Center for Tropical and Subtropical Aquaculture

2001 Accomplishment Report

Center for Tropical and Subtropical Aquaculture
Administration Center
The Oceanic Institute
41-202 Kalanianaole Hwy.
Waimanalo, Hawaii 96795
Phone: (808) 259-3167
Fax: (808) 259-8395
http://library.kcc.hawaii.edu/CTSA

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

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

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 the CTSA's programmatic functions, and an Executive Committee is responsible for the 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. Dean Smith, University of Hawaii, {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:

- Dr. Jeff Barcinas, College of Agriculture and Life Sciences, University of Guam;
- 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. Gary D. Pruder, The Oceanic Institute {Executive Committee Chairman};
- Dr. Singeru Singeo, Land Grant Program, College of Micronesia;
- Dr. Dean Smith, University of Hawaii, {Board Chairman}.

INDUSTRY ADVISORY COUNCIL

Members of the Industry Advisory Council include commercial aquaculture farmers, aquaculture suppliers and members of government bodies and financial institutions. Members are appointed by the Board of Directors for three-year, renewable terms. 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. David Barclay, Aquatic Culture and Design;
- Dr. Paul Bienfang, CEA Tech USA Inc.;
- Mr. Dennis Bishop, Kona Mariculture;
- Ms. Rebecca Bishop-Yuen;
- Ms. Mary Brooks, Pacific Aquaculture;
- Mr. Steve Chaikin, Molokai Sea Farms;
- Mr. Michael Crisostomo, Kurumaya Seahorse Restaurant;
- Mr. Richard Croft, Pohnpei Natural Products;
- Mr. Yimnang Golbuu, Palau Community College;
- Mr. John Gourley, Micronesia Clam Company;
- Ms. Linda Gusman, Island Aquaculture;
- Mr. Steve Katase, Royal Hawaiian Sea Farms;
- Mr. Robert Kern, Tropical Ponds of Hawaii;
- Mr. Jeff Koch, Mokuleia Aquafarm;
- Mr. Andrew Kuljis, Aquatic Farms;
- Mr. Richard Masse, Mangrove Tropicals;
- Mr. Toshiuki Rudolph, Nukuoro Municipal Government;
- Mr. Neil Sims, Black Pearl, Inc.;
- Dr. Richard Spencer, Hawaiian Marine Enterprises {Industry Advisory Council Chairman and *ex officio* member of the BOD}
- 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};
- Dr. Maqsudul Alam, University of Hawaii;
- Ms. Kristen Anderson, Hamilton Library, University of Hawaii;

- Mr. Richard Bailey, Sea Grant Extension Service, University of Hawaii;
- Dr. John Brown, College of Agriculture and Life Sciences, University of Guam;
- Mr. David Coleman, Hamilton Library, University of Hawaii;
- Mr. David Crisostomo, University of Guam Cooperative Extension Service;
- Mr. Simon Ellis, Land Grant College Program, College of Micronesia;
- Dr. Robert D. Howerton, Sea Grant Extension Service, University of Hawaii;
- Mr. Tom Iwai, Anuenue Fisheries Research Center;
- Dr. Christopher Kelley, Hawaii Institute of Marine Biology;
- Dr. PingSun Leung, University of Hawaii;
- Dr. Shaun Moss, The Oceanic Institute;
- Dr. Anthony Ostrowski, The Oceanic Institute;
- Dr. James Szyper, Sea Grant Extension Service, University of Hawaii at Hilo;
- Mr. Howard Takata, Sea Grant Extension Service, University of Hawaii
- Dr. Clyde Tamaru, Sea Grant Extension Service, University of Hawaii.

Executive Summary

PROGRAM SCOPE

During 2001, the Center for Tropical and Subtropical Aquaculture completed work on projects funded under its Eleventh Annual Plan of Work and continued work on projects funded under its Twelfth and Thirteenth Annual Plans of Work. In addition, in July 2001, CTSA initiated work on projects developed under its Fourteenth Annual Plan of Work and began developing its Fifteenth Annual Plan of Work.

