Center for Tropical and Subtropical Aquaculture

2003 Accomplishment Report

In cooperation with

Center for Tropical and Subtropical Aquaculture

2003 Accomplishment Report

The Center for Tropical and Subtropical Aquaculture
Waimanalo and Honolulu

Center for Tropical and Subtropical Aquaculture

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Table of Contents

Introduction

Mission

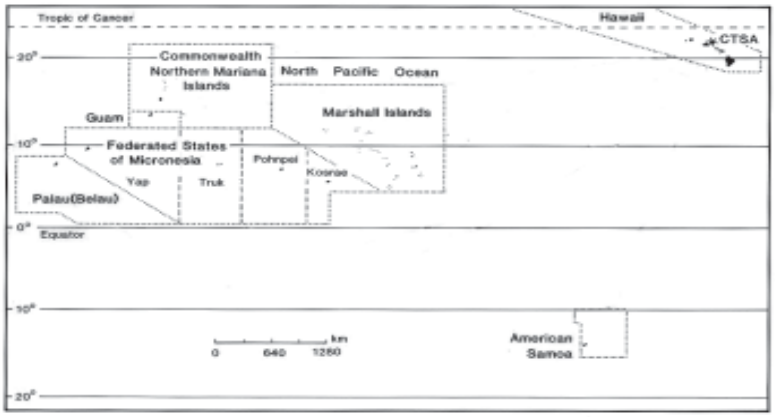
The mission of the Center for Tropical and Subtropical Aquaculture (CTSA) is to support aquaculture research, development, demonstration and extension education to enhance viable and profitable U.S. aquaculture.

Background

Title XIV of the Agriculture and Food Act of 1980 and the Food Security Act of 1985 authorized establishment of five regional aquaculture 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.

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

CTSA is jointly administered by the University of Hawaii and The Oceanic Institute. The Center has offices at both the University of Hawaii's Manoa campus and The Oceanic Institute's Makapuu point site on windward Oahu.



The CTSA region comprises:

American Samoa
Commonwealth of the Northern Mariana Islands
Federated States of Micronesia
Guam
Hawaii
Republic of Palau
Republic of the Marshall Islands

Objectives

The RACs encourage cooperative and collaborative aquaculture research and extension education programs that have regional or national applications. The Centers' 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 as follows:

1. 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;

2. 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.

Organizational Structure

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

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

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

Administrative Center

CTSA 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. A second office is located at the University of Hawaii's Manoa campus, also on the island of Oahu. CTSA staff provide 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, Executive Director, supervises operation of the Center.

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:

- 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 are:

- Mr. John Corbin
  Hawaii State Dept. of Agriculture Aquaculture Development Program
- Dr. E. Gordon Grau
  University of Hawaii Sea Grant College Program
- Dr. Andrew Hashimoto
  College of Tropical Agriculture and Human Resources,
  University of Hawaii at Manoa
- Dr. Jo-Ann Leong (Chair)
  Hawaii Institute of Marine Biology, University of Hawaii at Manoa
- Dr. Gary D. Pruder
  The Oceanic Institute
- Dr. Singeru Singeo
  Land Grant Programs, College of Micronesia
- Dr. Lee S. Yudin
  College of Natural and Applied Sciences, University of Guam

Executive Committee

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

- Dr. Gary D. Pruder (Executive Committee Chair)
  The Oceanic Institute
- Dr. Jo-Ann Leong (Board of Directors Chair)
  Hawaii Institute of Marine Biology, University of Hawaii at Manoa

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. As an advisory body, the IAC's capacity 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 IAC:

- identifies 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. Richard Bailey, Common Heritage House
- Dr. Paul Bienfang, Ceatech USA, Inc.
- Dr. James Brock, Moana Technologies, Inc.
- Mr. J. Randy Cates, Cates International, Inc.
- Mr. Richard Croft, Pohnpei Natural Products
- Mr. John Gourley, Micronesian Environmental Services
- Ms. Linda Gusman, Island Aquaculture

- Mr. Steve Katase, Royal Hawaiian Sea Farms
- Mr. Robert Kern, Tropical Ponds of Hawaii
- Mr. Jeff Koch, Mokuleia Aquafarm
- Mr. Andrew Kuljis, Aquatic Farms
- Mr. Richard Masse, Mangrove Tropicals
- Mr. Dennis Mitchell, The Fish Shack
- Mr. Ramsey Reimers, Robert Reimers Enterprises
- Mr. Neil Sims, Black Pearls, Inc.
- Dr. Richard Spencer, Hawaiian Marine Enterprises (IAC Chair and *ex officio* member of the BOD)
- Dr. Albert Tacon, Aquatic Farms
- Mr. Howard Takata
- Mr. Frank Toves, Aquaculture Culturists
- Mr. Ron Weidenbach, Hawaii Fish Company
- Dr. Leonard Young, Hawaii State Dept. of Agriculture 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 research institutions. The TC provides research expertise to address priorities set by the Industry Advisory Council. The Board of Directors appoints members for three-year, renewable terms. The TC:

- evaluates the technical merit of proposals submitted to CTSA;
- 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, Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa (TC Chair and *ex officio* member of the BOD)

- Ms. Kristen Anderson, Hamilton Library, University of Hawaii at Manoa
- Dr. John Brown, College of Natural and Applied Sciences, University of Guam
- Mr. David Crisostomo, Cooperative Extension Service, University of Guam
- Dr. Maria Haws, Pacific Aquaculture and Coastal Resources Center (PACRC), University of Hawaii at Hilo
- Dr. Kevin Hopkins, PACRC, University of Hawaii at Hilo
- Dr. Robert D. Howerton, Sea Grant Extension Service, University of Hawaii
- Mr. Tom Iwai, Anuenue Fisheries Research Center
- Dr. Charles Laidley, Marine Finfish Program, The Oceanic Institute
- Dr. PingSun Leung, Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa
- Dr. Anthony Ostrowski, U. S. Marine Shrimp Farming Program, The Oceanic Institute
- Mr. Vernon Sato
- Dr. James Szyper, Sea Grant Extension Service, University of Hawaii at Manoa
- Dr. Clyde Tamaru, Sea Grant Extension Service, University of Hawaii at Manoa

Executive Summary

PROGRAM SCOPE

During 2003, the Center for Tropical and Subtropical Aquaculture completed work on projects funded under its Thirteenth Annual Plan of Work and continued work on projects funded under its Fourteenth and Fifteenth Annual Plans of Work. In addition, in November 2003, CTSA initiated work on projects developed under its Sixteenth Annual Plan of Work and began developing its Seventeenth Annual Plan of Work.

Eleven projects were funded under CTSA's 16th year program, which was approved by CTSA's Board of Directors on February 11, 2003. Six were continuations of projects begun under the programs of previous years and five are new projects.

Since the inception of CTSA in 1986, it has funded 172 research, demonstration, development and extension projects. Twelve projects were active during 2003. These projects fall into five categories:

- National Aquaculture Priorities
- Information Dissemination
- Extension Support to Further Industry Development
- Development of New Technologies
- Demonstration and Adaptation of Known Technologies

This project addresses national aquaculture priorities:

- National Aquaculture Extension Conference

These projects address information dissemination:

- Library Aquaculture Workstation

- Publications

These projects address extension support to further industry development:

- Disease Management in Hawaiian Aquaculture
- Transitioning Hawaii's Freshwater Ornamental Industry
- Development of Black-Lip Pearl Oyster Farming in Micronesia
- Aquaculture Extension and Training Support for the U.S.-Affiliated Pacific Islands

These projects address development of new technologies:

- Aquaculture of Marine Ornamental Species
- Reproduction and Selective Breeding of the Pacific Threadfin
- Aquaculture of Hawaiian Marine Invertebrates for the Marine Ornamental Trade

These projects address demonstration and adaptation of known technologies:

- Evaluation of Tilapia Species and Varieties for Establishment of a Tilapia Hatchery in Guam
- Improving Sturgeon Farming in Hawaii
- Transitioning Hawaii's Freshwater Ornamental Industry

A brief listing of the principal accomplishments of the active projects in these categories during 2003 is presented below. Details on each project's funding, participants, objectives, anticipated benefits, progress and future plans are presented in the Progress Reports section.

National Aquaculture Priorities

National Aquaculture Extension Conference

This project allowed CTSA, like the other four USDA Regional Aquaculture Centers, to be a co-sponsor of the National Aquaculture Extension Conference, which is held every five to six years. This project also enabled two representatives from the CTSA region (Hawaii and Guam) to attend the conference, which was held in Tucson, Arizona from April 7-11, 2003. The two participants' abilities to perform their extension missions were enhanced through their receiving updated information on nationwide and international trends in the industry and in extension work.

Information Dissemination

Library Aquaculture Workstation

During the Year 14 reporting period, there were 47,892 queries. PRAISE staff responded to 918 requests for direct assistance by returning to users 2,011 articles totaling 26, 549 pages delivered almost exclusively by e-mail.

In the Year 15 reporting period, there was a total of 27,644 queries. PRAISE staff responded to 543 requests for direct assistance by returning to users 1,160 articles totaling 15,220 pages delivered almost exclusively by e-mail.

Publications

The quarterly newsletter was printed and disseminated in March, June and September 2003. The staff assisted with the editing and layout of one publication and distributed numerous other CTSA and RAC publications throughout the region.

Extension Support to Further Industry Development

Disease Management in Hawaiian Aquaculture

Diagnostic support and pathogen testing and disease surveillance services continued to be provided. Two cell lines have been established from Pacific threadfin and two cell lines were prepared from body muscle and fins of bluefin trevally and are currently being used for the investigation of a disease occurring in marine fish culture in Hawaii. They will also be available for future isolation and study of bluefin trevally viruses. Samples of chilled and frozen shrimp were collected from various markets on Oahu and tested for selected shrimp viruses. None of the samples tested positive for TSV or YHV, however, the majority of the samples tested positive for WWSV and IHHNV.

Transitioning Hawaii's Freshwater Ornamental Industry

The minimum effective dosage of Ovaprim for use in the induction of final maturation and spawning was determined for both the redtail and rainbow sharks. Likewise, the suitability of commercially available formulated diets were tested amongst a variety of first feeding fish larvae. The results clearly demonstrate that the ability to utilize an artificial feed as an intial food item for first feeding larvae is species specific. Project staff also field tested the artificial insemination technique to produce swordtails with the lyretail characteristic, and it clearly demonstrated that the lyretail trait is a dominant genetic trait that is being inherited in Mendelian fashion. However, the most intriguing part about the ability to cross a lyretail male and a lyretail female is that a small percentage (i.e., 25%) of the offspring will have the homozygous lyretail genotype. Project staff successfully demonstrated this twice.

The significance of the homozygous individual is that it will theoretically be able to produce progeny that are 100% lyretails. Although the characteristics and mode of inheritance of the lyretail trait was postulated many years ago, to our knowledge, this is the first time that homozygous lyretail swordtails have actually been documented to exist. The project work group published numerous journal articles and newsletter articles on various project topics as well as a manual on the artificial insemination of the lyretail swordtail.

Development of Black-Lip Pearl Oyster Farming in Micronesia

In Pohnpei, a small pearl oyster demonstration farm was established at the Ponape Agriculture and Trade School (PATS), which continues to serve as a training site for PATS students and community members. Four staff biologists from the Marine Environmental Research Institute of Pohnpei (MERIP) received full training in pearl oyster spawning, larval rearing, land-based nursery techniques and microalgae culture. Fourteen Micronesian students from MERIP also received training in these techniques through organized classroom activities and practical demonstrations.

In the Marshall Islands, individuals from government and private agencies received training in hatchery operations, algal culture and larval rearing. Although spawning pearl oysters proved to be difficult likely due to unseasonably warm lagoon waters that might have been related to El Niño, one successful spawn was achieved. Spat from this spawn were used by researchers at the College of the Marshall Islands demonstration pearl farm. A hatchery operations manual was also completed and utilized by hatchery technicians and the new pearl oyster hatchery specialist. It represents the first significant reference for hatchery operations in Micronesia. As a result of the hatchery work at PATS and in the Marshalls, useful data was collected on larval biology and culture. Significant gaps in the knowledge base for pearl oyster hatchery and nursery methods were identified, and these serve as the basis for research currently sponsored by CTSA to improve hatchery and nursery methods at PATS.

The bioeconomic model was completed. Publications for peer-reviewed journals are now being completed, and results of the work have been presented to farmers. This work is being continued by tracking data from regional farms and hatcheries to develop a more extensive database and refine the model.

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

Project staff succeeded in producing 1.07 million settled spat during the project's first hatchery run. The spat were distributed to two local companies and each received 357,000 spat ranging in size from 2-2.5 mm. This was three times the amount initially requested. The remaining spat were used for research conducted at the College of the Marshall Islands' demonstration pearl farm in Arrak.

Development of New Technologies

Aquaculture of Marine Ornamental Species

Several colonies of captive yellow tang were established during project year 1. However, in year 2, egg fertilization rates were problematic and larvae supply was sufficient to carry out only preliminary rearing trials. Also in year 2, significant progress was made in flame angelfish larviculture within CTSA- and NOAA-funded projects and led to the production of the world's first captive-reared juvenile flame angelfish. Year 3 work is directed toward the development of pilot-scale rearing methods for flame angelfish. Established populations of yellow tang and flame angelfish are continuing to be maintained for seed production using current best husbandry practices. Tank commissioning allowing for expansion of flame angelfish broodstock population was achieved in May, and tanks were stocked in June. Holding facilities for angelfish broodstock were stocked in June and harem size experiments are in progress. Early in the project, a trial was carried out to compare semi-intensive versus intensive rearing of flame angelfish larvae. Survival rate was observed to be higher in the intensive rearing tanks than in the semi-intensive rearing tanks.

Reproduction and Selective Breeding of the Pacific Threadfin

Successfully strip-spawning Pacific threadfin broodstock in pairs or small groups has proved to be unusually difficult. The project work group will revert to the original protocol based on obtaining eggs from larger group spawns, attempting to stock runs from multiple spawn days to increase genetic variability in each line. The Pacific threadfin genetic selection project is progressing well behind schedule due to the slower than anticipated maturation and initiation of spawning of select and control animals. Hatchery runs are scheduled for late Fall 2003. Data suggest that portions of the Pacific threadfin broodstock population reach the female stage of development between 11 and 16 months of age and initiate spawning around 2.5 years.

Aquaculture of Hawaiian Marine Invertebrates for the Marine Ornamental Trade

The project staff conducted growth trials on wild caught juvenile feather duster worms. The data clearly show that the groups fed *Chaetoceros* grow significantly faster than the ones receiving only water directly from Kaneohe Bay. Apparently, feather duster worms can utilize only phytoplankton in their diet, and by the fact that the group fed only Kaneohe Bay seawater also grew during the same time period, the minimum amount of food required to sustain feather duster worms need not be so high. Using this data, it was projected that the feather duster worms grow at a rate where they can be harvested at market size within a year.

The project staff also successfully induced spawning in feather duster worms by ablating the worms at the bottom where the coelomic contents could be viewed under a compound microscope. Three days after ablation, the feather duster worms

in both the treated and control tanks were observed to be spewing gametes. The entire experiment was repeated in November and the same results were obtained. These two successful induction of spawning trials provided project staff with the opportunity to document the early life history of the feather duster worm.

The project staff conducted an experiment from January to April 2003 to compare growth of three varieties of soft corals on varying kinds of substrate and exposed to three different means of water motion. One species (*Zoanthus sp.*) produced significantly more polyps than the other two species under all of the conditions. Improvement in growth for the other two species (*Protopalythoa* sp. and *Z. pacifica*) requires further investigation. Lastly, a mass growout trial of *Zoanthus sp.* was conducted. Currently, there are at least 10,00 polyps of this species, and it appears to be adding approximately 20 polyps per month.

Demonstration and Adaptation of Known Technologies

Evaluation of Tilapia Species and Varieties for Establishment of a Tilapia Hatchery in Guam

After some initial setbacks due to Supertyphoon Pongsona, project staff successfully imported two varieties, 'Chitralada' and 'Philippine selected,' of *Oreochromis niloticus* fry and broodstock from Thailand. Project staff have been evaluating the growth rates of the two varieties in nursery tanks and of the 'Chitralada' variety in an earthen pond.

Improving Sturgeon Farming in Hawaii

Project staff have tried and are continuing to try to obtain sturgeon eggs from various sources. The staff was able to distribute materials for the standardized hatchery systems to the participating farms, and four out of six systems have been assembled and are currently operational. Training activities included a sturgeon reproduction and hatchery workshop held in March 2003.

Transitioning Hawaii's Freshwater Ornamental Industry

See page 10.

A Look Ahead at Year 17

Development

The development of the Year 17 program was initiated in March 2003 at the annual meeting of the Industry Advisory Council (IAC). The IAC reviewed the progress of funded projects and recommended research priorities that would aid industry development based on the 37 concepts submitted by farmers and researchers from the region. Members identified twelve project areas for funding priority, from which a call for pre-proposals was disseminated. Fifteen pre-proposals were submitted in response to the call, and fourteen were then asked to submit a full proposal. Twelve full proposals, which addressed ten of the twelve project areas, in addition to the Publications, Information, and Library proposal were submitted. The Board of Directors approved eight proposals for inclusion in CTSA's Year 17 Plan of Work.

Proposals

1. Evaluation and Propagation of Tilapia Strains for a Self-Sufficient Tilapia Industry on Guam , Year 2
2. Aquaculture of Marine Invertebrates for the Marine Ornamental Trade, Year 3
3. Disease Management in Pacific Aquaculture, Year 11
4. Marine Ornamentals Phase II, Year 2: Mass Culture Techniques for Pygmy Angelfish and Broodstock Management of Hawaiian Wrasses
5. Aquaculture Extension and Training Support for the U.S.-Affiliated Pacific Islands with a Special Emphasis on Hatchery Propagation of the Black-Lip Pearl Oyster (*Pinctada margaritifera*), Year 15
6. Amberjack Fingerling Production, Year 2
7. Optimal Harvesting Strategies for Farmed Fish and Shrimp
8. Publications, Information, and Library

Review

In September, CTSA began its three-month review process. All proposals were first subjected to internal and external peer reviews by at least three experts in the project topic area. Proposals were then reviewed for technical merit by the Technical Committee during their annual meeting on November 25, 2003. The Industry Advisory Council Chair presented the proposals to the Center's Board of Directors for approval on January 15, 2004. These proposals will be incorporated into the Seventeenth Annual Plan of Work and will be submitted to the U.S. Department of Agriculture Cooperative State Research, Education and Extension Service for final approval.

Progress Reports

An individual summary of the principal accomplishments of the active projects in these categories during 2003 is presented in the following pages. Details on each project's funding, participants, objectives, anticipated benefits, progress, work planned, impact and publications are presented. Information and results from previous years can be found in the correlating year's annual accomplishment report.

National Aquaculture Extension Conference

General Information

Reporting Period August 1, 2002 - September 30, 2003 (final report)

Funding Level

Year	Amount
1	**$9,580**

Participants

James P. Szyper, Ph.D., Extension Specialist
Sea Grant Extension Service, University of Hawaii at Manoa

David Crisostomo, Extension Agent III
Cooperative Extension Service, University of Guam

Objectives

The overall goal of this project was to arrange CTSA participation and representation at the National Aquaculture Extension Conference which was held in Tucson, Arizona during April 7-11, 2003.

1. To support the travel and reporting activities of two representatives from the CTSA region to the conference.
2. To co-sponsor the cost of the conference as agreed among the RACs and other partners in the conference.

Principal Accomplishments

Objective 1 - To support the travel and reporting activities of two representatives from the CTSA region to the conference.

One representative from Hawaii and one from Guam attended the third National Aquaculture Extension Conference from April 7-11, 2003 in Tucson, Arizona.

Project leader Dr. James Szyper of the University of Hawaii (UH) and David Crisostomo of the University of Guam traveled to the conference from Hilo and Guam, respectively, on tickets purchased through the contract's account at UH Sea Grant. The travelers' other expenses have been settled and reimbursed. The Sea Grant fiscal office will provide complete accounting of the budget upon project completion.

