Center for Tropical and Subtropical Aquaculture

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Accomplishment Report

In coorperation with

Center for Tropical and Subtropical Aquaculture

2002 Accomplishment Report

Center for Tropical and Subtropical Aquaculture
Administration Center
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Introduction

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.

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 (RAC's) funded by the U.S. Department of Agriculture. Research projects span the American Insular Pacific, using its extensive resource base to meet the needs and concerns of the tropical aquaculture industry.

The RACs encourage cooperative and collaborative aquaculture research and extension education programs that have regional or national applications. Center programs complement and strengthen existing research and extension educational programs provided by the U.S. Department of Agriculture and by other public institutions. The Center's objectives are to:

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

CTSA is jointly administered by the University of Hawaii and The Oceanic Institute. The Center offices and staff are located at The Oceanic Institute's Makapuu Point site on windward Oahu.

Organizational Structure

CTSA funds aquaculture research, development and demonstration projects. Each year's program is the result of several groups working together for many months. A Board of Directors oversees 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. The Administrative Center staff provides all necessary support services for the Executive Committee, the Board of Directors, the Industry Advisory Council, the Technical Committee, various project review panels and delegations and project work groups. Dr. Cheng-Sheng Lee, Center Director, supervises operation of the Center.

EXECUTIVE COMMITTEE

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

- Dr. Gary D. Pruder, The Oceanic Institute, {Executive Committee Chairman};
- Dr. Jo-Ann Leong, Hawaii Institute of Marine Biology {Board of Directors Chairman}.

BOARD OF DIRECTORS

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

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

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

The members of the Board of Directors are:

- Mr. John Corbin, Hawaii State 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;
- Dr. Jo-Ann Leong, Hawaii Institute of Marine Biology, University of Hawaii {Board Chairperson}
- Dr. Gary D. Pruder, The Oceanic Institute {Executive Committee Chairman};
- Dr. Singeru Singeo, Land Grant Program, College of Micronesia;
- Dr. Lee S. Yudin, College of Agriculture and Life Sciences, University of Guam.

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 Industry Advisory Council'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 Industry Advisory Council:

- 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.;
- Ms. Rebecca Bishop-Yuen; Moana Technologies, Inc.;
- Dr. James Brock, Moana Technologies, Inc.;
- Mr. Randy Cates, Cates International Undersea Farms;
- Mr. Richard Croft, Pohnpei Natural Products;
- Mr. Simon Ellis, Mid Pacific Marine Consultants;
- Mr. John Gourley, Micronesia Clam Company;
- Ms. Linda Gusman, Island Aquaculture;
- Mr. Steve Katase, Royal Hawaiian Sea Farms;
- Mr. Robert Kern, Tropical Ponds of Hawaii;
- Mr. Jeff Koch, Mokuleia Aquafarm;
- Mr. Andrew Kuljis, Aquatic Farms;
- Mr. Richard Masse, Mangrove Tropicals;
- Mr. Dennis Mitchell, The Fish Shack;
- Mr. Ramsey Reimers, Robert Reimers Enterprises;
- Mr. Neil Sims, Black Pearl, Inc.;
- Dr. Richard Spencer, Hawaiian Marine Enterprises {Industry Advisory Council Chairman and ex officio member of the BOD}
- Ms. Anita Suta, Palau Community College
- Dr. Albert Tacon, Aquatic Farms
- Mr. Frank Toves, Aquaculture Culturists
- Mr. Ron Weidenbach, Hawaii Fish Company;
- Dr. Leonard Young, Hawaii State Aquaculture Development Program.

TECHNICAL COMMITTEE

The Technical Committee's members represent participating research institutions and state extension services, other state or territorial public agencies as appropriate, and non-profit private research institutions. The Technical Committee provides research expertise to address priorities set by the Industry Advisory Council. The Board of Directors appoints members for 3-year, renewable terms.

The Technical Committee:

- 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, University of Hawaii {Technical Committee Chairman and ex officio member of the BOD};

- Ms. Kristen Anderson, Hamilton Library, University of Hawaii;
- Dr. John Brown, College of Agriculture and Life Sciences, University of Guam;
- Mr. David Crisostomo, University of Guam Cooperative Extension Service;
- Mr. Simon Ellis, Land Grant College Program, College of Micronesia;
- Dr. Maria Haws, Pacific Aquaculture and Coastal Resources Center;
- Dr. Kevin Hopkins, Pacific Aquaculture and Coastal Resources Center;
- Dr. Robert D. Howerton, Sea Grant Extension Service, University of Hawaii;
- Mr. Tom Iwai, Anuenue Fisheries Research Center;
- Dr. Charles Laidley, The Oceanic Institute;
- Dr. PingSun Leung, University of Hawaii;
- Dr. Shaun Moss, The Oceanic Institute;
- Dr. Anthony Ostrowski, The Oceanic Institute;
- Mr. Vernon Sato
- Dr. Robin Shields, The Oceanic Institute;
- Dr. James Szyper, Sea Grant Extension Service, University of Hawaii at Hilo;
- Dr. Clyde Tamaru, Sea Grant Extension Service, University of Hawaii.

Executive Summary

PROGRAM SCOPE

During 2002, the Center for Tropical and Subtropical Aquaculture completed work on projects funded under its Twelfth Annual Plan of Work and continued work on projects funded under its Thirteenth and Fourteenth Annual Plans of Work. In addition, in August 2002, CTSA initiated work on projects developed under its Fifteenth Annual Plan of Work and began developing its Sixteenth Annual Plan of Work.

Eleven projects were funded under CTSA's 15th year program, which was approved by CTSA's Board of Directors on January 22, 2002. Six were continuations of projects begun under the programs of previous years and five are new projects.

Since the inception of CTSA in 1988, it has funded 161 research, demonstration, development and extension projects. Twelve projects were active during 2002. These projects fall into six categories:

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

These projects address 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

This project addresses marketing and economics:

* Marine Food Fish Seedstock Production

These projects address development of new technologies:

* Marine Food Fish Seedstock Production
* Aquaculture of Marine Ornamentals
* Reproduction and Selective Breeding of the Pacific Threadfin

This project addresses demonstration and adaptation of known technologies:

* Transitioning Hawaii's Freshwater Ornamental Industry

A brief listing of the principal accomplishments of the active projects in these categories during 2002 is presented below. Details on each project's funding, participants, objectives, anticipated benefits, progress and future plans are presented in the Progress Reports section.

Information Dissemination

Library Aquaculture Workstation

During the Year 13 reporting period, there were 23,668 queries. PRAISE staff responded to 730 requests for direct assistance by returning to users 1,762 articles totaling 22,720 pages delivered almost exclusively by email.

In the Year 14 reporting period, there was a total of 14,525 queries. The PRAISE staff responded to 256 requests for direct assistance by returning to users 838 articles totaling 8,401 pages delivered almost exclusively by email.

Publications

The quarterly newsletter was printed and disseminated in December, March, June and September. The CTSA staff assisted with the creation, production and distribution of three CTSA manuals. The homepage was redesigned to make it more user-friendly and to allow users to download any CTSA publications they choose.

Extension Support to Further Industry Development

Disease Management & Virology Service for Hawaiian Aquaculture

Project work group members developed primary cell lines for moi, (Polydactylus sexfilis), the green swordtail (Xiphophorus helleri), the angelfish (Pterophyllum scalare), the gray mullet (Mugil cephalus), and the snakehead, (Channa striatus).

Fish viral diagnostic support continued to be provided and two fish disease workshops were conducted.

Project work group members also controlled an outbreak of Prymnesium parvum from a large lake stocked with mullet and an outbreak of Ich. They also assisted commercial high health shrimp farms to establish a new founder stock of SPF Penaeus japonicus.

Through work conducted by work group members, it was also demonstrated that warm water temperature (>31 ºC) is an effective way to control TSV.

Transitioning Hawaii's Freshwater Ornamental Industry

During the reporting period, project work group members demonstrated 1) docosahexanoate (DHA) is not essential for maturation and spawning of freshwater ornamental fish; 2) high density culture of Moina using Chlorella and "green water" from recirculating culture systems; and 3) stocking density effects on growth, survival and sex ratio on swordtails.

Development of Black-Lip Pearl Oyster Farming in Micronesia

A pearl oyster hatchery was set up in Pohnpei, and 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, three technicians from the Marshall Islands Marine Resources Authority and Black Pearls of Micronesia (BPOM), one aquaculture extension agent from the College of the Marshall Islands / University of Hawaii at Hilo, and one land grant aquaculture researcher received training in hatchery operations, algal culture and larval rearing. Pearl oyster spawning was unsuccessful and the hoped for quantity of pearl oyster spat was not produced, but the opportunity to work with a consultant and test the BPOM hatchery was a valuable experience. An effort is being made to document the hatchery's operational procedures and assess its worth.

The economic modelling work is projected to be finished in early 2003. The data has been collected and the model is in the process of being developed and tested.

Marketing and Economics

Marine Food Fish Seedstock Production

During the three-year project, the project has successfully met its major goal of assisting in the establishment of a marine food fish industry in the Pacific region. Several notable achievements were made that contributed to this effort including the establishment of a year-round supply of fertilized Pacific threadfin eggs and the transferring of broodstock technologies to the local industry. Threadfin technology was also further refined through the development of an economic model and the transfer of fingerlings to commercial operations. Transfer was completed under a phased-out program that delivered 273,449 fingerlings in Year 1, 215,896 fingerlings in Year 2, and 178,514 fingerlings in Year 3. A commercial operator is now supplying fingerlings to the local industry. Additionally, as a direct result, multiple small-scale growout operations were established in the islands and large-scale, offshore production of threadfin was initiated. Captive farmed threadfin products are now appearing in local restaurants and retail markets and are being sold both to mainland and international markets.

Development of New Technologies

Marine Food Fish Seedstock Production
See above.

Aquaculture of Marine Ornamental Species
Centralized broodstock populations of yellow tang and flame angelfish for seed production continue to be maintained at The Oceanic Institute, which enabled the project to obtain natural spawning of yellow tang and increase its fertility rates with isolated spawns being up to 88% fertile.

Flame angelfish broodstock husbandry protocols have been optimized with the completion of studies demonstrating a strong relationship between tank size and both fecundity and spawn fertility.

Reproduction and Selective Breeding of the Pacific Threadfin
Natural spawning experiments have not been successful at this point and experiments are currently being revised in attempts to derive natural spawning. Data from the genetic selection project suggests that size, rather than environmental or behavioral conditions appear to be important in determining the timing of sexual development and sex change in captive stocks of Pacific threadfin.

Demonstration and Adaptation of Known Technologies

Transitioning Hawaii's Freshwater Ornamental Industry
See above.

A Look Ahead at Year 16

The development of the Year 16 program was initiated in February 2002 at the annual meeting of the Industry Advisory Council (IAC). The IAC reviewed the progress of funded projects and recommended Year 16 research priorities based on concepts submitted by farmers and researchers from the region and beyond that would aid industry development. Members identified several project areas for funding priority, from which a call for pre-proposals was disseminated. Forty-three pre-proposals were submitted in response to the call and thirteen were then asked to submit a full proposal. Fifteen proposals were reviewed by the Technical Committee in October 2002 and will be forwarded to the Board of Directors as the Year 16 Plan of Work in February 2003:

1. Addressing some of the Critical Bottlenecks to Commercially Viable Hatchery and Nursery Techniques for Black-Lip Pearl Oyster Farming in Micronesia - Year 1
2. Aquaculture of Hawaiian Marine Invertebrates for the Marine Ornamental Trade - Year 2
3. Aquaculture of Marine Ornamental Species - Year 4
4. Aquaculture Potential of the River catfish Pangasius sutchi - Year 1
5. Disease Management in Hawaiian Aquaculture - Year 10
6. Economic Feasibility for Freshwater Ornamental Fish Growers in Hawaii to Market their Products Directly to West Coast Retailers - Year 1
7. Grant Opportunities for Hawaii Aquaculture Businesses: Information Resources, Proposal Preparation Training, and Practical Assistance - Year 1
8. Improvement of Growout Techniques for Hatchery-Based Blacklip Pearl Oyster Farming in Pohnpei State, FSM, and in Other Micronesian Regions - Year 1
9. Improving Sturgeon Hatchery Efficiency in Hawaii - Year 2
10. Library Aquaculture Workstation - Year 16
11. Reproduction and Selective Breeding of Pacific Threadfin - Year 2
12. Pacific Threadfin Fingerling Transport Technology Development - Year 1
13. Population Genetics of the Black-Lipped Pearl Oyster (Pinctada margaritifera) - Year 1
14. Postharvest Handling and Storage of Limu - Year 1
15. Publications - Year 14

In August, CTSA began its two-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 October 30 and 31, 2002. Once the final versions of the proposals are received and reviewed by the Program Review Delegation in January

2003, they will be incorporated into the Sixteenth Annual Plan of Work, and presented to the Center's Board of Directors for approval in February 2003. Following Board approval, the plan will be submitted to the U.S. Department of Agriculture Cooperative State Research, Education and Extension Service for final approval.

Progress Reports

An individual summary of the principal accomplishments of the active projects in these categories during 2002 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.

Disease Management in Hawaiian Aquaculture, Year Eight

Reporting Period

October 1, 2001 – September 30, 2002 (Year 8)

Funding Level

Year 1	$41,638
Year 2	$63,725
Year 3	$45,956
Year 4	$44,030
Year 5	$66,451
Year 6	$51,934
Year 7	$81,991
Year 8	$67,902
TOTAL	$463,627

Participants

Dr. Robert Bullis, Technical Director of Animal Health, The Oceanic Institute

Dr. Yuanan Lu, Assistant Researcher, Retrovirology Research Laboratory, The University of Hawaii at Manoa

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

Objectives

1. Establish a continuous cell line from each of the following fishes: the clownfish (Amphiprion ocellaris), the snakehead (Channa striatus) and the omilu (Caranx melampygus).

2. To provide culture diagnostic services for the isolation of fish viruses from 10 case submissions of marine or freshwater fishes.

3. Conduct two fish health training classes and provide effective communication of the class schedule.
4. Provide technology transfer to the industry.

Anticipated Benefits

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.

