report
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 form 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.

About 80 percent of the program has been completed with the exception of the cash flow module and the online help. It will be tested by four farmers, then adjusted before being distributed.

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 six months of this project, investigators will:

Supply an additional 125,000 threadfin fingerlings to farmers;
Supply an additional 20,000 milkfish fry to farmers;
Develop a draft technical pamphlet for threadfin growout;
Develop a draft technical pamphlet for milkfish semi-intensive rearing;
Complete the first version of the enterprise budget and implement at cooperating farm sites;
Conduct additional site visits to provide technical oversight to farms on Hawaii and Oahu;
Conduct additional promotional events for threadfin marketing in Hawaii;
Conduct European marketing tests of threadfin in cooperation with Nautique Import & Export, Inc. and Pacific Harvest;
Manufacture and distributed plastic gill tags for promotional efforts;
Publish and distributed promotional brochure.

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.

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.

Development of Improved Growout Culture for Chinese Catfish Through Ploidy and Feed Applications
termination report

Dates of Work
May 1995 through August 1998

Funding Level
$110,000

Participants
Dr. Arlo W. Fast and Dr. Jianguang Qin, Hawaii Institute of Marine Biology, University of Hawaii;
Dr. Harry Ako, Department of Environmental Biochemistry, University of Hawaii.

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

Objectives
The overall goal of this two-year project, which was initiated under the CTSA Eighth Annual Plan of Work, is to develop improved Chinese catfish culture techniques that result in reduced production costs, greater growth and survival rates, lower feed conversion ratios (FCR) and more reliable production. Specific objectives related to that goal are to:

develop methods to reliably produce triploid catfish using cold shock treatment of fertilized eggs;
compare survival, growth FCR and condition of normal diploid catfish to those of triploid catfish;
determine intrinsic, diel feeding patterns of diploid and triploid catfish during growout to market size using demand feeders and data loggers;
conduct subsequent feeding trials based on observed feeding patterns. These trials will allow catfish to feed either in synchrony with their intrinsic feeding patterns or at times that differ significantly from those intrinsic feeding patterns. Growth rates, survival and FCR of both will be compared;
compare growth rates, survival, FCR and disease resistance of Chinese catfish fed with Rangen floating trout chow, Rangen sinking trout chow and a Moore-Clark mahimahi chow formula, the chemical composition of which will be analyzed;
conduct technology transfer to the Hawaii aquaculture community through workshops, collaborative research and publications.

Principal Accomplishments
Objective: Develop methods for reliably producing triploid catfish using cold shock treatment of fertilized eggs.

Broodstock Chinese catfish were obtained Broodstock Chinese catfish were obtained from Aquatic Culture & Design. Spawning trials were conducted July 11, 1995 at Hawaii Institute of Marine Biology (HIMB). Male brood fish were identified by the conical shape of the genital papilla and weighed from 250 to 400 grams each. Females were identified by the rounded shape of the genital papilla; those selected for induced spawning had a soft distended

abdomen and protruding genital papilla. Females chosen weighed from 300 to 500 grams. A total of 16 female and 12 male fish were used and divided into four groups for inducing diploid and triploid fish. Female fish were injected with human chorionic gonadotrophin (HCG) to induce ovulation. Injections of HCG, reconstituted with sterile physiological saline, were administered with tuberculin syringes using a 25 gauge needle. These injections were given intramuscularly in the dorsal region, just lateral to the dorsal fin and posterior to the pelvic fins. A single injection with 4 international units per gram of fish was given to the fish 18 hours before egg stripping at 25 C.

Fish were spawned the day following injection, and eggs were manually stripped and stored in 1-liter covered plastic containers until fertilized. Males were sacrificed, and their testes were removed. The testes were then cut into tiny pieces with a razor in a petri dish. The resulting homogenate was rinsed through a coarse nytex screen with a washing bottle and mixed with eggs. After mixing for approximately 1 minute, more water was added to enable complete mixing of eggs using a gentle swirling motion. Three minutes after insemination, cold shocks were carried out by immersing the fertilized eggs in 5 C water for 15-20 minutes. The cold treatment would result in the second polar body retention to yield triploid fish (3N). Treated eggs were then placed on a frame with screen on the top, and incubated in 50-liter plastic containers with a flow rate of approximately 10 liters per hour at 25 C in the same condition as non-shocked eggs. One milliliter of eggs was counted and the volume of the remainder recorded to compute total egg number per batch (four females). Hatching rates for diploid and triploid eggs were measured by placing 100 fertilized eggs in petri dishes (10-centimeter diameter) with three replicates. Six hours after insemination, fertilization rate was calculated based on the number of white eggs, which were considered not fertilized. Forty-eight hours after insemination, hatchlings were counted and the hatch rate was calculated as the relative percentage of the initial eggs incubated. Ploidy determination was carried out on juvenile fish (10-15 g). Blood samples were taken from 25 fish for measuring the size of erythrocytes nuclei. The major and minor axes of the nucleus of 20 erythrocytes of each fish were measured and the nucleus volume was calculated as 4/3(Trab), where “a” and “b” were major and minor semiaxis, respectively.