Seven projects were funded under CTSA's 14th year program, which was approved by CTSA's Board of Directors on January 11, 2001. Six were continuations of projects begun under the programs of previous years and one was a new project.

Since the inception of CTSA in 1988, it has funded 139 research, demonstration, development and extension projects. Fourteen projects were active during 2001. 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 Coordinator for New Animal Drug Applications

These projects address information dissemination:

* Library Aquaculture Workstation
* Publications
* Aquaculture Extension and Training Support for the U.S. Affiliated Pacific Islands

These projects address extension support to further industry development:

* Aquaculture Extension and Training Support for the U.S. Affiliated Pacific Islands
* Disease Management for Hawaiian Aquaculture
* Transitioning Hawaii's Freshwater Ornamental Aquaculture Industry

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

These projects address demonstration and adaptation of known technologies:

* Transitioning Hawaii's Freshwater Ornamental Industry
* Aquaculture Extension and Training Support for the U.S. Affiliated Pacific Islands
* Best Management Practices for Hawaiian Aquaculture

A brief listing of the principal accomplishments of the active projects in these categories during 2001 is presented below. Details on each project's funding, participants, objectives, anticipated benefits, progress and future plans are presented in the Progress Reports section.

National Aquaculture Priorities

National Coordinator for Aquaculture New Animal Drug Applications

New INADs are now in place at NIO to develop efficacy data on florfenicol and AQUI-S , and CRADAs are also in place with the sponsors for AQUI-S , copper sulfate, chloramine-T and florfenicol. In the latter half of 2000, new sponsors replaced the original sponsors for chloramine-T (Axcentive bv for Akzo Nobel Chemicals, Inc.) and oxytetracycline (Phibro Animal Health for Pfizer Inc.).

Information Dissemination

Library Aquaculture Workstation

In the Year 12 reporting period, there were 31,236 queries. PRAISE staff responded to 461 queries by returning to our users 5,514 journal citations, delivering 1,184 documents totaling 13,553 pages, and answering 166 miscellaneous queries. Staff delivered 78 article reference submissions to Cambridge Scientific Abstracts for inclusion to the ASFA database.

During Year 13's reporting period there were a total of 18,435 queries to the ASFA database. The PRAISE staff responded to an average of 33 research or journal requests per month by forwarding to our users 6,277 journal citations, delivering 1,048 documents totaling 13,022 pages, and answering 198 miscellaneous queries. A "Find It Yourself Online" page for aquaculturists has been developed and features links to free databases, online publications and full text materials for aquaculture and marine science.

Publications

The quarterly newsletter was printed and disseminated in March (double issue), June, September, and

December. The CTSA staff assisted with the creation, production and distribution of three CTSA publications and is currently working on production of three more. The homepage was updated monthly and maintained as needed.

Extension Support to Further Industry Development

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

The extension specialist distributed over 145 literature packets on a variety of aquaculture topics to various entities throughout the region during the reporting period. Work was completed on the applied research project on the culture of eight hard and soft corals. A manual has been written and will be printed soon. The agent also conducted 11 workshops or training sessions for private and government agencies.

Disease Management & Virology Service for Hawaiian Aquaculture

To assist aquaculture operations, 82 trips were made to the field during the reporting period. There were 403 case submissions of aquatic animals received for diagnostic laboratory analysis services. Samples of *Penaeus vannamei, P. stylirostris, P. japonicus* and *Halocaridina rubra* were evaluated for pathogens.

Development of New Technologies

Marine Foodfish Seedstock Production

During the Year 2 reporting period, the project produced and delivered 215, 896 Pacific threadfin and 3,600 milkfish fingerlings to farmers on the islands of Oahu, Maui, Molokai, and Hawaii and 1.7 million Pacific threadfin and 1.34 million milkfish eggs were distributed to three growout and research operations on Oahu and Hawaii. The project also developed the first select line of Pacific threadfin.