Disease losses have been high in some groups of ornamental and food fishes in Hawaii. Sometimes, the causes for the these losses have been identified and in other cases the cause is elusive. We regularly encounter cases of "new" diseases. Two recent examples are an idiopathic, diffuse myopathy in the body musculature of cultured juvenile clown fish and sea horses and a high mortality disease of fry and juvenile goldfish with nuclear inclusion bodies and intranuclear virus-like particles.

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. Without appropriate cell culture systems, development of preventative strategies for viral diseases and the inspection of batches of juvenile fish for health certification are severely limited. Same fish host cell lines are an essential first line of defense for the detection and surveillance of viruses important to fish health programs. For the work in Year 8, we proposed to continue this effort and to develop three more new cell lines from three different fish species.

In 1999, new, probably 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 8 project.

Work Progress and Principal Accomplishments

Objective 1 - Establish a continuous cell line from each of the following fishes: the clownfish (Amphiprion ocellaris), the snakehead (Channa striatus) and the omilu (Caranx melampygus).

Clownfish cell culture

Thus far we have tried two times to establish cell culture from clownfish. We observed some minor cell growth from the explants. However, primary clown cell culture grows very slowly and no successful passage has been made. It is necessary to optimize the growth condition for this species' cell cultures.

Snakehead cell culture

There have been three attempts to establish cell culture from snakehead fish. So far we have successfully sub-cultured cells from the heart and kidney (13th passage), snout and muscle (19th passage) and swim bladder (14th passage). Although we observed primary cell growth from the spleen and the liver, these cells did not survive after initial passage. We have preserved sub-cultured cells in liquid nitrogen from their early passages. In addition to the subculture of snakehead cells, we are currently characterizing these cell lines in terms of their optimal growth condition, chromosome, plating efficiency and viral susceptibility. A manuscript is in preparation.

Omilu cell culture

We have successfully established two cell lines from omilu: OMMS (muscle) and OMF (fins). Since the initiation of the primary cultures on June 25, 2002, these cells have been sub-cultured over 17 times. We have characterized these cell lines in terms of their biological properties, including plating efficiency, stability in liquid nitrogen storage, chromosomal typing and viral sensitivity. A manuscript is in preparation.

Objective 2 - To provide culture diagnostic services for the isolation of fish viruses from 10 case submissions of marine or freshwater fishes.

Three cases of diseased fish suspected or known to be infected by a pathogenic fish virus were evaluated by cell culture method in the period covered by this report. The species submitted was the Chinese catfish (Clarius fuscus). No evidence of a viral infection was detected in any of the cells recovered from the sick fish.

Estimated economic impact:

Control of an outbreak of *Cryptocaryon* sp. $ 32,000

Control of a mortality caused by gas bubble disease $ 210,000

Control of an outbreak of *Neobenedenia* $ 81,000

Objective 3 - Conduct two fish health training classes and provide effective communication of the class schedule.

Two fish health training classes were held during this reporting period. The first class was held on the island of Maui on November 10-11, 2001. The second class was held in Hilo on October 26, 2002.

Objective 4 - Provide technology transfer to the industry.

Two articles were provided to CTSA during the reporting period, Establishment and Growth of Cell Lines from Aquacultured Fish in Hawaii for Diagnosis and Isolation of Pathogenic Viruses and The Reduced Impact of Taura Syndrome Virus on Litopenaeus vannamei Held Under Hyperthermic Conditions.

Impacts

The estimates shown on the previous page regarding economic impact were made by the staff from the companies that received assistance from this program. The estimates of economic impact are based on the known or expected gain, or the reduction in the anticipated loss, once the disease problem 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 examples listed below, the farm personnel implemented the strategies that were recommended. To a great extent, the success of a given strategy reflects their efforts. The establishment of a new shrimp species in Hawaii is the direct result of quarantine facilities provided by the Aquaculture Disease Prevention program. 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

Publications in Print

Lu, Yuanan, Lijun He, Dee Montgomery-Brock, Jim Brock, Robert Bullis and Richard Yanagihara. Establishment and Growth of Cell Lines from Aquacultured Fish in Hawaii for Diagnosis and Isolation of Pathogenic Viruses. CTSA Regional Notes, Vol. 12. No. 4, Fall 2001.

Montgomery-Brock, Dee, Ron Y. Shimojo and Robert A. Bullis. The reduced impact of Taura Syndrome Virus on Litopenaeus vannamei held under hyperthermic conditions. CTSA Regional Notes, Vol. 13. No.1, March 2002.

Papers presented

Montgomery-Brock, Dee and Robert A. Bullis. Disease Prevention Through Proper Husbandry and Management. Fourth Annual Hawaii Aquaculture Conference 2002. Windward Community College, May 8, 2002.

Transitioning Hawaii's Freshwater Ornamental Industry, Years One & Two

Reporting Period

January 1, 2000 - May 1, 2002 (Year 1 - final report)
October 1, 2001 - September 30, 2002

Funding Level

Year 1	$100,000
Year 2	$70,000
TOTAL	$170,000

Participants

Year 1

Dr. Clyde Tamaru, Extension Agent, Sea Grant Extension Service

Kathleen McGovern-Hopkins, Extension Agent, Sea Grant Extension Service

Dr. Harry Ako, Professor, University of Hawaii at Manoa

Dr. James Brock, Aquaculture Development Program, Department of Agriculture

Jennifer Olson, Graduate Student, University of Hawaii

Matt Lyum, Graduate Student, University of Hawaii

Lena Asano, Graduate Student, University of Hawaii

Eri Shimizu, Graduate Student, University of Hawaii

Year 2

Dr. Clyde Tamaru, Extension Agent, Sea Grant Extension Service

Kathleen McGovern-Hopkins, Extension Agent, Sea Grant Extension Service

Dr. Harry Ako, Professor, University of Hawaii at Manoa

Dr. James Szyper, Sea Grant Extension Agent, University of Hawaii at Hilo

Dr. Robert Howerton, Sea Grant Extension Agent, University of Hawaii

Brandon Avegalio, Graduate Student, University of Hawaii

Justin Iwai, Graduate Student, University of Hawaii

Eri Shimizu, Graduate Student, University of Hawaii

Kelly DeLemos, Graduate Student, University of Hawaii

Objectives

Year 1

1. Conduct laboratory and field studies on sex determination in live bearers.

2. Demonstrate live feeds production systems to practicing freshwater ornamental aquafarmers.

3. Investigate the role of Highly Unsaturated Fatty Acids (HUFAs) in the culture of freshwater ornamentals.

4. Provide technical assistance in the form of verbal consultations, written information, site visitations, workshops, manuals and production handouts.

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 cartenoids 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.

Anticipated Benefits

The Center for Tropical and Subtropical Aquaculture, Sea Grant Extension Service, Department of Environmental Biochemistry, Aquaculture Development Program, Windward Community College, Hawaii Institute of Marine Biology and recently, the Pacific Business Center, have collaboratively supported the development of an ornamental fish culture industry since 1993. In addition to providing an ornamental fish specialist for technical assistance, previous projects focused on broodstock and fry amplification of species that reflected the varied life histories (e.g., livebearers, spontaneous egg layer, egg layer requiring hormonal induction of spawning, hatchery operations) that would be encountered in the freshwater ornamental fish industry. The activities under the current project both address constraints that were identified during previous projects (e.g., sex ratio of swordtails) or are obvious challenges that face the expansion and diversification of Hawaii's industry. Project workgroup members have always felt that addressing challenges before they impact the industry (e.g., moina, rotifer and recirculating system research) should also be part of these projects. Lastly, providing technical assistance is the mainstay of all of the previous projects and replacement of the fish specialist allows for the continued flow of information to the appropriate end users. All of the activities are focused on the development of a strong industry in the state producing freshwater ornamentals that are noted for their quality and consistency.

Year 1

Objective 1: Conduct laboratory and field studies on sex determination in livebearers.

The main benefit of completing this objective would be understanding the factors that are involved in determining sex ratio in swordtails. Current market demands a 2:1 female to male ratio which is quite different from reports from the field where female to male sex ratios as skewed as 9:1 have been reported. Understanding the contributing factors to sex ratio allows extension and outreach personnel to make recommendations as to appropriate best management practices that will avoid the over production of the female sex.

Objective 2: Demonstrate live feeds production systems to practicing freshwater ornamental aquafarmers.

There were two desired outcomes for the activities conducted under this objective. The first was to find alternative food organisms that could replace brine shrimp in the larval rearing and nursery stages. This is in light of the increasing prices and variation in availability of brine shrimp cysts. The second desired outcome was to introduce fish farmers to live feeds that were smaller than brine shrimp nauplii. Use of these smaller prey items allows for the rearing of tropical ornamentals that cannot eat the brine shrimp nauplii due to their initial mouth size. Alleviation of this constraint results in the continued diversification of the number of kinds of fishes that can be cultured by Hawaii's fish producers.

Objective 3: Investigate the role of Highly Unsaturated Fatty Acids (HUFAs) in the culture of freshwater ornamentals.

Understanding the nutritional requirements and the consequences of not meeting those requirements is one of the major achievements that has resulted in the growth of the aquaculture industry in general, particularly in the marine arena. The major benefit from the activities under this objective is that the preliminary HUFA requirements for freshwater ornamental fishes will be known. With this understanding , the proper recommendations for a suitable maturation diet, larval diet and growout diet can be made to farmers.

Objective 4: Provide technical assistance in the form of verbal consultations, written information, site visitations, workshops, manuals and production handouts.

No matter what the research findings are, it is of little value if the results are not disseminated in a timely manner. The challenge and desired outcome for extension and outreach personnel would be to provide the information in a format that will result in a behavioral change (e.g., use of a recommended feed or feeding strategy).

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 foodfish 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 proposed project seeks to have "experts" provide the latest marketing information to be delivered in a conference/workshop format. The information presented will allow farmers, researchers, and administrators to decide on how to best approach marketing of freshwater ornamental fish products and anticipate and identify potential technical constraints.

Objective 2: Investigate use of cartenoids for color enhancement.

The results of this objective will provide the foundation to make sound recommendations as to the feeds and use of color enhancers that result in the highest quality freshwater ornamental fish. Insuring the fishes 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. There is also another aspect of this research. Breeding practices currently rely on the chance mating of male and female individuals. With artificial insemination, selected individuals can be mated on demand and undoubtedly provide a more effective means to produce new varieties of hybrids. This would 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 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. The activity being proposed is to improve the dependability of the spawning technique by incorporating the latest development in inducing cultured fish to spawn.

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

The morphology and coloration of the male sex of the swordtail is in large part its main attraction. Culture methods that result in a preponderance of females ultimately results in lower profitability. Uncovering the parameters that influence the sex ratio of the swordtails will allow for establishing a best management practice that is 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.

Work Progress and Principal Accomplishments

Year 1

> Results at a glance...
>
> Demonstrated docosahexanoate (DHA) is not essential for maturation and spawning of freshwater ornamental fish.
>
> Demonstrated high density culture of *Moina* using *Chlorella* and “green water” from recirculating culture systems.
>
> Demonstrated stocking density effects on growth, survival and sex ratio on swordtails.
>
> Held 10 workshops.

Objective 1 - Conduct laboratory and field studies on sex determination in livebearers.

Based on an experiment we conducted with one-week old pineapple swordtails that were at a mean weight of 21 mg, average survival appeared to be negatively correlated with the increase in stocking density. However, only the tanks that were stocked at a density of 6 fish/L were found to be statistically smaller in size. Average body weight was found to be negatively correlated with stocking density with each treatment density resulting in significantly different average body weight. With regard to the resulting sex ratio in response to stocking density, the data is extremely misleading if one were to simply score the number of females versus males that resulted. A preponderance of females results in all groups because fish that are smaller than 30 mm in total length are scored as females irrespective of the stocking density. If the diagnostic characteristic of males was to include the presence of the “sword” on the caudal fin, it is clear that these individuals are only found in size classes of fish that are greater than 30 mm in total length. If the individuals that are smaller than 30 mm in total length are removed from the scoring of the sex ratio, statistically there are no differences in the sex ratio in response to stocking density.

Based on the results of these experiments, it would appear that stocking densities between 1-3 fish/L would be the highest ratio one would use for the culture of swordtails in a recirculating system.

To confirm the results, a large-scale (2000-L) outdoor rearing trial with the red velvet strain of swordtails was conducted using a stocking density of 3 fish/L. This activity was conducted during the reporting period at WCC. This experiment was coupled with the testing of bioremediation systems. Overall survival was very poor (13%) due to a disease outbreak and as anticipated, the control tanks (no biofilter) and one of the treatments (submerged biofilter) did very poorly; the apparent cause being the amount of fish and the feeding regimen overcame the capacity to control water quality parameters in tanks with no biofilter (control) and those with submerged biofilters. Of the surviving fish, sex ratios were consistent with those reported for the aquaria where the sex ratio apparently is related to the size of the fish. The percentage of males present in groups of fish that are greater than 45 mm in total length approach a sex ratio of 1:1. The implication of the results are that the growout systems for swordtails will need to maximize growth to result in suitable quantities of males.

Objective 2 - Demonstrate live feeds production systems to practicing freshwater ornamental aquafarmers.

Small-scale (8/L) trials using a variety of "green water" (tilapia, guppy, recirculating systems) were tested and very high densities were achieved using the resulting water present in tanks with recirculating systems.

Objective 3 - Investigate the role of Highly Unsaturated Fatty Acids (HUFAs) in the culture of freshwater ornamentals.

Earlier investigations demonstrate that there are similarities in essential fatty acids for various live maturation or conditioning feeds used in the ornamental industry. Arachidonic acid appears to play an essential role as opposed to the Docosahexanoate which is in striking contrast to what has been reported for marine fishes. The results obtained from these experiments focused on determining the effects of essential fatty acids on maturation and spawning.

Objective 4 - Provide technical assistance in the form of verbal consultations, written information, site visitations, workshops, manuals and production handouts.

Kathleen McGovern-Hopkins was hired as an assistant extension agent with the University of Hawaii Sea Grant Extension Service in September 2000. During the reporting period, the completion of the repairs to the Windward Community College (WCC) aquaculture complex were made and the facility is in compliance with the UH Animal Care and Use office and that of the UH Safety Office.

The manual for the commercial production of swordtails was completed during the reporting period and was the main reason for the no cost extension. Two other manuals are in the planning stages and they are: 1) An artificial insemination of livebearers manual and 2) a live feeds production manual.

During the reporting period a total of 10 workshops were conducted on various islands.