The project leader and the Guam representative attended and participated in all sessions of the conference (to the extent possible - some sessions were concurrent) and received ring-binder copies of the conference schedules. The conference proceedings were included in the binder on a CD-ROM. Also included were two other CD-ROMs, which carried materials used for two of the four workshop sessions on the third day of the conference. The PI produced and presented the poster: "Aquaculture Extension in Hawaii," which is available on a separate CD-ROM.

During the early months of the project period, the project leader participated in six of eight conference calls (with the exceptions being due to absences from the state) held among the National Steering Committee (of which the project leader is a member) and the Conference Advisory Committee, during which the conference was planned in terms of target dates for various accomplishments and tasks were taken up by individuals.

The work statement in the contract also specified that the project leader would write an article for the AquaTips section of the CTSA newsletter, when requested by CTSA. This article was delivered in June 2003 and subsequently published in Aqua Tips by CTSA. The article consisted of background on the conference's history and development and an account of the proceedings.

Objective 2 - To co-sponsor the cost of the conference as agreed among the RACs and other partners in the conference.

This objective was completed when the conference organizer, the University of Arizona, invoiced the UH Sea Grant fiscal office for the agreed upon amount of $4,500.

Impacts

The nature of this project was such that the impacts are difficult to assess directly, but may be reasonably seen as accruing over extended time periods. The two participants' abilities to perform their extension missions were enhanced through their receiving updated information on nationwide and international trends in the industry and in extension work. The extension efforts in Hawaii and Guam were exposed to the extension community through personal contact and the presentations made by the two participants in this project.

The content of the conference sessions was recorded on a CD-ROM and described in the Aqua Tips article. The latter was widely distributed by CTSA; it informed the recipients of industry and extension trends. The project leader described the conference in his own newsletter, Big Island Aquaculture News, which was distributed to more than 300 recipients in the state of Hawaii and on the U.S. mainland. This article resulted in three in-state requests for copies of the University of Arizona CD-ROM, "Aquaculture in the Classroom," which was part of the conference proceedings.

During the conference, the project leader discussed a design for a fluidized bed sand biofilter with a conference participant from the University of Florida, and later received this design from the colleague and built a working model in Hawaii. This will serve as a demonstration of low-cost biofiltration, which has the potential to assist farmers. It is expected that this effort will be reported to an international conference in the year 2004.

Publications in Print, Manuscripts, and Papers Presented

Szyper, J. 2003. Aquaculture extension agents absorb new techniques and ideas at national conference. CTSA Regional Notes Vol. 14, No. 2.

Library Aquaculture Workstation (PRAISE), Years 14 & 15

General Information

Reporting Period

October 1, 2001 - September 30, 2003 (Year 14 - final report)
October 1, 2002 - September 30, 2003 (Year 15)

Funding Level

Year	Amount	Year	Amount	Year	Amount
1	$7,000	6	$14,100	11	$24,000
2	$6,700	7	$28,000	12	$23,000
3	$6,000	8	$49,000	13	$28,850
4	$7,000	9	$25,000	14	$25,230
5	$20,000	10	$30,000	**15**	**$25,000**
				TOTAL	$318,880

Participants

Kristen Anderson, Reference Librarian
University of Hawaii at Manoa

Lois Kiehl-Cain, Assistant
University of Hawaii at Manoa

Objectives

Year 14

1. Continue to provide established services.
2. Develop a Web page aggregating free/non-subscription sites to facilitate the ability of PRAISE users to conduct their own aquaculture research.
3. Develop an innovative user education Web presence. Incorporating the research page created in Objective 2, create a tutorial to instruct users in the process of locating information in the field of aquaculture.

4. Technology transfer.

Year 15

1. Develop innovative user education products. For Year 15, testing and review of the tutorial developed in Year 14, translating the tutorial to streaming video, and burning the video onto CD for distribution.
2. Develop a Web form to request delivery of species or subject specific bibliographies via CD.
3. Continue to provide established services.
4. Technology transfer.

Anticipated Benefits

Providing practitioners the resources to develop the skills needed to obtain information makes them self-sufficient. Providing alternative resources where they can develop their skills no matter where they are profits the entire community. Swift and accurate dissemination of information allows practitioners to be aware of the latest progress in all phases of the industry.

Work Progress and Principal Accomplishments

Year 14

Objective 1 - Continue to provide established services.

Dissemination of information via online research and document delivery is ongoing.

Objective 2 - Develop a Web page aggregating free/non-subscription sites to facilitate the ability of PRAISE users to conduct their own aquaculture research.

A Web page aggregating databases and document sources is available at: http://lama.kcc.hawaii.edu/praise/findit.html.

Objective 3 - Develop an innovative user education Web presence. Incorporating the research page created in Objective 2, create a tutorial to instruct users in the process of locating information in the field of aquaculture.

The PI and assistant have completed a tutorial on how to do research on the Web and posted it on the PRAISE Web site. It may be found on the Find-it-Yourself-Online page.

Objective 4 - Technology transfer.

We have the capability to provide the Gray Literature database as a CD on demand. Full text is not available because we have not yet received permission to digitize the materials.

Year 15

Objective 1 - Develop innovative user education products. For Year 15, testing and review of the tutorial developed in Year 14, translating the tutorial to streaming video, and burning the video onto CD for distribution.

The PI and assistant are working on this project. The tutorial requires some changes to make it attractive on video. We are investigating alternative formats as well.

Objective 2 - Develop a Web form to request delivery of species or subject specific bibliographies via CD.

The format for the form has been developed, but it has not yet been implemented.

Objective 3 - Continue to provide established services.

Dissemination of information via online research and document delivery is ongoing.

Objective 4 - Technology transfer.

We have the capability to provide subject bibliographies and the Gray Literature database as a CD on demand. Full text of our gray literature is not yet available because we have yet to receive permission to digitize the materials. PRAISE staff has digitized about 1/6 of the titles listed in the Gray Literature Bibliography in anticipation of permission to load on the Web.

Work Planned

The PI and assistant will enhance the tutorial to make it adaptable to video. The form will be finalized and posted to the Web site under services offered. The PRAISE staff will continue to request permission to digitize gray literature documents. Provision of established services is ongoing.

Impacts

The PRAISE Web site (http://library.kcc.hawaii.edu/praise/index.html) is an excellent resource for the region, and it specializes in efficient document delivery services.

The value of the PRAISE service is staggering. Based on rates one would pay to the information industry's major suppliers (Dialog Information Service, Inc. for access to ASFA, plus document delivery charges based on the average cost per article from Ingenta, Inc.) the dollar value for our primary service may be presented as follows:

Year 14
47,892 queries averaging 3 minutes each or:
2,395 hours online @ $60/hr = $143,700
2,011 articles @ $18.00 ea. = $36,198
Total $179,898

In replying to 918 requests for direct assistance, 8,989 of those queries were e-mailed to PRAISE patrons. The 2,011 articles represent 26,549 pages delivered almost exclusively by e-mail. In addition, the staff responded to 576 miscellaneous requests.

Year 15
27,644 queries averaging 3 minutes each or:
1,382 hours online @ $60/hr = $82,920
1,160 articles @ $18.00 ea. = $20,880
Total $103,800

In replying to 543 requests for direct assistance, 3,441 of those queries were e-mailed to PRAISE patrons. The 1,160 articles represent 15,220 pages delivered almost exclusively by e-mail. The staff responded to 302 miscellaneous requests.

The PRAISE Web site allows users to make requests online, publicizes research being done in the Pacific via the Gray Literature Bibliography and gives local vendors a venue to advertise themselves to the world. Web-based instructional products are invaluable, especially to people in locations with limited resources. Understanding how research works and how to locate materials gives the enduser confidence in the quality of their work and lifelong learning skills. Our patrons in remote locales have expressed positive results from utilization of the tutorial.

Publications, Year 13

General Information

Reporting Period October 1, 2002 - September 30, 2003

Funding Level

Year	Amount	Year	Amount
1	$10,000	8	$18,000
2	$10,000	9	$18,000
3	$12,000	10	$18,000
4	$15,000	11	$33,600
5	$38,000	12	$33,600
6	$18,000	**13**	**$33,600**
7	$18,000	TOTAL	$275,800

Participants

Cheng-Sheng Lee, Ph.D., Executive Director
Center for Tropical and Subtropical Aquaculture

Kai Lee Awaya, Information Specialist
Center for Tropical and Subtropical Aquaculture

Alcian Clegg, Administrative Assistant
Center for Tropical and Subtropical Aquaculture

Debra Sasaki, Publications Specialist
Center for Tropical and Subtropical Aquaculture

Objectives

1. Inform the people involved in education and from the industry, of pertinent aquaculture information and the status of aquaculture in the region through various forms of media.

2. Through dissemination of our and other publications, inform the aquaculture community and interested parties of CTSA's projects' progress in relation to our mission.

Anticipated Benefits

The main benefit of this project is the enhancement of communications it provides regarding aquaculture activity within the region by functioning as a nucleus for information exchange between the aquaculture industry and ongoing research programs. This, in turn, will aid in the technological advancement of aquaculture.

By disseminating research results and other information related to commercial aquaculture production, the project also helps to overcome the limited information available in the region.

Work Progress and Principal Accomplishments

Objective 1 - Inform the people involved in education and from the industry of pertinent aquaculture information and the status of aquaculture in the region through various forms of media.

The *Regional Notes* was published in March, June and September of 2003. The staff participated in editing and formatting one publication, CTSA Publication No. 150, *Overview of Aquaculture Permitting Issues in the Commonwealth of the Northern Mariana Islands, USA*, which is available on our Web site. It was not printed in hard copy format because it is anticipated that the information will be periodically updated. Project staff has also continued to work with editors on a chapter titled "Giant clam (Bivalvia: Tridacnidae)" submitted for publication in the book *Aquaculture in the 21st Century.* The Web site has also continued to be updated and maintained as needed.

Objective 2 - Through dissemination of our and other publications, inform the aquaculture community and interested parties of CTSA's projects' progress in relation to our mission.

The Annual Accomplishment Report was completed and distributed in February 2003. Project staff responded to requests from throughout the CTSA region and the continental U.S. for CTSA publications, which included over 12 different print publications and two videos. Project staff also automatically disseminated our lat-

est print publication, CTSA Publication No. 149, *Procedural Guide for the Artificial Insemination of the Lyretail Swordtail,* Xiphophorus helleri. Project staff also responded to regional requests for publications published by the other RACs.

Work Planned

The staff will produce and publish the December 2003 and March 2004 issues of the quarterly newsletter, maintain the Web site, prepare the2003 Annual Accomplishment Report and continue to disseminate information.

Impacts

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

Publications in Print, Manuscripts, and Papers Presented

Lee, C-S and K. Awaya. 2003. Suggestions for viable aquaculture development in the U.S. Affiliated Pacific Islands - lessons from giant clam farming and sponge farming. Aquaculture Economics & Management 7 (1/2):125-135.

Disease Management in Hawaiian Aquaculture, Year 9

General Information

Reporting Period October 1, 2002 - September 30, 2003

Funding Level

Year	Amount	Year	Amount	Year	Amount
1	$41,638	4	$44,030	7	$81,991
2	$63,725	5	$66,451	8	$67,902
3	$45,956	6	$51,934	**9**	**$89,529**
				TOTAL	$553,156

Participants

Dee Montgomery-Brock, Aquatic Health Associate
Aquaculture Development Program, Hawaii State Department of Agriculture

Yuanan Lu, Ph.D., Assistant Researcher
Retrovirology Research Laboratory, University of Hawaii at Manoa

Objectives

1. (a) Establish cell lines from Pacific threadfin (moi) for use in the isolation and early detection of pathogenic viruses and (b) Early detection of pathogenic viruses in moi and other marine and fresh-water fish;
2. Test frozen commodity shrimp for selected shrimp viruses;
3. Investigate the relationship between commercially raised moi and fatty livers using on-farm surveys;
4. Continue to provide diagnostic support and pathogen testing and disease surveillance services to fish and shrimp farms and commercial hatcheries in Hawaii.

Anticipated Benefits

Same fish host cell lines are an essential first line of defense for the detection and surveillance of viruses important to fish health programs. Viruses are important pathogens to many species of aquacultured fishes. Increasingly, new probable virus diseases have been encountered in groups of ornamental freshwater fishes imported into Hawaii. Appropriate cell lines are necessary for the cultivation and isolation of pathogenic viruses from aquaculturally important fishes.

Cell lines will be used as laboratory tools for disease diagnosis.

Without appropriate cell culture systems, development of preventative strategies for viral diseases and the inspection of batches of juvenile fish for health certification will be severely limited. Once developed, the new cell lines will be used as laboratory tools for disease diagnosis when, in disease outbreaks, members of this species are submitted for laboratory examination. Additionally, the use of host-specific cell lines will provide a significant means to check batches of the listed species for viral pathogens, an important consideration for export certification of specific-pathogen-free status. For the work in Year 9, we proposed to continue this effort and to develop new cell lines from moi.

Early detection will enable farmers to react quickly to stop the spread of disease.

In 1999, new, probable viral diseases caused losses in groups of ornamental fish. A recent example was the high mortality of cultured fry and juvenile goldfish due to a putative systemic virus infection. The present diagnostic service program offered by the Aquaculture Development Program does not have appropriately trained personnel to conduct fish virus culture, isolation and identification work on case submissions of fish submitted for diagnostic examination. However, trends in case submissions indicate a strong need for fish diagnostic virology for aquaculture producers in the state. Service needs for fish virology will be addressed with the virology work proposed in the Year 9 project.

Knowing possible vectors for disease transmision will help shrimp growers establish more effective biosecurity policies.

The tests on commodity shrimp sold in Hawaii will clarify if commodity shrimp are a possible source of shrimp pathogens introduced into the State of Hawaii. In 1994, Hawaii was hit with a new type of shrimp virus, Taura Syndrome Virus (TSV), which up until that time had only been seen in Central and South America. One theory on how the shrimp in Hawaii were infected was that frozen shrimp imported for human consumption may have somehow brought the virus into the state. Birds feeding on discarded shrimp heads could have possibly been a vector for transmitting this virus into nearby shrimp ponds. The results of the tests will confirm whether these products are carrying OIE reportable pathogens such as White Spot Virus (WSSV), Yellow Head Virus (YHV) or TSV.

Farmers will benefit from knowing whether fatty livers in moi are a bonus or a problem and if feed is the cause.

The trials with moi will ideally give us information on three questions: How widespread is the presence of fatty livers in commercially-raised moi? Do fish fed a diet with a lower percentage fat, have less fatty livers than those fed a diet high in fat? Is high fat in the liver a negative characteristic, or will it contribute to a tastier, moister product? High levels of fat have routinely been seen in the livers of cultured moi. These observations have caused concern among some of the farmers who

raise moi commercially. It is assumed that the commercial feed is the reason for the high fat content in the livers, but this has not been proven.

This project will continue to provide diagnostic services, which is critically important to the aquaculture industry.

The routine diagnostic support and pathogen inspection services provided to the aquaculture community provides a continuous line of communication between the members in this project and the farmers. This ongoing relationship has provided the members of this project with a better understanding of the diseases in particular to aquaculture facilities in the Pacific region. This increased understanding has enabled us to react quickly to disease issues, specific to the species being raised and working around the limitations that hinder production in a given site.

The aquaculture of freshwater ornamentals, food fish, marine fish and shrimp has received increased attention for industry development in recent years in Hawaii. In the past five years, we have evaluated an average of 300 case submissions each year for pathogen inspection or disease diagnosis. The types of warm water aquatic animals cultured include freshwater and marine fish, marine shrimp and marine mollusks. Some submissions are for the determination of the cause of animal morbidity and mortality. In other cases, we conduct tests for specific pathogens and provide to the client documentation of the pathogen status of the product. The inspection documents are necessary as quality control measures for national and international animal transfer.

Work Progress and Principal Accomplishments

Objective 1a - Establish cell lines from Pacific threadfin (moi) for use in the isolation and early detection of pathogenic viruses.

Primary moi cell culture: To develop a protocol for establishment and growth of moi cells *in vitro*, we tried two times to initiate primary cell cultures from moi.

For the first cell culture trial, we used three young-aged fishes (40+5 g) with apparently healthy appearances. We were able to collect snout and fins for primary cell culture, but not internal organs because of the small size of the donor fishes. Following the treatment in antibiotics incubation medium for two hours at room temperature (23+1°C), tissue fragments were minced into small pieces and seeded in cell culture flasks to allow tissue explants to attach and grow. Both media, L15 and M199, were supplemented with 20% fetal bovine serum (FBS) and growth factors. Initiation of cell growth was observed around the edges of some seeded explants at day 4 incubation at 25°C. Cell numbers increased during the following week, but were very limited mainly due to limited tissue specimens.

The second moi cell culture trial was conducted with the use of three big fish (150 g). We collected snout, fins, heart and kidney for primary culture this time. Using the same explant culture method and cell culture media as the first time, we were able to see the cell growth from snout and fins at day 4 incubation and from kidney and heart at day 7. Primary snout and fin cells grew rapidly this time and formed some cell colonies by day 8, while cells derived from heart and kidney grew rather slowly.

Two cell lines have been established from Pacific threadfin (moi).

Subculture of moi cells: Since the initiation of the primary culture of moi in September 2002, two cell lines have been established from this fish, including the cell line derived from snout (MoiSN) and fins (MoiFN). These cells grow well at 25°C in medium L15 supplemented with 20% FBS and various antibiotics. The MoiSN and MoiFN grew more slowly in comparison to other fish cell lines, such as EPC. However, these two moi cell lines have been subcultured 16 and 20 times, respectively, without any apparent morphological alterations.

Although we were able to grow cells from heart and kidney at the primary culture, these cells did not grow well, and we were not be able to subculture these cells. It may be necessary to use larger moi as organ or tissue donors in order to establish cell cultures from kidney and heart.

Characterization of moi cell lines: We have preserved some early stage of moi cells in liquid nitrogen, and these cells exhibit a high stability (>90%) for storage at –196°C.

Morphological examination of established moi cell cultures revealed that moi snout cells are fibroblastic while fin-derived cells are a mixed cell population containing both epithelial and fibroblastic cells.

Comparison of two cell culture media, L15 and M199, used in this study shows that the former is superior to the latter in terms of initiation of primary cell growth and subsequent cell proliferation. The growth of these moi-derived cells are serum-dependent; cells grow optimally at 25°C in medium L15 supplemented with 20% FBS and epidermal growth factor (50 ng/ml).

We plan to further characterize these two cell lines in terms of their biological properties. This work will include testing for viral susceptibility and genomic typing (chromosomal analysis and moi's gene amplification, i.e., 16S rRNA).

Objective 1b - Early detection of pathogenic viruses in moi and other marine and freshwater fish.

From omilu fish (Weight 148g, BL 18cm, BH 7.8cm / Case # 02-146): To establish a cell culture system to study potential viral diseases of omilu (bluefin trevally, *Caranx melampygus*), two cell lines were prepared from body muscle

Two cell lines have been established from bluefin trevally.

(OMM) and fins (OMF). Primary culture of these cells was conducted at 25°C using L15 medium supplemented with 20% FBS and various antibiotics. These cells have been serially subcultured 17-19 times since their initiation on June 25, 2002. Growth of the omilu cells were serum-dependent and their plating efficiencies ranged from 11-28.2%. Comparative analysis showed that these omilu cells grew equally well in the media L15, RPMI 1640, M199 and MEM, which are commonly used for cultivation of animal and mammalian cells. Examination of the early passage cells stored at –196°C revealed a large percent (nearly 98%) of cell viability following a six-month storage in liquid nitrogen. Karyotyping analysis indicated that these bluefin trevally-derived cell lines remained diploid with a chromosome count of 48 at their early passage (passage 7-12). These cell lines shared the same pattern of viral susceptibility, and they were sensitive to all six fish viruses (CCV, IHNV IPN, SVCV, SHRV and SHRV) in this study. These newly established cell lines are currently being used for the investigation of a disease occurring in marine fish culture in Hawaii and will be available for future isolation and study of omilu fish viruses.

From Chinese catfish fry (Weight of ten specimens ranged from 0.12-0.69g, / Case # 03-186): Several Chinese catfish fry were submitted from a farm where the fish were experiencing mortalities caused by an unknown disease. Infected animals showed unusual swimming behavior associated with an infection in the central nervous system. Since viral infections can occur in the brains of fish, we felt it was important to rule out the possibility that a viral pathogen was responsible for this disease.