Spawning trials proved successful, with oocytes easily stripped from the injected females. Fertilized eggs hatched within 48 hours after insemination. The average number of oocytes per female was 22,500. The average fertilization rate of diploid eggs was 71.4% (±18.9% s.d.), which was significantly higher than that of triploid eggs (58.8% +12.8%, $P<0.05$, Table 1). The average hatching rate of diploid eggs was 69.6% (±30.3%), which did not differ from that of triploids (67.5% +30.6%, P,0.85).

Cold shock treatment yielded 100% triploid fish. The nucleus shape of triploid fish was oval, while that of diploids was almost round. The means of major axis and the volume of erythrocyte nuclei of triploid fish were greater than those of diploids ($P<0.01$), but the lengths of minor axis were not different between triploids and diploids ($P>0.05$). The ratios of minor axes, major axes and volume of triploids to those of diploids were 1.11, 1.55, and 1.92, respectively.

Triploidy was successfully induced in Chinese catfish by cold shock at 4 C for 15 to 20 minutes, started 3 minutes after insemination. It should be noted that 100% triploids were achieved by cold shock, although the fertilization rate of cold shock was lower than the untreated eggs. This result suggests that the pressure method for inducing triploids might be worth trying to increase the fertilization rate of triploid eggs in the future. The sizes of erythrocyte nuclei between the diploids and triploids were distinctly different, but the number of chromosomes of both types must be confirmed in future research. Much of interest in triploid induction is based on the assumption that triploids are profitable for commercial cultivation due to their sterile nature and the rapidity of their growth. Investigators began conducting the growout experiment to collect data on the growth, feed conversion efficiency and feeding patterns. If the triploid fish prove to be of significant advantage in terms of commercial fish farming, the technique will be introduced to farmers throughout Hawaii.

In addition to the trials at HIMB outlined above, a second set of spawning trials was conducted at HIMB during August 1995. Shocked and non-shocked eggs from these spawns were reared at a cooperating commercial farm, Hawaii Fish Company. Triploidy induction with shocked fish from these trials was also 100%. Two additional spawning trials were conducted at another cooperating commercial farm, Kualoa Ranch, during August and October 1995. Survival of shocked fish from the first trial was very low, with less than 56 of the shocked eggs hatching. The second trial was more successful, producing sufficient numbers of 2N and 3N fish to conduct growout trials at Kualoa Ranch. Two attempts to produce triploid fish at Aquatic Culture & Design failed to produce enough 3N fish for growout trials there.

Objective: Compare survival, growth rates, FCR and condition of normal diploid (2N) and triploid catfish.

On July 11, 1995, a total of 16 female and 12 male Chinese catfish broodstock were spawned manually at Hawaii Institute of Marine Biology following HCG injection with 4 international units per gram of female fish at 25 C. About half the fertilized eggs were subject to cold-shock at 4 C for 15 minutes within two minutes post-fertilization, while the other half were held at ambient temperature of 25°C. Treated eggs were then placed on a frame with screen on the top, and incubated in 50 liter plastic containers with continuous aeration and water flow through (10 L/h) under same conditions as non-shocked eggs. Fertilized eggs hatched within 36 h at 25°C.

Fry were held in the same plastic containers for 30 days and fed with *Artemia* nauplii in the first week and subsequently with salmon and trout start feed (Rangen, Inc.). Examination of erythrocyte diameters using Giemsa stain from 100 cold-shocked fish and 20 non-shocked fish revealed that controls were all diploid (2N), while 95 percent of cold-shocked fish were triploid (3N). Diploid and triploid fish were separately reared in two 18-foot-diameter tanks with Rangen feed until they could accept the special formulated feed.

The comparative growth and survival between diploid and triploid fish were evaluated in 18 fiberglass tanks at the Hawaii Institute of Marine Biology. The tanks, which were arranged in five rows and could be conveniently sampled from elevated walkways, were 1.5 meters in diameter and 0.9 meters high with a volume of approximately 1.5 milliliters. A 5-centimeter-diameter standpipe is centered in each tank. Water enters from the top and flows out through the standpipe.

To evaluate the effect of temperature on fish growth and survival, diploid and triploid fish were reared at both low and high temperatures. The low temperature was maintained with tap water at 21.5°C, while the high temperature was elevated to 25.0°C in a 5.5-meter-diameter tank with a plastic sheet cover and pumped through a biofilter into each high temperature tank. Three replicates were used for each treatments, which included diploidy at low temperature (DL), diploidy at high temperature (DH), triploidy at low temperature (TL), and triploidy at high temperature (TH). Three replicates were used for each treatment. Fish were fed with New Age Pacific feed (Moore Clark, Canada) which contains 49 percent crude protein and 18 percent crude lipid.