Year 3, although only recently started, has already delivered 130,714 Pacific threadfin and 10,900 milkfish fingerlings and 2.3 million Pacific threadfin and 250,000 milkfish eggs to farmers and state agencies.

Aquaculture of Marine Ornamental Species

Centralized broodstock populations of yellow tang and flame angelfish were successfully maintained for over 18 months. The first recorded spawning of yellow tang in captivity was achieved by this project. Work group members also established new protocols for handling eggs and maintaining yolk-sac larvae of flame angelfish, increasing early survival rates to nearly 70%.

Demonstration and Adaptation of Known Technologies

Transitioning Hawaii's Freshwater Ornamental Industry

During the reporting period, the extension specialist supported by this project made 26 site visits. Work group members conducted a total of 10 workshops.

Best Management Practices for Hawaiian Aquaculture

This project was terminated this year because it had accomplished all of its objectibes. A manual outlining the findings of research conducted by the PI was written, printed, and distributed.

A Look Ahead at Year 15

The development of the Year 15 program was initiated in February 2001 at the annual meeting of the Industry Advisory Council (IAC). The IAC reviewed the progress of funded projects and recommended Year 15 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. Twenty-three pre-proposals were submitted in response to the call and 14 were then asked to submit a full proposal. Twelve proposals were reviewed by the Technical Committee in October 2001 and will be forwarded to the Board of Directors as the Year 15 Plan of Work in January 2002:

1. Library Aquaculture Workstation – Year 15 *(continuing priority)*;
2. Aquaculture Extension & Training Support in the U.S. Affiliated Pacific Islands – Year 14 *(continuing priority);*
3. Disease Management & Virology Service for Hawaiian Aquaculture – Year 9 *(continuing priority)*;
4. Transitioning Hawaii's Freshwater Ornamental Aquaculture Industry – Year 3 *(continuing priority)*;
5. Aquaculture of Marine Ornamental Species – Year 3 *(continuing priority);*
6. Black Pearl Culture in the Pacific – Year 2 *(new priority);*
7. Marine Invertebrates *(new priority);*
8. Improving Hatchery & Nursery Methods for Sturgeon in Hawaii *(new priority)*;
9. Reproduction & Selective Breeding of Pacific Threadfin *(new priority);*
10. Evaluation of Tilapia Species & Varieties for Establishment of a Tilapia Hatchery in Guam *(new priority);*
11. Culture of the Mullet, *Moolgarda seheli*, in Palau *(new priority);*
12. Publications *(continuing priority)*.

In September, CTSA began its four-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 in October 2001. Once the final versions of the proposals are received and reviewed by the Program Review Delegation in December, they will be incorporated into the Fifteenth Annual Plan of Work, and presented to the Center's Board of Directors for approval in January 2002. 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 2001 is presented in the following pages. Details on each project's funding, participants, objectives, anticipated benefits, progress, impact, work planned and publications are presented. Information and results from previous years can be found in the correlating year's annual accomplishment report.

Disease Management and Virology for Hawaiian Aquaculture, Years 7 & 8

Reporting Period

October 1, 2000 – October 1, 2001 (Year 7)
August 1, 2001 – October 1, 2001 (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. James Brock, Anuenue Fisheries Research Center

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

Mrs. Dee Montgomery-Brock, Aquatic Health Associate, ADP, DOA

Objectives

Year 7

1. To establish a continual cell line from each of the following fishes, the green swordtail (*Xiphophorus helleri*), and the angel fish (*Pterophyllum scalare*), the grey mullet *(Mugil cephalus*),and Pacific threadfin (*Polydactylus sexfilis*).