Year 2

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

The Center for Tropical and Subtropical Aquaculture, the University of Hawaii Sea Grant Extension Service and the Aquaculture Development Program collaborated together to organize a series of workshops titled, "Transitioning Hawaii's Freshwater Ornamental Fish Project, Initiative for Future Agriculture and Food Systems." The workshops focused on the marketing of aquatic products and were held at the Univeristy of Hawaii at Manoa and Windward Community College from September 12-14, 2002. The overall goal of the workshops was to introduce marketing concepts to participants through the interactive process of developing a marketing plan and the presentation of 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 so that one can be more competitive? What are the thought and planning processes that enable one to do so? Lastly, regarding a specific industry like the freshwater ornamental fish industry, two wholesalers (one from the mainland and one in Hawaii) discussed their respective companies, what their expectations are if they are to be repeat customers and what it takes to survive business in Hawaii.

Objective 2 - Investigate use of cartenoids for color enhancement.

During the reporting period, feed laced with 1% NatuRose was prepared and sent 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 male dwarf gouramis fed treated and untreated feed.

Objective 3 - Validate techniques for artificial insemination of livebearers.

Initial results confirm and validate the inheritance of the lyretail trait, and the use of artificial insemination techniques 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 lyretial trait, mark a milestone in fish breeding.

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

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. This was a step above what has been previously described. Both the rainbow sharks and redtail sharks underwent final maturation and spawned naturally overnight using Ovaprim according to the manufaturer'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 exhibitied 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 the manual stripping and fertilizing of the gametes.

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.

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 becomes apparent that the larger males are the ones with this characteristic (effect of stocking density), and there is also a higher percentage of male individuals that possess it.

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 rates at higher densities. The lower survival rate also translates into larger individuals at the end of the growout period.

Objective 6 - Conduct technology transfer activities.

Site visits and workshops were conducted on a regular basis In addition, three experiments are currently being conducted with the hopes of transferring the findings to the public: "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."

Work Planned

Year 2

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

An article summarizing the workshop's main points has been submitted to CTSA Aquatips and also to the Hawaii Aquaculture Association for printing in their next newsletter. A follow-up with C.L. Cheshire of the Pacific Business Center and Rick Spencer regarding the marketing survey for freshwater ornamentals is to be conducted. The intent is to work collaboratively to result in a handout that summarizes the findings of the survey and then distribute them to the aquarium community.

Objective 2: Investigate use of cartenoids for color enhancement.

Additional on-site farm tests are to be conducted with interested persons. One request has been generated from Kauai as a result of the collaborative work with the Pacific Tropical Ornamental Fish Project.

Objective 3: Validate techniques for artificial insemination of livebearers.

It has just been recently confirmed that three individuals are homozygous for the lyretail trait and that they were created through artificial insemination. A workshop series on each of the islands is being planned during the next reporting period and is to be conducted in collaboration with the USDA supported Initiative for Future Agriculture and Food Systems. The first workshop is to be implemented on December 14, 2002 and is designed to provide an explanation of the genetics of the lyretail trait and hands-on training in the technique of artificial insemination. In addition, a short manual is being produced on the artificial insemination technique and will be submitted for printing during the next reporting period. At the workshops, farms that are interested in the technique will be solicited to conduct on-farm trials. Results of the insemination work are being summarized for submission to the World Aquaculture Society Conference in Brazil. A summary manuscript is being planned for submission to the journal, Progressive Fish Culturists.

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

The results of the spawning trials with the redtail and rainbow sharks that were conducted under this objective will be summarized in a newsletter article to the Honolulu Aquarium Society. A joint Sea Grant manual on the induction of spawning is being discussed with non-paid project work group members and a draft should be available in early 2003. Discussions have already been made between project work group members and the USDA IFAFS project work group on the production of a workshop series during 2003 on all islands that covers induction of spawning of freshwater fishes. During the next spawning season (2003), the tinfoil barbs will be investigated again. During these trials, establishing the time of ovulation will be determined in order to utilize manual stripping and artificial fertilization to produce tinfoil barb larvae. It is being planned to submit a summary manuscript of the spawning results for the redtail and rainbow sharks next year to the journal Progressive Fish Culturists.

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

Discussions are ongoing among Sea Grant Extension staff and project work group members to develop a flyer summarizing the results of the research conducted.

Objective 6: Conduct technology transfer activities.

This activity will remain as the core activity for the project. Three manuals are targeted to be produced during the next reporting period. One will be on the production of Moina which will be a culminating deliverable for work conducted during previous projects and also include information on the latest availabe type of algal feeds. The second will be a step-by-step guide on artificial insemination of the lyretail swordtail. The third will be a manual on induction of spawning of freshwater fishes. Two manuscripts (artificial insemination and induced spawning) will be submitted to peer reviewed journals for publication. In addition, a workshop series is being planned for all islands in collaboration with the USDA supported IFAFS project. It is anticipated that there will be a total of six workshops conducted in 2003. Project work group members will also continue to support the PTOFP in review and awarding of projects.

Impacts

The impacts for the marketing workshop will probably not be felt immediately after the conclusion of the project. It is designed to provide the most up-to-date information for Hawaii's producers in what the expectations of the ornamental fish market is on the mainland. From that information, it remains to be determined how farmers will work collaboratively to meet the expectations of the mainland market.

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 that Hawaii's fish are perceived as being of "good" quality by buyers on the US mainland. 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 a homozygous individual can be produced using this technique. The workshops are designed to provide growers with a tool to improve their production of lyretail swordtails and ultimately, their profits. A more long-term impact that is being considered is to generate interest among hobbyists and others in beginning trials to produce new varieties of livebearers. In that way, Hawaii's mark on the production of fancy livebearers can be built upon.

One of the goals of current and previous projects has been for our project working group to serve as a forum for discussion regarding challenges that face the industry. Our collaborative relationship with the PTOFP project personnel is one indication that this goal has been accomplished to some degree. It is now the major forum in which the process and progress of the PTOFP project is evaluated. It is anticipated that the outreach and education components of the project will remain as one of the mainstays of the project and continue to have a lasting impact on the growth of the freshwater ornamental fish industry.

Publications in Print, Manuscripts and Papers Presented

Year 1

Publications in Print

Ako, H., L. Asano and C.S. Tamaru. 2000. Factors affecting sex ratios in the swordtail, Xiphophorus helleri. University of Hawaii Sea Grant College Program, Makai, Vol. 22, No. 10.

Asano, L., H. Ako, E. Shimizu and C.S. Tamaru. 2001. Bioremediation production systems for ornamental fish. Aquaculture Research (Submitted).

Tamaru, C.S, H. Ako, L. Asano, L. Pang and K. McGovern-Hopkins. 2001. Growth and enrichment of Moina macrocopa (Straus) for use in freshwater ornamental fish culture. Aquaculture Research (submitted).

Tamaru, C.S., H. Ako and L. Pang. 2001. The effects of three maturation diets on the spawning of the armored catfish Corydoras aeneus (Gill). Aquaculture Research (submitted).

Tamaru, C.S. and M. Lyum. 2000. Anesthetizing freshwater ornamental fishes using clove oil. University of Hawaii Sea Grant College Program, Makai, Vol. 22, No. 11.

Tamaru, C.S., K. McGovern-Hopkins, G. Takeshita, F. Morita and M. Yamamoto. 2001. The breeding of lyretail swordtails. I'a O Hawai'i. Volume 2001. Issue 6.

Papers Presented

Asano, L., H. Ako, E. Shimizu and C.S. Tamaru. 2001. Bioremediation production systems for ornamental fish. AQUARAMA 2001, 2nd World Conference on Ornamental Fish Culture. Singapore, May 31 - June 2, 2001. Book of Abstracts, pp. 34.

Shimizu, E., L. Asano, H. Ako and C.S. Tamaru. 2001. Enhancing aquaculture profitability through the use of bioremediation. University of Hawaii, College of Tropical Agriculture and Human Resources, Undergraduate Symposium. (Best Undergraduate Oral Presentation).

Tamaru, C.S. 2001. Tropical freshwater ornamental fish industry in Hawaii. Progress and future prospects. Third Annual Hawaii Aquaculture Association Conference. Windward Community College, Kaneohe, Hawaii. March 2, 2001.

Tamaru, C.S. 2001. Fancy Livebearer Production. Monthly Meeting, Honolulu Aquarium Society, Honolulu, Hawaii. January 5, 2001.

Tamaru, C.S., H. Ako, L. Asano and K. McGovern-Hopkins. 2001. Growth and enrichment of moina for use in freshwater ornamental fish culture. Aquarama 2001. 7th International Aquarium Fish and Accessories Exhibition and Conference. May 31 - June 3 2001. Singapore.

Tamaru, C.S., L. Pang and H. Ako. 2001. The effects of three maturation diets on the spawning of the armored catfish Corydoras aeneus . Aquarama 2001. 7th International Aquarium Fish and Accessories Exhibition and Conference. May 31 - June 3 2001. Singapore.

Year 2

Publications in Print

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. Volume 2002, Issue 6.

McGovern-Hopkins, K., G. Takeshita and C.S. Tamaru. 2002 On the use of artifical insemination for the commercial production of lyretail swordtails. Aquatips, Regional Notes, Center for Tropical and Subtropical Aquaculture. Vol. 13, No. 2, June 2002.

McGovern-Hopkins, K. and C.S. Tamaru. 2002. Raising boesemani rainbow, Melanotaenia boesemani, larvae using an artificial feed. I'a O Hawai'i. Volume 2002. Issue 5.

Tamaru, C.S., K. McGovern-Hopkins and Mike Yamamoto. 2002. Can an artificial feed be used to raise siamese fighting fish Betta splendens larvae? I'a O Hawai'i. Volume 2002, Issue 9.

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. Volume 2002, Issue 7.

Papers Presented

Shimizu, E., H. Ako and C.S. Tamaru. 2002. Behavioral limitations for high intensity ornamental fish culture. Fourth Annual Hawaii Aquaculture Conference 2002. Windward Community College, May 8, 2002.

Library Aquaculture Workstation (PRAISE), Years 13 & 14

Reporting Period

June 1, 2000 - July 8, 2002 (Year 13 - final report)
October 1, 2001 - September 30, 2002 (Year 14)

Funding Level

Year 1	$7,000
Year 2	$6,700
Year 3	$6,000
Year 4	$7,000
Year 5	$20,000
Year 6	$14,100
Year 7	$28,000
Year 8	$49,000
Year 9	$25,000
Year 10	$30,000
Year 11	$24,000
Year 12	$23,000
Year 13	$28,850
Year 14	$25,320
TOTAL	$293,970

Participants

Year 13

Kristen Anderson, Reference Librarian, University of Hawaii at Manoa

Lois Kiehl-Cain, Assistant, University of Hawaii at Manoa

Year 14

Kristen Anderson, Reference Librarian,University of Hawaii at Manoa

Lois Kiehl-Cain, Assistant, University of Hawaii at Manoa

Objectives

Year 13

1. Continue to provide established services.
2. Maintain ongoing programs for user education.
3. Develop innovative Web pages with an educational focus.
4. Provide technology transfer enhancements.

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.

Anticipated Benefits

Swift and accurate dissemination of information allows practitioners to be aware of the latest progress in all phases of the industry. Teaching them the skills needed to obtain this information makes them self-sufficient. Providing a consolidated resource where they can utilize their skills profits the entire community.

Work Progress and Principal Accomplishments

Year 13

Objective 1: Continue to provide established services.

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

Based on rates one would pay to the information industry's major suppliers, the dollar value for our primary service may be presented as follows:
23,688 queries averaging 3 minutes or
1,183 hours online @ $60/hr = $70,980
1,762 articles @ $18.00/ea. = $31,716
Total $102,696 (Year 13)
14,525 queries averaging 3 minutes or
726 hours online @ $60/hr = $43,560
838 articles @ $25.00/ea. = $20,950
Total $64,510 (Year 14)

Objective 2: Maintain ongoing programs for user education.

The Principal Investigator attended the Pacific Islands Association of Libraries and Archives Conference on Guam. The PI participated in the pre-conference workshop on digitization in preparation for work on the gray literature as well as providing a demonstration during exhibit periods of the services PRAISE offers. The PI and the Assistant attended the Hawaii Aquaculture Association Conference at Windward Community College on Oahu and passed out brochures and ran a PRAISE PowerPoint presentation. Instruction sessions were also given on Oahu, including ongoing participation with the MASSIP (Micronesia and American Samoa Student Internship Program). The Principal Investigator and the Project Assistant continue to develop instructional enhancements for the website.

Objective 3: Develop innovative Web pages with an educational focus.

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

Objective 4: Provide technology transfer enhancements.

PRAISE staff are continuing to scan the abstracts from the proceedings of the PACON conferences for submission to Cambridge Scientific Abstracts. We have the capability to provide the Gray Literature database as a CD on demand. Full text is not yet available as receiving permission to digitize materials has not happened.

Year 14

Objective 1: Continue to provide established services.

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

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 are working on this phase of this year's project. Objective 2 is still a work in progress and developing a tutorial for utilizing it is being tested. We have been experiencing technical difficulties with the University's network and our internal system. We also lost our server support manager, but work is still underway.

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.

Work Planned

Provision of established services is ongoing. The PI and Assistant are working to develop the tutorial and will continue to request permission to digitize gray literature documents.

Impacts

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 13

23,668 queries averaging 3 minutes each or:

1183 hours online @ $60/hr =	$70,980
1,762 articles @ $18.00 ea. =	$31,716
Total	$102,696

In replying to 730 requests for direct assistance, 9,607 of those queries were emailed to PRAISE patrons.

The 1,762 articles represent 22,720 pages delivered almost exclusively by email. In addition, the staff responded to 353 miscellaneous requests. The PRAISE Web site is a bonus. It allows users to make requests online, publicizes research being done in the Pacific via the Gray Literature Bibliography and gives local vendors a venue to advertise themselves to the world.

Year 14

14,525 queries averaging 3 minutes each or:

726 hours online @ $60/hr =	$43,560
838 articles @ $25.00 ea. =	$20,950
Total	$64,510

In replying to 256 requests for direct assistance, 4,444 of those queries were emailed to PRAISE patrons. The 838 articles represent 8,401 pages delivered almost exclusively by email. In addition, the staff responded to 236 miscellaneous requests. The remote access to ASFA at CTSA and other sites as well as the PRAISE Web site are a bonus. The website 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.