To evaluate the effect of feed protein contents on fish growth and survival, both diploid and triploid fish were fed with New Age Pacific feed (see above for formulation) and catfish feed that contains 36 percent crude protein and 23 percent crude lipid. Treatments include diploidy fed with catfish feed (DC), diploidy fed with New Age Pacific feed (DN), triploidy fed with catfish feed (TC), and triploidy fed with New Age Pacific feed (TN). Three replicates were used for each treatment, and water temperature averaged 21.5°C.

Both experiments initiated on January 10 and ended on June 26, 1996. Weights of diploid and triploid fish at stocking averaged 54.1 and 56.2 grams, respectively, but were not significantly different ($P>0.05$). Both diploid and triploid fish were stocked at 180 fish per tank (100 per square meter) and fed once a day at 3 to 5 percent of body weight. The water flow rate was about 4 liters per minute in each tank. Fish mortality was recorded daily; fish growth was checked every 6 to 8 weeks by measuring 30 fish for length and 50 for weight. At the end, fish survival, condition factor, and gonadal development were checked. Food conversion ratio was estimated by dividing the amount of feed provided with net fish production in each tank. The data analysis was performed with ANOVA using SAS program.

Weight differences between diploid and triploid fish reared at different temperature did not show in the first 40 days ($P<0-05$). After 80 days, significant differences appeared between diploid and triploid fish ($P<0.005$), or between low and high temperature ($P<0.006$). Catfish weights in the DL, DH, TL, and TH treatments averaged 112.1, 116.5, 120.0, and 128.9 grams, respectively.

After 175 days of culture, weight differences between diploid and triploid fish and between low and high temperature remained significant ($P<0.001$), with diploids averaging 250.1 grams and triploids averaging 262.5 grams at low temperature; and diploids averaging 272.0 grams and triploids averaging 300.5 grams at high temperature.

Weight differences between diploid and triploid fish fed with different feeds did not appear in the first 90 days ($P>0.05$). After 120 days, feed type had a significant effect on fish weight ($P<0.003$) . Fish given catfish feed weighed

more than those given New Age Pacific feed. Fish weights in the DN, DC, TN, and TC treatments reached 170.8, 189.1, 176.9, and 198.2 g, respectively. By the end of the culture, catfish weights in the DN, DC, TN, and TC treatments averaged 253.0, 272.3, 257.4, and 282.9 grams, respectively. Both diploid and triploid fish given catfish feed weighed more compared with those given New Age Pacific feed ($P<0.03$), while triploid fish weighed more than diploid fish only when both fish fed catfish feed ($P<0.05$).

Triploid fish had a lower feed conversion ratio (FCR) than diploid fish ($P<0.0002$), while both diploid and triploid fish had a lower FCR at high temperature than at low temperature ($P<0.0005$). Catfish feed exhibited a lower FCR than New Age Pacific feed for both fish ($P<0.0001$), whereas triploid fish had a lower FCR than diploid fish only when both fish fed Catfish feed ($P<0.0001$). Neither fish survival nor fish condition factor was different among treatments. Fish wet weights (W) and total lengths (L) were closely related and could be expressed as the following equation: w 82 .32 - 13 .71L + 0. 7OL' (N = 1790, r = 0. 99)

Triploid *C. fuscus* grew significantly faster than diploids cultured from the same spawns, under similar conditions This difference was even more pronounced at high temperature (25°C) or the fish were given catfish feed. After about 6 months, the average weight of triploids was 10 percent greater than that of diploids at high temperature, while triploids weighed 5 percent more than diploids at low temperature. With the other species in the same genus, Fast et al. (1995) found that the weight of triploid *C. macrocephalus* was 48.7 percent greater than with diploids after 8-months of culture at 28.6° to 29.4°C. These results indicate that the growth of triploid Chinese catfish may be temperature dependent. Further comparisons on the growth of diploid and triploid fish at high temperature (above 25°C) will be conducted for a longer duration.

The results also indicate that catfish feed with 36 percent crude protein and 23 percent crude lipid exhibited a lower feed conversion ratio than New Age Pacific feed, which contains 49 percent crude protein and 18 percent crude lipid. Traditionally, feeds are thought to be in high quality if they contain more protein. However, protein percentage can be maintained in feeds with soy or canola meal rather than fish meal. High levels of plant protein can be less palatable than feeds whose protein is comprised of high quality fish meal. Protein levels in New Age Pacific feed are mainly composed of plant protein, while the major protein source in catfish feed is fish meal. It is likely that the catfish feed was more palatable to the fish than New Age Pacific feed and therefore gave a lower feed conversion ratio.

Feed conversion ratio was lower at high temperature than at low temperature. Investigators observed that fish feeding was more active at high temperature. Because most pellet feeds would be dissolved two hours after releasing into water, less feed is wasted if fish take the feed sooner. This is partly the reason that low feed conversion ratio was found at high temperature.

Fish survival was not different between diploid and triploid fish, indicating triploid Chinese catfish is as viable as diploids. Although triploid fish grow faster than diploids, fish condition factors were not different between diploids and triploids. The similar body shape and fast growth make triploids superior to diploids for aquaculture.