No viral-induced EPC were observed during the course of the infection period (three weeks), and both infected and control cells looked the same. To confirm this observation, the infection assay was repeated once more using unfiltrate homogenate. We observed the cytotoxic effect when the undiluted sample was inoculated, and cells became detached and began floating. The EPC cells infected with diluted specimens showed no cytotoxic effect, and they looked like the control cells inoculated with MEM-0.

Our experimental results clearly showed that no virus was detectable when EPC cells were used for virus isolation. These data may suggest that a virus is not responsible for causing the disease/mortality. However, EPC cells are not the most permissive cell line for the primary isolation of the virus. It would be better if cell lines derived from catfish could be used. We do not have these cell lines available at this time in our laboratory.

Objective 2 - Test frozen commodity shrimp for selected shrimp viruses.

As of October 27, 2003, 11 samples of chilled and frozen shrimp were collected from various locations on Oahu. Chilled (previously frozen) *Penaeus monodon*

samples were collected from three separate vendors in Chinatown. The other three samples were collected from the seafood sections in Foodland, Star Market and Safeway Supermarket. Two of these three samples were from previously frozen *Litopenaeus vannamei* from Mexico. The third sample of frozen (peeled) shrimp collected from Star Market was from an unknown species of shrimp originating from China. The seventh sample was collected from Safeway Supermarket. These shrimp in this last sample were *P. monodon* that originated from Thailand.

Five shrimp per vendor were selectively chosen for shrimp viral screening, based on the gross appearance of the shrimp, i.e., discoloration or small harvest size, suggesting an emergency harvest due to the presence of disease. For the first sampling conducted on the commodity shrimp from Chinatown, the shrimp were pooled into groups of five separate shrimp samples per vial and the samples were preserved in 95% ethanol. For the commodity shrimp sold in the local supermarkets, individual shrimp were placed into separate vials and also preserved in 95% ethanol. The samples were submitted to the diagnostic laboratory at the University of Arizona in Tucson, Arizona for PCR assay, using Organization International de Epizooties (OIE) approved diagnostic procedures. A total of 16 vials containing shrimp tissues were submitted for PCR assay. The PCR results are listed in Table 1.

TABLE 1a. PCR results of commodity shrimp collected in Hawaii

Source	ADP Case #	WWSV	IHHNV
Seafood Market (Chinatown)	03-30 A1	POSITIVE	POSITIVE
Oahu Market (Chinatown)	03-30 B1	Not Detected	POSITIVE
NC Seafood (Chinatown)	03-30 C1	Not Detected	POSITIVE
Foodland Supermarket	03-141 A1	POSITIVE	Not Detected
	03-141 A2	POSITIVE	POSITIVE
Star Market	03-142 A1	POSITIVE	Not Detected
	03-142 A2	Not Detected	POSITIVE
Safeway	03-327 A1	POSITIVE	POSITIVE
	03-327 A2	POSITIVE	POSITIVE
	03-327 A3	POSITIVE	POSITIVE
	03-327 A4	POSITIVE	POSITIVE

TABLE 1b.

Source	ADP Case #	TSV	YHV
Seafood Market (Chinatown)	03-30 A2	Not Detected	Not Detected
Oahu Market (Chinatown)	03-30 B2	Not Detected	Not Detected
NC Seafood (Chinatown)	03-30 C2	Not Detected	Not Detected
Foodland Supermarket	03-141 B1	Not Detected	Not Detected
	03-141 B2	Not Detected	Not Detected
Star Market	03-142 B1	Not Detected	Not Detected
	03-142 B2	Not Detected	Not Detected
Safeway	03-327 B1	Not Detected	Not Detected
	03-327 B2	Not Detected	Not Detected
	03-327 B3	Not Detected	Not Detected
	03-327 B4	Not Detected	Not Detected

Objective 3 - Investigate the relationship between commercially raised moi and fatty livers using on-farm surveys

A total of 30 moi from two separate sites have been examined thus far. The livers from each individual fish were grossly analyzed for fat content. Each fish was then weighed and the livers dissected out from the fish. Individual livers were then weighed and the liver/somatic weight ratio was determined for each fish. Results from this trial will be compiled in the Year 9 final report that is scheduled for release at the end of January 2004.

The feed/growth studies are currently in process at the Anuenue Fisheries Research Center. Two, 12-ft tanks have been stocked with 20 moi each. The fish in one tank are being fed a standard Moore Clark diet with a fat content of 14%. The other group of fish is being fed the same Moore Clark diet, which has been coated with fish oil. At the end of December 2003, the fish will be sacrificed and weighed, and a blind taste test will be conducted. This will help to determine if the fish with higher fat in their system have an improved flavor, due to the higher fat content in the fish.

Objective 4 - Provide diagnostic support and pathogen testing and disease surveillance services to fish and shrimp farms and commercial hatcheries in Hawaii.

During the period covered by this report, there were 417 case submissions to the Disease Prevention Program. A total of 91 trips were made into the field. Thirty-eight individuals or organizations received assistance from this project; 628 histology blocks and slides were prepared during this reporting period. In addition, 98 samples were submitted for the culture, isolation, and identification of bacteria.

During the reporting period, surveillance sampling for shrimp pathogens was carried out on shrimp samples collected from 12 facilities in Hawaii. The following shrimp/prawn species were evaluated: *Litopenaeus vannamei*, and *Macrobrachium rosenbergii*. The samples were evaluated by histology or PCR method. A total of 4,127 shrimp were monitored by PCR or histology.

There were a total of 364 documents certifying the health of shrimp or fish exported from Hawaii during this reporting period.

Work Planned

We plan to continue work on the development of cell lines for moi, continue to provide viral isolation tests on fish from selected disease outbreaks, finalize the objective to survey farm-raised moi for fatty livers, and continue to provide pathogen screening to the local aquaculture industry.

Impacts

The work that this project's staff does is invaluable because the consequences of not having it would be economically and environmentally devastating. Estimates of economic impact were made by staff from the companies that received assistance from this program. The estimates were based on the known or expected gain, or the reduction in the anticipated loss, once the problem

Estimated economic impact:

Control of an outbreak of ectoparasites on koi
$5,000

Control of an external bacterial infection on freshwater ornamental fish
$30,000

was brought under control. In each case, our recommendations for control were the result of our evaluation of the problem in the field, trials or studies conducted in the field, and our interpretation of the laboratory results. In the highlighted examples, the farm personnel implemented the strategies that were recommended. To a great extent, the success of a given strategy reflects their efforts. These examples demonstrate the positive outcome that has occurred from the effective relationship that the Disease Management Project enjoys with the aquaculture community in Hawaii and the Pacific islands.

Publications in Print, Manuscripts, and Papers Presented

Montgomery-Brock, D. R., R. Y. Shimojo and K. Cochrane. 2003. Increased water temperature improves survival of TSV-exposed shrimp. Global Aquaculutre Advocate 6(4).

Zhao, Z., D. Montgomery-Brock, C.S. Lee and Y. Lu. In press. Establishment, characterization and viral susceptibility of 3 new cell lines from snakehead *Channa striatus* (Blooch). Methods in Cell Science.

Zhao, Z., D. Montgomery-Brock, C.S. Lee and Y. Lu. Submitted. Establishment, growth and preservation of two cell lines derived from muscle and fins of omilu fish (*Caranx melampygus*). In Vitro Cell Dev. Biol. Animal.

Transitioning Hawaii's Freshwater Ornamental Industry, Years 2 & 3

General Information

Reporting Period

October 1, 2001 - March 31, 2003 (Year 2 - final report)
October 1, 2002 - September 30, 2003 (Year 3)

Funding Level

Year	Amount
1	$100,000
2	$70,000
3	**$70,000**
TOTAL	$240,000

Participants Years 2 & 3

Clyde Tamaru, Ph.D., Extension Specialist
Sea Grant Extension Service

Kathleen McGovern-Hopkins, Extension Agent
Sea Grant Extension Service

Justin Iwai, Graduate Student
University of Hawaii at Manoa

Harry Ako, Ph.D., Professor
University of Hawaii at Manoa

James Szyper, Ph.D., Extension Specialist
Sea Grant Extension Service

Robert Howerton, Ph.D., Extension Specialist
Sea Grant Extension Service

Participants
Year 2 only

Brandon Avegalio, Eri Shimizu and Kelly DeLemos, Graduate Students
University of Hawaii at Manoa

Objectives

Year 2

1. Invite industry expert(s) to participate in a conference on marketing trends in the freshwater ornamental fish industry.
2. Investigate use of carotenoids for color enhancement.
3. Validate techniques for artificial insemination of livebearers.
4. Determine minimum effective dosage of Ovaprim for induction of spawning.
5. Validate factors affecting the sex ratio in swordtails.
6. Conduct technology transfer activities.

Year 3

1. Produce a position paper summarizing the current status of Hawaii's freshwater ornamental fish industry.
2. Field test the artificial insemination technique to increase efficiency in the commercial production of lyretail swordtails.
3. Demonstrate the cost-effectiveness of specialization in the production of freshwater ornamental fishes.
4. Provide technical assistance in the form of workshops, verbal communication, written material and site visits.

Anticipated Benefits

Year 2

Objective 1 - Invite industry expert(s) to participate in a conference on marketing trends in the freshwater ornamental fish industry.

In comparison to Hawaii's food fish commodities, the markets for the freshwater ornamental fish are much larger and extend out of Hawaii. This difference in markets has left Hawaii's growers with different sets of challenges. The information farmers,

researchers and administrators obtained from the workshop will allow them to decide on how best to approach marketing their freshwater ornamental fish products and how to anticipate and identify potential technical constraints.

Objective 2 - Investigate use of carotenoids for color enhancement.

The results of this objective will provide the foundation to make sound recommendations about the feeds and use of color enhancers that result in the highest quality freshwater ornamental fish. Insuring fish are of the highest quality is essential if Hawaii is to become a major player in the freshwater ornamental fish trade.

Objective 3 - Validate techniques for artificial insemination of livebearers.

The lyretail trait consistently results in a higher farm gate price irrespective of the swordtail variety. Successful development of techniques to improve the quantity of this fancy livebearer will have an immediate impact on the industry. Another aspect of this research is that breeding practices currently rely on the chance mating of male and female individuals. With artificial insemination, selected individuals can be mated on demand and will undoubtedly provide a more effective means to produce new varieties of hybrids. This will ultimately result in an increase in the number of varieties of ornamental fishes.

Objective 4 - Determine minimum effective dosage of Ovaprim for induction of spawning.

Inclusion of egg layers to the kinds of freshwater ornamental fishes being produced in Hawaii is an important part of the expansion and diversification of the local industry. This is one area that has not become an established protocol being used by Hawaii's growers, and the reasons are many, but one in particular is the dependability of the hormonal induction spawning techniques that have been previously used. Incorporating the latest developments in inducing cultured fish to spawn will improve the dependability of spawning techniques.

Objective 5 - Validate factors affecting the sex ratio in swordtails.

The morphology and coloration of male swordtails are in large part the main attraction. Culture methods that result in a preponderance of females ultimately result in lower profitability. Uncovering the parameters that influence the sex ratio of the swordtail will allow for establishing a best management practice that has yet to be clearly defined for the swordtails.

Objective 6 - Conduct technology transfer activities.

No matter what technology is developed in the laboratory, it has no practical value unless it can be used to improve the productivity of a farm. Dissemination of information comes in the form of brochures, pamphlets, manuals, newsletter articles and workshops. All of these methods augment the classical extension technique of one-on-one discussions and site visits that are already being done by Sea Grant Extension Service (SGES) agents and specialists. The alliance of the SGES with CTSA provides a cost-effective use of resources that can be brought to focus on alleviating constraints to the expansion and diversification of the freshwater ornamental fish industry.

Year 3

Objective 1 - Produce a position paper summarizing the current status of Hawaii's freshwater ornamental fish industry.

Over the past seven years, CTSA, the University of Hawaii Sea Grant Extension Service (SGES), UH Department of Molecular Biosciences and Bioengineering (MBBE) and the Hawaii State Aquaculture Development Program (ADP) have pooled their resources to support the development of a freshwater ornamental fish culture industry in Hawaii. These initial projects have been the focal point for the development of the industry to this point in time. It would appear that the success of Hawaii's fledgling industry to carry on after public funds are withdrawn will hinge on whether a sufficient foundation of collaborative partners will result from the proposed activities. The project provides a forum for this activity to begin because it will identify the current challenges the industry faces.

Objective 2 - Field test the artificial insemination technique to increase efficiency in the commercial production of lyretail swordtails.

One means of expansion and diversification of the fledgling freshwater ornamental industry in Hawaii is the inclusion of the lyretail swordtail into the inventory of fishes produced locally. The lyretails fetch a price that ranges between three to four times that of the common color varieties. Mass production of lyretail swordtails is hampered by the inability of the lyretail male to mate with a female. The lyretail trait is a dominant genetic characteristic, and this genetic change in the fins results in the inability of the male swordtail to mate due to the over-development of the gonopodium. Following simple rules of Mendelian inheritance and using the method of backcrossing of individuals, theoretically, a maximum of only 50% of the next brood would possess the lyretail trait. Artificial insemination is a method that can circumvent the problem of lyretail males not being able to mate with lyretail females or ensure that a specific cross between a selected pair occurs because mating of individuals is no longer left to chance. In this fashion, lyretail males and lyretail

females can be crossed, and the percentages of lyretail individuals increases to a theoretical value of 75% of the offspring that will have the genotype that results in the lyretail trait. What is most intriguing is that with the ability to cross a lyretail male and a lyretail female, a small (25%) percentage of individuals will result in a homozygous lyretail genotype. These homozygous individuals will become the most sought after individuals because, theoretically, all of their progeny will be lyretails. To clarify, a homozygous lyretail female can be mated with a normal male and all of their offspring will have a heterozygous genotype, which means that their phenotype will be lyretail. The key is to produce and identify these homozygous individuals.

Objective 3 - Demonstrate the cost-effectiveness of specialization in the production of freshwater ornamental fishes.

By conducting the activity under the auspices of the project, a substantial amount of risk to the private sector will be alleviated. Most importantly, there will be a reasonable real-life estimate of the associated costs involved for each of the particular species that is examined. The information will be gathered in an unbiased fashion, and the model can be presented in a non-threatening way in order to stimulate discussion. The potential to obtain growout information on a large number of species in a short time frame is equally important. This will prove to be invaluable because this is often the kind of information that is not available when trying to make projections or construct a business plan.

Objective 4 - Provide technical assistance in the form of workshops, verbal communication, written material and site visits.

The role of the Sea Grant Extension Service (SGES) Aquaculture Extension Program is to serve as a bridge between researchers and Hawaii's aquafarmers by transferring the technologies researchers develop for improving production capabilities, hatchery operations, husbandry practices and identification of new species. All of the activities are focused on developing the aquaculture industry in Hawaii to become a significant contributor to the State's economy. The project provides funding support for conducting the necessary experiments and demonstration projects in the form of manpower and supplies. Without these components, SGES would not be able to conduct the activities outlined in the proposal, which are considered necessary to enhance Hawaii's freshwater ornamental fish industry. The proposed project allows the integration of both institutions' resources to insure that the laboratory results get field tested and that the information is disseminated in a user-friendly format.

Work Progress and Principal Accomplishments

Year 2

Objective 1 - Invite industry expert(s) to partcipate in a conference on marketing trends in the freshwater ornamental fish industry.

Two wholesalers and a university business development specialist shared their advice with ornamental fish producers and hobbyists at a September 2002 conference.

The objective was completed during the previous reporting period and is summarized again in this report. This project, in collaboration with the USDA's Initiative for Future Agriculture and Food Systems project, the University of Hawaii Sea Grant Extension Service and the Hawaii State Aquaculture Development Program, organized a workshop series that focused on the marketing of aquatic products. It was held at the University of Hawaii's Manoa campus and at Windward Community College from September 12-14, 2002.

The overall goal of the workshop series was to introduce marketing concepts to participants through the interactive process of developing a marketing plan and the practical exercise of examining case studies from the aquatic product industry. Some of the questions discussed were: What is marketing and what does it entail? What are the techniques and methods that one can use to be more competitive? What are the thought and planning process that enable one to do so?

Three main speakers and a total of 26 participants attended the workshop. Two of the speakers were wholesalers: Mr. Mark Taylor of African Northwest Pets and Supply in Seattle and Mr. Ron England of Worldwide Aquatics in Hawaii. England and Taylor discussed their respective companies, what their expectations were if they were to become repeat customers and what it took to survive doing business in Hawaii. In addition, Dr. C.L. Cheshire of the University of Hawaii Pacific Business Center presented the results of a marketing survey conducted under the auspices of the Pacific Tropical and Ornamental Fish Project. A summary of the workshop was also disseminated in CTSA's *Regional Notes* newsletter and in the Hawaii Aquaculture Association newsletter.

Objective 2 - Investigate use of carotenoids for color enhancement.

During the previous reporting period, it became apparent that the use of natural sources of beta carotene and astaxanthin were more effective in enhancing fish color. In a collaborative effort, both Sanders Brine Shrimp Co. and Rolf C. Hagen Inc. incorporated 2% of Spirulina Pacifica™ and 1% NatuRose™ into flake feeds. The amount of the two algae used would characterize these as acute dosages where an effect would be noticeable within two to three weeks. These were tested with a variety of freshwater ornamental fishes courtesy of our freshwater ornamental fish growers in Hawaii. Some of the fishes that were tested were: red velvet swordtails, rainbowfish, 24K mollies, topaz cichlids, neon swordtails, discus, rainbow

Promising results were achieved using prepared feeds for the majority of the fish that were tested.

sharks, pink gouramis and rosey barbs. Only a subjective test of whether the treated feeds work was used, and in all cases, the treated feeds resulted in a noticeable change in color in fish provided the feed. However, it is also clear that what colors appear in a fish is dependent on the species, sex and state of maturity. In other words, the prepared feeds enhance the natural coloration of the target fish and do not add color as with dyeing a fish (e.g., glass fish).

During the reporting period, feed laced with 1% NatuRose was prepared by project work group member Harry Ako and sent to Bob Kern (Tropical Ponds of Hawaii) for field testing. Fish were fed *ad libitum* for a minimum of two weeks and sent to Oahu for visual examination and photographing. Three species were tested during the on farm trials and they were: red wag swordtails, rosey barbs and dwarf gouramis. No differences could be detected by project work group members for both the rosey barbs and redwag swordtails. However, a clear difference could be detected between treated and untreated male dwarf gouramis.

Breeders of Siamese fighting fishes, *Betta splendens*, are also interested in the use of the color enhanced feed produced by the project work group and have been providing it on a trial basis during the reporting period. Elizabeth Hahn, an avid breeder, utilizes the feed to enhance the color of her fishes which she enters in the International Betta Congress shows and which have done very well. Her Web site is at http://planet-hawaii.com//liz/betta/.

Objective 3 - Validate techniques for artificial insemination of live bearers.

Project staff successfully used artificial insemination to produce swordtails with the homozygous lyretail genotype.

During the previous reporting period, the activities on validating the use of artificial insemination were presented. To demonstrate the validity of the technique and its possible utility in the mass propagation of lyretail swordtails, the project work group members embarked on the production of a homozygous individual for the lyretail trait because this individual can only be produced via artificial insemination. According to the Mendelian Law of Segregation, the expected ratio of a mating between a common swordtail to a lyretail individual is 1 common : 1 lyretail. This was statistically confirmed, and the observed ratio was consistent with the expected 1:1 ratio (chi square = 0.06, $P>0.50$) of how the transmission of the lyretail genes to the next generation is being realized.

Progeny from the experiment were then grown out and virgin females that possessed the lyretail trait were selected for artificial insemination trials. Using the artificial insemination technique, male lyretail and female lyretail crosses were made between November and December of 2001, and offspring were produced during January through March 2002. These were grown out to be large enough so that their phenotypes could be scored confidently. From the data available, three clutches were found to have lyretail to common swordtail phenotypes that are consistent

with the expected 3:1 ratio. Equally important is that 25% of the lyretail individuals should possess a homozygous genotype for the lyretail trait.