Aquaculture of Marine Ornamental Species, Years Two & Three

Reporting Period

October 1, 2001 - September 30, 2002 (Year 2)
June 1, 2002 - September 30, 2002 (Year 3)

Funding Level

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

Participants

Year 2

Anthony C. Ostrowski, Ph.D., The Oceanic Institute

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

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

Karen Brittain, B.A., Waikiki Aquarium

Bruce A. Carlson, Ph.D., Waikiki Aquarium

Cynthia L. Hunter, Ph.D., Waikiki Aquarium

Year 3

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

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

Karen Brittain, B.A., Waikiki Aquarium

Cynthia L. Hunter, Ph.D., Waikiki Aquarium

Objectives

The solicitation for this research effort called on the resources of two leading groups engaged in marine ornamental fish culture in Hawaii, The Oceanic Institute and Waikiki Aquarium.

Overall Project Objectives

1. Establish broodstock husbandry, maturation and spawning protocols for selected high value ornamental finfish and invertebrate species.
2. Establish centralized broodstock populations for project use.
3. Determine appropriate husbandry conditions for maturation and spawning.
4. Resolve bottlenecks in mass culture of larvae.
5. Determine appropriate first feed items.
6. Define nutritional basis for improvements in production.
7. Develop appropriate larval rearing and live feeds production techniques for technology transfer.

Year 2

The Oceanic Institute

1. Maintain centralized broodstock populations of yellow tang (Zebrasoma flavescens) and flame angelfish (Centropyge loriculus).
2. Examine natural spawning of yellow tang. Determine the usefulness of hormones to induce spawning.
3. Expand existing flame angelfish populations, and determine appropriate husbandry conditions for optimum natural spawning.

4. Compare various live food organisms as first-feeds for angelfish, yellow tang, and/or other identified larvae.

5. Determine the usefulness of treated water in culture of ornamental larvae, and examine interactions of microbial populations on overall growth and survival of larvae.

The Waikiki Aquarium

6. Collect and identify wild zooplankton species from in-shore waters in South Oahu.

7. Determine uptake rate, survival and growth of flame angelfish larvae, and/or other available species fed wild zooplankton.

Year 3

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

5. Determine uptake rate, survival and growth of angelfish and yellow tang larvae, and/or other available species fed wild zooplankton collected from in-shore 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

Year 2

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

Centralized broodstock populations of both yellow tangs and flame angelfish were maintained throughout year 2 as a source of seed stock for larval rearing research. Yellow tang holdings included eight tanks, each containing 8 to 12 fish in approximately equal ratio of males to females, while flame angelfish holdings comprised four tanks of production pairs that spawned daily.

Objective 2: Examine natural spawning of yellow tang. Determine the usefulness of hormones to induce spawning.

Research on yellow tang continued to make progress during year 2, with the continued demonstration and documentation of daily spawning activity that began in January 2001. Peaks in spawning activity were observed in April and August 2001, although overall fertility rates were generally low. Fertility rates tended to increase over time, with isolated spawns being up to 88% fertile.

It was originally anticipated that yellow tang would not spawn in captivity without supplemental hormone administration, therefore a series of hormone-induction experiments was planned for project year 2. However, having obtained natural spawning (a species-first), it was decided to reassign the research resources to monitoring the spawning stocks at OI.

Objective 3: Expand existing flame angelfish populations, and determine appropriate husbandry conditions for optimum natural spawning.

Investigations to optimize tank size in terms of broodstock performance and minimizing setup costs were initiated in April 2001. Fifteen pairs of flame angelfish were purchased from a local supplier, quarantined and stocked, in triplicate, into each of 15 broodstock tanks ranging in volume from 40 L up to 750 L. The fish were maintained and monitored for egg production and spawn fertility on a daily basis beginning in early April through to experiment termination in early October 2001. A clear positive relationship was found between tank volume, onset of spawning and reproductive output of flame angelfish, with the 750 L holding tanks providing the best spawning performance.

Objective 4: Compare various live food organisms as first-feeds for angelfish, yellow tang, and/or other identified larvae.

The attainment of natural spawning by yellow tang provided small amounts of larvae for an initial diet trial during project year 2. One thousand yellow tang eggs were stocked into a 200 L rearing tank. On day 3 post-hatch, Tetraselmis sp microalgae was added at a density of 50,000 cells/ml and nauplii of calanoid copepods were added at 1.3/ml. Larvae were sampled for gut contents analysis on days 4 and 5 post-hatch. Copepod nauplii were identified in the yellow tang larvae's digestive tract on both sampling days. However, survival rate to day 5 post-hatch was low (1.8% of hatched larvae), preventing continuation of the trial beyond day 5. Further work will be required to establish the environmental requirements of this species during early development, before ascertaining the larvae's dietary requirements.

Objective 5: Determine the usefulness of treated water in culture of ornamental larvae, and examine interactions of microbial populations on overall growth and survival of larvae.

Results at a glance . . .

Successfully maintained centralized broodstock populations of yellow tang and flame angelfish for seed production.

Increased fertility rates of yellow tang, with isolated spawns being up to 88% fertile.

Optimized flame angelfish broodstock husbandry protocols with completion of studies demonstrating a strong relationship between tank size and both fecundity and spawn fertility.

Obtained natural spawning of the yellow tang.

A series of small-scale experiments was conducted with flame angelfish to determine appropriate egg handling methods and environmental conditions for yolk sac larvae, in preparation for egg disinfection trials. Experimental treatments were evaluated in terms of hatch rate and survival rate of larvae to day 4 post-hatch.

Flame angelfish embryos were found to be tolerant of mechanical handling up to approximately 1 hour prior to hatch. No significant difference was found in larval survival rate between containers stocked with newly-hatched larvae and those stocked with embryos. Yolk sac larvae could be maintained at densities up to 100/L without any detrimental effect on survival. Aeration had an adverse effect on survival in the experimental containers (250ml and 1L volume); insertion of water quality probes was also detrimental in the smallest containers (250ml). Addition of Nannochloropsis sp had no significant effect on larvae survival at a cell density of 100,000/ml, however at cell densities of 200,000/ml and above, survival rates were reduced (this may have been related to high levels of oxygen saturation). Survival rates did not differ among larvae reared in UV-sterilized well water, aged seawater, or artificial seawater.

Based on these findings, standardized protocols were established enabling survival rates of circa 70% to day 4 post-hatch. These protocols were then applied in experiments to define a safe and effective egg disinfection protocol for flame angelfish. Two disinfectants were tested: peroxyacetic acid and hydrogen peroxide.

Angelfish embryos were exposed to different disinfectant concentrations and contact times and the effects on surface sterility, hatch rate and larval survival rate measured. Embryos could be exposed to a 600ppm solution of peroxyacetic acid for up to 1 min, without reduction in hatch rate. Higher concentrations of peroxyacetic acid were required to obtain full surface sterility, but this was accompanied by high embryo mortality. Immersion of embryos in a 3% solution of hydrogen peroxide for 5 minutes reduced more than 99% of bacterial loading without adversely affecting hatch rate. More reproducible sterilization and survival results were obtained with hydrogen peroxide than with peroxyacetic acid, therefore hydrogen peroxide was adopted for routine use. With both types of chemical, angelfish larvae exposed to disinfectant survived longer in small, static rearing containers than control groups rinsed in clean seawater.

Studies were also carried out 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 receiving matured water plus T. Isochrysis sp. Nannochloropsis sp 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.

Objective 6. Collect and identify wild zooplankton species from inshore waters in South Oahu.

A student assistant was hired and underwent preliminary training. A literature review was also initiated of the coastal plankton. The plankton pump and screening system were also designed.

Objective 7. Determine uptake rate, survival and growth of flame angelfish larvae and/or other available species fed wild zooplankton.

Staff continued collection of Genicanthus personatus eggs and larval culture trials.

Year 3

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. Spawning stocks are being further expanded during year 3, to increase angelfish larvae supply for pilot-scale rearing studies.

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 8 tanks during year 2, however the quantity and consistency of egg production and egg fertilization rates were problematic, and larvae supply was sufficient to carry out only preliminary rearing trials. Broodstock management of yellow tang therefore forms an important focus of research during year 3 of the Marine Ornamentals project.

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 1,500L 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 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 towards 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 Waikiki Aquarium (WA) researchers during 2001, with the first ever production of juvenile masked angelfish, Genicanthus personatus, using cultured prey. WA researchers also provided a zooplankton identification service for the GADC during project year 2, of samples collected by in Guam during year 1.

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.

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

Commissioning of new broodstock holding facilities is underway for this work.

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

Commissioning of new broodstock holding facilities is underway.

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

A trial was carried out during this grant period 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.

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

No progress reported.

Work Planned

The following research work is planned for the remainder of project year 3:

1. Continue husbandry of adult flame angelfish and yellow tang for seedstock production.

2. Commission flame angelfish broodstock holding facilities and carry out harem size experiment

3. Commission yellow tang broodstock holding facilities and carry out stocking density and sex ratio studies.

4. Continue flame angelfish pilot-scale rearing trials, with emphasis on improving postlarval survival rates.

A research plan has also been submitted for year 4 of the Marine Ornamentals project, incorporating the following objectives:

1. Validate husbandry procedures developed for large-scale production of flame angelfish eggs.

2. Assess the reproducibility of preliminary mass rearing techniques developed for flame angelfish larvae.

3. Develop appropriate weaning protocols for juvenile flame angelfish.

4. Disseminate information on flame angelfish culture techniques and rearing performance.

5. Isolate, culture and test alternative zooplanktonic prey for marine ornamental fish larvae.

Impacts

Year 2

The ultimate goal of this project is to facilitate the development of a new marine ornamental aquaculture industry in Hawaii and the Pacific. Research on the zootechnical aspects of juvenile fish production made good progress during year 2 of the project. In particular, the method of holding and spawning flame angelfish was improved and a technique was developed for disinfecting the eggs of this species. These practical designs and procedures are suitable for immediate transfer to industry. Useful information was also obtained on the most appropriate husbandry methods for early larval stages of flame angelfish. The continued spawning of captive yellow tang provided an important first opportunity to rear larvae of this species.

In summary, the year 2 accomplishments provide important new applied information on holding and rearing techniques for marine ornamental fish that will assist the development of a new regional aquaculture sector. While culture techniques are not yet sufficiently developed to allow the transfer of a technology package to industry, the project is on course to deliver reliable rearing protocols as planned. In the meantime, those farmers interested in beginning work with the high value fish species in question will benefit immediately from the information generated during project year 2.

Year 3

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

Papers Presented

Laidley, C.W. Captive reproduction of yellow tang and pygmy angelfish. Presented at the annual conference of the Hawaii Aquaculture Association, May 8, 2002.

Laidley, C.W., A. F. Burnell and A.C. Ostrowski. 2001. Captive reproduction of yellow tang and pygmy angelfishes at the Oceanic Institute in Hawaii. Marine Ornamentals ’01 meeting, November 27 to December 1, 2001 in Lake Buena Vista, Florida.

Publications, Year 13

Reporting Period

October 1, 2001 - September 30, 2002

Funding Level

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

Participants

Cheng-Sheng Lee, Ph.D., 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

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 ours 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

Since 1989, the Center has developed and published a newsletter four times a year which is distributed to approximately 1,000 individuals, organizations and universities worldwide. The Center has also created Project Updates, technical bulletins that are distributed to the CTSA Board of Directors, Industry Advisory Council, Technical Committee, and to extension agents and other interested parties upon request. Additionally, the Publications project has produced two videos which provided the latest results from the Center-funded projects at the time.

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 2002. The staff participated in the production (editing, layout, and/or printing coordination, etc.) of three manuals, A Manual for Commercial Production of the Swordtail, Xiphophorus helleri, The Basic Methods of Pearl Farming: A Layman's Manual, and Recent Advances in Lagoon-based Farming Practices for Eight Species of Commercially Valuable Hard and Soft Corals - A Technical Report. In addition, project work group members submitted for publication in Aquaculture Economics and Management a paper titled "Viable Aquaculture in the U.S. Affiliated Pacific Islands - Lessons from Giant Clam and Sponge Farming."

We have updated the look and content of our website to be more content-driven and user-friendly. Information on all of our projects and all of our publications are now available at www.ctsa.org.

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

During 2002, staff automatically disseminated varying numbers of copies of 13 publications throughout Hawaii and the U.S. Affiliated Pacific Islands. We also responded to a multitude of requests for CTSA publications.

Work Planned

The staff will continue to produce and publish the quarterly newsletter, continue developing our website, disseminate information, produce articles for other publications to further propagate the accomplishments of our projects and provide editing, layout and printing assistance with publications as needed.

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.

Marine Food Fish Seedstock Production, Years One, Two & Three

Reporting Period

January 1, 1999 - September 30, 2002 (termination report)

Funding Level

Year 1	$110,000
Year 2	$135,000
Year 3	$135,000
TOTAL	$380,000

Participants

Year 1

Anthony C. Ostrowski, Ph.D., The Oceanic Institute

James A. Brock, Ph.D., Aquaculture Development Program, State of Hawaii

Christopher Kelley, Ph.D., Hawaii Institute of Marine Biology, University of Hawaii

PingSun Leung, Ph.D., University of Hawaii at Manoa

Year 2

Brad J. Argue, Ph.D., The Oceanic Institute

Anthony C. Ostrowski, Ph.D., The Oceanic Institute

James A. Brock, Ph.D., Aquaculture Development Program, State of Hawaii

Christopher Kelley, Ph.D., Hawaii Institute of Marine Biology, University of Hawaii

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

PingSun Leung, Ph.D., University of Hawaii at Manoa

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

Year 3

Anthony C. Ostrowski, Ph.D., The Oceanic Institute

Brad J. Argue, Ph.D., The Oceanic Institute

James A. Brock, Ph.D., Aquaculture Development Program, State of Hawaii

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

Robert A. Bullis, Ph.D., The Oceanic Institute

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

Reason for project termination

The CTSA-funded "Marine Food Fish Seedstock Production" project has now closed, having successfully met its major goal of assisting in the establishment of a marine food fish industry in the Pacific region. To this end the project has successfully:

- Established a year-round supply of fertilized Pacific threadfin eggs and transferred broodstock technologies to local industry.
- Completed threadfin hatchery technology refinement, including development of an economic model, and transfer to commercial operations. Transfer was completed under a phased-out program that delivered 273,449 fingerlings in year one, 215,896 fingerlings in year two, and 178,514 Pacific threadfin fingerlings in year three of the project. A commercial operator is now supplying fingerlings to the local industry.
- Established multiple small-scale growout operations on the islands and enabled large-scale, offshore production of threadfin to be initiated.
- Initiated health assurance and disease monitoring programs.
- Generated captive farmed threadfin products that are now appearing in local restaurants, retail markets, and are being sold both to mainland and international markets.