Objective: Determine intrinsic, diel feeding patterns of diploid and triploid catfish during growout to market size using demand feeders and data loggers.

Investigators installed demand feeders, thermocouples, solar cells and a data logger system on three 18-foot- (5.5-meter-) diameter tanks at HIMB. The demand feeders were suspended from individual load cells, allowing feed consumption estimations on a nearly continuous basis. The experimental set-up in the three tanks included: triploid fish under ambient light in Tank 1; and diploid fish under ambient light in Tanks 2 and 3. The preliminary data showed that the feeding activities peaked from 8:00 to 9:00 and from 18:00 to 19:00. Further testing was conducted to determine whether the feeding pattern will change if a constant light is provided. Continuous monitoring of feed consumption will last until December 31, 1996.

Objective: Based on intrinsic feeding patterns observed during Year 1 using demand feeders and data logger recordings, alter feeding patterns to coincide with those commonly used at commercial production facilities. Observed comparative growth rates, survival and FCR of fish with altered versus intrinsic feeding patterns.

During Year 1, juvenile Chinese catfish were found to have two distinct feeding peaks. An experiment was begun February 1, 1997, at HIMB to test whether fish growth and survival will be affected by altered feeding schedules other than coinciding with their intrinsic feeding patterns. Three feeding schedules will be included: (1) feed once daily at noon, (2) feed once daily at midnight, and (3) equally divide the amount of feed and deliver to the fish twice daily at 8 a.m. and 6 p.m.

Three 1.5-meter-diameter, 0.8-meter-high tanks will be used for each feeding schedule with 150 fish (20 g) stocked in each tank. Daily ration will be 5 percent of fish weight and this amount will be adjusted monthly according to fish weight gain. This experiment will last 6 months.

Objective: Confirm reliability of triploid (3N) catfish production using cold shock treatment of fertilized eggs, and compare with pressure shock treatments.

The method of cold shock for triploid induction was further evaluated under two temperature regimes (4°C or 5°C) with six shock durations (0, 5, 10, 15, 20, 25, and 30 minutes). Hatch rates were not different between temperature treatments ($P>0.05$), but reduced with increased time of cold treatment. There were no significant changes in the hatch rate between 20 and 30 minute cold shocks, which yielded >90% triploid fish at 4° or 5°C when shock duration was 20 or 30 minutes. Based on this experiment and results from Year 1, the cold shock treatment at 4° to 5°C for 20 to 30 minutes seems to be most effective to induce triploid Chinese catfish.

To compare the methods between the cold shock and pressure shock in inducing triploid fish, we used 4°C for temperature treatment and 8000 psi for pressure treatment. After eggs were mixed with sperm for 3 minutes, the fertilized eggs were then divided into three equal portions (i.e., control, cold shock, and pressure shock) for treatments with four replicates for each. The cold shock and pressure shock lasted 20 and 8 minutes, respectively. The control and treated eggs were incubated in circulated hatching jars at 26° to 27°C. Hatch rates in the control group were greater than either in the cold shock or in the pressure shock treatment ($P<0.001$). Pressure shock produced a greater hatch rate than temperature shock ($P<0.001$).

Objective: Compare survival, growth rates, FCR, and condition of normal diploid (2N) and triploid catfish produced by the two shock treatment.

The poor survival of triploid fish for both temperature and pressure treatment after hatching did not allow us to compare survival, growth rates, FCR, and condition of normal diploid (2N) and triploid catfish produced by the two shock treatments. The comparison will be made in spring and summer of 1997.

Objective: Using cold and/or pressure shock, attempt to produce tetraploid (4N) Chinese catfish and rear these fish to reproductive age. If successful, tetraploids could be cross-bred with diploids to produce 100% triploids.

Fertilized eggs were cold shocked at 5° to 6°C for 17 minutes at 3, 15, 30, 45, 60, 75 and 105 minutes post-fertilization. A control group was not shocked. The nuclear diameters of red blood cells (rbc) in the developing larvae were then measured. As expected, control fish (c0) all had rbc nuclear diameters of <35pt. More than 70 percent of fish shocked at 3 minutes post-fertilization (c3) had rbc nuclear diameters of >35 , while those shocked at 15 minutes post-fertilization (c15) were similar to the control group. Eggs shocked at 30 minutes post-fertilization (c30) indicated >50% with nuclear diameters >35 .

These data indicate the following. Control fish were normal diploids (2N), while 70 percent of those shocked at 3 minutes post-fertilization were triploids (3N) due to polar body retention. Shocking at 15 minutes post-fertilization resulted in normal diploids since the polar body was lost, but the first cell division was not affected. Shocking at 30 minutes post-fertilization resulted in production of tetraploids (4N) due to disruption of first cell division after

chromosome replication. To a lesser extend, this may have also occurred with fish shocked at 45 minutes post-fertilization but not at 60 minutes post-fertilization. Shocking later than 60 minutes probably resulted in mosaics, with some cells being 2N and some 4N.