During the reporting period, one clutch (n=20) of lyretail offspring resulting from a lyretail : lyretail cross, was placed with common male swordtails at a ratio of 3F:1M on June 28, 2002. The group was allowed to mate randomly with common swordtails for one month. The females were then separated into separate aquaria on July 22, 2002 and clutches of fry that were produced from each female were reared separately and allowed to grow out to the point when their phenotype could be scored confidently. One of the problems that we have consistently encountered with lyretails is that it is very difficult to score individuals confidently for either sex or for the lyretail trait unless they are grown old and/or large enough. Of the 20 supposed females that were placed in spawning trays, six were found to be male after growing larger. As of August 6, one female had not produced any clutches and was removed from the testing program which resulted in 13 known females that have been producing clutches since late July. At this writing, there have been 30 clutches that have been produced and that are being grown out to be scored. Of the lyretail females that we have been tracking, we have found three females (F#2, F#9 and F#17) that have produced clutches that are 100% lyretail. The expected number of homozygous individuals from 13 individuals is 3 and is consistent with the observed ratio of homozygous individuals. Additional broods are still being grown out and have not attained the size at which the lyretail trait can be scored confidently. However, F#17 has had two clutches (n=6 offspring, n=58 offspring), both of which are 100% lyretail. The initial results confirm and validate the inheritance of the lyretail trait and the use of the artificial insemination technique for livebearers is a means to generate the homozygous individual. While the inheritance of the lyretail trait has long been proposed to follow the dominant recessive model, to our knowledge, the generation of actual homozygous individuals for the trait has never been reported. The three females that have been determined to be homozygous, by progeny testing, for the lyretail trait marks a milestone in fish breeding.

Using females resulting from a completely separate lyretail to lyretail cross, we repeated the entire mating and progeny testing. In this instance, we had a total of 22 females that underwent progeny testing and we found five homozygous lyretail females. The expected number of homozygous females (25% of 22 = 6) is six females, which is consistent with the expected number of homozygous individuals. All of these results clearly demonstrate that the lyretail trait is a dominant characteristic and is being inherited in Mendelian fashion.

Objective 4 - Determine minimum effective dosage of Ovaprim for induction of spawning.

In January 2002 we began monitoring broodstock maturation of three freshwater ornamental egg layers (tinfoild barbs, redtail sharks and rainbow sharks). Redtail

sharks and rainbow sharks were found to be sexually mature beginning in June 2002 and the tinfoil barbs in July 2002. At that time, all females possessed the same size oocytes (approximately 1 mm in diameter) and expression of milt from males could be accomplished by applying slight pressure to their abdomen. Tinfoil barb males possessed planter tubercles, which resulted in a sandpaper-like skin condition. Induction of spawning trials were initiated in August 2002. Only females that possessed oocytes that averaged 1 mm in diameter and in which the germinal vessicle were centrally located were used in the trials. Likewise, only males from which milt could be extruded after applying slight pressure to the abdomen were used in the spawning trials.

Five female rainbow sharks were injected with saline and placed with milting males in a 40 gallon tub equipped with an airstone and sponge filter. This group served as the control for the spawning trial and was conducted once during the testing period. A spawning trial consisted of using the same doseage of Ovaprim on five individual females with five males, beginning with the manufacturer's recommended dosage for use of Ovaprim (0.5ml/kg body weight). The entire experiment was repeated with redtail sharks.

The minimum effective dosage of Ovaprim for use in the induction of final maturation and spawning was determined to be the manufacturer's recommended dosage for both the redtail and rainbow sharks.

Ovaprim, the induction agent that was investigated during the reporting period, represents the latest development in spawning agents. It was decided that the criteria for success of the spawning trials was to achieve induction of final maturation and spawning without having to intervene by stripping and artificially fertilizing the gametes. Both the rainbow sharks and redtail sharks underwent final maturation and spawned naturally overnight using Ovaprim according to the manufacterer's recommended dosage of 0.5 ml/kg body weight. Fertilization was consistently high >80% and fecundity ranged between 1,000 - 8,000 eggs per female. While the rainbow sharks could also be spawned at a dosage of 0.25ml/kg body weight, a more varied response in spawning was observed for the redtail sharks. At the 0.125 ml/kg dose, both the redtail and rainbow sharks exhibited variable responses in ovulation, and none of the fish spawned naturally.

From the results of the spawning trials, it is clear that the manufacturer's recommended dosage of 0.5 ml/kg body weight should be used for both the rainbow and redtail sharks. In addition, the spawning can be conducted naturally, and it is not necessary to rely on manual stripping and fertilizing of the gametes. The successful spawning of the redtail and rainbow sharks allowed for the production of newly hatched larvae and additional testing of hatchery procedures.

Tinfoil barbs were also investigated for their responsiveness to Ovaprim. Once again, the criteria for success was induced final maturation and natural spawning and fertilization. Assessment of the state of maturity of both females and males was conducted as described for the rainbow and redtail sharks. Spawning trials were conducted similarly as with the sharks, but larger spawning tanks (1200 L) needed to be used for the spawning trials because of the size of the broodstock. As with

the shark trials, the recommended dosage was first used, and five females were injected and placed in separate tanks and kept there overnight. While all of the females ovulated at this dosage, none of them spawned naturally. At a dosage of 0.25ml/kg body weight, none of the females ovulated.

Objective 5 - Validate factors affecting the sex ratio in swordtails.

Results indicate that lower stocking densities will result in a higher percentage of males with the caudal sword.

This objective was completed during the first reporting period . In summary, when the possession of a gonopodium is used as a defining characteristic, there is no significant difference that could be detected between sex ratio and stocking density. However, when the presence of the caudal sword is used, it became apparent that males with the sword were more abundant among the larger size individuals. These results indicate that processes that result in the larger individuals (e.g., stocking density) will also result in a higher percentage of male individuals possessing the caudal sword.

The data was summarized in conjunction with density trials with other freshwater ornamental fish species (gourami, guppy, topaz cichlids, rainbowfish and golden tilapia). Interestingly, it appears that variations observed in overall survival and growth at high densities is related to the species of fish being cultured. Tilapia, topaz cichlids and the rainbowfish were found to be tolerant to high density situations, whereas, guppies, swordtails and rainbow sharks were observed to result in lower survival at higher densities. The lower survival also translates into large individuals at the end of the growout period.

Objective 6 - Conduct technology transfer activities.

Technology transfer activities included site visits, workshops and experiments.

Site visits and workshops were conducted on a regular basis. In addition, the three experiments mentioned in the last reporting period were completed and the findings were transferred to the public. The experiments were titled "First Feeding Experiments with Freshwater Ornamental Egg Layer Fishes," "Culture of the freshwater cladoceran *Moina macropoda*" and "Large-scale recirculation using red tilapia as model species." Project work group members also collaborated with Susan Matsushima of the Pacific Tropical Ornamental Fish Project (PTFOP). PTOFP is funded through the Department of Commerce and overseen by the National Sea Grant Program. Funding to Hawaii is through the Economic Development Alliance of Hawaii (EDAH). It is anticipated that this will be the beginning of a multi-year program, and for Year 2, there is $350,000 available in funding support. A total of 55 concept statements were received and 35 invitations for full proposals were sent out. During the reporting period, the 35 invited proposals were reviewed by project work group members and 25 awards were distributed.

Year 3

Objective 1 - Produce a position paper summarizing the current status of Hawaii's freshwater ornamental fish industry.

A summary of the main points that were made by the speakers in the workshop held to fulfill objective 1 in Year 2 was printed in both the Hawaii Aquaculture Association newsletter and CTSA's *Regional Notes*.

Objective 2 - Field test the artificial insemination technique to increase efficiency in the commercial production of lyretail swordtails.

During the previous reporting period, we began using a technique called progeny testing by separating 13 virgin lyretail females from a single clutch that were the product of a lyretail male to lyertail female cross. The cross had been achieved through artificial insemination. Three common males were allowed to mate randomly for one month. The females were then separated in separate aquaria, and clutches of fry that each female produced were reared separately and allowed to grow out to the point when their phenotype could be scored confidently. Of the 13 females that have been producing fry since late July, three females have produced clutches that are 100% lyretail. This is consistent with the expected ratio of 3 out of 13 females if the lyretail gene is being inherited in Mendelian fashion. During the current reporting period, females resulting from a completely separate lyretail to lyretail cross went through the entire mating process and progeny testing. In this instance, a total of 22 females underwent progeny testing, and five homozygous lyretail females were identified. The expected number of homozygous females (25% of 22 = 6) is six females, which is consistent with the expected ratio. These results validate the mode of inheritance of the lyretail trait in swordtails and also demonstrates that the use of the artificial insemination technique is a means to generate a higher percentage of lyretail offspring and also produce the homozygous lyretail genotype.

Objective 3 - Demonstrate the cost-effectiveness of specialization in the production of freshwater ornamental fishes.

Rainbow shark broodstock that are present at the Windward Community College Aquaculture Complex were targeted as a model for this objective. After consulting a local wholesaler for price information to determine what the farm gate price of this species would be, a target of $0.15 was selected for this excercise. The size at purchase would be two inches. We made an estimate of the starting target production volume based on these values and based on what needed to be met in order to attain a target income of $50,000 annually. However, some assumptions needed to be made in order to obtain an estimate of the number of fry that would

be needed to meet the production goal. The assumptions were that the percent survival would be 75% and 50% during the growout and larval rearing phases, respectively. From those values, and by using the farm gate price, the estimated quantity of two inch rainbow sharks needed would be 334,000. Calculating for a mortality of 25% during the growout phase, the number of two week old fry that would need to be delivered to the growout phase is estimated at 417,000. Using the 50% criterion for survival during the hatchery phase, we would need a total number of 625,000 hatched larvae. A harvest density of 25 fry/L was obtained from the spawning and two week larval rearing results conducted during the reporting period. These were based on the use of two females injected with Ovaprim. Using the harvest density, a working volume for the hatchery can then be obtained, and that was calculated at 25,000 L. Likewise, at least 420 females would need to be spawned based on these calculations. If we assume a 1:1 male to female ratio, then the total number of broodstock animals would be approximately 1,000 males and females. One clear result is that economies of scale will dictate that the output of a hatchery specializing in a particular species with a low overall market value will need to be very high.

Output would have to be very high for a hatchery to become cost-effective.

Objective 4 - Provide technical assistance in the form of workshops, verbal communication, written material and site visits.

Technology transfer activities included site visits, workshops, tours and one-on-one communications.

Seven workshops were held during the reporting period and are summarized below.

1. December 14, 2002. Windward Community College, Artificial insemination of lyretail swordtails. Kathleen McGovern-Hopkins and Clyde Tamaru.
2. February 15, 2003. Komohana Complex, University of Hawaii at Hilo. Artificial insemination of lyretail swordtails. Kathleen McGovern-Hopkins and Clyde Tamaru.
3. March 1, 2003. Windward Community College, Fish Health Workshop. Dee Montgomery-Brock and Kathleen McGovern-Hopkins.
4. March 22, 2003. Windward Community College, Pacific Tropical Ornamental Fish Project- Call for Proposals. Clyde Tamaru, Kathleen McGovern-Hopkins and Susan Matsushima.
5. April 2, 2003. Natural Energy Laboratory of Hawaii, Kailua-Kona. Pacific Tropical Ornamental Fish Project- Call for Proposals. James Szyper and Susan Matsushima.
6. April 5, 2003. Komohana Complex, University of Hawaii at Hilo. Pacific Tropical Ornamental Fish Project- Call for Proposals. James Szyper and Susan Matsushima.
7. April 12, 2003. Maui Community College, Agriculture Building Room 101. Pacific Tropical Ornamental Fish Project - Call for Proposals. Robert Howerton and Susan Matsushima.

During the reporting period, eight site visits and 19 one-on-one communications were conducted. A total of ll tours of the Windward Community College Aquaculture

Complex were conducted. Fish were supplied on eight occasions during the reporting period.

Work Planned

Year 3

Objective 1 - Produce a position paper summarizing the current status of Hawaii's freshwater ornamental fish industry.

A collaborative effort will be undertaken with Dr. C.L. Cheshire of the University of Hawaii Pacific Business Center in an effort to assess case studies of several freshwater ornamental fish businesses. Additional information will also be obtained during the next reporting period as part of the granting cycle for the Pacific Tropical Ornamental Fish Project. Using the data obtained from that project and what was obtained from the previous workshop, a position paper is to be generated for review and dissemination.

Objective 2 - Field test the artificial insemination technique to increase efficiency in the commercial production of lyretail swordtails.

It has been demonstrated that the homozygous lyretail swordtail can be produced using artificial insemination. At present, the only known living homozygous lyretail swordtail females are present at the Windward Community College Aquaculture Complex, and these are being held separately. Even though a manual has been produced and several workshops and presentations have already been completed, there are no reported farms that are attempting to use the technique to produce lyretails. Clearly, the artificial insemination technique is too cumbersome for general farm use at this point in time. A much simpler method that can be incorporated into current farm practices is needed to mass produce lyretail swordtails. A project to develop such a method has been approved by USDA CSREES and work is scheduled to begin in October 2003.

Objective 3: Demonstrate the cost-effectiveness of specialization in the production of freshwater ornamental fishes.

A cost breakdown of the hatchery operation will be completed during the next reporting period based on the results obtained for the rainbow sharks. In addition, the hatchery output is also being obtained for the dwarf gourami, and a separate assessment will be made either with the dwarf gourami alone or in combination with the rainbow shark.

Objective 4: Provide technical assistance in the form of workshops, verbal communication, written material and site visits.

The last workshop on artificial insemination of lyretail swordtails is scheduled to take place on Maui during the next reporting period. Other extension and outreach activities will continue as requested.

Impacts

Year 2

As reported during the previous reporting period, the impacts for the marketing workshop will probably not be felt immediately after the conclusion of the project. It was designed to provide the most up-to-date information to Hawaii's producers regarding the expectations of the ornamental fish market on the mainland. From that information, it remains to be determined how farmers will work collaboratively to meet the expectations of the mainland market as well as the changes that are ongoing in the transportation industry.

One market expectation that is already a given is that the fish produced will have to be of the highest quality. The results of the color enhancers are a proactive approach to address the issue of quality. Unfortunately, insuring that the fishes are of the right color alone is only one aspect that defines good quality. It is, however, a means to inform growers that there will need to be a great deal of attention given to quality if this industry is to penetrate markets and sustain itself against outside competition. It should be mentioned however, that there is clear evidence (C.L. Cheshire, Mark Taylor and Bob Kern) that Hawaii's fish are perceived as being of "good" quality by buyers on the US mainland. Likewise, betta producer Liz Hahn employs the use of color enhancing feed to insure that the fish she shows at the International Betta Congress are of the highest quality, and her fish have succeeded in winning several best in show awards. This modest beginning should be built upon to penetrate the freshwater ornamental fish market.

The immediate impact of the lyretail work is the validation and demonstration that homozygous individuals can be produced using this technique. The extension activities (manual, workshops) provide an opportunity for farmers to become engaged in the techniques used to improve and/or diversify their own stocks. In order for a significant economic impact to be realized, it will clearly require the development of additional technologies. Currently, the main constraint for lyretail swordtail production is the method used to identify homozygous individuals. Classical progeny testing, which was used in this project, is too cumbersome and

time consuming to be of commercial value. What is clearly needed is gene technology that can detect homozygous lyretail swordtails with a simple test, and clearly, it is an area for future investigation. The incentive, however, is quite clear because the lyretail swordtail is between three to five times higher in value than the common swordtail, and the development of populations that produce 100% lyretails will clearly have a significant economic impact.

The use of Ovaprim to induce spawning in various egg layers is a far more simplified procedure than the traditional methods that employed a combination of human chorionic gonadotropin and carp pituitary extracts. With a single injection, the same results can be achieved. It is anticipated that the results of the investigations will lead to a larger number of producers engaging in the production of egg layers, and thereby diversifying the industry.

Validated factors affecting the sex ratio in swordtails have clearly indicated that the size of the growout facility and stocking density will dictate the resulting sex ratio of the swordtails produced. As the swordtails are one of the major freshwater ornamental fish species being produced in Hawaii, it already has impacted the industry by setting an industry standard that is being used in Hawaii. A greater understanding of the development of simplified recirculating systems that can be used in the production of freshwater ornamental fishes has also been acquired. As freshwater resources become increasingly taxed (e.g., drought conditions), there will clearly be a need to have additional strategies on the bioremediation of freshwater for the production of the ornamental fishes.

Developed technologies that are produced under the auspices of the various projects' activities are of little value unless they are transferred to appropriate end users. Likewise, the end users are an important source of feedback regarding the utility of the developed technology as well as the other areas that are constraints to the continued expansion and diversification of the industry. For that reason, the extension component of the project remains as one of the major activities being undertaken.

Year 3

A realization of what it takes to develop a freshwater ornamental industry in Hawaii, without the projects being in a leadership role, is beginning to formulate. Clearly, a collaborative effort will need to be developed and maintained if the industry expects to capture a share of the market. It is anticipated that the information obtained from the current project will shed light on the various stages of producing and marketing various kinds of ornamental fishes. This information should be used to place everyone on a level playing field and to form the basis for developing collaborative partnerships.

The work on the production of the homozygous lyretails has proceeded to the point where it is no longer a question of whether it can be done, but whether it gives a discrete advantage to Hawaii's growers. Developing a line of homozygous lyretail individuals can help several farmers in the short term because their production output of lyretails would increase dramatically. However, in the long term, it may be more advantageous to market the homozygous lyretail broodstock as a commodity much like seed corn and specific-pathogen-free shrimp. Clearly, to accomplish this will require a means to cost effectively identify the homozygous individuals and may rely on the tools available in emerging in biotechnology, the property rights of which will need to be protected.

Publications in Print, Manuscripts, and Papers Presented

Year 2

Publications in Print

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McGovern-Hopkins, K., P. Maloney, N. Maloney, and C.S. Tamaru. 2002. Can an artificial feed be used to raise Buenos Aires tetra, *Hemigrammus caudovittatus*, larvae? I'a O Hawai'i Vol.2002, No. 6.

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Tamaru,C.S. and K. McGovern-Hopkins. 2002. Use of marine microalgae concentrates to grow the freshwater water flea, *Moina macropoda.* I'a O Hawai'i. Vol. 2002, No. 7.

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Year 3

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Development of Black-Lip Pearl Oyster Farming in Micronesia

General Information

Reporting Period October 1, 2001 - September 30, 2003 (final report)

Funding Level

Year	Amount
1	**$56,428**

Participants

FSM Component

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Senator Gerson Lekka and Chief Magistrate George Stephens
Nukuoro Pearl Farm

Objectives

This project has three basic components:

1. Hatchery technology development and training in the Federated States of Micronesia (FSM)
2. Hatchery technology and training in the Republic of the Marshall Islands (RMI)
3. Bioeconomic study of Micronesian pearl farms

Hatchery technology development and training in the FSM

The overall goal of this component was to introduce pearl oyster hatchery technology into Pohnpei via a demonstration and training hatchery that will also provide spat to potential farmers and researchers.

1. **Demonstration and seed supply.** Installation of a simple pearl oyster hatchery into the existing MERIP facility for seed supply, demonstration and training purposes. The hatchery is intended to be of appropriate size and technology level that it can be operated and replicated in the Micronesia context.

2. **Training and technology transfer.** Transfer pearl oyster hatchery methodology to marine science students, marine resource management personnel and private sector individuals on Pohnpei and throughout the region.

Hatchery technology and training in the RMI

The overall goal of this component was to both produce pearl oyster spat to supply the industry as a bridging strategy until a permanent hatchery operation could be re-established and to make local operation and oversight of the hatchery possible in the future so as to eliminate the dependence on foreign entities.

1. **Demonstration and seed supply.** Provide a means to rescue the Marshall Islands pearl industry through a short term effort to revive hatchery operations and document procedures for hatchery operation and production.

2. **Training and technology transfer.** Transfer pearl oyster hatchery methodology to local aquaculturists, marine science students, marine resource management personnel and private sector individuals in Majuro with the goal of creating sustainable local capacity to operate pearl oyster hatcheries.

Bioeconomic study of Micronesian pearl farms

The overall goal of this component was to gain a clearer understanding of the economics of pearl farming to better guide development and research efforts.

1. Model the economics of Hawaii / Micronesian pearl farms to inform decision making, management practices and financial strategies.