2. To provide culture diagnostic services for the isolation of fish viruses from 10 case submission or marine or freshwater fishes.
3. To provide diagnostic support for the aquaculture sector in Hawaii and the Pacific Islands
4. To communicate to the industry the findings from the studies conducted in this project

Year 8

1. Establish a continuous cell line from each of the following fishes: the clownfish (*Amphiprion ocellaris*), the omilu (*Caranx melampygus*) and the snakehead (*Channa striatus*);
2. Provide fish virus culture and identification support services to the freshwater and marine fish aquaculturists in Hawaii;
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 services available through the Disease Management Project benefit aquaculture producers through the reduction of disease impacts in farmed aquatic animals and thus minimizing loss of animals and profits.

Work Progress and Principal Accomplishments

During the duration of this project, the participants have assisted the aquaculture community in controlling product losses due to disease. This assistance has been provided in various ways. On-site visits have been the most frequent method used to assist farmers. Experimental trials supported by the CTSA grant, made it possible to determine alternative and more efficient ways for farmers to overcome financially devastating losses caused by an aquatic disease. This program also has played a very important role in adding value to the aquaculture products sold from Hawaii, by providing routine Health Screening of the shrimp and fish populations produced here in the islands. The Health documents accompanying the shipments of these products increase the value of the product and the reputation of the aquaculture farmers in Hawaii.

Year 7

Objective 1

Experiments were carried out to establish primary cell lines from three of the four species: mullet, swordtails, and angelfish. Snout and caudal fin derived cell lines from the swordtail and angelfish are established and are in passage. Muscle derived

cell lines from the angelfish are established and in passage. Also, snout derived cell lines are in passage for Pacific threadfin.

Objective 2

One case of diseased fish suspected or known to be infected by a pathogenic fish virus was evaluated by cell culture method in the period covered by this report.

Objective 3

During the period covered by this report 82 trips were made into the field. There were 403 case submissions of aquatic animals received for diagnostic laboratory analysis service. Of these 403 cases, 100 cases involved assisting the producers and/or sellers of tropical fish. One hundred and twelve cases were requests for export documents certifying the health of the animals originating from aquaculture facilities in Hawaii.

During the reporting period surveillance for shrimp pathogens was carried out on shrimp tissue samples collected from 10 companies and/or facilities in Hawaii. The following shrimp samples were evaluated: *Penaeus vannamei, Penaeus stylirostris, Penaeus japonicus* and *Halocaridina rubra*. The samples were evaluated for pathogens by PCR method and/or histopathology. For PCR assay the samples were submitted to the University of Arizona, Tucson, Arizona.

Objective 4

A current technical report is being prepared, describing the progress made on developing fish cell lines. Two oral presentations were given that reported results from work conducted in this project. One article that reported on the results from work conducted in this project was accepted for publication.

Year 8

Objective 1

Work on this objective has not started. We will begin collecting samples in November, starting with the snakehead (*Channa striatus*).

Objective 2

No samples have been collected for this objective. There have not been any cases of fish viral disease submitted to our program.

Objective 3

Arrangements have been made for the first fish health training class to be held on the island of Maui on November 10-11, 2001.

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 4

A technical report is being prepared on the progress of developing fish cell lines.

Work Planned

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.

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 eight project.

In year eight we will continue to work on the development of cell lines for warm water fish, provide virus isolation tests for selected fish disease outbreaks and will provide two workshops on aquatic animal health.

Impacts

The following estimates of the economic impact were made by the staff of the companies that received assistance from this program. The estimates of the economic impact are based on the known or expected gain, or the reduction in the anticipated loss, once the 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 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

Brock, J.M. and Bullis, R.A**.** 2001. Disease Prevention and Control for Gametes and Embryos of Fish and Marine Shrimp. Aquaculture, 197(1-4):137-149.

Bullis, R.A. and D.C.M. Stake, eds. 2001. The U.S. Marine Shrimp Farming Program Annotated Bibliography: 1985-2001. The Oceanic Institute, Waimanalo, HI, 383 pp.

Bullis, R.A. Aquatic Animal Health & Biosecurity for Marine Ornamental Fish. Aquarium Sciences and Conservation. xxx: 000-000. 2001. In press.