Due to low survival of these fish, trials are being continued and efforts are being concentrated on shocking during the period from 25 to 40 minutes post-fertilization. If this succeeds in producing sufficient quantities of fish that appear to be 4N, their condition will be verified by chromosome spread (count) techniques.

Objective: Conduct technology transfer to the Hawaii aquaculture community through workshops, collaborative research and publications.

In August 1998, the investigators completed an eight-page extension fact sheet titled *Triploid Chinese Catfish*. It will be published as CTSA publication number 134. The sheet outlines the cold shock method of inducing triploidy and the conclusions of growout experiments. The investigators concluded that triploid Chinese catfish grow 13 percent faster and consume about 24 percent less feed than diploid Chinese catfish. All male triploid catfish could reach market size almost two months earlier than diploids. In addition, the fatty acid profiles of triploids demonstrated that they have more favorable food quality for consumers.

Impacts

Significantly greater growth of triploid fish at higher temperatures was observed. These higher water temperatures are perhaps representations of typical water temperature at most Hawaii aquaculture sites. However, triploid growth was only about 10 percent greater on average than that of diploids. This is less than expected based on earlier results with *Clarias macrocephalus*, but still would be an advantage to farmers because triploids would reach marketable size earlier than diploids.

Triploid fish also exhibited significantly greater fat content when cultured at both high and low temperatures and on different feeds. Those fat profiles are considered “healthful” and could add to their market appeal. These and other results are still being analyzed.

Support

This project received support from the Center for Tropical and Subtropical Aquaculture (CTSA), the University of Hawaii (UH) and cooperating commercial producers (Industry).

Year	CTSA	Other Support			TOTAL
		UH	Industry	Total Other	
One	$55,000	$32,695	$16,000	$48,695	$87,711
Two	$55,000	$32,695	$16,000	$48,695	$87,711
TOTAL	**$110,000**	**$65,390**	**$32,000**	**$97,390**	***$175,422***

Publications, Manuscripts or Papers Presented

Fast, A. W. 1998. *Triploid Chinese Catfish.* Center for Tropical and Subtropical Aquaculture Publication Number 134. Waimanalo, Hawaii.

Public Policy Impact on Aquaculture Development in Guam

Dates of Work

October 1996 through January 1998

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;

Objectives

The overall goal of this one-year project, initiated under the CTSA Ninth Annual Plan of Work, is 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 are to:

prepare a case study of permitting problems experienced by Guam aquaculture farmers;
develop policy and regulatory solutions to those problems and present those solutions to decision makers;
educate existing and prospective farmers about the permitting process with a forum, a video and extension activities.

Anticipated Benefits

This project will benefit the aquaculture industry by garnering participation of relevant agencies in the consideration and formation of sound aquaculture policy. Specifically, benefits will be achieved through the distribution of accurate and current information affecting aquaculture development worldwide.

Principal Accomplishments

Objective: Prepare a case study of the permitting process experienced by Guam aquaculture farmers.

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. Investigators expect to choose one or more of these four farms for the case study, but none of the farmers has agreed to the extensive interviews necessary for a proper case study. Investigators expect to gain their cooperation in the near future.

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.

1998 report

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. 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 summary of all the available permitting information was prepared and is available to all interested parties at the Department of Commerce and GADTC. Investigators plan to update the existing booklet on permitting, "An Introduction to Aquaculture on Guam: Prospects, Permits and Assis tance," pending the outcome of the "One-Stop Aquaculture Permit."

A video was prepared of the GADTC facility, including GADTC site visits by Governor Gutierrez and Jean Michel Cousteau, the Flores farm site and its owners, and the Scope farm site. The script objectives are being finalized with assistance from David Crisostomo, who will also assist in the final production and editing of the video, which will include an aerial survey of Guam's aquaculture operations.

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.

Investigators scheduled an August 1997 forum on the aquaculture permit process to educate aquaculture farmers and policy makers about the permitting process. The first day will focus on education. Investigators plan to develop the framework for an action plan to ameliorate possible differences between lead agencies involved with the approval of the "One-Stop" program. The possibility of an executive order or legislative sponsorship for the implementation of the "One-Stop Aquaculture Permit" program during the forum is possible. The second day of the forum will address the immediate status and future prospects of the aquaculture industry on Guam. The governor will speak, emphasizing his "Vision 2001" plan, which includes long-term development commitments to the aquaculture industry on Guam. The forum will also include extension activities such as workshops or site visits, multimedia presentations about public policy regarding aquaculture permit processes. If the "One-Stop" program is approved, the presentations will help smaller farms to become more easily established and perhaps more common place.

1998 Report

The Guam Aquaculture and Environmental Awareness Forum was held August 28-29, 1997, at the University of Guam. The governor of Guam issued a proclamation declaring August 27 through September 3, 1997, as "Aquaculture and Environmental Week" on Guam. Approximately 90 individuals attended the forum, including 60 potential and current aquaculture producers, 20 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.