Anticipated Benefits

FSM component

This work was intended to transfer hatchery technology to the FSM through demonstration and training. Capacity building was a key element and was intended to build a cadre of trained individuals who can contribute to industry development through research, training, education or farming in the future. Long-term benefits will also include hatchery and farm facilities at PATS that are open to all members of the public who wish to learn more about pearl farming technology.

RMI component

Due to the closure of the BPOM pearl oyster hatchery in 2000, the industry greatly needed a temporary source of pearl oyster spat until a permanent hatchery operation could be re-established. The BPOM hatchery was the RMI's principal source of pearl oyster spat, and local pearl farms are already looking at a two year gap in pearl harvests in the near future. Furthermore, the BPOM hatchery considered all their technology to be proprietary, and no local group of trained individuals existed who could continue hatchery operations.

Bioeconomic Study component

Pearl farming has been a risky, yet potentially lucrative endeavor in the South Pacific, Australia, Japan and other countries. The industries are now suffering from a host of problems impacting their long-term economic feasibility due to recent changes and trends. The Hawaiian and Micronesia industries are significantly different, but little information exists to inform farmers, researchers or technical assistance providers. The results of this work will help farmers choose better management strategies, reduce costs, plan financial strategies and identify areas of sensitivity for future focus. This work targets only the production aspects of the industry, but will be integrated into a wider study of global economics and marketing.

Work Progress and Principal Accomplishments

FSM component

Objective 1 - Demonstration and seed supply. Installation of a simple pearl oyster hatchery into the existing MERIP facility for seed supply, demonstration and training purposes. The hatchery is intended to be of the appropriate size and technology level so that it can be operated and replicated in the Micronesian context.

A simple pearl oyster hatchery was installed at MERIP.

A simple pearl oyster hatchery was set up at the existing MERIP facility. A new seawater system specifically for delivery to the larval rearing tanks was installed. All supplies and equipment were ordered, and the protocols were put into place. A storeroom at MERIP was converted into an algae room and in August 2001, it was stocked with four species of microalgae from CSIRO in Hobart, Australia (*Isochrysis galbana, Chaetocerus muelleri*, *Pavlova salina* and *Tetraselmis suecica*). Algae room operations were initiated utilizing 500 ml, 2-L and 15-L containers.

One successful spawn was obtained in February 2002.

Spawning was attempted on three separate occasions in January and February 2002 with only one successful spawn in February 2002. A total of 46 broodstock were collected from the wild and kept in three separate locations in the lagoon. Fertile eggs totaling 22.98 million were obtained from the successful spawn and 3.6 million D-stage larvae were stocked into six 300-L rearing tanks 24 hours after spawning occurred (the maximum for the available tank space). The larvae were reared to metamorphosis using the newly designed hatchery and algae room. Survival to day 27 was 5 % and did not differ appreciably from survival rates reported in the literature (Southgate and Ito, 1998; Alagarswami et al 1989). Approximately 100,000 larvae were taken through metamorphosis. Once eyed larvae were transferred to setting tanks, they were disturbed as little as possible, and population estimates were conducted infrequently. On day 71 post-spawn, 46,000 spat were removed from setting tanks and placed in spat bags. The majority of these spat were placed on a longline in the lagoon for further growout. Some spat were then moved into spat bags in raceways with flow-through seawater and supplemental drip-feeding of algae for further land-based nursery training and education purposes.

Objective 2 - Training and technology transfer. Transfer pearl oyster hatchery methodology to marine science students, marine resource management personnel and private sector individuals on Pohnpei and throughout the region.

Pearl oyster hatchery methodology was transferred to MERIP staff biologists and local students.

During this period, four MERIP staff biologists (three expatriates and one Micronesian) received full training in all aspects of design, installation and operation of a pearl oyster hatchery including microalgae culture, pearl oyster spawning techniques, larval rearing and land-based nursery techniques. Two Micronesian technicians were also trained to assist the biologists in all of these tasks. Fourteen Micronesian students from MERIP also received training in these techniques through organized classroom and practical demonstrations. Seven senior students were able to observe larval and spat development and observe rearing techniques during months 7-10, until they graduated in May 2002. Another group of seven students (class of 2003) had similar exposure to the project in months 7-10, plus hands-on training in axenic algae culture, flask preparation, autoclave operation, spawning induction, egg sampling and enumeration, measurements using an ocular micrometer and stage micrometer and spat husbandry in months 13-14.

Total staff hours spent on this project was estimated at 120 hours per week for biologists and 30 hours of assistance from technicians. This labor included all algae culture, larval rearing, data collection, cleaning, seawater system maintenance, training, and aquaculture education. Members of the local community routinely visited the laboratory, especially on Sundays. Staff and students explained to community members what this project was about and asked that the tanks, which are exposed to the public, not be tampered with in any way. No tampering or vandalism occurred during this larval rearing run.

This project was initially going to conduct a workshop to teach spawning and hatchery techniques for black-lip pearl oysters. Due to the loss of the regional aquaculture extension position in Pohnpei, this segment of the work was cancelled. There were no other personnel available to execute this work. A new MERIP staff member, Tomoaki Yamada, was trained during this time. Mr. Yamada is teaching the aquaculture course at MERIP as well as providing laboratory and field training exercises for the students in the afternoon. One former MERIP staff member, Matang Ueanimatang, has taken a position as Aquaculture Extension Agent at the College of the Marshall Island/University of Hawaii at Hilo and is currently using the knowledge gained from this project to raise black-lip pearl oysters at the college aquaculture facility. He is working with both a Land Grant researcher and a biologist from Robert Reimers Enterprises on a joint hatchery project. He will also be responsible for training college students.

RMI component

Objective 1. Demonstration and seed supply. Provide a means to rescue the Marshall Islands pearl industry through a short-term effort to revive hatchery operations and to document procedures for hatchery operation and production.

The original focus of this project had been to develop a means to reduce predation by *Cymatium* snails on pearl oyster spat and biofouling removal costs. When the BPOM pearl oyster hatchery closed, these experiments were no longer possible due to the lack of oyster spat. Efforts were then made to revive operations at the BPOM hatchery. To complicate matters, the hatchery had been out of operation for nearly two years and had been suffering from certain infrastructure and operational problems even before its closure. Thus, a great deal of planning and coordination was required to determine how to get the hatchery back into operation as much as possible before the consultant arrived in Majuro so that his time could be used as efficiently as possible. The hatchery and algae lab were cleaned, equipment and laboratory supplies were repaired or replaced, and the water and air systems were put back into operation. No uncontaminated algae culture existed at Majuro, so cultures were imported from PATS, The Oceanic Institute and UH Hilo. Local personnel assisted in all efforts.

Additionally, it was necessary to develop a series of agreements between private sector partners and public sector partners regarding use of the facility, contributions of matching funds and in-kind match, and allocation of pearl oyster spat. It was also necessary to find strategic ways to execute the work given the limited availability of funds and support for this effort. It should be noted that the private sector partners (RRE, BPOM and Mid-Pacific Pearls) and the public sector partners (CMI, Land Grant and PRTP) dedicated a significant amount of funds and effort to the initiative. Many individuals who were not obligated to do so, voluntarily spent a great deal of time preparing for and participating in this work.

Broodstock pearl oysters were collected and spawning attempts made. Local personnel assisted in all efforts. Pearl oysters are rare at many Micronesian atolls, and obtaining sufficient broodstock for spawning attempts is often difficult. Complicating this, is the desirability of using only broodstock from the same atoll at which farming is to take place given that significant genetic heterogeneity may exist. In the case of Majuro, pearl oysters have been imported into Majuro from other countries and farmed there, so farmed and even wild stocks of pearl oysters from Majuro have uncertain heritages. Stocks at the Majuro farm also suffer from chronic mortality of an unknown origin. In the case of Arno atoll, the farmed pearl oysters are sourced from Jaluit. In order to adhere as closely as possible to recommended guidelines to use local stock and because it was so difficult to obtain wild stocks in Majuro with known origins, it was decided to use broodstock from Arno.

Spawning pearl oysters proved to be difficult, but one successful spawn was achieved in August 2002, and the spat were used for research at CMI.

Arrangements were made to visit the RRE Arno farm in early July and attempt spawning. This was planned to coincide with the time around the full moon, when spawning attempts usually yield the best results. Six spawning attempts were made during July, August and September, and all but the final one were unsuccessful because of lack of gonad development. Pearl oysters from Arno, Majuro and Jaluit were tried. Additionally, the team also attempted to source pearl oysters from Likiep and other atolls, but failed due to transportation difficulties. Conditioning of the collected pearl oysters was also attempted using methods previously used by Wise and Horbushko. The final and only successful spawning attempt was made on August 26 using collected pearl oysters. The quality of this spawn was rather poor. On day 6, 6 million larvae remained. Wise supervised larval rearing until he was forced to leave Majuro on September 2 due to having another assignment pending. Care of the hatchery was then taken up by Sebastian Horbushko, Rod Bourke and Manoj Nair. The CMI/UHH Aquaculture Extension Agent, Matang Ueanimatang, arrived and took over principal hatchery duties. Ueanimatang had been previously employed at the pearl oyster hatchery in Kiribati, after completion of his teacher training at PATS where he was trained in the FSM component of this project.

Pearl oyster spawning has not been observed to be so difficult in any of the situations previously encountered by the members of the technical team. Successful spawns

were reported for this season by BPOM. The universally poor spawning conditions of pearl oysters from several Marshallese atolls and the fact that regeneration of gonadal material did not occur during the consultant's three-month stay caused great concern among members of the technical team because this was a previously unobserved phenomena. Spawning tends to peak with water temperature, and regeneration of gonadal material occurs within a month or so. Development of gonad material also has been empirically observed to coincide with the monthly lunar cycle. One possible hypothesis is that the beginning of El Niño may have raised water temperatures for a prolonged period thus resulting in the extended barren period. At the same time, coral bleaching was observed by a CMI survey team on several Marshallese atolls. Previously, the Marshalls and the FSM have been largely unaffected by bleaching events believed to be provoked by high water temperatures that have so severely impacted the South Pacific coral reefs. If this hypothesis holds, then it indicates the cyclic climatic events, perhaps exacerbated by global climate warming, may be a threat to the pearl oyster industry and other marine resources. Further research is needed on the topic, as well as development of cost-effective methods for conditioning pearl oysters to avoid future delays in hatchery production.

Difficulties were also encountered at the larval rearing stage due to several reasons. The hatchery's water system was poorly designed and allowed pumping only at certain hours and did not allow for sufficient storage of reserve water. The quality of water in the area surrounding Woja was dubious because of contamination from human and agricultural activities. High mortality began on Day 8 and continued. Antibiotics were administered after Day 8 in an attempt to curb mortality. High and sudden mortality appears to have been a chronic problem during previous years at the BPOM hatchery and no successful larval run had ever been done without the use of antibiotics. Given that antibiotic use is not common in pearl oyster hatcheries, it indicates that there is a serious underlying problem. By Day 20, 300,000 larvae remained and these were smaller than would be expected at this age. Metamorphosis occurred over a prolonged period as is typical with *P. margaritifera*. By the time the spat were between 0.5 and 1.0 cm DVM, only about 1,000 remained (Day 42). It was decided jointly by the partners that since so few spat were produced, it did not make sense to divide them among the industry partners. Instead, the spat were donated to CMI to begin the demonstration farm sponsored by the MSI Sea Grant and the USDA IFAFS projects.

Objective 2 - Training and technology transfer. Transfer pearl oyster hatchery methodology to local aquaculturists, marine science students, marine resource management personnel and private sector individuals in Majuro with the goal of creating a sustainable local capacity to operate pearl oyster hatcheries.

All phases of the hatchery renovation, preparation for spawning, spawning and larviculture were used for the dual purpose of training and demonstration. The

Pearl oyster hatchery methodology was transferred to individuals from government and private agencies.

consultant trained three technicians from MIMRA and BPOM, the CMI/UHH Aquaculture Extension Agent and the Land Grant Aquaculture Researcher in hatchery operations, algal culture and larval rearing. The consultant also taught at CMI and trained marine science students. Fifteen CMI students were involved in some phase of this work and six of them worked as interns at MIMRA before they left to attend college in the U.S. in early 2003.

Industry members from RRE, BPOM and Mid-Pacific Pearls also received training and participated in these efforts. Significant exchange among biologists who work in aquaculture and pearl culture also occurred, and this contributed greatly to efforts in the region. The gathering and sharing of information among so many biologists at this level may be among the first of its kind in the Pacific region, where opportunities to exchange information is rare and often prohibited by the tendency towards secrecy among workers in this area.

The dire situation that resulted from closure of the BPOM hatchery was partially due to the fact that the operators of this hatchery maintained all information related to its operation as an industrial secret. While hatchery methods for pearl oysters are not otherwise unknown, this information has not been widely disseminated. There is usually a large body of information related to any specific hatchery that needs to be known in order to operate that particular facility. The BPOM hatchery had many quirks, and an apparent tendency for pearl oyster spawns to require special treatment to assure survival. Thus, part of this effort included the drafting and publishing of an operations manual specific to the BPOM hatchery to be used for reference by future operators. Wise wrote a description of the procedures used for spawning and larviculture, while Haws and Ellis assisted with literature research, editing and graphics. This was completed in October 2003, although numerous copies of the draft document had been widely disseminated to stakeholders so that the information could be made available to them in a timely manner.

Bioeconomic Study component

Objective 1 - Model the economics of Hawaii/Micronesian pearl farms to inform decision making, management practices and financial strategies.

Final data collection occurred in January 2002 when Dr. Fong, M. Haws, S. Ellis and other collaborators in this effort were able to visit the RMI and FSM under funding from the USDA/IFAFS project. They were assisted by the Reimers family, Virgil Alfred and Bobby Muller in the RMI, and the Nukuoro Pearl Farm representatives, Senator Gerson Lekka and Chief Magistrate George Stephens, in the FSM. The final data collection was somewhat impeded by the serious illness of Virgil Alfred (BPOM farm manager) and the long absence of the Nukuoran

The bioeconomic model was completed and the results were presented to farmers.

Pearl Farm representatives who spent several long periods on Nukuoro Island. Communication with Nukuoro Island is nearly impossible, and this hindered data collection and review. Additionally, marketing study data collected under the IFAFS project was incorporated into the model so that post-production returns would be as accurate as possible. This work was concluded in August 2003, and the model was finalized shortly thereafter. The model was developed and has been reviewed and tested by the work group. A final model using Excel has been produced and can be easily used by any person adept at use of this software.

Work Planned

FSM component

All objectives of the FSM component of the work were met and exceeded in 2002, when the final work was completed.

RMI component

All objectives of the planned work were met and exceeded as of the end of 2002, with the exception of the publication of the hatchery operations manual, which was completed in October 2003.

Bioeconomic Study component

While all CTSA-funded work has been completed, this effort is being continued under funding provided by USDA/IFAFS and contributions from the University of Alaska Fairbanks at Kodiak. Marketing studies that will contribute to the post-production side of the economic model were conducted on the Eastern Seaboard of the U.S. and in Europe in July-August 2004. Data on prices were incorporated into the final version of the model. Some marketing studies will continue using non-CTSA funding during 2004 to keep the model updated as world pearl markets fluctuate.

Impacts

FSM component

It has been clearly established that a small-scale, cost-effective hatchery can be successfully operated in the FSM using local personnel.

RMI component

CTSA has recently placed a consultant in the RMI to continue with production of pearl oyster spat for the RMI farms. Technical personnel who provide services to the region under other programs (E. Ellis, S. Ellis and M. Haws) and local staff trained by David Wise are working to support the new CTSA effort. This new RMI effort is based at two facilities: 1) the College of the Micronesia Arrak Marine Science Center, which has a small, multipurpose hatchery, and 2) the Wotje hatchery, which is currently being put back into operation. Both of these facilities benefited from the work of David Wise and his local counterparts. The RMI-based and visiting personnel, which include Danny Wase (MIMRA), Don Hess (CMI), Simon Ellis (UHH), Maria Haws (UHH), Rod Bourke, Sebastian Horbuskcho and Ramsey Reimers (RRE), Bobby Muller (BPOM), Matang Ueanimatang (CMI) and Manoj Nair (CMI-Land Grant) are currently working with the new CTSA consultant, Rand Dybdahl, to provide support and guidance for his efforts to spawn pearl oysters to supply the RMI pearl farms. These individuals have also worked to provide financial resources to support the CTSA hatchery effort in the RMI.

The hatchery manual authored by David Wise, Simon Ellis and Maria Haws was printed by CMI and has been used extensively in the RMI by the new hatchery operators, Rand Dybdahl, Manoj Nair and MIMRA staff.

Bioeconomic Study component

The model has contributed significantly to the knowledge and ability of pearl farmers and of technical assistance providers to make business management decisions because this model is the first of its kind. The outcomes of the model predict that pearl farms will generally tend to break even in three to five years with profits accruing thereafter. This is using the most conservative predictions and world market price data. In reality, local markets, which are currently absorbing all of the Micronesia production, range from three to four times higher than this with the result that farms become financially self-sufficient earlier than predicted by the model. This information has already been helpful in finding support and garnering interest in pearl farm development from prospective pearl farmers, funding agencies and private investors. Key information such as labor costs, prices and equipment costs have revealed key points of sensitivity that are helping pearl farmers become more efficient and cost-effective.

The results of the model and the process involved in its development were presented to regional stakeholders during the regional IFAFS Collaborative Alliance Meetings in January 2003 (Pohnpei) and July 2003 (Kodiak, Alaska). Further outreach will be conducted under other funding in 2004.

Publications in Print, Manuscripts, and Papers Presented

Wise, D., S. Ellis and M. Haws. 2003. Hatchery Manual. College of the Marshall Islands.

Publications resulting from this work will be submitted to peer-reviewed journals in January 2004.

Literature Cited

Alagarswami, K., S. Dharmaraj, A. Chellam and T. S. Velayudhan. 1989 Larval and juvenile rearing of black-lipped pearl oyster, *Pinctada margaritifera* (Linnaeus). Aquaculture 76:43-56.

Southgate, P. C. and M. Ito. 1998. Evaluation of a partial flow-through culture technique for pearl oyster (*Pinctada margaritifera* L.) larvae. Aquaculture Engineering 18:1-7.

Aquaculture Extension and Training Support for the U.S. Affiliated Pacific Islands, Year 14

General Information

Reporting Period April 1, 2003 - September 30, 2003

Funding Level

Year	Amount
1	**$70,000**

Participants

Rand Dybdahl
College of the Marshall Islands

Manoj Nair R., Ph.D.
College of the Marshall Islands Land Grant Program

Donald Hess, Chair
College of the Marshall Islands

Objectives

1. Establish and implement protocols for spawning and rearing sufficient number of pearl oyster spat per year for research, extension, demonstration and evaluation.
2. Provide training to local people (Marshallese and other Micronesians) on all aspects of the pearl industry from spat production to establishment of farms.

3. Design and conduct practical research on hatchery prodcution of spat and farm growout technologies in collaboration with other scientists from local and regional institutions.
4. Assist and advise in hatchery designs and construction.
5. Initiate and maintain algal cultures. Supervise and train at least five local hatchery staff to be able to independently perform all required activities.
6. Perform other related duties as may be needed and appropriate as may be determined by the CTSA and the College of the Marshall Islands.

Anticipated Benefits

The black-lip pearl oyster, *Pinctada margaritifera*, is endemic throughout most of the tropical Pacific islands. Pearl culture is an important currency earner regionally. For example, black pearl culture is already well established in French Polynesia where it is the second largest foreign revenue-earner at over US$140 million per annum. Pearl culture in the Republic of the Marshall Islands (RMI) started in the 1990s. There are currently four small pearl farms that are considering expanding their operations to other atoll farm sites. The main bottleneck preventing their expansion, however, is the inability to acquire sufficient quantities of wild pearl oysters. Surveys of the RMI's wild stocks indicate that population numbers are insufficient to support commercial pearl farming. These stocks are reportedly only present on four or five atolls. The expansion of the still small cultured black-lip pearl oyster industry in the RMI is also hindered by the likelihood that collection of wild pearl oyster spat will never be a viable option. Unlike the more established pearl industries in French Polynesia and the Cook Islands, dependence on wild spat collection is not feasible on most atolls and islands in the RMI (N. Sims, S. Ellis, M. Haws, personal communication). Therefore, the black-lip pearl oyster farms in the RMI must rely on hatchery propagated spat. This project supports the only black-lip pearl oyster hatchery in the RMI, and project staff will train local individuals so that they will be able to take over hatchery operations and ensure industry sustainability.