Efforts to diversify with the milkfish and crimson snapper did not progress to the same degree as the Pacific threadfin. There is still interest in establishing a commercial hatchery for milkfish on the island of Molokai although economics of this lower value species has slowed its commercial development in Hawaii. Efforts to resolve critical bottlenecks in the life cycle of the crimson snapper have proved more challenging and now form the basis of a DLNR-funded bottom-fish project being conducted at HIMB.

Objectives

Year 1 (January 1999-June 30, 2000)

1. Maintain broodstock and distribute a total of 250,000 Pacific threadfin fingerlings to qualified, participating farmers.
2. Maintain broodstock and provide 100,000 milkfish fingerlings to qualified farmers.
3. Initiate a health assurance program for Pacific threadfin and milkfish.
4. Initiate and maintain a broodstock development program for crimson snapper (Pristipomoides filamentosus).
5. Determine the size of a Pacific threadfin hatchery and costs required for various levels of production.
6. Initiate a phased payment schedule for Pacific threadfin and milkfish seedstock distributed to farmers statewide, and provide technical advice and assistance for growout operations.

Year 2 (October 2000-December 2001)

1. Maintain Pacific threadfin, milkfish, and crimson snapper broodstock.
2. Produce 250,000 threadfin and 100,000 milkfish fingerlings for distribution to qualified farmers.
3. Refine disease-free certification program for finfish larvae and improve fry quality.
4. Begin to domesticate Pacific threadfin for aquaculture and produce a selected line for increased growth.
5. Determine production cost structure and profitability of milkfish growout in Hawaii.
6. Continue phased fry payment schedule and expand program to other areas of the Pacific taking into account site-specific, economic, and genetic considerations.

7. Evaluate other bottom fish species as potential aquaculture candidates and develop broodstock capabilities as money and fish become available.

Year 3 (April 2001-September 30, 2002)

1. Improve threadfin fry quality by use of rotifer enrichment methods.

2. Determine role of enhanced natural productivity in semi-intensive production ponds on improved survival and growth of larval milkfish.

3. Continue the disease-testing program to insure the disease-free status of threadfin fry provided to the industry and refine it by establishing a cell line to screen for potential viruses.

4. Develop pair spawning methods for Pacific threadfin for genetic selection work.

5. Domesticate Pacific threadfin for aquaculture and produce a selected line for increased growth.

6. Confirm transfer of reliable fry production technologies to commercial hatcheries.

Work Progress and Principal Accomplishments

Year 1

1.0 Supply Pacific Threadfin Seedstock

Objective 1: Maintain broodstock and distribute a total of 250,000 Pacific threadfin fingerlings to qualified, participating farmers.

At the request of farmers, the OI hatchery initiated quarterly threadfin fry production runs during year 1, to allow farmers to better manage their growout operations and provide a more even flow of product into the marketplace. OI scheduled hatchery runs in April, June, September, and October 1999. All hatchery runs followed the methods developed by Ostrowski and Molnar (1998).

A total of 273,449 threadfin fingerlings were supplied to eight threadfin farms over the project period. The April 2000 run, (two, 5-ton production tanks) yielded 31,088 fish; the June run (two tanks) yielded 28,400 fish; the September run (three tanks) yielded 87,116; and the fourth run (four tanks) in October yielded 84,078 fish. An additional 42,767 threadfin were supplied to farmers from the OI hatchery from other ongoing research. These fish were raised in the nursery to 30 days of age (D30) and approximately 0.05 g each, packed, and air freighted to participants on Hawaii and Molokai. While shipping at this age reduced costs by allowing more animals to be packed in a box, farmers had to implement protocols required during the Nursery I phase (D25 – D40) to control cannibalism. Local farmers on Oahu received their fish on D40 (1.0 g) to lessen their burden of implementing the Nursery I phase. New packing protocols based on 24-hour pack-out experiments conducted at OI have increased the survival (98%) of landed fry to the outer islands. The new packing regimen includes reduced handling, incorporation of ammonia pillows in each bag, square bottom shipping bags, and reduced transfer/air time.

In addition to fry, 1,190,000 threadfin eggs were shipped to local farmers and researchers from broodstock maintained at OI. The five participants that received eggs included Ben Krause, Ryan Murashige, Romy Aquinaldo, Anuenue Fisheries Research Center (AFRC), and Hawaii Institute of Marine Biology (HIMB). In addition to fry, farmers were given the OI hatchery manual for threadfin production as a guide through rearing.

Results of the on-farm hatchery runs were mixed. Often-cited problems included lowered priority for the run, which lead to neglect in some instances, difficulty in scaling-down of the OI methods, and inconsistency in rotifer supply. In short, successful larval culture from the farmers has been limited due to the lack of experience in this field. Additional on-farm training in larval rearing and live feeds management through this project should improve success.

Follow-up conversations over the course of the year indicated that out of all of the fingerlings sent to farmers, approximately 65% (177,741 fish) would reach market during 1999-2000. Farmers currently sell 0.5 – 1.25 lb fish for prices ranging from $4.50 – 7.00/lb in the round. Given a mode 0.75 lb/fish harvested weight (133,306 lbs total) and a $5.00/lb price, the total economic impact is estimated to be $666,529 in off-the-farm sales. Mortality on growout farm sites is primarily due to catastrophic reasons (e.g. water shut off, oxygen depletion), and the gill parasite, amyloodinium. Recently, the use of restricted light to control the growth of the parasite has proved promising on one farm that has had a history of outbreaks.

2.0 Supply Milkfish Seedstock

Objective 2: Maintain broodstock and provide 100,000 milkfish fingerlings to qualified farmers.

During 1999, OI initiated research to develop a semi-intensive hatchery method for production of milkfish fry in conjunction with production activities. Semi-intensive methods of milkfish production are commonly practiced in Taiwan and other regions of the world, but the techniques have not been extended to Hawaii. Such methods would significantly lower production costs for fry, and improve profitability of milkfish culture in the state. Last year, six production runs were conducted in 12 ft x 35 ft, concrete ponds (40-ton capacity) that yielded an overall survival rate of 5% from stocked eggs. Problems with gas supersaturation (over 104%) due to high levels of oxygen production from algal blooms were responsible for heavy mortality of larvae around D11. Efforts this year were intended to refine techniques and improve overall survival rates.

Overall survival from four semi-intensive hatchery runs completed between July and November 1999 yielded an average 24% survival from stocked eggs (150,000/tank) to D40. This survival rate was nearly 5 times greater than achieved last season. Reasons for the improvement included better management techniques and more stable rotifer densities in tanks. The use of 80% shade-cloth over tanks to restrict ambient light intensity in the outdoor systems dramatically improved survival of larvae through the first 11-12 days of development. Algae blooms were better controlled with use of the shade-cloth, which prevents large increases in oxygen and subsequently total gas supersaturation levels. Algae densities were maintained at a secchi disk reading of between 20-30 cm. Temperatures ranged from 24.3 – 27.8°C and pH was maintained between 8.1 – 9.0 using water exchange. Rotifer production from the hatchery was also more consistent, yielding better control of live feed densities in tanks during critical periods. Rotifer densities in tanks were consistently maintained between 3-5/ml.

Production from the semi-intensive runs was better than expected (144,071); yielding 44% (44,071) more fry than originally targeted. Fry were distributed to 27 farmers on the islands of Oahu, Molokai, Maui, and Hawaii. Fry were shipped between D38 and D40 (0.05-0.08 g each), and packed at 1,000/shipping bag. Survival rates of fish to outer islands ranged from 96-99%. Approximately 54.5% of the fry went to freshwater facilities (78,571) while 45.5% of the fish

Results at a glance . . .

Established a year-round supply of fertilized Pacific threadfin eggs and transferred broodstock technologies to local industry.

Completed threadfin hatchery refinement.

Established multiple small-scale growout operations in the islands and enabled large-scale, offshore production of threadfin to be initiated.

Initiated health assurance and disease monitoring programs.

Generated captive farmed threadfin products that now appear in local restaurants and retail markets and are being sold to mainland and international markets.

went to saltwater farms (65,500). In addition, 300,000 eggs were provided to Ryan Murashige to conduct hatchery runs.

Follow-up conversations indicated that nearly 70% of fish sent to farmers would reach market size during 1999 – 2000. Currently, farmers are able to receive up to $3.50/lb of 5-6" (3-4 oz.) or 1 lb. or greater milkfish. The former can be produced within four months growout. This season, farmers have noted an increase in market demand for the smaller product which is prized by the local Filipino community. Given that demand and a conservative $3.00/lb price of fish, it is estimated that economic impact from the project this year would yield approximately $75,600 in off-the-farm sales.

Results from the production runs indicate that methods of semi-intensive milkfish fry production can be achieved in Hawaii and that fry can be produced reliably. On average, tanks yielded 1 fry/liter (i.e., ca. 42,000 fry/40-ton tank). Preliminary estimates from this and CTSA-funded milkfish research are that it costs between $0.03 – 0.05/D40 fry produced, including broodstock holding costs. This evidence should help support the creation of commercial, Hawaii-based milkfish fry production facilities for industry expansion.

3.0 Design and Implement a Health Status Program

Objective 3: Initiate a health assurance program for Pacific threadfin and milkfish.

Largely because of favorable survival at harvest and the virtual absence of episodes of disease in the hatchery phase, the health status of threadfin and milkfish has not been systematically evaluated for the fingerlings previously produced through CTSA-funded projects. It is now well understood that the provision of healthy and robust fry from the hatchery is a critical component to success of any fish production industry, and that establishment of fry quality must be at the foundation of industry transition. The IAC recommended that more aggressive surveillance of the fish should be initiated to begin to document the health condition of the fingerlings produced by the project prior to being distributed to participating farms.

Threadfin and milkfish fingerlings (30 from each sp.) from each production and research run conducted were delivered to Dr. Jim Brock at the Anuenue Fisheries Research Center (AFRC) for examination. Each group of fish was killed with an overdose of MS-222, weighed, necropsied, and tissues from major organ systems (gill, heart, liver, spleen, caudal kidney, digestive tract and attached abdominal viscera) were collected and preserved in Davidson fixative. The specimens were blocked and processed by routine histopathology methods and slides were prepared. The tissue sections were stained with hematoxin and eosin and inspections were conducted.

According to Dr. Brock, results of necropsy and histopathology found no gross change in the threadfin or milkfish fry that would suggest the presence of an infectious disease problem in the batches of fish. Fatty vacuolation of the livers was found in 33% of the threadfin. This was related to a common microscopic change present in cultured threadfin. Its appearance suggested an excess of lipid in the diet of the fish. The clinical impact of this was considered apparently minimal, based on the relatively high (ca. 85%) survival rate of nursery fish at OI.

4.0 Develop Crimson Snapper Broodstock Population

Objective 4: Initiate and maintain a broodstock development program for crimson snapper (Pristipomoides filamentosus).

The original design of this trial was to split remaining fish from the previous growout trial into broodstock groups that would be monitored for maturational development and eventually spawned. Additional fish were to be collected from the wild to supplement stocks. Unfortunately, similar problems encountered with the growout trial precluded set up of the broodstock trial. It was decided it was not warranted to collect additional fish and place them in tanks until remedies for exopthalmia and swim bladder inflation were found. A total of 47 fish remained from the original 184 fish stocked into OI tanks in July 1998. Of these, 21 fish had exopthalmia (popeye) or were blind, and were immediately culled. The remaining 26 fish (448.9 ± 122.8 g, 27.7 ± 1.7 cm, fork length (FL)) were stocked into a 50-ton (37.5-ton working volume) square (6.1m x 6.3m x 1.0 m) concrete tank. Fish were fed combinations of squid, smelt, krill, and supplemental vitamins and minerals at a rate equal to 2-3% body weight daily. An artificial structure comprised on concrete blocks and 6" PVC-pipe was also placed in the center of the tank for refuge. In addition, 95% shade cloth was placed over the tank and around tank sides to minimize light input and disturbance to fish. The purpose was to provide an environment to minimize stress in fish.

Despite changes in holding protocols, fish continued to exhibit exopthalmia and/or swim bladder inflation almost on a weekly basis and died throughout the summer months. After 3 months, only 14 fish remained. These fish were then placed in a 20-ton (7.3 m diameter) circular tank with a 3.0 m inner ring. This donut-shaped tank was covered twice with 80% shade cloth, and supplied high current and water exchange. Fish were held for 9 months in this system, but continued to die. In November 1999, only 4 fish remained.

The remaining four fish were then transferred to a 3 m diameter HDPE tank (8-ton working volume) located indoors in an a photoperiod control lab and placed under short (8 hours light; 16 hours dark) photoperiod regimen. Fish appeared to adapt better to the long photoperiod regimen and no cases of expothalmia occurred. Fish were euthanized in March 2000, weighed and measured. Fish grew to a range of 2.4 – 4.0 kg, and 35 – 45 cm FL. No signs of pairing or reproductive behavior were observed in any fish.

Late during the project period it was decided that onshore holding of fish was not possible. Newly caught adult snapper were stocked into 28m^3 floating cages moored near Coconut Island. Each cage was 3m in depth. Fish appeared to adjust better and the incident of exopthalmia was reduced dramatically. Viable spawns have yet to be recorded.

Results indicate that crimson snapper are a challenging species to domesticate and maintain in onshore tanks. Difficulties remain with the acclimation process of newly captured fish, as well as fish that have been maintained on site for extended periods of time. Problems believed to be stress-related from the change in environment lead to exophthalmia, inflated swim bladders, cessation of feeding, and finally death. Holding of broodstock in nearshore cages appears promising. These holding methods might be linked to onshore techniques of spawning and eventually larval rearing.

5.0 Determine Economics of Hatchery Production

Objective 5: Determine the size of a Pacific threadfin hatchery and costs required for various levels of production.