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.

Work Planned

Investigators are seeking a new employee because the individual who handled most of the day-to-day tasks of the project resigned. After the new employee is hired and becomes familiar with the politics of aquaculture and the environment on Guam, he or she will complete the case study interviews, revise the brochure "Aquaculture on Guam: Prospects, Permits and Assistance," and complete the video.

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

No publications or presentations were produced during the reporting period.

Diversification of Species for Aquaculture in Guam
termination report

Dates of Work

May 1995 through October 1997

Funding Level

$73,050

Participants

Dr. John Brown, Dr. Robert Barber, David Crisostomo and Dr. Ilse Silva-Krott, College of Agriculture and Life Sciences, University of Guam;
Dr. Robert Richmond, Marine Laboratory, University of Guam.

Reason for Termination

This project was terminated because all the objectives were completed.

Objectives

The overall goal of this two-year project, which was initiated under the CTSA Eighth Annual Plan of Work, was to improve the profitability of Guam's aquaculture industry by diversifying the product mix to reach new local and export markets. Specific objectives related to that goal are to:

develop a protocol to identify and select candidate indigenous species for quick commercial development;
develop commercial seedstock production techniques for selected species, including
- developing commercial seedstock production techniques for mangrove crabs;
- developing commercial seedstock production techniques for hard corals;
- developing an artificial settling substrate for hard corals;
- determining the growth rates of cultured hard corals and transport methods that provide the highest survival rates;

determine commercial feasibility for selected species;
develop extension publications and videos to transfer the technology to the aquaculture industry;

Anticipated Benefits

The growth of the aquaculture industry on Guam has declined due to increased competition from imported milkfish. The addition of marine shrimp helped to offset this dilemma somewhat. However, the need for a diversity of species remains a priority. Adding mangrove crabs to the list of species cultured on Guam for food would greatly add to the overall health of the industry. The work accomplished in this project will help to provide needed products for commercial producers.

Guam's aquaculture industry is constrained by at least five factors: a narrow diversity of products, limited local ethnic markets for many current products, a lack of export markets, and producer uncertainty about the profitability of new methods and products, and regulatory difficulties in obtaining permits and importing new species. This project addresses the first four constraints. The first year of work is divided into three components: an economics component that will develop a protocol for selecting and prioritizing indigenous species for aquaculture and will perform preliminary feasibility analyses on selected species; a component that will develop technology to produce larval mangrove crabs and growing them out; and a component that develop technology to spawn and grow out hard corals for local and export markets.

The cultivation of corals for the aquarium trade would allow a new industry to develop while preventing damage to reefs and related fisheries. The development of techniques for the cultivation of corals will not only provide a means of economic development for the tropical islands of the Pacific and Caribbean but also will enable the protection of natural resources.

Principal Accomplishments

Objective: Develop a protocol to identify and select candidate indigenous species for quick commercial development and determine commercial feasibility of culturing the selected species.

A literature search on species selection was completed. The final report on the preliminary economic analysis of mangrove crab culture is being prepared.

Objective: Develop commercial seedstock production techniques for mangrove crabs.

A memorandum of understanding was signed with the Guam Department of Commerce, and the remaining mangrove crab broodstock was transferred to the DOC facility to investigate whether the crabs were not spawning because of stress. The crabs that were obtained from Guam rivers had been held in the quarantine facility at the UOG Marine Laboratory for more than six months. Additional juvenile crabs were imported and grown out to determine growth rates and feeding requirements.

Objective: Develop commercial seedstock production techniques for hard corals.

Coral gametes were collected from several species after spawning that took place following the June full moon. The majority of species being studied spawned following the July full moon. Using sperm concentrations of approximately 1 million cells per milliliter, egg fertilization rates ranging from 80 to 95 percent were achieved. Cultivation of fertilized eggs in static, bottle and aerated pyramid tanks was successful, and thousands of larvae were raised to a competent planula stage.

During the second year of the project, full moon occurred on July 1 and July 31, and as a result, some Acroporids released their gametes in early July while others waited until early August. This provided two opportunities for experimentation. During the summer spawning events, investigators experimented with modified techniques for mass fertilization and growout of corals and were able to simplify methods substantially. Protocols were developed for culturing coral larvae in 3-gallon plastic tubs, and the amount of labor needed to grow larvae through settlement and metamorphosis was reduced. Simplified methods of calculating appropriate sperm concentration were developed.

As the previous data demonstrated, the hard coral species *Pocillopora damicornis* releases fully developed planulae larvae between the new moon and the lunar first quarter each month of the year. The larvae are competent soon after release from the parent colony. Unlike the planulae of the spawning corals studied, larvae of *P. damicornis* contain a full complement of zooxanthellae upon release.