Montgomery-Brock, D.R., V.T. Sato, J.A. Brock and C.S.Tamaru. 2001. The application of hydrogen peroxide as a treatment for the ectoparasite *Amyloodinium ocellatum* (Brown, 1931) on the Pacific Threadfin, *Polydactylus sexfilis.* Journal of the World Aquaculture Society, 32; 250-254.

Montgomery-Brock, D.R., J.A. Brock. The utilization of low light as a means for controlling Amyloodinium sp. on the Pacific threadfin. Presented at the World Aquaculture Society Conference 2001 in Orlando, Florida

Moss, S.M., B.J. Argue, F.L. Castille, S.M. Arce, J.M. Lotz, V.M. Breland, D.V. Lightner, B.L. White, C.L. Browdy, S.Y. Prior, A.L. Lawrence, H. Bowers and R.A. Bullis. 2001. Family Survival of Pacific White Shrimp *Litopenaeus vannamei* to Taura Syndrome Virus in Field and Laboratory Challenges. World Aquaculture'01, Orlando, Florida, World Aquaculture Society, p.459.

Newman, S.G. and R.A. Bullis. Immune Mechanisms of Shrimp: Form, Functions, and Practical Application. 2000. In: C.L. Browdy and D.E. Jory (Eds.), The New Wave. Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture, p.226.

National Coordinator for Aquaculture New Animal Drug Applications, Year 5

Reporting Period

October 1,2000 - October 1, 2001

Funding Level

Year 1	$5,000
Year 2	$10,000
Year 3	$10,000
Year 4	$10,000
Year 5	$10,000
TOTAL	$45,000

Participants

Rosalie (Roz) Schnick, National Aquaculture NADA Coordinator

Objectives

The overall goal of this project is for the National NADA Coordinator to coordinate activities for investigational new animal drug exemptions (INADs) and new animal drug applications (NADAs) to expedite approval from the Center for Veterinary Medicine (CVM) for the use of various drugs in aquaculture. The specific objectives for the fifth year of this project are to coordinate approval activities for these drugs: amoxicillin, AQUI-S™, calcein, chloramine-T, crude carp pituitary, copper sulfate, Earth Tec™, erythromycin, 17-beta estradiol, florfenicol, formalin (extension), fumagillin, hydrogen peroxide, 17 alpha-methyltestosterone, MS-222, Ovaplant™, Ovaprim™, oxytetracycline, pet fish therapeutants, potassium permanganate, Pyceze™, Romet-30™, ReproBoost™, sea lice control agents, strontium chloride, and trichlorfon. The general objectives are to address antimicrobial resistance and aquaculture effluents issues, help develop minor species legislation, and promote international harmonization and cooperation on aquaculture drug approvals.

Anticipated Benefits

New or amended approvals of aquaculture drugs will help reduce mortalities from disease and improve production efficiency; thus, the aquatic species producer can deliver more and healthier animals for consumption and recreational purposes and compete with foreign producers who can use many drugs without regulation. The National NADA Coordinator will ensure that progress is made toward NADA approval and that resources (funds, people, and facilities) are used effectively.

Work Progress and Principal Accomplishments

Since May 1995, two drugs (formalin and human chorionic gonadotropin) have been approved involving three label claims for all fish, six drugs are nearing completion for approval of 11 label claims, and 41 drugs have been addressed for use and potential approval in aquaculture. Major efforts have centered on the Federal-State Aquaculture Drug Approval Partnership Project (known as the IAFWA Project) with its eight drugs and crop grouping research, gaining sponsors for high priority drugs, achieving internationally standardized sensitivity tests, gaining Minor Use Minor Species legislation, addressing the proposed national EPA Effluents Guidelines Plan for drugs and chemicals, and dealing with antimicrobial resistance issues.