A spreadsheet model was developed by Dr. PingSun Leung and staff over the project period to determine a viable scale for a commercial threadfin hatchery in Hawaii. Dr. Leung and staff met with OI hatchery personnel, OI engineers, and engineers at the Natural Energy Laboratory of Hawaii Authority (NELHA) to establish basic model assumptions. The model characterized finfish production parameters and permitted ease of calculation of the costs associated with variations in production design and scale of production. The base production scheme was modeled after current hatchery practices performed at OI. Production requirements were determined according to demand and the estimates for survival over each phase of production including the spawning (broodstock), larval rearing, and early nursery phases. Site-specific requirements including construction, site survey and preparation were based on estimates from NELHA. The engineering designs for plumbing, drilling and aeration assumptions were based on estimates from OI.

Results indicated that for a hatchery enterprise producing 1.2 million fry per year, the cost associated with raising each 1-g fry is estimated at 20.13¢. The largest variable costs were labor and supplies, which comprised 54% and 10% of the total production cost. The combined annualized fixed costs for development and equipment was approximately 10% of total production costs. At a sale price of 25¢ per fry, the 20-year internal rate of return (IRR) was 47.70%. In comparison to the 20.13¢ unit cost for 1.2 million fry production, analyses of smaller enterprises producing 900,000 and 600,000 fry per year reflected significant size economies with unit costs of 25.25¢ and 35.98¢, respectively. Additional analyses revealed that increased profitability is possible by shortening the nursery period, but at a diminishing rate. Increasing the nursery length exhibits decreasing daily costs per fry. The cost savings resulting from the elimination of live feed production was also calculated and serves as a decision aid for management when considering outsourcing or investing in commercial substitutes.

6.0 Implement Technology Transfer

Objective 6: Initiate a phased payment schedule for Pacific threadfin and milkfish seedstock distributed to farmers statewide and provide technical advice and assistance for growout operations.

Prior to the beginning of fry distribution, prospective threadfin and milkfish farmers were contacted to inform them of the phased payment plan. Prices were set at $0.07/threadfin and $0.01/milkfish fry supplied, roughly one-third the estimated OI fry production cost for each species. Farmers were urged to pay for the fingerlings as soon as possible but were allowed to make payments over a period of up to eight months to allow income received from the harvest of fish to accumulate. In addition, outer island farmers were allowed to pay for only 80% of fish sent to them, minus mortality recorded within two days post-shipment, in the interest of parity. The rationale was a concern expressed by outer island farmers that fish they received from the project were still within the cannibalistic phase (ca. D30), while those received by farmers on Oahu were past this stage (D40), and thus less prone to subsequent losses on the growout farm. Outer island farmers also noted that Oahu farmers did not experience mortality due to air shipment. A similar arrangement was made with milkfish farmers.

The income received was used to supply additional threadfin and milkfish fry to farmers, and to increase information exchange efforts and onsite visits. The project this year distributed 9.4% more threadfin and 44.1% more milkfish fry to farmers than was originally targeted as deliverables. A greater exchange of information occurred this year over the Internet, but onsite visits proved valuable to refine methods to preclude problems on existing farms and assist new farms in start-up. Activities coordinated with farmers included system design, feeding strategies, disease, stocking densities, ordering supplies (i.e., identification of sources), fish transport, and handling. An average 2.3 site visits occurred monthly that included 15 outer island visits and 13 visits on Oahu. Staff aided new threadfin and milkfish farmers on the outer islands of Kauai, Maui, and Hawaii. Oahu site visits covered a broader area and new farmers as well in Kahuku, Waipahu, Laie, and Kaneohe.

A key contribution by OI staff has been the coordination of feed shipment with threadfin farmers and the local feed import and storage facility, Land-O-Lakes (LOL), located on the leeward coast of Oahu. The majority of feed used by farmers is the Marine Grower, a diet formulation researched by the project PI, Dr. Ostrowski, and developed in concert with Moore-Clarke, Co. Canada. Current cost of the diet is $0.65/lb FOB, LOL. OI staff met with Moore-Clarke to discuss ways to support industry development. Moore-Clarke agreed to lower costs of the feed with bulk orders, and OI staff coordinated needs with local farmers. Recent bulk orders through LOL reduced the price to $0.58/lb FOB, LOL, and larger shipments are targeted to reduce the price to $0.52/lb. Previous economic work (see draft publication Martinez-Cordero et al. in Appendix 3) indicated feed costs for threadfin growout are the largest variable cost, ranging from 23 – 27% of total operating costs. At a feed conversion of 1.3, a $0.12 reduction in price of feed translates into a 20% reduction in total feed costs.

Research efforts to further reduce feed costs were also coordinated with Ben Krause of Pacific Harvest, Kona, Hawaii, and the largest threadfin producer in the state. A new formulation based on past research of the PI was developed with Moore-Clark. This new formulation incorporated use of a lowered dietary protein level and soybean meal as a fishmeal substitute. The new diet was tested in a single tank of threadfin in growout at Pacific Harvest. Preliminary estimates are that, if successful, the price of feed could drop to near $0.45/lb, FOB, LOL.

OI also provided threadfin eggs to the State of Hawaii's Anuenue Fisheries Research Center (AFRC) to aid their efforts in transition to support industry needs. A recent bill passed in the Hawaii State Legislature, supported by OI, which included provisions for the AFRC to produce fry to support the growing marine foodfish industry in the state. OI staff through the CTSA project will continue to assist the AFRC.

Year 2

1.0 Maintain Broodstock

Objective 1: Maintain Pacific threadfin, milkfish, and crimson snapper broodstock.

OI continued to maintain Pacific threadfin and milkfish broodstock for the project. Pacific threadfin spawned on regular monthly intervals throughout the report period. Milkfish began spawning in May 2001. Eggs were collected routinely and many have been distributed to local farmers to attempt mass culture. The distribution of eggs is summarized in Task 2.0 below.

By the time this phase of the project was initiated, there were only four broodstock crimson snapper remaining at OI. It was decided to cull these animals rather than risk any potential disease transference by combining them with established animals at HIMB. Broodstock snapper at HIMB continued to grow and develop well in the net-pens. It was further decided by the Project Work Group not to collect additional animals to avoid overstocking the net-pens. Animals are currently being monitored and plans are to send eggs to the OI hatchery if and when they become available to attempt mass culture. During this period, Dr. Kelley resigned his position at HIMB and currently works for the NMFS in Honolulu.

2.0 Supply Threadfin and Milkfish Seedstock

Objective 2: Produce 250,000 threadfin and 100,000 milkfish fingerlings for distribution to qualified farmers.

The provision of Pacific threadfin and milkfish seedstock to support industry development remains a critical aspect of these emerging industries. OI is the primary supplier of threadfin and milkfish seedstock in the State of Hawaii and supports both growout and baitfish production operations at more than 30 farms on the islands of Oahu, Maui, Molokai, and Hawaii. Since 1996, the OI hatchery has conducted large-scale research during these production runs to improve efficiency and overall fry quality. Last year all milkfish fry were produced using semi-intensive methods by first inoculating outdoor tanks with shrimp effluent water, and supplementing natural phyto-and zooplankton. This season the target was to test the usefulness of a product called AquaMats™ which purportedly improves semi-intensive production methods by providing biologically active surface area and enhancing natural biological processes to grow phytoplankton, detrital bacteria, and sessile zooplankton. In addition, efforts were targeted to improve threadfin fry survival, growth, and overall robustness by testing the usefulness of rotifer enrichment methods. The method originally developed for threadfin fry production included only enrichment of Artemia, the second live food item for this species. Rotifer enrichment methods are a new phenomenon and were not available during the hatchery development phase of this project.

The final two threadfin production runs scheduled for this phase of the project were completed in October 2000, and January 2001. In total, 215,896 fingerlings and over 3.6 million eggs were shipped to five facilities across the state. The majority of eggs were shipped to Pacific Harvest, which has established a pilot larval rearing facility. OI staff has assisted with technical advice and visits to this facility to aid hatchery development as well. No other milkfish trials were conducted over this report period due to the lack of spawns. All production and research trials scheduled to yield fingerlings for distribution have been completed as planned.

Results of trials in 200-L tanks demonstrated significantly greater survival rates to metamorphosis for threadfin larvae receiving "Algamac 2000" – enriched rotifers (Aquafauna Biomarine, Inc.) compared to those receiving Nannochloropsis-enriched rotifers. However, there was little difference in opercular deformity rates. Rotifer enrichment is recommended to improve survival and overall production of threadfin fry.

A total 14,100 fry were distributed. A drop in algal production at the OI facility that resulted in low rotifer output hampered production efforts. Efforts were directed to correct the situation and additional runs were re-establihed in October. Results of the Aquamat™ trials were inconclusive.

In addition to fry, the OI hatchery also provided threadfin and milkfish eggs to support industry development. Several farmers this year have begun hatchery efforts in earnest in response to increasing demands for fry. During this project phase, the OI hatchery supplied 1.7 million threadfin and 1.34 million milkfish eggs to three growout and research operations on the islands of Hawaii and Oahu.

3.0 Health Assurance Program

Objective 3: Refine disease-free certifications program for finfish larvae and improve fry quality.

Threadfin and milkfish fingerlings (30 from each species) from each production run at OI were delivered to Dr. Brock at AFRC for examination. Each group of fish were killed with an overdose of MS-222, weighed, necropsied, and tissues from major organ systems (gill, heart, liver, spleen, caudal kidney, digestive tract, and attached abdominal viscera) were collected and preserved in Davidson fixative. The specimens were blocked and processed by routine histopathology methods and slides were prepared. The tissue sections were stained with hematoxin and eosin, and inspections were conducted.

The necropsy and histopathology findings showed no gross change in the fry threadfin or milkfish, which disputes any presence of an infectious disease problem in the batches of fish. Fatty vacuolation of the livers was found in 33% of the threadfin. This is a common microscopic change present in cultured threadfin. Its appearance suggests an excess of lipid in the diet of the fish. The clinical impact of this is apparently minimal. Documented results of these findings are in Appendix 1.

Before the project end, Dr. Brock resigned his position as the State of Hawaii's Aquatic Veterinarian. Dr. Robert Bullis from OI assumed temporary responsibility for this task. Due to the lag time in transition, Dr. Bullis did not complete the processing and reading of the remaining cases before the end date of this project.

4.0 Develop Selected Broodstock Lines

Objective 4: Begin to domesticate Pacific threadfin for aquaculture and produce a selected line for increased growth.

Efforts to implement a selected breeding program for Pacific threadfin have been initiated with the development of a domesticated broodstock group in which 10 females collected from the wild have been stocked with 10 F1 males. These animals reached reproductive condition in September with small spawns in September and October.

Spawns from semi-domesticated broodstock populations were reared through the larval hatchery in February 2001 producing 4,000 fingerlings that were then transferred from nursery to 30-ton growout tanks. These fish are grown to market size at which time control and selected top growers are PIT-tagged and stocked into broodstock tanks. Preliminary tissue samples were sent to Kent Sea Tech, Biotechnology division for preliminary microsatellite marker development. Costs associated with this effort were much higher than originally quoted and the effort was curtailed.

5.0 Milkfish Economics

Objective 5: Determine production cost structure and profitability of milkfish growout in Hawaii.

Delays in obtaining information from farmers and write-up caused a request to extend the project deadline. The model developed for milkfish was similar to the one developed for threadfin growout under a previous CTSA project. The synthetic enterprise budget was based on the design and operating parameters (feed conversion, mortality, yield) from over twenty existing farms that receive fry from the project. Budgets were developed for pond and tank systems in fresh, brackish, and saltwater, currently practiced in Hawaii. Capital costs, overhead expenses, and variable operating costs were estimated using the current costs faced by commercial aqua farmers in Hawaii. A computerized budget generator designed for milkfish growout production was developed under the Windows 95 environment for ease of use.

Evaluations of Hawaii milkfish pond and tank production systems were made. Spreadsheet models were developed for each system based on input from Hawaii farmers. Based on the observed practice of milkfish culture as a secondary or tertiary crop, capital costs and several operation costs of the farm were pro-rated to accurately depict the current situation. The study estimated the total production cost for milkfish production at $3.31/lb for the pond system and $3.81/lb for the tank system. At a sale price of $3.00/lb and seed cost of $0.25/fingerling, neither system is profitable under a 20-year project life. Based on the cost structure developed for each system, fixed costs including administrative overhead, equipment depreciation, and insurance comprise an estimated 40% of the total production cost. Discussions with milkfish farmers support the view of milkfish production serving as supplemental revenue to regular farm activities and legitimize the need to focus on variable expenses incurred. When considering variable costs alone, the production cost is $1.79/lb for the pond system and $2.31/lb for the tank system, yielding returns on variable costs of 68% and 30% respectively. The sentiment of Hawaii milkfish farmers is consistent with the results of this study: considering the market conditions for input requirements and product (milkfish) demand in Hawaii, milkfish production is only secondary to core species. Based on the information and assumptions used in this study, projected revenues from milkfish sales are not a premise for investment in dedicated farm infrastructure (i.e. a start-up venture in milkfish production) and the analysis of additional production scenarios.

6.0 Expand Program

Objective 6: Continue phased fry payment schedule and expand activities to other areas of the Pacific taking into account site-specific, economic, and genetic considerations.

Seedstock provision to farmers was logged for each shipment. A cost of $0.14/fingerling for threadfin and $0.03/fingerling for milkfish were assessed for each shipment (67% of actual cost for each fry produced from the OI hatchery). Costs to farmers for milkfish were based upon OI's costs of $0.05/fry to raise fry extensively to 25 days of age (D25). Costs to farmers for threadfin were based upon OI's costs of $0.21/fry to raise fry intensively to D25.

Site visits were conducted on the islands of Hawaii and Maui to assist in hatchery and growout set-up. One farmer is currently setting up a hatchery and conducting trial, larval rearing runs using eggs provided by OI. In addition, a total 30 hours were spent by staff on the phone arranging shipments of fry and eggs, and advising on receiving fish, tank design, feed requirements, and stock densities. There were no requests for fry from Pacific island areas other than Hawaii.

7.0 Develop Other Bottom Species

Objective 7: Evaluate other bottom fish species as potential aquaculture candidates and develop broodstock capabilities as money and fish become available.