Studies were performed to determine if the planulae larvae prefer certain substrates. Experiments using a variety of natural and artificial substrates were performed. *P. damicornis* was found to be non-specific, and larvae would routinely settle and metamorphose on 3-day-old biological films composed primarily of diatoms and bacteria. The Acroporid corals, as well as *Goniastrea retiformis,* demonstrated marked preferences for particular species of crustose coralline algae. The preferred algal species can be grown on artificial sub-strata. Attempts to induce larvae to undergo metamorphosis on Plexiglas discs were successful. These discs are convenient for shipping and attachment to aquaria substrata.

During the second year of the project, the crustose coralline alga *Hydrolithon reinboldii* was found to induce settlement and metamorphosis in a variety of commercially valuable species of corals, particularly *Goniastrea retiformis* and several species of *Acropora*. This alga was induced to coat artificial substrate, including 1-inch-diameter Plexiglas discs, through direct contact as well as by distribution in seawater tables with algal thalli.

In ongoing growout experiments, most corals exhibit non-linear growth rates. Initial growth was relatively slow, on the order of one to three millimeters per month. Once the colony reaches a size greater than 1 centimeter in diameter, growth rates appear to increase. Survival of *P. damicornis* is high, often reaching 100 percent. The survival of newly settled recruits of the spawning species was found to depend on the incorporation of zooxanthellae. Experiments on growth and survival are ongoing.

During the second year of the project, colonies of corals cultured during Year One were measured to provide growth rates and survival data. Growth rates of colonies of both *P. damicornis* and *Acropora* spp. were found to be non-linear, with larger colonies growing at faster rates. *P. damicornis* survival was variable, depending on weather conditions. In several experiments, survival through the first month was greater than 85 percent. Due to a seawater system shutdown during a passing typhoon, survival of colonies through the first month in that trial was only 30 percent. This demonstrated the need for dependable seawater flow, an issue that is being addressed by installation of a seawater well above the wash zone to back up the beach-side system that must be dismantled when typhoons pass the coast. Two corallivorous gastropods were found, and the coral was inspected weekly for this predator.

Experiments are being conducted to determine the optimal size for donor colonies from which to harvest coral fragments. Data is being collected on growth rates, repair rates and survival of donors and collected fragments.

Investigators made a break-through on increasing the growth rate of corals through juvenile colony fusion. The time to marketable size and an appropriate size for harvesting branches was reduced by half to five months by fusing several small larval recruits. This has valuable implications for the success of the project.

A cooperative agreement between the University of Guam Marine Laboratory and the Guam Aquaculture Development and Training Center, Guam Department of Commerce, allowed enlarging the production of corals from larvae. In addition, the Guam Department of Agriculture approved permits to produce and sell cultivated corals.

In 1997, two typhoons severely damaged the laboratory and hatchery facility and all the coral died.

Egg-sperm clusters were collected on 80 m nytex screens, separated and prepared for fertilization experiments. Sperm densities of 10^5 sperm per ml were found to be optimal. A haemocytometer is normally used to determine sperm density in the containers used to collect the filtrate form the egg-sperm clusters. The concentrated sperm is then added to the cultivation containers to reach the calculated optimal concentration.

To simplify the procedures, the sperm density of gamete clusters was determined for *Goniastrea retiformis* and several species of *Acropora*. The sperm densities ranged from 2×10^6 to 6×10^6. Fertilization experiments found that adding 100 gamete clusters (50 from each of two corals) to one liter of 0.45 millipore-filtered seawater resulted in more than 90 percent fertilization. The additional advantage of this simplified technique is the production of 800 to 1,200 larvae of known parentage in one liter of water. A larval density of 1 larva per milliliter was found to be proper for early developmental stages.

After eggs were fertilized, water was changed daily. UV-sterilized seawater is sufficient for this. Development time to the competent planula larval stage ranged from 18 hours for the smaller *Goniastrea* larvae to 72 hours for the larger *Acropora* larvae. Once the larvae are competent to settle and metamorphose, suitable substrate were added to the cultivation basins. The substrata were checked daily for the presence of recruits, and those with settled corals were moved to flow-through seawater tanks for growout.

The eggs and planula of all the spawning species under study lack symbiotic zooxanthellae. We have found the simplest technique for initiating inoculation is by placing the newly settled and metamorphosed recruits in tanks with adult colonies of the same species. The common fouling sea anemone *Aptasia* was also found to be a suitable donor. Other, more complex techniques were developed for acquiring specific genetic lines but proved too labor intensive to be used in a commercial culture program. Since February 1997, all colonies of *Pocillopora damicornis* collected form Agana bay were tagged with numbered plexiglass square. The total number of planulae produced by each colony, the general condition of the colony (percentage of bleaching), the date of collection and the date of return were recorded. Seventy-seven colonies were tagged and returned to Agana Bay. The purpose of tagging is to identify colonies that produce large numbers of planulae, return the colonies to the ocean so that they recover from the stress of being

maintained in the laboratory, then gather them several months later for larval collection. The data will help to determine the best, non-destructive way to use parent or source colonies.