Approval status for IAFWA Drugs

All the technical sections (except efficacy) for which the IAFWA Project is responsible will be submitted by 2002 to allow for broader label claims when efficacy data are generated beyond 2002 for chloramine-T, formalin, hydrogen peroxide, and oxytetracycline. The sponsor of florfenicol is completing the technical sections on florfenicol for salmonids and catfish but the Drug Approval Working Group decided not to allow IAFWA Project funds to be expended to extend the label claims to cool and scaled warm water fish. Amendments to broaden initial or existing aquaculture drug approvals will be possible after pivotal and supporting efficacy data are generated and accepted to substantiate label claims beyond those mentioned above. At the present time, adequate efficacy data exist mainly for salmonids but are lacking for cool water and warm water fish. This lack of efficacy data jeopardizes the addition of these species to label claims in original or amended NADAs.

New INADs are now in place at NIO to develop efficacy data on florfenicol and AQUI-S™, and CRADAs are also in place with the sponsors for AQUI-S™, copper sulfate, chloramine-T and florfenicol. In the latter half of 2000, new sponsors replaced the original sponsors for chloramine-T (Axcentive bv for Akzo Nobel Chemicals, Inc.) and oxytetracycline (Phibro Animal Health for Pfizer Inc.).

Highlights for Individual Drugs-October 1, 2000 through October 1, 2001

Amoxicillin **(oral antibacterial)**

Kent SeaFarms Corporation, the new United States representative GB Research, Inc., for the development of its amoxicillin product has recently interacted with CVM and the National Coordinator for Aquaculture NADA to write a development plan.

AQUI-S™ **(anesthetic)**

On November 29, 2000, CVM permitted the use of AQUI-S™ under FWS's INAD to treat up to 100 million fish with 5 to 34 mg/L AQUI-S™ in a static bath for one to ten minutes.

The sponsor, AQUI-S New Zealand LTD., reversed a business decision to reformulate their product. The product to be developed in the United States is the same formulation that the company has approved as a fish anesthetic in several countries.

Chloramine-T (external antibacterial)

Axcentive bv held a meeting with CVM and UMESC on November 29, 2000 to discuss the development of its EA on its chloramine-T product Halamid™.

A meeting was held on December 14, 2000 with CVM to discuss the draft labels for chloramine-T; to identify any remaining data gaps for the label claims. Residue chemistry data requirements were clarified for an Aall finfish@ label claim. Applications of chloramine-T in continuous-flow systems will be verified by FWS

Label claims nearing completion:

Chloramine-T
Copper sulfate
Florfenicol
Florfenicol
Formalin
Hydrogen peroxide
Hydrogen peroxide
Hydrogen peroxide
Oxytetracycline
Oxytetracycline
Oxytetracycline

All holders of chloramine-T INADs were sent notices in March 2001 that CVM has concerns for the possible carcinogenicity of p-TSA, the marker residue of chloramine-T and will not renew slaughter authorizations (including release of fish) after a certain point (depends upon the date of INAD renewal). This policy will continue until CVM receives new information from the sponsor that addresses CVM's mammalian safety concerns for p-TSA. The sponsor is actively engaged in responding to concerns raised by CVM.

Common Carp Pituitary (CCP)

Mississippi State University submitted a target animal safety study on grass carp to CVM. Funding has been requested from NRSP-7 to do research on walleye, catfish, and white bass.

Diquat Dibromide (external microbicide)

The potential sponsor, Syngenta, has expressed an interest in developing their diquat product for use as a drug in aquaculture.

Florfenicol (oral antibacterial).

A Cooperative Research and Development Agreement (CRADA) between Schering-Plough Animal Health and USGS was signed on April 10, 2001. The in-life phase of a target animal safety study for florfenicol in channel catfish has been completed under that CRADA.

Hydrogen peroxide (external microbicide)

CVM accepted pivotal efficacy data for treatment of salmonid eggs to control mortalities associated with saprolegniasis by a 15-minute treatment at 500 mg/L of hydrogen peroxide; and a 60-minute treatment at 50 mg/L or 30-minute treatment at 100 mg/L of hydrogen peroxide to control mortalities associated with BGD on salmonids. CVM accepted as supporting data, 60-minute treatments to control mortalities associated with external columnaris disease in yellow perch.