Accomplishment of this task was dependent upon the supply of eggs from the CTSA (Task 1.0 above), DLNR, or NOAA funded projects, and the amount of funds remaining in this project. There were no spawns from any snapper species under any project.

Year 3

Objective 1: Improve threadfin fry quality by use of rotifer enrichment methods.

Two sets of experiments were conducted to examine the effects of diet on the quality of Pacific threadfin fry during the year-three project period. The first experiment compared rotifer and Artemia enrichments and the second experiment specifically examined the effects of vitamin C supplementation.

Experiment 1: Rotifer and Artemia enrichments. An experiment was conducted comparing the survival and growth rates and morphological characteristics of threadfin larvae receiving two different diet enrichment products (Algamac 2000®, Aquafauna Biomarine Inc; Aquagrow® Advantage, Martek Biosciences Corp.). Both of these DHA-rich products are prepared from dried protist cells (Schizochytrium sp and Crypthecodinium sp respectively).

The experiment was carried out in 4,000L larvae rearing tanks, 2 tanks per treatment. Each tank was stocked at a density of 40 threadfin eggs/L. Water exchange rates, aeration rates and diet transition regimen followed standard OI procedures. Larvae received the experimental enrichments throughout the rotifer phase, (day 2 to day 15 post-hatch), according to the manufacturers' directions. During the Artemia phase (day 12-15 post-hatch), 50% of the daily Artemia ration was enriched using the experimental product and 50%

with DHA Selco (Inve Aquaculture NV).

Samples of enriched rotifers and Artemia were collected for biochemical analysis while larvae samples were collected at 5-day intervals throughout the experiment for size measurement and biochemical analysis. At the end of the experiment an additional sample of 60 fish per tank was examined for opercular morphology ('normal', 'wrinkled' or 'missing' operculum). This involved close inspection of each fish using a dissecting microscope.

Survival rate of threadfin to day 25 was variable between tanks with no clear relationship to enrichment type. Threadfin from the Algamac group were 18% larger (standard length) on average than those from the Aquagrow group at the end of the experiment. Opercular characteristics were highly conservative within groups. 71.7% of threadfin receiving Algamac 2000 did not exhibit any opercular abnormalities at all compared to just 49.1% of Aquagrow-fed fish. Both 'wrinkled' and 'missing' operculae occurred with higher incidence in the Aquagrow group. Biochemical analyses and intermediate growth measurements are to be completed. While neither of the test products was effective in eliminating threadfin opercular abnormalities, the strong treatment effects provide evidence for a nutritional component to this phenomenon. On a practical basis, the Aquagrow Advantage product is not recommended for threadfin larviculture under the intensive rearing conditions tested.

Experiment 2: Effects of vitamin C supplementation. A second experiment was conducted to specifically examine the effects of dietary vitamin C supplementation on survival, growth and morphology of intensively reared Pacific threadfin larvae. The hypothesis being investigated was that dietary deficiency of vitamin C impairs collagen formation in developing threadfin larvae leading to skeletal abnormalities including malformed operculae.

Rotifers and Artemia were supplemented with ascorbyl palmitate (AscP, a fat-soluble ester of vitamin C), delivered via lipid emulsion and particulate enrichments. The experiment was conducted in 6 production scale (4,000L) rearing tanks, each stocked at a density of 35 threadfin larvae/L.

Larvae were sampled for size measurement and biochemical analysis at 5-day intervals from day 1 to day 20 post-hatch. Numbers of surviving threadfin in each tank were also counted during transfer to the nursery on day 25 and samples collected for size determination, biochemistry and morphology assessment.

No significant diet-dependent differences in survival or growth rates were observed over the experimental period. However, threadfin receiving ascorbyl palmitate-supplemented rotifers and Artemia suffered a significantly higher incidence of malformed operculae relative to those in the control group (mean 71.6% "wrinkled" + "shortened" operculae, versus 44.9%). Interpretation of these results is complicated by the provision of DHA Selco-enriched rotifers only to the supplemented group; nonetheless these findings demonstrate a clear influence of larval diet on threadfin opercular characteristics. It can be speculated from this data that vitamin C deficiency is not the determining factor for abnormal opercular development in threadfin, however further interpretation must await analysis of tissue levels of vitamin C.

Objective 2: Determine role of enhanced natural productivity in semi-intensive production ponds on improved survival and growth of larval milkfish.

This objective was switched to a comparison of intensive versus semi-intensive methods of threadfin production, with emphasis on fingerling quality from the different rearing systems. Milkfish fingerlings for distribution to farmers were instead produced in a single intensive hatchery run.

The semi-intensive rearing units for threadfin were located outdoors and consisted of rectangular concrete tanks, 9.6m by 3.7m, with operating volume 20,000L. The intensive rearing units were 4,000L circular black GRP tanks, located indoors. OI's standard threadfin rearing protocols were used for the indoor 4,000L tanks involving daily addition of microalgae and enriched rotifers/Artemia. Threadfin eggs were stocked into the 4,000L tanks at a density of 40/L. The 15,000L tanks were filled with seawater and fertilized to obtain 2mg/L total ammonia nitrogen (TAN), then inoculated with Nannochloropsis sp (300,000 cells/ml). Once TAN levels had dropped below 1mg/L, s-type rotifers were inoculated at a density of 2/ml and 1 day old threadfin larvae at 5/L. Water exchange was adjusted within the range 5-15% of tank volume per day. After stocking, microalgae and rotifers were added to the 15,000L tanks as required.

A preliminary trial was carried out comparing 3 intensive versus 2 semi-intensive rearing tanks. Threadfin larvae were sampled from each system at 4-day intervals for size measurement and an additional sample of 60 fish per tank was sampled for operculae assessment on day 25.

Mean survival rate from the intensive 4,000L rearing system was 19.6%, equivalent to 24,817 fingerlings per tank. Of the two 15,000L semi-intensive rearing tanks, one did not yield any fish, while the second produced 399 fingerlings (= 0.4% survival). Surviving fingerlings from the 15,000L tank were smaller than those reared intensively (mean standard lengths 8.6mm and 13.9mm respectively). Despite substantial differences in stocking density, feeding regimen and physical environmental conditions, the incidence of opercular deformity was comparable between the two rearing systems. The total opercular deformity rate among semi-intensively reared fingerlings was 52.5% (comprising 21.2% wrinkled plus 31.2% missing), compared to 42.8% for those reared intensively (20.0% wrinkled plus 22.8% missing).

We conclude that the semi-intensive husbandry techniques applied in the 15,000L tanks were sub-optimal for Pacific threadfin larvae. The relatively small size of fingerlings harvested from this system indicates that the larvae's energetic requirements were not adequately met. Unlike their intensively reared counterparts, the outdoor population did not benefit from Artemia or from the early introduction of formulated feed. As for the low overall survival rate in the semi-intensive rearing system, it remains to be seen whether physical or biological constraints were the most significant. Despite the low survival rate obtained, this first documentation of semi-intensively reared threadfin provides a valuable new tool for studying fingerling quality issues in this species.

Objective 3: Continue the disease-testing program to insure the disease-free status of threadfin fry provided to the industry and refine it by establishing a cell line to screen for potential viruses.

During the report period both Dr. James Brock, long-term state aquatic veterinarian, and Dr. Robert Bullis of OI, who was assisting the State during the transition period, resigned their posts. In the interim, fish from hatchery production runs were supplied to Dee Montgomery-Brock with the State of Hawaii for disease-free certification. During this period there were no positive reports of disease in hatchery-reared fry although one batch obtained as a by-product of the vitamin C supplementation study and stocked in an offshore cage showed an unusually high-incidence of chronic mortality that could not be diagnosed. This reinforces the requirement for disease diagnosis and treatment expertise in the Islands, a need that is made even more critical with the massive scale-up of offshore cage culture activities in the Islands.

Objective 4: Develop pair spawning methods for Pacific threadfin for genetic selection research.

Pacific threadfin from OI production tanks with a proven record of "group-spawning" were individually picked for experimentation. Eight pairs (one female and one male) confirmed by biopsy were stocked into separate 5,000-L broodstock tanks in an attempt to get natural pair spawns. Tanks were fed daily to satiation with the standard OI broodstock diet, and tank effluents were monitored for the appearance of fertilized eggs for a period of three months (February through April) and covering two expected spawning periods. During this interval, none of the selected pairs spawned. However, the stock tank from which pairs were derived (i.e., external control) also failed to spawn during this period, likely attributed to the stress of handling and movement.

A second experiment using experimental stocks allocated into twelve 5 m^3 tanks at densities of 2, 4 and 8 fish with equal number of male and females is currently under progress. Fish will be implanted with LHRHa implants (100 ug/kg) and monitored for egg production in the tank effluent.

A final approach using hCG injection and strip-spawning will be adopted as the last resort. These trials were scheduled under the end of year 3 activities and in the beginning of the "Reproduction and Selective Breeding of Pacific threadfin" year 1 activities and will be complete prior to adoption for pair spawning of select and control stocks in late January 2003.

Objective 5: Domesticate Pacific threadfin for aquaculture and produce a selected line for increased growth.

The Pacific threadfin genetic selection project is by necessity a multi-year project due to the time requirement necessary to domesticate stocks, select animals and complete life cycles.

The first round of selection was completed in August 2001 from a post-growout population of 604 fish at approximately 6 months of age at a mean weight of 358g. From this population, 50 controls (mean weight of 378g) and 50 "select" fish (mean weight of 516g) were established and stocked into 25m^3 broodstock tanks for growth and sexual maturation.

January 2002 maturation checks (at about 12 months of age) revealed that in addition to maintaining significant size difference between groups (686g vs. 896g), 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 have entered the male phase of sexual maturation.

June maturation checks of these same stocks again revealed a significant size difference (730g vs. 980g) and the appearance of the first female animals. Interestingly, the majority of the select group has rapidly proceeded to the female stage of 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.

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.

Somewhat surprisingly, despite the appearance of females in both the control and select populations, there has not been any spawning activity to date. The original project work plan had anticipated somewhat earlier sexual development and the initiation of natural spawning activity with the appearance of a significant number of female stage animals. This has forced us to abandon natural tank spawning activity as a source of seed stock and increase efforts on hormone induced, and possibly strip-spawning approaches to generate seed stock lines for selection evaluations.

Objective 6: Confirm transfer of reliable fry production technologies to commercial hatcheries.

A total of three threadfin hatchery runs and one milkfish run were carried out during the third year of project activities. Qualified farmers were asked to submit requests in advance of each run and the total available fingerlings were then distributed on a proportional basis.

Impacts

The overall project goal of assisting in the development of a sustainable marine food fish industry has clearly been met. Some of the specific accomplishments include:

- An emerging marine food fish industry is now present in Hawaii with both threadfin and milkfish farms having been established under CTSA assistance.
- During the 3-year project, almost 700,000 fingerling threadfin and 170,000 fingerling milkfish were distributed to over 30 farms on the islands of Oahu, Maui, Molokai, and Hawaii to meet industry needs.
- Developments in hatchery technology (now transferred to industry) have enabled the recent establishment of a commercial cage culture operation with production capacities of over 1 million threadfin per annum.
- Captive farmed products now appear in local restaurants, retail markets, and sales are continuing to grow both on the mainland and in international markets. Health assurance and disease monitoring programs have been initiated.
- Industry has begun to examine other marine food fish species in efforts to diversify the market and broaden economic expansion in the Islands.

CTSA-funded research has provided the cornerstone for this growing industry to date, and expected developments of new project funding will further assist in securing requisite fingerling supplies to meet the needs of both on-shore and offshore production and support industry diversification.

Recommended Follow-up Activities

The establishment of the Marine Food Fish Industry in Hawaii has opened the door to broad suites of economic opportunity that will not only provide a new source of revenue to the islands, but does so in a manner that is both environmentally and socially appropriate. However, it is important to note that the industry is still very early in its development and will need continued support and nurturing to ensure long-term viability and profitability. Some areas for recommended follow-up include:

- Establish domesticated disease-free stocks with selection for performance-enhancing traits to establish and maintain industry competitiveness.
- Examine production efficiencies at all stages of the production cycle including hatchery, nursery, transport, and growout technologies to allow farmers to increase productivity from available facilities and labor resources.
- Implement water re-circulation technologies to reduce water consumption, gain better control over water quality, and reduce effluent discharge. It is important that the aquaculture industry become good stewards of the environment.
- Implement quality assurance measures for farmed products, to ensure consumer confidence. This is to include research to optimize physical traits (morphologically normal) and nutritional quality of Hawaiian farmed marine fish.

- With industry growth, species diversification and intensification, will also come increasing challenge from diseases. Other areas (and sectors) have been hard hit by catastrophic disease outbreaks and we should be proactive in the areas of disease detection, disease prevention, and treatment. Included in this category is the need to develop, certify, and maintain disease-free broodstock and strict disease prevention protocols statewide.
- Continue development of both on-shore and offshore growout technologies with emphasis on greater intensification. Both approaches to industrial scale-up should be investigated and evaluated since either has benefits and problems and it is likely that both approaches will be part of aquaculture development in the Islands.

Publications in Print, Manuscripts and Papers Presented

Publications in Print (Year 1)

Martinez-Cortero, F.J., P.-S. Leung, A.C. Ostrowski and M.D. Chambers. 2001 Profitability analysis of the commercial growout of moi (Polydactylus sexfilis) in Hawaii under different production systems. Journal of Aquaculture in the Tropics 16:101-112.

Manuscripts (Year 1)

Kam, L.M., P.-S. Leung, A.C. Ostrowski and A. Molnar, in press. Economics of a moi hatchery in Hawaii. Journal of the World Aquaculture Society 33(4):xxxxx. (pages not yet designated).

Publications in Print (Year 2)

Kam, L.E.Y.W., P.-S. Leung, A.C. Ostrowski and A. Molnar. 2001. Economics of a Pacific threadfin (Polydactylus sexfilis) Hatchery in Hawaii. Center for Tropical and Subtropical Aquaculture. Publication no. 146. 50 pp.

Ostrowski, A.C. and A. Molnar. 1998. Pacific Threadfin Polydactylus sexfilis (Moi) Hatchery Manual. Center for Tropical and Subtropical Aquaculture. Publication no. 132. 96 pp.