Work Progress and Principal Accomplishments

Objective 1 - Establish and implement protocols for spawning and rearing sufficient number of pearl oyster spat per year for research, extension, demonstration and evaluation.

The first hatchery run was successful and produced enough spat for local industry needs and research.

Project staff succeeded in producing 1.07 million settled spat during the project's first hatchery run (June 1 to July 16, 2003). The spat were distributed to two local companies, Robert Reimers Enterprises and Black Pearls of Micronesia (BPOM), and each received 357,000 spat ranging in size from 2-2.5 mm. This was three times the amount initially requested. The remaining spat were suspended from a submerged long-line at the CMI demonstration pearl farm in Arrak to evaluate nursery methods.

Objective 2 - Provide training to local people (Marshallese and other Micronesians) on all aspects of the pearl industry from spat production to establishment of farms.

Trainees have not yet been hired. The hatchery run mentioned in Objective 1 was completed by the PI and Dr. Nair with the help of whomever happened to be available among the other CMI staff.

Objective 3 - Design and conduct practical research on hatchery production of spat and farm grow-out technologies in collaboration with other scientists from local and regional institutions.

When the PI arrived in March 2003, there was no working hatchery for him to use. His first order of business was to set one up (see Objective 4). A pearl oyster spat growout demonstration farm was also set up at the CMI Arrak campus with the help of staff from the University of Hawaii at Hilo.

Objective 4 - Assist and advise in hatchery designs and construction.

The hatchery run was conducted at the experimental pearl oyster hatchery at CMI's Arrak campus. The improvised hatchery run was conducted at the Arrak hatchery because fewer modifications were required. For example, this hatchery already had electric power and working seawater intake pumps, etc. It had initially been built in 2001 to propagate sea cucumbers, and it was recently remodeled to accommodate the oysters. Project staff are now focusing their time on upgrading the larger commercial hatchery at Woja, owned by the Marshall Islands Marine Resources Authority (MIMRA).

Objective 5 - Initiate and maintain algal cultures. Supervise and train at least five local hatchery staff to be able to independently perform all required activities.

Three microalgal species were acquired from the Woja hatchery and a fourth species was brought in from Hawaii. During the hatchery run, these starter cultures were then used to initiate food production for the pearl oyster larvae and settled spat. However, food production was limited by the available glassware / culture

flasks on site as well as by the lack of trainees who would have been able to take over some of the tasks.

Objective 6 - Perform other related duties as may be needed and appropriate as may be determined by the Center for Tropical and Subtropical Aquaculture or College of the Marshall Islands.

The PI had initially been asked to make the hatchery propagation of pearl oyster spat his priority. With that objective accomplished with the initial hatchery run, he will be able to devote more of his time to fulfilling the remaining objectives during subsequent hatchery runs.

Work Planned

The next main task for the remainder of Year 14 will be to upgrade the Woja hatchery to working order because continuing to supply hatchery propagated spat is seen as critical for the survival of the pearl oyster industry in the RMI. Spat production will restart at the Woja hatchery once it is fully operational, and this will ensure a stable supply for the local industry. Extension training will begin in earnest for newly recruited hatchery staff, and this will promote self-sufficiency and industry sustainability. At the same time, project staff will conduct selected experiments to gain valuable information on larval rearing, spawning and nursery techniques. The project's original function as a source of regional and general extension support will also continue on a contract basis. This way, appropriately qualified individuals will run the various workshops.

Impacts

In May 2002, The Center for Tropical and Subtropical Aquaculture (CTSA) redirected the focus of its regional aquaculture extension project from a general, multi-species approach, to a concentrated effort to stabilize the black-lip pearl oyster spat supply in the Republic of the Marshall Islands (RMI). CTSA moved the project from Pohnpei, the Federated States of Micronesia (FSM), to Majuro, RMI, where it would make the biggest impact. The project's goal is to propel the RMI's struggling cultured pearl industry forward before local businesses ran into too many insurmountable problems and were forced to close down. Unreliable spat supply has been a major constraint to the development of the pearl oyster industry in the RMI. Due to past overharvesting, the RMI does not have sufficient numbers of pearl oysters in the wild to supply pearl farms with required pearl

oyster spat. The collection of wild spat also does not appear to be a viable option. In response to this problem, the private company Black Pearls of Micronesia (BPOM) constructed a pearl oyster hatchery in the late 1990s at Woja on Majuro Atoll. However, due to financial reasons, BPOM closed its hatchery operations in 2000. This left the industry without a source of spat throughout 2001 and 2002. In March 2003, this project was initiated. Without this project, the industry would still not have had a source of black-lip pearl oyster spat, and its future would have been very precarious.

Aquaculture of Marine Ornamental Species, Year 3

General Information

Reporting Period June 1, 2002 - September 30, 2003

Funding Level

Year	Amount
1	$128,735
2	$104,135
3	**$102,325**
TOTAL	$335,195

Participants

Robin J. Shields, Ph.D.
The Oceanic Institute

Charles W. Laidley, Ph.D.
The Oceanic Institute

Cynthia L. Hunter, Ph.D.
Waikiki Aquarium

Karen Brittain
Waikiki Aquarium

Objectives

The Oceanic Institute

1. Maintain and expand centralized broodstock populations of yellow tang (*Zebrasoma flavescens*) and flame angelfish (*Centropyge loriculus*).
2. Evaluate the effects of harem size on reproductive activity and spawning performance of angelfish broodstock.
3. Determine appropriate stocking densities and sex ratio for yellow tang broodstock.
4. Test pilot scale rearing system for mass production of angelfish fry.

The Waikiki Aquarium

Determine uptake rate, survival and growth of angelfish and yellow tang larvae, and/or other available species fed wild zooplankton collected from inshore waters in South Oahu.

Anticipated Benefits

Successful completion of this project will immediately affect the aquarium industry by providing hatchery techniques to culture several species of marine ornamentals, thereby offering a more environmentally sustainable alternative to wild collection practices. Consistency in production would ensure a solid base for development of an industry and transfer of reliable technologies. Techniques to mature and spawn the species chosen could be transferred to other highly desired ornamental fish, allowing for the rapid development of new aquacultured species. As expressed in the June 1997 newsletter of the American Marine Life Dealers Association, several benefits, apart from cost savings, will accrue to the industry from the financial investment in research and development of captive propagation, including new economic development, job creation, and an increased emphasis on the importance of maintaining coastal resources. Additional economic benefits will flow throughout the industry, strengthening aquarium and pet retail stores and benefiting consumers with healthier fish.

Work Progress and Principal Accomplishments

Project Years 1 and 2

During years 1 and 2 of this project, broodstock populations of two highly popular marine ornamental species, flame angelfish and yellow tang, were established at OI for seedstock production. Experiments were carried out to determine optimal tank configuration for adult flame angelfish, in terms of system volume and topography to induce spawning behavior. Systems and husbandry protocols were developed based on 750-L holding tanks, mimicking essential parameters in the fishes natural reef environment, in combination with a natural photoperiod and temperature regime and varied broodstock diet. This approach enabled the rapid onset of reproductive activity, continuous daily spawning, and record levels of egg production from captive flame angelfish stocks. Installation of multiple broodstock holding units based on this design generated sufficient flame angelfish larvae to begin small-scale hatchery rearing trials.

Several colonies of captive yellow tang were established during project year 1. These stocks began releasing unfertilized eggs that year and subsequently proceeded to produce fertile spawns, enabling the first description of yellow tang yolk sac larvae. Examination of spawning rhythmicity indicated a lunar spawning cycle for this species. Yellow tang stocks were expanded to eight tanks during year 2, however the egg fertilization rates were problematic, and larvae supply was sufficient to carry out only preliminary rearing trials.

A breakthrough occurred in Year 1 when a feeding strategy was identified that kept flame angelfish larvae alive for 19 days post-hatch.

In an effort to identify suitable diets for small marine ornamental fish larvae, a series of replicated experiments was carried out at OI during project year 1, comparing the survival and growth rates and feed uptake of flame angelfish larvae offered different zooplanktonic prey-types. None of the tested diets (ss-type rotifers, oyster trochophores and dinoflagellate/protozoa combinations) enabled survival beyond yolk exhaustion, indeed the angelfish larvae appeared to be starving even when the experimental diets were seen to be ingested. Better progress was made by applying a semi-intensive feeding strategy to rear flame angelfish larvae in 1,500-L tanks. Using a combined diet of dinoflagellates, ciliated protozoa and rotifers, angelfish larvae were reared to 19 days post-hatch, which represented a breakthrough at the time.

Researchers from the Guam Aquaculture Development Center (GADC) collected adults of several ornamental fish species, including clown coris, during project year 1, but were unable to obtain spawns.

Planned experiments to compare intensive versus semi-intensive "green water" rearing methods for a variety of fish larvae were not accomplished during project Year 1 (University of Hawaii, UH Sea Grant). Owing to the departure of the relevant investigator, Year 1 research to develop culture methods for feather-duster

worms (University of Hawaii and UH Sea Grant) was transferred out of the CTSA Marine Ornamentals project.

During project year 2, larvae research at OI focused on examining the effects of different microbial conditions on flame angelfish rearing performance. A small-scale rearing system was established, allowing rigorous testing of multiple experimental treatments. This system was used to develop a disinfection technique that reduced surface bacterial loading of angelfish embryos by more than 99%, involving immersion in a 3% solution of hydrogen peroxide for 5 minutes.

Studies were also carried out at OI during year 2 to examine the effects of water source and microalgae type on the survival rate of flame angelfish yolk sac larvae. Larvae were reared in 'matured,' UV-sterilized water, or in water that had only received mechanical filtration, with or without addition of microalgae. Highest mean survival rate (70% to day-4 post-hatch) was obtained in groups of larvae receiving matured water plus *T. Isochrysis* sp microalgae. *Nannochloropsis* sp algae was not beneficial to early larval survival, while larvae reared in "clear," mechanically filtered water exhibited much lower mean survival (23%) than all other groups. Based on these findings, the use of matured water plus *T. Isochrysis* sp was adopted as standard for first-feeding flame angelfish larvae.

In Year 2, progress from CTSA- and NOAA-funded projects led to the production of the world's first captive-reared juvenile flame angelfish.

In parallel to these year 2 CTSA studies, a breakthrough was made within a NOAA-funded project at OI in culturing a suitable zooplanktonic diet (calanoid copepod) for flame angelfish and other tropical marine fish species that produce very small larvae. The world's first captive-reared juvenile flame angelfish were produced during 2001 using this diet. Thanks to the combined progress in flame angelfish larviculture within the CTSA- and NOAA-funded projects during 2001-2002, it has been possible to direct year 3 CTSA funding toward the development of pilot-scale rearing methods for this desirable ornamental fish species.

Good progress in rearing new marine ornamental fish species was also made by WAQ researchers during 2001, with the first ever production of juvenile masked angelfish, *Genicanthus personatus*, using cultured prey. WAQ researchers also provided a zooplankton identification service for the GADC during project year 2, of samples collected by in Guam during year 1.

Current Project Year (Year 3)

Objective 1: Maintain and expand centralized broodstock populations of yellow tang (Zebrasoma flavescens) and flame angelfish (Centropyge loriculus).

Established populations of yellow tang and flame angelfish have been maintained for seed production using current best husbandry practices. Daily measurements of fecundity and egg fertilization rate are made for each spawning stock at OI and

developing embryos are supplied for hatchery rearing trials as required. Tank commissioning allowing for expansion of flame angelfish broodstock population was achieved in May and tanks were stocked in June. Commissioning of the yellow tang system is nearing completion and should be available for stocking in early November.

Objective 2: Evaluate the effects of harem size on reproductive activity and spawning performance of angelfish broodstock.

Commissioning of new broodstock holding facilities for harem size experiments was achieved in May 2003, and the system was stocked in June. Although stocks are just beginning to acclimate after 5 months in the system, a pattern is beginning to emerge with the single-female tanks currently generating an average of eight spawns/month, the two-female tanks generating an average of 14 spawns/month, and the three-female tanks generating an average of 25 spawns/month. Tank fecundity also shows a similar relationship between harem size and egg production with the single-female tank averaging only 41 eggs/spawn, the two-female tank yielding 174 eggs/spawn, and the three-female tanks producing 762 eggs/spawn. Fertility rates are near 0% in the one- and two-female systems and the still relatively low (23%) in the three-female systems.

Objective 3: Determine appropriate stocking densities and sex ratio for yellow tang broodstock.

Commissioning of new broodstock holding facilities is nearing completion and will be stocked early next month.

Objective 4: Test pilot scale rearing system for mass production of angelfish fry.

Early in the project a trial was carried out to compare semi-intensive versus intensive rearing of flame angelfish larvae. Larvae were reared either semi-intensively in 4,000 L tanks, or intensively in 200 L tanks (two tanks per method). For the semi-intensive approach, the rearing tanks were inoculated with *Isochrysis* sp microalgae and cultured calanoid copepods (all developmental stages), before stocking with 2 day old flame angelfish larvae at a density of 0.7 to 0.9 /L. Larvae grazed on the tanks' endogenous copepod populations and rotifers were introduced only when copepod supplies were becoming exhausted. In contrast, the intensive 200-L tanks were stocked at a higher density (~10 larvae/L) and the angelfish larvae were fed a defined copepod ration each day. Rotifers were introduced to the intensive rearing tanks from day-10 post-hatch. Survival rate to 3-4 weeks post-hatch was higher (2.1%, 6%) in the intensive rearing tanks than in the semi-intensive rearing tanks (0%, 1.4%). Fish from both groups were reared to metamorphosis, although chronic mortality during the late postlarval phase was problematic. Subsequent larval rearing experiments have been delayed while the new broodstock populations

were established and conditioned to generate sufficient numbers of eggs to meet experimental requirements.

Objective 5: Determine uptake rate, survival and growth of larvae fed wild zooplankton collected from in-shore waters in South Oahu.

At WAQ, *Genicanthus personatus* stocks produced only very small batches of eggs, preventing rearing trials from being carried out. Small spawns of *C. fisheri* and *C. multicolor* were also obtained. Trials were conducted with *C. fisheri* larvae supplied by Frank Baench, using rearing methods developed in 2001. None of these larvae survived for more than 10 days. Wild plankton was collected in off-shore tows on three occasions and by hand from near-shore reef areas on three other occasions. These samples were screened, sorted, and identified and cultures were started with several of the smaller copepod species. Cultures were maintained for up to two weeks. A starter culture of *Oithona* sp was also obtained from HIMB and culture trials are ongoing. The project culturist, Karen Brittain, has recently departed from WAQ causing potential delays in planned project activities.

Work Planned

The following research work is planned for the remainder of project:

- Continue husbandry of adult flame angelfish and yellow tang for seedstock production.
- Continue harem size experiment.
- Commission yellow tang broodstock holding facilities and carry out stocking density experiment.
- Continue flame angelfish pilot-scale rearing trials, with emphasis on improving postlarval survival rates.
- With the departure of Karen Brittain from WAQ, planned work at WAQ prior to project completion is uncertain and will be reassessed.

Impacts

The ultimate goal of this project is to assist in the development of a marine ornamental aquaculture industry in Hawaii and the Pacific. This represents a key economic opportunity for farmers in the State of Hawaii and Pacific Island affiliates such as Guam for several reasons. Firstly, there is a worldwide void in aquaculture production of marine ornamental species. It is estimated that less than 5% of all marine ornamental species traded on the open market are aquacultured, and that the actual numbers of cultured fish traded is miniscule compared to those traded by collectors. This is unlike the situation currently faced by freshwater ornamental farmers in Hawaii, who compete in markets with well-established foreign and other domestic producers. Secondly, it is well known that the health of coral reef ecosystems around the world is being severely degraded, and that wild collection practices are likely unsustainable unless alternatives are sought. Moreover, the Hawaiian Islands are home to over 85% of the coral reefs in the United States, well-positioning the region to develop an aquaculture-based industry. Success of this project will not only provide new economic opportunity to farmers, but will also help ensure the long-term sustainability of the marine ornamental trade by providing alternatives to wild collection practices, and a means to practice resource conservation.

Publications in Print, Manuscripts, and Papers Presented

Invited presentation at MACNA XV on "Research Development on yellow tang and pygmy angelfishes at The Oceanic Institute in Hawaii." September 5-7, 2003 in Louisville, Kentucky.

Reproduction and Selective Breeding of the Pacific Threadfin

General Information

Reporting Period June 1, 2002 - September 30, 2003

Funding Level

Year	Amount
1	**$100,000**

Participants

Charles W. Laidley, Ph.D.
The Oceanic Institute

Robin J. Shields, Ph.D.
The Oceanic Institute

Objectives

1. Complete methods development for pair spawning of Pacific threadfin for application to the genetic selection efforts.
2. Establish and maintain domesticated and selected Pacific threadfin broodstock lines.
3. Conduct controlled spawning of select broodstock lines to generate select seedstock for growth performance evaluation.
4. Complete life cycle of growth-selected and control lines of Pacific threadfin and determine direct effects of selection on growth performance, and indirect effects on survival, reproductive development, and generation time.
5. Gain estimate of heritability for growth and indirect effects on survival, dressing percentage, and reproduction in Pacific threadfin.

6. Initiate research on water reuse technology to protect selected broodstock lines from pathogenic exposure and to decrease on-site water consumption.

Anticipated Benefits

Available estimates of heritable improvements in fish growth performance through genetic selection typically range from 10 to 23% per generation of selection amongst species examined to date. It is not unusual for these programs to require external support during early years due to the inherent time lags between program initiation and delivery of improved seedstock to farmers. However, the potential benefits to commercial aquaculture production in terms of improved growth and reduced production costs are significant. Most costs, with the exception of feeds, are tied to rates of production or growth. Thus the anticipated improvements in growth performance (i.e., 10 to 23% per round of selection) will reduce time to market in the order of 18 to 43 days and yield overall gains in farm profitability in the range of 6.5 to 15%. Based on even modest gains in the range of 15% per generation of selection, the resulting improvement in industry efficiency would lead to increased profits of over $100,000 based on farm gates of approximately $1 million. These benefits will be further enhanced with industry expansion and with further rounds of selection.

Research also targets tracking reproductive development as a critical parameter in the development of a selective breeding program. The overall generation time is a critical parameter that effects the rate of genetic improvement. Although, existing research has suggested that some female broodstock become mature by about one year of age, our experience to date and anecdotal experience by others suggests a somewhat slower generation time. A fundamental understanding of all stages of the species life cycle is needed to proceed with selective breeding efforts. In addition, selective breeding can reduce the turnover time of successive filial generations. OI has evidence that wild or domesticated animals, raised more quickly under aquaculture conditions, also mature earlier than wild counterparts. The potential for the selection of faster generation time, potentially as a serendipitous result of selecting for faster growth, holds promise for speeding up the selection process and reducing turnover time.

Work Progress and Principal Accomplishments

Objective 1 - Complete methods development for pair spawning of Pacific threadfin for application to the genetic selection efforts.

Strip-spawning Pacific threadfin in pairs or small groups has proven to be difficult. Eggs will therefore be obtained from larger group spawns.

The first objective was to examine methods of spawning Pacific threadfin broodstock in pairs or small groups to gain more control over the parental input to genetic lines, as opposed to current methods based on group spawning on larger broodstock tanks. During this project work period we completed three new experiments including (1) the use of LHRHa implants to tank-spawn groups of 2, 4, or 8 broodstock; (2) the use of hCG to induce tank spawning of paired broodstock; and experiments (3) and (4) in which hCG was used to induce final maturation and ovulation in conjunction with strip-spawning of females and artificial fertilization of eggs. Both hCG and LHRHa were successful in inducing egg-release from induced females but spawns were not fertile. Experiments using hCG to strip-spawn females allowed the delineation of the time course for final oocyte maturation but none of the females (total 10 tested) successfully ovulated, precluding completion of the strip-spawning protocol. Through these experiments we have made steady progress, although the species appears to be unusually difficult to successfully strip-spawn. Based on this data, we will revert to the original protocol based on obtaining eggs from larger group spawns, attempting to stock runs from multiple spawn days to increase genetic variability in each line.