A target animal safety technical section on all fish was submitted on October 19, 2000 to CVM by UMESC.

A meeting was held on December 14, 2000 with CVM to discuss the draft labels for hydrogen peroxide to identify any remaining data gaps for the label claims. Target animal safety and efficacy data requirements were clarified for an all finfish label claim. Applications of hydrogen peroxide in continuous-flow systems will be verified by FWS.

UMESC has continued to expand its coordination and collaboration to develop additional efficacy data to support the use of hydrogen peroxide by initiating three compassionate INADs. Participation in the three INAD protocols has increased immensely over the past year from 24 INAD cooperators in 2000 to 115 in 2001.

MelaFix™ (external microbicide)

CVM met with the potential sponsor to discuss the development of MelaFix™ on December 13, 2000.

17 alpha-methyltestosterone (gender manipulation aid)

Environmental safety-Auburn University is working on a response to comments by CVM on the environmental assessment that they wrote on the use of MT for tilapia. It has been determined that an environmental study is needed and Auburn has secured a USDA laboratory to do the study. This study will be conducted next spring. Auburn developed a study protocol now that they submitted on October 4, 2001 to CVM for review and acceptance.

Target animal safety (TAS)-Auburn is working on a TAS technical section for tilapia. Southern Illinois University (SIU) completed a TAS study on percids that was funded by NCRAC and reviewed by CVM. The TAS study was found to be inadequate by CVM; SIU needs to respond to the comments by CVM to resolve the issues so that the technical section can be declared complete for percids by CVM.

Efficacy-Auburn is working on a technical section to complete it for tilapia. The University of Wisconsin is working on developing the efficacy technical section for percids.

Oxytetracycline (OTC, oral antibacterial)

CVM accepted residue chemistry studies on oxytetracycline (OTC) for use on salmonids below 9EC, and established a withdrawal time of three days for juvenile salmonids treated with OTC medicated diet.

Two separate residue depletion studies in representative cool water fish, walleye and northern pike, were submitted to CVM on October 4, 2000.

A meeting was held on December 14, 2000 with CVM to discuss the draft labels for OTC to identify any remaining data gaps for the label claims. Residue chemistry data requirements were clarified for an all fish label claim. An environmental assessment will have to be developed for any new label claims. CVM accepted the human food safety technical section for juvenile salmonids at 9EC and established a three-day withdrawal period.

Praziquantel (trematode and cestode control) and Pyceze™ (external microbicide)

The potential sponsor of praziquantel expressed an interest in developing their product for our domestic aquaculture at a meeting in August 2001.

Pyceze™ (external microbicide)

Vericore Limited sold its Pyceze™ to Novartis Animal Health LTD who is interested in moving forward more rapidly in developing their product for our domestic aquaculture as stated at a meeting in August 2001.

Romet-30™ (oral antibacterial)

The National Aquaculture NADA Coordinator met with the product manager for Romet-30™ and UMESC on November 1-2, 2000 in La Crosse, Wisconsin and also on January 24, 2001 in Orlando, Florida and in August 2001 in Bozeman, Montana to discuss potential extensions and expansions of the NADA for publicly cultured finfish.

Sea Lice Control (various drugs and pesticides)

Various drugs and pesticides (azamethiphos or Salmosan™, cypermethrin or Excis™) are being pursued by the United States and Canada and are at various stages of registration and approval. These uses are being challenged on the East coast, particularly in Maine. An INAD for Slice™ (emamectin benzoate) was allowed by CVM as a result of great need for a control that could not be challenged to the extent that the others have been.

Trichlorfon (external parasite control)

Some interest on the part of potential sponsor in a U.S. NADA approval; has approvals in several countries; several Special Local Need (SLN) registrations obtained in 1998. The SLN registration in California is in jeopardy because the state wants more data.