Publications in Print (Year 3)

None

Reproduction and Selective Breeding of the Pacific Threadfin - Year One

Reporting Period

June 1, 2002 - September 30, 2002

Funding Level

Year 1	$100,000
TOTAL	$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

1.0 Completion of pair spawning methods development.

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

Experimental Pacific threadfin for pair spawning experiments have not initiated group-spawning activity in larger 25m^3 holding tanks to date. As an alternative, eight pairs of Pacific threadfin broodstock (one female and one male confirmed by biopsy) from the OI production tanks were stocked into separate 5,000-L broodstock tanks in an attempt to get natural pair spawns. During this interval, none of the selected pairs spawned, nor did the stock tank from which the pairs were derived (i.e., external control).

> Results at a glance . . .
>
> Experiments suggest 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.
>
> No spawning activity to date.

In late October 2002, experimental Pacific threadfin stocks were allocated into twelve 5,000-L tanks at quadruplicate densities of two, four, and eight fish per tank with equal male/female sex ratios. The stocks will be acclimated to experimental tanks for approximately one month prior to experimentation. Subsequent to the next full moon, stocks will be lightly anaesthetized and implanted with LHRHa/cholesterol implants to induce tank spawning. If this procedure is unsuccessful, then fish from the 25m^3 stock tank will be subjected to hormonal injection and strip spawning procedures used to obtain fertilized eggs. Spawning control will need to be in place prior to scheduled hatchery runs in late January 2003.

2.0 Maintain broodstock lines

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

The Pacific threadfin genetic selection project is slightly 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 January/February 2003.

Control and fast-growth fish were selected in August 2001 from a post-growout population of 604 fish at approximately 6 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.

January 2002 maturation checks (at about 12 months of age) revealed that in addition to maintaining significant size difference between groups (686g vs. 896g), 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 have entered the male phase of sexual maturation.

June maturation checks of these same stocks again revealed a significant size difference (730g vs. 980g) and the appearance of the first female animals. Interestingly, the majority of the select group has rapidly proceeded to the female stage of 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.

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.

Somewhat surprisingly, despite the appearance of females in both the control and select populations, there has not been any spawning activity to date. The original project work plan had anticipated somewhat earlier sexual development and the initiation of natural spawning activity with the appearance of a significant number of female stage animals. This has forced us to abandon natural tank spawning activity as a source of seed stock and increase efforts on hormone induced, and possibly strip-spawning approaches to generate seed stock lines for selection evaluations.

3.0 Spawn select lineages

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

This portion of the work plan is now scheduled during the natural reproductive phase of the lunar cycle toward the end of January 2003.

4.0 Monitor performance of select and control lines

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 should be initiated in March 2003 with completion of the growth data collection phase at the end of August 2003.

5.0 Analyze genetic improvement in growth and other attributes.

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 is tentatively now scheduled for August/September 2003.

6. 0 Water reuse

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

The principal investigator recently attended the hands-on program put on jointly by the Freshwater Institute and Cornell University to acquire current state-of-the-art training in water reuse technologies and is currently soliciting cost quotes and developing designs for application to captive threadfin stocks. These designs should be complete and implemented in time for growout of select and control threadfin lines early in 2003.

Work Planned

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. 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 UH/OI 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

Publications in Print

Ostrowski, A.C., A. Molnar. 1998. Pacific Threadfin, Polydactylus sexfilis (Moi), Hatchery Manual. Center for Tropical and Subtropical Aquaculture. Publication no. 132. 96 pp.

Development of Black-Lip Pearl Oyster Farming in Micronesia - Year One

Reporting Period

October 1, 2001 - September 30, 2002

Funding Level

Year 1	$56,428
TOTAL	$56,428

Participants

FSM Component

Dr. Maria Haws, Pearl Research and Training Program, The University of Hawaii at Hilo (UHH), Hawaii

Eileen Ellis, Researcher, Marine and Environmental Research Institute of Pohnpei (MERIP), Pohnpei, FSM

Simon Ellis, Aquaculture Development Agent, College of Micronesia (COM)-Land Grant, Pohnpei, FSM

Fr. Gregory Muckenhaupt, Director of the Ponape Agriculture and Trade School and MERIP, Pohnpei, FSM

Stacy Maenner, Suzanne O'Connor, Matang Ueanimatang, Tomoaki Yamada, biologists, MERIP, Pohnpei, FSM

Kustine Silbanuz , and Joachim Wasan, staff members, MERIP, Pohnpei, FSM

RMI Component

David Wise, Pearl Oyster Hatchery Consultant

Simon Ellis, Pacific Coordinator, Pacific Aquaculture and Coastal Resources Center (PACRC) Initiative for Future Agriculture and Food Systems (IFAFS) project

Peter Fuchs, CFO, Robert Reimers Enterprises, Inc. (RRE)

Ramsey Reimers, President, RRE

Sebastian Horbushko, RRE

Junior DeBrum, Marshall Island Marine Resource Authority (MIMRA)

Virgil Alfred, Manager, Black Pearls of Micronesia, Inc. (BPOM) and Owner, Mid-Pacific Pearls

Don Hess/Dr. Dean Jacobsen, College of the Marshall Islands (CMI)

Dr. Manoj Nair, Aquaculture Researcher, CMI Land Grant

Matang Ueanimatang, UH H/CMI Aquaculture Extension Agent

Pearl Farming Bioeconomic Study

Dr. Quentin Fong, Resource Economist, University of Alaska Fairbanks-Kodiak

Simon Ellis, Pacific Coordinator, PACRC IFAFS project

Virgil Alfred, Manager, BPOM and Owner, Mid-Pacific Pearls

Dr. Maria Haws, Pearl Research and Training Program, UHH, Hawaii

Peter Fuchs, CFO, RRE

Senator Gerson Lekka and Chief Magistrate George Stephens, Nukuoro Pearl Farm

Objectives

This project has three basic components:

- Hatchery technology development and training in the Federated States of Micronesia
- Hatchery technology and training in the Republic of the Marshall Islands
- Bioeconomic study of Micronesian pearl farms

Hatchery technology development and training in the Federated States of Micronesia

1. 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.

2. 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 Republic of the Marshall Islands

1. 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. 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.

Bioeconomic study of Micronesian pearl farms

1. Create a production oriented economic model based on Hawaiian / Micronesian pearl farms and use the data to guide decision making, management practices, financial strategies, and research and development efforts. A model farm possessing the characteristics of typical Micronesian pearl farms will be developed for analytical purposes. Economic modeling will be done using Crystal Ball Software.

2. The methods and outcomes of the model will be transferred through workshops, a layman oriented publication and peer reviewed publications.

Anticipated Benefits

FSM component

The hatchery technology developed from this project will be transferred to the FSM through demonstrations and training. Our goal is to establish a cadre of skilled individuals who can contribute to industry development through research, training, education and farming. Long-term benefits will also include a hatchery and farm facility at PATS that will be 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 the hatchery operations. Therefore, it is essential to establish local operation and oversight of any proposed hatchery in order to reduce the dependence on foreign entities.

Pearl Farming Bioeconomic Study

Pearl farming has been a risky, yet generally 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 Micronesian industries are significantly different, but little information exists to inform farmers, researchers or technical assistance providers of these industries. 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: 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 Micronesia context.

A simple pearl oyster hatchery was set up at the existing MERIP facility. A new seawater system designed specifically for delivery to the larval rearing tanks was installed. All equipment and supplies were ordered, and the protocols were put into place. A storeroom at MERIP was converted into an algae room and 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.

Spawning was attempted on three separate occasions in January and February 2002 with only one successful spawn in February 2002. A total of 46 pearl oyster broodstock were collected 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 one day after the spawn (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. Approximately 100,000 larvae were taken through metamorphosis. Once eyed larvae were transferred to setting tanks where 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 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: Transfer pearl oyster hatchery methodology to marine science students, marine resource management personnel and private sector individuals on Pohnpei and throughout the region.

During this period, four MERIP staff biologists (three expatriates and one Micronesia) received full training in pearl oyster spawning, larval rearing, land-based nursery techniques and microalgae culture. They participated in all aspects of the design, installation and operation of the pearl oyster hatchery. Fourteen Micronesian students from MERIP also received training in these techniques through organized classroom activities and practical demonstrations.

RMI Component

Objective 1: 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.

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 suffering from certain infrastructure and operational problems even before its closure. 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 cultures 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 should be noted that the private sector partners (RRE, BPOM and Mid-Pacific Pearls) and the public sector partners (CMI, Land Grant, PRTP/ UH-Hilo) dedicated a significant amount of funds, time and effort to the initiative. Many individuals who were not obligated to do so, volunteered their time preparing for and participating in this work.

Broodstock pearl oysters were collected and spawning attempts were made. It was decided to use broodstock from Arno in order to adhere as closely as possible to recommended guidelines to use local stock. Also, wild stocks from Majuro have uncertain origins and stocks from the Majuro farm tended to suffer from chronic mortality of an unknown origin. Arrangements were made to visit the RRE Arno farm in early July and attempt spawning. Six spawning attempts were made during July, August and September and all but the final one were unsuccessful because of the lack of gonad development. Pearl oysters from Arno, Majuro and Jaluit were tried. The team also attempted to source pearl oysters from Likiep and other atolls, but failed to 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.

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 and this caused them great concern. 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. An unusually prolonged hot spell may also have produced this affect. If this hypothesis holds, then it indicates that 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 the development of a cost-effective method for conditioning pearl oysters to avoid future delays in hatchery production.

Difficulties were also encountered at the larval rearing stage due to the poor design of the water system and the poor quality of the water in the area surrounding Woja which was contaminated 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 1000 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.

The description of operational procedures for the BPOM hatchery was documented and will soon be published. 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 of future operators.

Objective 2: 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, spawning and larviculture were used for the dual purpose of training and demonstration. Three technicians from MIMRA and BPOM, plus the CMI/UHH Aquaculture Extension Agent, and the Land Grant Aquaculture Researcher were trained in hatchery operations, algal culture and larval rearing. Fifteen CMI students were involved in some phase of this work and now six of them have internships at MIMRA. Industry members from RRE, BPOM and Mid-Pacific Pearls also participated in a technology exchange. Significant exchange among biologists who work in aquaculture and pearl culture also occurred, and this contributed greatly to efforts in the region.

Pearl Farming Bioeconomic Study

Objective 1: Create a production oriented economic model based on Micronesian pearl farms and use the data to guide decision making, management practices, financial strategies, and research and development efforts. A model farm possessing the characteristics of Micronesian pearl farms will be developed for analytical purposes and economic modeling will be done using Crystal Ball Software.

Dr. Fong visited the RRE and BPOM pearl farms in June, 2002 accompanied by Haws and S. Ellis. The Mid-Pacific Pearl Farm at Arno was not visited, but the owner, Mr. Virgil Alfred was interviewed. Managers and owners of the Marshallese pearl farms, Frankie Pedro, Virgil Alfred, Peter Fuchs and Ramsey Reimers provided data and information to Dr. Fong on the operations and strategies of the farms.

Prior to this event, Fong had visited the FSM PATS pearl oyster hatchery and demonstration farm and collected data from this effort, funded through the UH-Hilo IFAFS project. This information is key because it provides good data on hatchery economics which will be entered into the farm model as spat costs. Additional spat production information was collected during the CTSA RMI hatchery work.

The model was developed and its assumptions were reviewed by Haws and Ellis. Ellis checked back with key stakeholders to verify assumptions. This work continues and is projected to be finished in early 2003.

Objective 2: The methods and outcomes of the model will be transferred through workshops, a layman oriented publication and peer reviewed publications.

This will commence once the model has been fully developed and tested and the data analysis completed.

Work Planned

RMI component

The hatchery operation manual will be completed. However, given the serious problems with the hatchery, continued production at this site will entail a high level of investment. Even if the infrastructure shortcomings were to be addressed, it may not be possible to resolve problems related to the lagoon water quality. The technical team will continue to move forward with development of a small research and training hatchery at the CMI Arrak Aquaculture facility.

Pearl Farming Bioeconomic Study

Testing of the model will continue and is projected to be finished in early 2003. Presentation of the model and the accompanying interpretative reports are also projected to be done in early 2003.

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. It is too early at this time to evaluate the full impacts of this project in regard to industry development.

The public has manifested a great deal of interest in pearl farming and the team will continue to support efforts to transfer technology to potential pearl farmers, educators and researchers. Seven PATS marine students have graduated with high school diplomas, seven new senior students have received further training, and two Micronesian technicians have received training to assist the project. The algae laboratory has remained in operation for future training and education. One former Micronesian staff member has obtained employment at the College of the Marshall Islands as a result of his black-lipped pearl oyster experience from this project. Many visitors to the MERIP laboratory (the majority of which have been Micronesian) have been given tours and explanations of the project.

RMI component

Although the hoped for quantity of pearl oyster spat was not produced, this project produced both intended and unintended benefits that will positively affect the course of industry development in the near future. First, the RMI now has a technical team, supported by members in the FSM and Hawaii that are knowledgeable about pearl oyster hatchery technology. The opportunity to learn from Wise, and the chance to work together and exchange information among the various pearl oysters specialists was invaluable. Secondly, several Marshallese technicians and students (some now employed at MIMRA) are able to raise pearl oyster spat and have gained a broad understanding of pearl oyster biology and culture. Thirdly, while the project team had been engaged in planning a long-term course of action for hatchery development, the opportunity to test the BPOM hatchery was valuable as it has become clear that serious problems exist that will be either costly or impossible to mitigate.

Of particular interest and relevance to future research directions are the following findings:

- Marshallese pearl oysters may not follow seasonal patterns documented for stocks in the South Pacific.
- Marshallese pearl oysters appear to have been uniformly affected by some environmental factor for a prolonged period of time that prevented accumulation of gonad material.
- The scarcity of pearl oysters and the difficulty in collecting enough local pearl oyster stocks of known origin clearly points out the need to establish guidelines regarding importation of foreign stocks and mechanisms to control inter-island transfers.
- Methods to hold and condition broodstock are clearly needed due to the unpredictability of spawning and the scarcity of appropriate broodstock.

Pearl Farming Bioeconomic Study

This work has yet to be completed, but is expected to assist industry members, technical assistance providers and researchers with information that will help guide industry development efforts.

Publications in Print, Manuscripts and Papers Presented

RMI component

Two publications (operations manual and methods) are pending publication by CMI; this is projected to be accomplished by the end of 2002.

Pearl Farming Bioeconomic Study

No publications have been issued to date, although the effort was described in an article submitted to the CTSA Aquatips.