Objective 2: Establish and maintain domesticated and selected Pacific threadfin broodstock lines.

Data suggest that Pacific threadfin females initiate spawning at around 2.5 years which is a much longer period of time than anticipated.

The Pacific threadfin genetic selection project is progressing well behind schedule due to the slower than anticipated maturation and initiation of spawning of select and control animals. Hatchery runs were previously scheduled for October 2002 and are now scheduled for late Fall 2003.

Control and fast-growth fish were selected in August 2001 from a post-growout population of 604 fish at approximately six months of age at a mean weight/length of 358g/25.6cm. From this population a 50 animal control group with mean weight/length of 378g/25.6cm and a 50 fish "select" group with mean weight/length of 516g/28.9cm were established (Table 1).

January 2002 maturation checks (11 months of age) revealed that in addition to maintaining significant size difference between groups (31%), that 10 out of 47 control animals had reached the male stage of sexual maturity (Pacific threadfin are protandrous hermaphrodites) whereas 41 out of 50 growth selected animals has entered the male phase of sexual maturation.

June maturation checks of these same stocks again revealed a significant size difference (34%) and the appearance of the first female animals. Interestingly, the majority of the select group has rapidly proceeded to the female stage of

TABLE 1. Survival, growth and reproductive development of control and select parental stocks of Pacific threadfin. Individual fish (identified by PIT tags) were weighed, measured and sexed at approximately 6, 12, and 16 months of age. Reproductive maturation is represented as the ratio of reproductively immature (I), male (M), and female (F) fish in each group.

Age	Date	Survival		Weight (g)		Length (cm)		Reprod. Matur. (I:M:F)	
(mth)		control	select	control	select	control	select	control	select
6	Aug 01	100%	100%	377	516	25.6	28.9	50:0:0	50:0:0
11	Jan 02	94%	100%	686	896	30.9	34.2	37:10:0	9:41:0
16	Jun 02	94%	98%	730	980	32.4	35.9	12:22:13	0:12:35
31	Sep 03	94%	84%	947	1173	35.6	38.2	11:22:14	1:10:31

development with 12 males and 35 females while the control group was slower to develop reproductively with 12 fish remaining immature, 22 as males, and 13 advancing to the female stage.

By this fall (Sept 2003) the growth-selected broodstock continued to maintain a distinct size advantage (24%) and a higher proportion of females than seen in control stocks. This data also suggests that size, rather than environmental or behavioral conditions appear to be more important in determining the timing of sexual development and sex change in captive stocks of Pacific threadfin.

The growth selected group initiated monthly spawning activity in May 2003 (27 months of age) and have now spawned in four of the last six months. The control group began spawning in September. Typical of first spawning fish, fertility rates are quite low (<15%) impairing hatchery stocking. Stocks will continue to be monitored and fertile spawns stocked in hatchery when available. If viable spawns are not achieved before the end of the year, then hormone implants will be tested as a method to stimulate spawning activity for egg production.

Objective 3 - Conduct controlled spawning of select broodstock lines to generate select seedstock for growth performance evaluation.

The original project work plan had anticipated a one year period for growth to sexual maturity and initiation of spawning. However, data obtained from this project suggests that portions of the Pacific threadfin broodstock population reach the female stage of development between 11 and 16 months of age, and initiate spawning around 2.5 years.

This new information on reproductive maturation and initiation of spawning activity for this species obliges us to extend the project past the original November 31, 2003 project completion date. A request of a no-cost extension has been submitted to the CTSA Board of Directors to allow sufficient time for spawning and grow-out phases of the project.

This portion of the work plan is now scheduled during the natural reproductive phase of the lunar cycle over the next few months. If stocks do not spawn viable eggs naturally, then fish will be dosed with LHRHa implants to stimulate spawning activity.

Objective 4 - Complete life cycle of growth-selected and control lines of Pacific threadfin and determine direct effects of selection on growth performance, and indirect effects on survival, reproductive development, and generation time

This portion of the project work plan will be initiated when select and control lines become available. However, due to increased efforts put toward pair-spawning components of this project and longer than expected broodstock maintenance period, there are not sufficient funds remaining to complete post-market size growth and reproductive development studies. We therefore request guidance from the CTSA with regard to project extension and potential availability for funds to complete reproductive development components of this project.

Objective 5 - Gain estimate of heritability for growth and indirect effects on survival, dressing percentage, and reproduction in Pacific threadfin.

This portion of the project is scheduled post-growout and as for Objective 4, will be initiated subsequent to completion of the growout phase of control and select lines.

Objective 6 - Preliminary evaluation of water reuse systems for maintaining Pacific threadfin broodstock.

The final objective of this project, evaluating "low-cost" water reuse systems for maintaining broodstock is in progress and is partially commissioned and will be installed to initiate testing in November. The system is designed from readily available parts and can be run using either airlift or mechanical pumps. The prototype system will run using airlifts to move water. The system will move 30gpm of bottom water through a cone filter to remove large suspended solids while 90 gpm of surface water will be run through a moving biofilter to remove dissolved wastes and a foam fractionator to remove small solids, proteins and lipids. A UV filter will help disinfect return flow. Systems will be evaluated over the six month project extension period and results will be available for the project final report.

Work Planned

1. Continue to maintain domesticated and selected Pacific threadfin broodstock lines.
2. Spawn control- and select-broodstock lines and rearing fingerlings for growth evaluations.
3. Fingerling growout to determine direct effects of selection on growth performance, and indirect effects on survival. Longer term examination of reproductive development and generation time may not be possible without long-term grant extension due to the much longer than expected (2.5 yrs vs. 1 yr) generation time for the species.
4. Gain estimate of heritability for growth and indirect effects on survival, and dressing percentage.
5. Complete water reuse system commissioning and begin evaluation as a tool in broodstock management, biosecurity, and water use reduction.

Impacts

The aquaculture development of the Pacific threadfin is gaining substantial momentum in Hawaii with the appearance of captive farmed product in local restaurants, retail markets, and sales to both mainland and international markets. Recent adoption of cage culture technologies based on the joint OI/SeaGrant Hawaii Offshore Aquaculture Research Project (HOARP) has further intensified production capability in the sector. CTSA-funded research has provided the cornerstone for this growing industry to date, and expected developments under current project funding will further assist in securing requisite fingerling supplies to meet the needs of both on-shore and offshore production. Current efforts to enhance aquaculture performance through genetic selection will provide new opportunities to increase industry efficiency through improved growth, reduced generation time, and greater resistance to stress and disease.

Publications in Print, Manuscripts, and Papers Presented

Laidley, C.W., R. J. Shields, M. Kaiwa, J. Kobashigawa, and A. Molnar. Emerging marine finfish species under culture development at The Oceanic Institute. International Sustainable Marine Finfish Culture and Workshop, October 9-10, 2003, Harbor Branch Oceanographic Institution, Fort Pierce, FL.

Aquaculture of Hawaiian Marine Invertebrates for the Marine Ornamental Trade, Year 1

General Information

Reporting Period October 1, 2002 - September 30, 2003 (final report)

Funding Level

Year	Amount
1	**$55,000**

Participants

Clyde Tamaru, Ph.D., Extension Specialist
Sea Grant Extension Service

David Bybee, Graduate Student
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Anthony Montgomery, Research Assistant
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Tom Ogawa, Research Assistant
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Ethan Morgan, Jennifer Hix
The Oceanic Institute

Shaun Moss, Ph.D.
The Oceanic Institute

David Ziemann, Ph.D.
The Oceanic Institute

Objectives

Feather Duster Worm

1. Determine the appropriate feed and feeding regimen to result in captive spawning of collected broodstock.
2. Document larval development stages and determine time to settling.
3. Facilitate larval settlement and determine settlement preferences on different substrates.
4. Determine growth time of settled worms to market size.
5. Summarize results obtained into technical bulletins, newsletter articles and where appropriate manuscripts to be submitted to peer reviewed journals for publication.

Soft Coral

1. Determine the appropriate type of substrate and water motion for culturing each target species. Also, the determination of an interaction effect from the two variables.
2. Document baseline growth rates for each of the target species.
3. Determine other critical variables that may contribute to optimal growth. This determination will be based on observations and experiences during the course of the first year.
4. Determine preliminary marketability and crude economic feasibility information for the target species.
5. Develop, in theory, a larger scale prototype system that would incorporate the optimal conditions and growth information obtained during year 1 also including any marketing information.

Anticipated Benefits

Hatchery technologies for some marine species are already at commercial scale and used as a fisheries management tool (stock enhancement) locally and abroad. Advancements in the artificial propagation of marine species have led to the belief that culturing marine ornamental organisms can alleviate some of the fishing pressure on wild stocks as well as create small- or large-scale industries. Commercially cultured feather duster worms would provide an alternative source of animals for the aquarium trade and would also ease the burden on coral reefs caused by

current collecting practices. High demand suggests that this type of aquaculture also has the potential to provide substantial economic benefits to commercial farmers (DLNR catch data 1986-1994). Realization of this potential, however, will hinge upon the successful development of culture technologies that are cost-effective enough to overcome the economic constraints of doing business in Hawaii.

With the increased attention to/awareness of the coral reefs and associated communities, it is felt that the research community needs to take a proactive role in the development of techniques for the propagation of other marine organisms other than fishes. It should be pointed out that regarding marine aquaria, invertebrates are as important to the industry as are the fishes and some are considered hardy additions to the home aquarium. With regard to other species to be investigated, the proposed project will target three Zoanthid soft coral species (*Zoanthus pacifica*, *Z. morgan* and *Protopalythoa* sp.). These species were chosen based on their availability in local waters, their aesthetic value, and the fact that there are no legal restrictions regarding their collection or use.

Work Progress and Principal Accomplishments

Feather Duster Worm

Objective 1 - Determine the appropriate feed and feeding regimen to result in captive spawning of collected broodstock.

The project began in October 2002. Based on preliminary information regarding the spawning season of the feather duster worm and on the only recorded spawning of these worms, which was observed during this month, an attempt was made to induce wild caught broodstock to spawn at the Hawaii Institute of Marine Biology. This objective is actually scheduled as part of the Year 2 activities, however, the work with the shrimp pond effluent at The Oceanic Institute was being undertaken during the same time period and would also be addressing this objective, albeit in a different format. The opportunity presented itself and the Principal Investigator decided the situation warranted the change.

Project staff successfully induced spawning in feather duster worms.

Spawning trials were conducted in four 15 gallon aquaria, and 20 feather duster worms were placed in each tank. The feather duster worms were collected from the surrounding waters of HIMB. Each tank was equipped with a continuous flow of seawater and a single airstone. To induce spawning, five individuals were ablated at the bottom of where the coelomic contents could be viewed under a compound microscope. The intent was to insure that there were both males and females and not to set a particular sex ratio. The ablated worms were placed in their respective tanks, and another tank was treated in the same way. Control tanks also consisted of the same number of worms, but the only difference was that the five worms

were handled, but not ablated before being placed back into their respective tanks. Three days after the ablation, the feather duster worms in both treatment tanks were observed to be spewing gametes. It was observed that not only were the ablated worms spawning, but those that were not ablated as well. The entire experiment was repeated in November, and the same results were obtained. In both trials, larvae were examined under a compound microscope to obtain the developmental series. Other trials (e.g., temperature shock and salinity) were also conducted, but did not yield any spawning results.

Initial activities at the Oceanic Institute revolved around the hiring of two assistants (Ethan Morgan and Jennifer Hix). Two hundred fifty feather duster worms were purchased from a local collector. The worms came from Dave Reinhardt and were collected in Kaneohe Bay. All animals were quarantined outside the Oceanic Institute's campus in order to determine whether any of the worms harbored any penaid shrimp pathogens. The majority of the work involved construction of 15 feather duster tanks. Each feather duster tank consists of a 75 L plastic container, 6 L egg collector, seawater supply, air supply, and shrimp pond water supply. Each tank was stocked with 15 broodstock-size feather duster worms and the experiment began in earnest in January 2003. Three tanks are receiving100% shrimp pond effluent, another three tanks are receiving 50% and the remaining tanks are receiving 25% pond effluent. Measuring growth has been problematic, and alternative ways of obtaining data is ongoing. Survival, however, is clearly being impacted by the amount of pond effluent. The tanks receiving the 100% pond effluent have suffered the highest mortalities and require constant tank maintenance due to the sludge build up that pond effluent brings. Growth is barely detectable in all of the treatments, and it is suspected that the amount of organic material in the shrimp pond effluent is too high for the culture of the feather duster worm. No spawning was detected in any of the treatments over the course of the reporting period.

Objective 2 - Document larval development stages and determine time to settling.

Larval development stages were documented. It was observed that larvae settled within 7-8 days.

The successful induction of spawning trials conducted at HIMB provided the opportunity to document the early life history of the feather duster worm. First cleavage was detected within 30 minutes after spawning at 26°C. Development was rapid with hatching occurring with 7 hours after spawning. Development of the free swimming larvae progressed over the course of a week and within 7-8 days the larvae settled to the bottom of the tank where the distinctive cetae were present. Elongation of the anterior portion of the body with the characteristic "feather" began soon after settling of the larvae. Photographs were taken to document the various stages. The induced spawning and developmental stages of the feather duster worm were summarized in three presentations that were given by David Bybee.

Objective 3 - Facilitate larval settlement and determine settlement preferences on different substrates.

Investigations were hampered by predation and may have to be moved into a laboratory.

Three different settling devices were constructed during the reporting period. Essentially, a meter square was constructed out of one inch PCV piping, and various kinds of material (e.g., nylon netting, black plastic netting and concrete) was stretched across it. These have been placed at strategic locations around HIMB and have been monitored bimonthly over the course of the reporting period. After almost one year of deployment, no settling of feather duster worms has been observed, despite being placed in areas where the density of feather duster worms exceeds 10 individuals per square meter. The level of predation on these worms is apparently extremely high, and a new device will need to be constructed. Alternatively, with the availability of feather duster larvae, the investigations may be conducted in the laboratory rather than out on the reef flats.

Objective 4 - Determine growth time of settled worms to market size.

In October 2002, young feather duster worms (e.g., 1 mm in tube diameter) were obtained in waters surrounding HIMB. Approximately five individuals were placed into 15 gallon aquaria that received a continuous supply of seawater from Kaneohe Bay. There were four tanks in total that were setup for this trial. Over the course of the next three months, two of the aquaria received 1 L of *Chaetoceros,* which was cultured in the same fashion as used by shrimp hatcheries. Once each month, the tube diameters of the worms from all tanks were measured, and these were plotted against the length of time they were in the tank. The data clearly show that the fed group grew significantly faster than the ones that received only water directly from Kaneohe Bay. Apparently, feather duster worms can utilize only phytoplankton in their diet, and by the fact that the group fed only Kaneohe Bay seawater also grew during the same time period, the minimum amount of food required to sustain feather duster worms need not be so high.

It was projected that it would take one year for settled worms to reach market size.

Using the growth data from the worms that were being fed, a regression analysis yielded a statistical model that summarized the rate of growth. The model: $Y=(0.039)*Days$, $r^2 = 0.72$, $P<0.001$ can be used to estimate the amount of time that will result in an adult feather duster worm that has an average tube diameter of 10 mm. Substituting 10 mm for Y and solving for Days we obtain 200 Days, which is the estimated time interval it would take to result in a feather duster worm to reach a market size of a 10 mm tube diameter. The data indicate that the feather duster worms grow at a rate where they can be harvested within a year.

Objective 5 - Summarize results obtained into technical bulletins, newsletter articles and where appropriate manuscripts to be submitted to peer reviewed journals for publication.

No progress was made during the reporting period because data are still being accumulated. Target productions will include induction of spawning feather duster worms, developmental stages and preliminary growth data for the soft corals and feather duster worms being fed algae. No written documents have been prepared. As mentioned previously under Objective 2, three presentations were made both locally and at an international conference.

Soft Coral

Objective 1 - Determine the appropriate type of substrate and water motion for culturing each target species. Also, the determination of an interaction effect from the two variables.

The soft corals, like the feather duster worms, underwent an intensive quarantine process. After the quarantine process was completed, coral were placed in tanks that consisted of a 2,000 L oval raceway with a seawater and air supply. Each coral tank had a different type of water motion. Tank 1 had a periodic surge motion created by two 110 L Carlson Surge Devices. Tank 2 had a semi-laminar flow created by two spray-bars at each end that can be angled to adjust the water velocity (note: the velocity of water is not being monitored). Tank 3 had a static water motion with the same turnover rate as the other two tanks. This was created by two spray-bars spread out evenly over the entire raceway. The only change in the experimental design was a change in the regime and type of substrates tested for the corals. The new regimes were as follows:

Species	Substrate 1	Substrate 2	Substrate 3
Protopalythoa sp.	Gravel	Coral chips	Gravel mixed with sand
Zoanthus pacifica	Gravel	Coral chips	Lave chips (cinder)
Zoanthus morgan	Gravel	Coral chips	Lave chips (cinder)

The substrates chosen were based on two different criteria. First, the availability of the substrate keeping in mind what is available and affordable for the commercial farmer. Secondly, growth patterns from the wild. For example, *Protopalythoa* sp. is found attached to rocks under the substrate (typically sand). The sand/substrate interface may be important to the asexual reproduction of this coral. This question will be answered with this experimental protocol.

With the completion of all construction, the animals were stocked into all experimental units. Each coral tank consists of 90 individually labeled trays. Each species is being tested with three substrates and ten replicates. The *Protopalythoa* trays have 3 - 5 polyps, while the *Zoanthus* species have 10 - 30 polyps due to their smaller size. One important note, *Anthelia edmondsoni* was not stocked into the experiment because not enough polyps were collected in time for the quarantine process. So, this species will take a much smaller role in the project

than originally described. However, as polyps are able to be collected, they will be integrated into the experiment.

Zoanthus morgan *grew remarkably faster than the other two species under all of the conditions.*

The experimental period extended between January 2003 to the end of April 2003. Clearly, interspecific variation as to the substrate and water motion conditions that would result in optimal growth could be detected. However, one result is that one species *Zoanthus morgan*, produces more polyps than the other two species under any of the conditions that it simply overshadows the differences observed for a particular species. Growth for both *Protopalythoa* sp. and *Z. pacifica* are possibly inhibited by intense sunlight exposure, or requires an additional source of food besides light energy. Improvement in growth for both these species clearly requires further investigation.

Objective 2 - Document baseline growth rates for each of the target species.

Four months of data have been obtained. As mentioned previously, one clear result is that *Zoanthus morgan* clearly grew at a remarkable rate of approximately 20 polyps per month under all conditions and had reached a plateau by the end of the growth trial. The slowing of growth is attributed to the lack of space for additional polyps to grow because it had overgrown the test baskets.

Objective 3 - Determine other critical variables that may contribute to optimal growth. This determination will be based on observations and experiences during the course of the first year.

As mentioned under objective 2, there does not appear to be a significant effect of water motion or substrate on the rate at which the various corals are growing. Because there clearly will not be a Year 2 portion of the project, the growth experiment was terminated in April. During that time, the growth experiment became a growout trial where the polyps of *Z. morgan* were pooled and placed on lava cinders to conduct a mass growout during the previous reporting period. This was done to have a significant enough biomass to allow for a suitable testing of the market and allow for the collaborative partner to start their own colonies. At the end of the current reporting period, there are at least 10,000 polyps of *Z. morgan* that will be shipped to Ocean Rider Inc. in Kailua-Kona during the next reporting period.

Objective 4 - Determine preliminary marketability and crude economic feasibility information for the target species.

Soft coral colonies are to be shipped during the next reporting period to Ocean Rider, Inc., located in Kailua-Kona, who have agreed to take on the test marketing of the soft coral produced under the auspices of the current project. They represent a high-end distributor as they exculsively market their product by internet sales

and command the highest prices. For an idea about the enterprise one can visit their website at: http://www.oceanrider.com/. A summary of the preliminary findings will be provided during the next reporting period.

Objective 5 - Develop, in theory, a larger scale prototype system that would incorporate the optimal conditions and growth information obtained during year 1 also including any marketing information.