From November 1996 to June 1997, planulae were collected from *P. damicornis* each month. Collections began a day or two before the new moon and lasted 6 to 12 days or until a drop in the number of larvae being released. With the exception of December, in which the brood colonies released very few larvae, 1,000 to 2,500 larvae were collected each month. The data indicate different populations (genotypes) consistently release their larvae on different lunar phases each month. The expanding database makes it possible to identify colonies from which larvae can be collected on most days of the month. Larvae were also collected from *Stylophora mordax*.

During January and February 1998, juvenile growth and survival experiments were repeated with two different cohorts of corals. The *P. damicornis* from the January cohort (n=83) was followed for 93 days. During the first two weeks, 2 percent mortality occurred; by the end of 28 days, a cumulative mortality of 20 percent was recorded; by Day 36, an additional 4 percent of the coral died. Cumulative mortality was 24 percent for the 93 day trial, and the mean number of polyps per juvenile was 39.

During the February trial, which lasted 45 days, mortality of the cohort (n=168) was only 45 percent, which corresponds to the improved survival during the last 57 days of growth of the January cohort and probably resulted from improved water quality. In addition, the February cohort exhibited improved growth rates with the mean number of polyps per juvenile at 97. Because colonies of *P. damicornis* receive most of their energy through photosynthesis performed by their symbiotic zooxanthellae, the increase in growth rate is not surprising.

Objective: Develop extension publications and videos to transfer the technology to the aquaculture industry.

Detailed records and photographs of all techniques have been kept for the development of a coral culture manual. Video of coral spawning, gamete collection, fertilization and growout have been taken in preparation for the extension video. The materials will be published with funding from another source in Fall 1998.

Impacts

This project clearly demonstrated that corals can be cultivated from both brooding and spawning species. Research advances enabled the techniques to be considerably simplified. High survival levels were achieved in the laboratory. With support form the Department of the Interior, Office of Insular Affairs, project personnel conducted a workshop during Summer 1998 to train individuals from Palau, Kosrae, Yap, Saipan, American Samoa, Guam and Washington D.C. in coral cultivation and its applications, including reef restoration.

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**	**UOG**	**Federal**	**Total Other**	**Total**
One	$50,000.00	$30,000.00	$0.00	$30,000.00	$80,000.00
Two	$23,050.00	$15,300.00	$200,000.00	$215,300.00	$238,350.00
TOTAL	**$73,050.00**	$45,300.00	$200,000.00	*$245,300.00*	**$318,350.00**

Publications, Manuscripts or Papers Presented

Richmond, R. H., Y. Golbuu and S. Leota. 1995. Fertilization, development and recruitment success in several species of mass-spawning corals. *American Zoologist.* 35(5):9A.

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Richmond, R. H., S. Leota, and J. Coleson. 1997. Cultivation of Corals for Reef Restoration and the Aquarium Trade. VIII Pacific Science Intercongress. July 1997. University of the South Pacific, Suva, Fiji.

Richmond, R. H. 1997. Coral Cultivation and its Application to Reef Restoration and Management: A Collaborative Approach. 15th Annual Pacific Island Coastal Zone Management Conference. September 1997. Maui, Hawaii.

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Marine Ornamental Fish Culture and Conservation

Dates of Work

May 1998 through October 1998

Funding Level

$49,200

Participants

- Dr. Christopher Brown, 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 will lay the foundation for development of a marine ornamental fish culture industry in the region by developing a species priority list and examining larval collection and growout.

Principal Accomplishments

Objectives: Collect larvae from the wild, return them to the laboratory and rear them to market size.

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 fork lift 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 attraction of 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 bar 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.

This objective will be successfully fulfilled by the end of the project term. A draft review of ornamental reef fish in Hawaii has been prepared for submission to a suitable journal and is being revised. This review includes evaluations of the culture potential of most of what are considered the most promising marine ornamental species as well as analyses of their distribution, environmental and economic concerns, reproductive biology, etc. The review will form the basis for a well thought-out species priority list that will be delivered by the end of the project.

Work Planned

During the remaining six months of the project, investigators will finish the priority list of marine ornamental species and refine egg and larval collection techniques.

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 September 1998

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 efficiciency.

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 preliminary literature review was conducted covering BMPs for trout and channel catfish.

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.

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.

Work Planned

During the next six months, the Project Work Group plans to:

- 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;
- Contact all Regional Aquaculture Centers to obtain documents concerning effluent discharge and BMPs, which will be evaluated;.
- 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 1998

Funding Level

$139,000

Participants

Dr. Kevan L. Main (through March 1997), Cheng-Sheng Lee (starting February 1997) and Patti Killelea-Almonte, 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 final 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 and positions 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 final 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 final 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 , layed out and oversaw printing of *Triploid Chinese Catfish*, an extension fact sheet resulting from a CTS-funded project.

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

The Center staff duplicated 11 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, selected project final reports and maintain the Web site.

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.

Year	CTSA	Total Support
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
TOTAL	**$ 157,000.00**	**$ 157,000.00**