No progress was made during the reporting period. Year 2 activities for this component were not supported and consequently, this objective was dropped from the project's work plan. The bulk of the activity will have to be developed by Ocean Rider Inc.

Work Planned

Feather duster worm: Because the spawning objective was switched between the year 1 and year 2 projects, preparations will take place to address the original objective in which various phytoplankton species will be investigated to assess their impacts on maturation and spawning. In addition, what was begun prior to the start of the current project is the histological analysis of worms collected throughout the year to determine the extent of the spawning season. Induction of spawning trials will be planned during Year 2 with the intent to focus on improved larval survival and to address the settlement on various substrates. The negative result of using artificial substrate placed on the reef flats does not appear to be working and an alternative plan will be to use the spawned larvae instead. With the availability of settled larvae, attempts at growout will be made using the various opportunities (shrimp pond effluent, Hawaiian fishponds, etc).

During the remainder of the project, a major attempt will be to grow as many of the soft corals as possible to obtain data on large-scale culture and to address the market acceptability. As there will no longer be any support for the soft coral work, the main focus will be to complete the extension and outreach material and have the information disseminated to appropriate end users. A final workshop summarizing the results will be planned during the next reporting period.

Impacts

Progress to date indicates that there well may be some new cultured products that can be developed as Hawaiian home grown marine invertebrates. This will undoubtedly add to the inventory of enterprises engaged in the production of marine ornamentals. From the marketing data that is to be obtained, the project work group will be better able to provide an estimate of the potential economic impact that the results of this project may bring.

Publications in Print, Manuscripts, and Papers Presented

Presentations During Reporting Period:

Bybee, D. 2003. Reproduction of the sabellid polychaete *Sabellastarte spectabilis* in Kaneohe bay, Oahu, Hawaii. Albert Tester Student Symposium, April 16-17, 2003, Department of Zoology, University of Hawaii at Manoa.

Bybee, D.R., J.H.Bailey-Brock and C.S. Tamaru. 2003. Observations on the spawning of the fan worm, *Sabellastarte spectabilis*, in Hawaii. Fifth Annual Hawaii Aquaculture Conference. Windward Community College, May 11, 2003.

Bybee, D.R., J.H.Bailey-Brock and C.S. Tamaru. 2003. Observations on the spawning of the fan worm, *Sabellastarte spectabilis*, in Hawaii. World Aquaculture 2003, Salvador, Brazil. May 19-23, 2003. Book of Abstracts, Page: 132.

Evaluation of Tilapia Species and Varieties for Establishment of a Hatchery in Guam, Year 1

General Information

Reporting Period October 1, 2002 - September 30, 2003

Funding Level

Year	Amount
1	**$50,200**

Participants

David Crisostomo, Extension Agent III
Cooperative Extension Service, University of Guam

Josh Golder, Francine Taitingfong and Kristen Cruz
Guam Aquaculture Development and Training Center (GADTC)

Objectives

1. Identify, obtain and import into Guam male seedstock of five strains of tilapia.
2. Evaluate the growth rate, survival, food conversion and dress out characteristics of these five strains under local conditions in both ponds and above-ground tank systems.
3. Obtain, import and raise to sexual maturity broodstock of the five strains for future determination of reproductive rates and estimation of the costs of fry production at the GADTC facility.

Anticipated Benefits

Guam will experience substantial direct benefit to the aquaculture industry by the eventual establishment of a proven fast growing stock of tilapia that will serve as the base stock of a tilapia hatchery. The eventual establishment of a tilapia hatchery will reduce or eliminate the need for farmers to import tilapia fry. This will reduce the potential of disease introduction and provide a year-round supply of tilapia fry for producers. This new hatchery will also assist the islands in the region. Additional benefits will occur in the overall economy of Guam when tilapia producers purchase locally produced fry, rather than sending money off-island.

Work Progress and Principal Accomplishments

Objective 1 - Identify, obtain and import into Guam male seedstock of five strains of tilapia.

Two varieties of Oreochromis niloticus *fry were imported into Guam from Thailand.*

This project was delayed after Guam was devastated in December 2002 by Supertyphoon Pongsona. This was due to the late recovery of power and water infrastructure at the GADTC and commercial farms and damage of some tanks at GADTC. However, in May 2003, we ordered and imported two varieties of *Oreochromis niloticus*, the 'Chitralada' variety (33,000 pieces of sex-reversed fry and 10 families of breeders) and the 'Philippine selected' variety (22,000 sex-reversed fry and 4 families of breeders) from the Asian Institute of Technology in Thailand. The fish arrived on June 27, 2003, and the sex-reversed fry were placed in 20 ton tanks with flow through water. Each family of breeders was stocked in 1 ton tanks with flow through water. Breeders came in separate, unrelated families consisting of between 50-200 individuals per family.

Objective 2 - Evaluate the growth rate, survival, food conversion and dress out characteristics of these five strains under local conditions in both ponds and above-ground tank systems.

Project staff have been evaluating the growth rate of two varieties in nursery tanks and of one variety in an earthen pond.

All fish were fed with trout fry feed (48% protein) from Silver Cup. Breeders were additionally fed with *Artemia* nauplii for the first three weeks. Initially, all fry were fed to satiation three times daily. After five days, fry were sampled for weight. The average initial weight for the 'Chitralada' strain was 0.048 g, while the 'Philippine Selected' averaged 0.035 g. Feeding rates were started at 20% body weight for the first week and then reduced to 10%. The 'Chitralada' variety grew at a rate of 0.09 g per day up to September 30, 2003 in the 20 ton GADTC nursery tanks. The 'Philippine Selected' variety grew an average of 0.08 g per day over an 86 day period in the 20 ton GADTC nursery tanks.

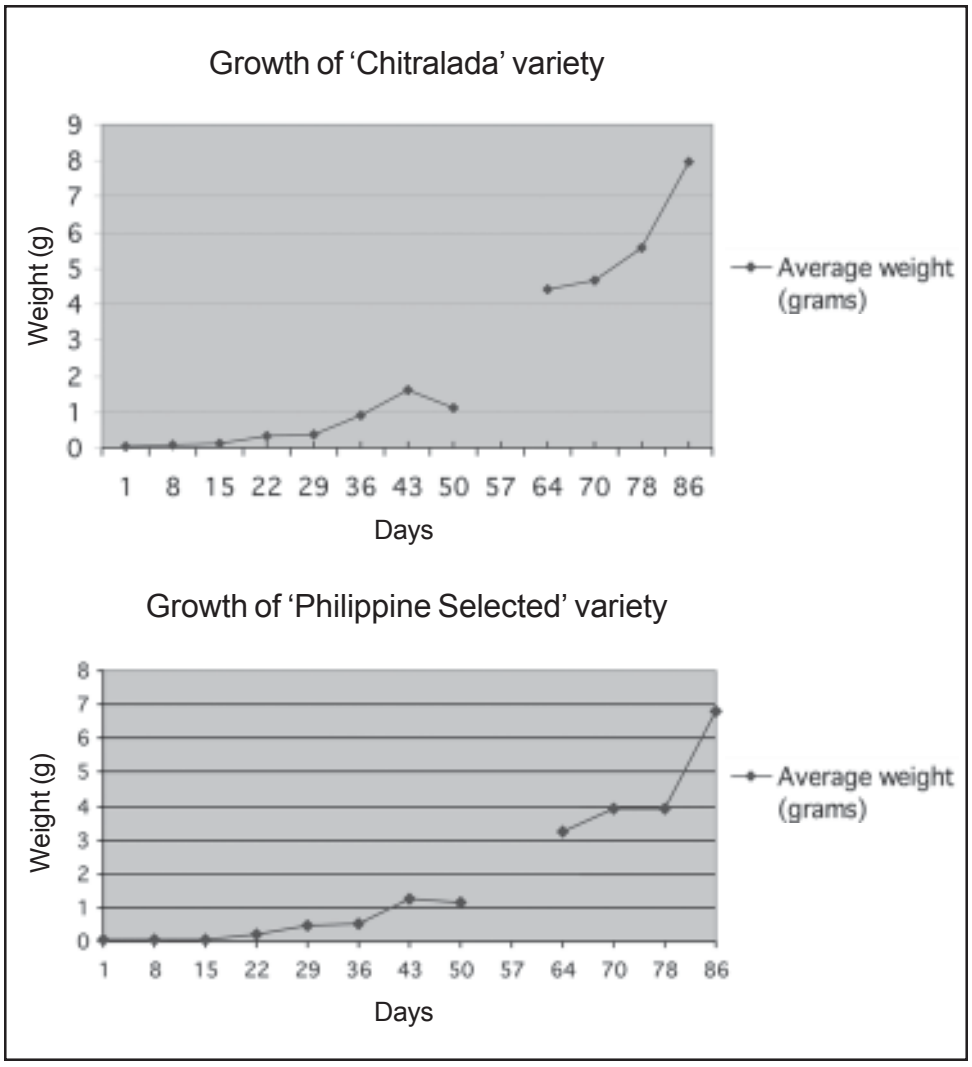

FIGURE 1. Growth of two varieties of *Oreochromis niloticus* in nursery tanks

Approximately 16,400 fry of the 'Chitralada' variety were stocked into an earthen pond at the SCOAP farm on July 29, 2003. The pond was stocked at a rate of 5.77 fish per m^2 (2,842 square meters). Fish were stocked at a weight of 0.38 g. The feeding regimen was left to the farmer's standard culture practice. Fish were sampled for growth every two weeks. Fish gained an average of 106.22 g in 25 days, yielding an average daily growth rate of 4.25 g (Figure 2).

Since the project was delayed and all the participating farmers were forced to modify their production schedules, they opted to stock their ponds when fry from their usual sources became available. Their usual source is in Taiwan, where fry are only available from April to September. Their loss of production time forced them to hurriedly stock ponds when they were available. This forced a delay in

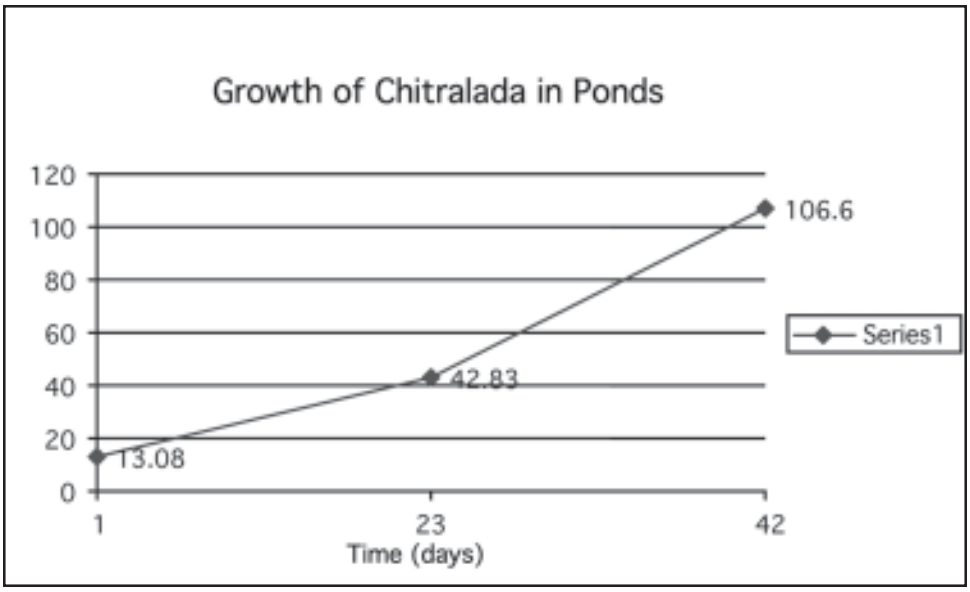

FIGURE 2. Growth of 'Chitralada' variety of *Oreochromis niloticus* in earthern pond

stocking the project fish due to a lack of pond space, and we subsequently had to change the method for evaluating the growout of the fish in the project. We decided to conduct growth trials at the GADTC by modifying the raceways, which measure 10 ft x 220 ft. One raceway will be divided into sections measuring 10 ft x 10 ft by using 1 inch PVC pipes for a frame and .25 inch square mesh plastic screen. Each variety will be stocked into four sections at a stocking rate of 10,000 fish per acre (23 fish per section).

Objective 3 - Obtain, import and raise to sexual maturity broodstock of the five strains for future determination of reproductive rates and estimation of the costs of fry production at the GADTC facility.

Two varieties of Oreochromis niloticus *broodstock were imported into Guam from Thailand.*

Two varieties of broodstock were imported in September 30, 2003. Approximately 200 fish in each of 10 unrelated families of the 'Chitralada' variety and 100 fish in each of 4 unrelated families of the 'Philippine Selected' variety were received at the same time as the other fish used for the growout trials. Each family of broodstock was stocked into separate 1 ton tanks. Broodstock were fed to satiation two to three times daily. Initially, they were fed with trout diet and *Artemia* nauplii for the first three weeks. They were then weaned to Rangen catfish diet (30% protein). Growth of the broodstock has not been recorded. Broodstock will be separated by sex and tagged to distinguish between males and females, and between families.

Work Planned

We will obtain and import three more strains of tilapia seedstock, and we will continue to evaluate growth rate, survival, food conversion and dress out characteristics for all of the strains as they become available. We will also obtain and import three more strains of tilapia broodstock, which we will raise to sexual maturity in the same manner as the inital two strains.

Impacts

Impacts from this project will not be realized until completion of the third year. Findings from this year and next year will determine the variety to use in expansion of the breeding program to commercial levels. To date, one farmer has stocked one variety into a pond. Growth has been good, but a comparison is not possible at this point. Future impacts are expected in the area of improved financial gains for the farms of large and small producers. Savings should be seen because large farms will not have to maintain fish in stunting ponds, and small producers will be able to purchase good quality fry on a year-round basis without having to wait because of availability or seasonal fluctuation in supply.

Improving Sturgeon Farming in Hawaii, Year 1

General Information

Reporting Period October 1, 2002 - September 30, 2003

Funding Level

Year	Amount
1	**$18,365**

Participants

Kevin D. Hopkins, Ph.D., Director
Pacific Aquaculture and Coastal Resources Center (PACRC),
University of Hawaii at Hilo (UHH)

Frank A. Chapman, Ph.D., Associate Professor
University of Florida

Howard Tanaka, dba Hawaiian Sturgeon & Caviar Company

Bob Kern and Ashley DeLoach, Tropical Ponds Hawaii LLC

Ron Weidenbach, Hawaii Fish Company

Jeff and Linda Koch, Mokuleia Aquafarms

Brent Burkott, Hawaii Farm Fresh Seafood

William Lansford and Jo Sosna, Aquatic Ventures Inc.

Objectives

1. **Training:** Improve the level of technical expertise of at least six Hawaiian fish farmers so that they can nurse, handle, stage, and if possible, spawn *A. gueldenstaedti*.

2. **Establish Sturgeon Stocks:** Establish at least three (six preferred) populations of *A. gueldenstaedti* and one other Russian species at farms throughout Hawaii.

3. **Reducing Deformity:** Determine if increasing calcium concentrations in incubation water will reduce the incidence of deformity in yolk sac larvae and fingerlings.

4. **Hatchery Manual:** Prepare a preliminary hatchery manual for Russian sturgeons in Hawaii.

Anticipated Benefits

This project will provide the basic information and fish stocks needed for the establishment of a local sturgeon industry in Hawaii. In particular, hatchery and nursery methods will be improved and multiple stocks established.

Work Progress and Principal Accomplishments

Objective 1 – Training: Improve the level of technical expertise of at least six Hawaiian fish farmers so that they can nurse, handle, stage, and if possible, spawn A. gueldenstaedti.

A workshop was held in March 2003 to help farmers, extension personnel and students determine the sex and ripeness of sturgeon.

A sturgeon reproduction and hatchery workshop was held at Hoowaiwai Farms and PACRC on the island of Hawaii from March 10-11, 2003. This training was co-sponsored by the USAID/IFAFS-funded project "Bridging Gaps to Insure Long-Term Viability of Small Tropical Mariculture Ventures in Hawaii and the U.S. Affiliated Islands." The workshop was attended by 22 persons (14 farmers and 8 extension/outreach persons) plus 12 students from UHH and a local high school. The instructor was Dr. Frank Chapman from the University of Florida. Day 1's hands-on activities included seining 8 year old *Acipenser gueldenstaedti* (up to 113 lbs), weighing, tagging with PIT tags, anaesthetizing, egg sampling via incision followed by suturing, and evaluation of the stage of maturation. During Day 2, a whole day of classroom lectures and discussions covered all aspects of sturgeon culture, concentrating on hatchery and nursery techniques. Also during

this reporting period, detailed diagrams and instructions for assembly of standardized nursery systems were prepared and distributed to the farmers.

Objective 2 - Establish Sturgeon Stocks: Establish at least three (six preferred) populations of A. gueldenstaedti *and one other Russian species at farms throughout Hawaii.*

Project staff have tried and are continuing to try to obtain sturgeon eggs from various sources.

An opportunity arose to cooperate with sturgeon farmers in Florida to obtain eyed eggs from Russia. However, an earlier attempt to transship them from Florida had not been successful. A test shipment (jointly-funded by the project and one of the farms) of 200+ *A. gueldenstaedti* fry was conducted in late February. The fish arrived with minimal mortality and were eventually distributed to two new sturgeon farmers and one existing farm. (Note: As the P.I. has a relationship with that existing farm, the project was reimbursed for the cost of the fish that the farm received).

Two additional attempts were made to import sturgeon eggs or fry during this time period. In the first case, arrangements were made to obtain part of a shipment made to Rokaviar in Florida from Italy. Before transshipment from Florida could be made, massive mortalities started to occur precluding transshipment to Hawaii. In the second attempt, a Russian supplier contracted to supply fish to both Dr. Chapman's associates in Florida and this project. The first part of the shipment to Florida was not successful. The supplier requested a delay to early winter. As the shipments were not successful, the project incurred no out-of-pocket expenses (Note: the $2,000 down payment to the Russian supplier was made by the Hawaii Sturgeon and Caviar Company because it was extremely difficult to prepay using government funds. The company will be reimbursed when the fish arrive. If the supplier does not provide the fish nor return the down payment, the company will absorb the loss). One of the farmers went to Russia at his own expense to locate suppliers of eggs/fry. Discussions are now underway to obtain eggs from at least three sources (the Russian supplier discussed above, a Russian government agency and a supplier in Europe).

The design of the standardized hatchery systems was finalized after modifications to enhance CO_2 removal and evaporative cooling. Materials were ordered and distributed to the farms. At least four systems are currently operating, and most of the remainder will be operational before the eggs/fry arrive. Two of the farms have undergone managerial changes since the systems were distributed. The new management is being asked whether they wish to participate in the project or return the systems to the University.

Objective 3 - Reducing Deformity: Determine if increasing calcium concentrations in incubation water will reduce the incidence of deformity in yolk sac larvae and fingerlings.

No activity during this period.

Objective 4 - Hatchery Manual: Prepare a preliminary hatchery manual for Russian sturgeons in Hawaii.

No activity during this period.

Work Planned

Objective 2 - Establish Sturgeon Stocks.

Orders are being placed for delivery of both *A. baeri* and *A. gueldenstaedti* eggs or yolk sac fry in early 2004. These eggs will be distributed to the six originally-identified farms for closely-monitored growout in the standardized culture systems which will be constructed at each farm. An additional three farms will receive yolk sac fry for culture in their own facilities. The fish will be nursed in accordance with standard protocols and subsequently grown for six months.

Objective 3 - Reducing Deformity.

This experiment will be conducted at the PACRC, although data from the commercial farms will also be included for comparison. Eyed eggs will be hatched and nursed at four hardness levels (10, 30, 60, and 90 mg $CaCO_3$/l) prepared with calcium chloride.

Objective 4 - Hatchery Manual.

Work on a preliminary hatchery manual for Russian sturgeons in Hawaii will begin shortly.

Impacts

This project will provide the basic information needed for the establishment of a local sturgeon industry in Hawaii. This addition to the list of culture species would have advantages of a larger market niche: the mainstream, white tablecloth restaurant trade for both meat and caviar and the international markets for smoked sturgeon and caviar. Additionally, sturgeon is well suited to cool freshwater which is abundant at higher elevations and on the windward sides of the various islands.