Meetings and Special Activities

IAFWA Project

The Drug Approval Working Group (DAWG) for the Federal-State Aquaculture Drug Approval Partnership Project (IAFWA Project) held two meetings to (1) discuss the progress being made on the IAFWA Project drugs, (2) work plans for the final year of the IAFWA Project, (3) discuss new funding proposals on florfenicol, AQUI-S™, disease model for external columnaris disease, and environmental assessment for oxytetracycline, and (4) the future of public drug approval efforts after 2002.

Antimicrobial resistance

A special session entitled "Aquaculture and drug resistance" was convened at Aquaculture 2001 on January 25, 2001, Lake Buena Vista, Florida. Topics included: (1) biology of antibiotic resistance, (2) antibiotic resistance in the salmonids and channel catfish industries, (3) standardization of susceptibility testing, and (4) the negligible public health risk from antimicrobial use in aquaculture.

EPA Effluent Guidelines Plan

The Joint Subcommittee on Aquaculture formed the Aquaculture Effluents Task Force (AETF) to coordinate and facilitate input of science-based information to assist in the development of national effluent limitation guidelines and standards for aquaculture facilities by EPA. The AETF met on October 17-18, 2001 to discuss the status of EPA's Effluent Guidelines Plan for aquaculture facilities. A conference call was convened on May 30, 2001 to discuss drug and chemical issues with EPA and a response was prepared after a conference call on October 11, 2001 with AETF members.

Minor Use/Minor Species (MUMS) legislation

A bill entitled "Minor Animal Species Health and Welfare Act of 2000" was introduced in the U.S. Congress into the House on June 27, 2000 (HR-4780) and into the Senate on October 5, 2000 (S-3169). The MUMS Act will facilitate and accelerate the approvals of aquaculture drugs. There is a great need for more co-sponsors. The bill includes provisions for early life stages that should help expedite the approvals of aquaculture drugs that are of interest to public and private fish production. A revised bill "Minor Use Minor Species Animal Health Act of 2001" was reintroduced into the House on May 24, 2001 (HR-1956) and into the Senate on August 2, 2001 (S-1346). The MUMS Coalition met on June 22, 2001 to coordinate the legislative effort on the bill, present information to legislative staff, and contact individual congressmen and senators for their support and sponsorship. Letters were written to follow-up on the contacts.

Internationally standardized sensitivity tests

A follow-up session on international harmonization of sensitivity testing was held in Dublin, Ireland in September 2001; an update is not available at this time. The EAFP Work Group is working with the National Committee for Clinical Laboratory Standards to develop protocols that will allow aquatic diagnostic and research laboratories to (1) test disease-associated bacterial isolates for antimicrobial susceptibility patterns and (2) recommend

appropriate therapy in a standard, internationally accepted manner. Added benefits include aiding the approval process for antibacterial agents and helping to determine the antimicrobial resistance of aquaculture pathogens worldwide.

Risk assessment of drugs and chemicals used in foreign aquaculture

CVM awarded a contract on the risk assessment of drugs and chemicals used in foreign aquaculture on September 28, 2001 to ICF Consulting. The objectives of this contract are to (1) create a database containing information on drug and chemical use is foreign aquaculture and (2) perform a human food safety risk assessment for each drug and chemical listed in the database. FDA will use the results of this contract to (1) prioritize the monitoring of drug and chemical residues in the edible tissue of imported aquaculture products, (2) prioritize the development of methods to be used in the monitoring program, and (3) provide a basis for promoting discussion with foreign countries regarding the hazard concerns identified by the risk assessment. The National Aquaculture NADA Coordinator is a subcontractor to this.

Potential sponsors for aquaculture drugs

On August 2-3, 2001, nine sponsors or potential sponsors invited by the National Aquaculture NADA Coordinator attended a meeting of the Federal-State Aquaculture Drug Approval Partnership Project.

Work Planned

Coordinate approval activities for amoxicillin, AQUI-S™, calcein, chloramine-T, crude carp pituitary, copper sulfate, erythromycin, 17 beta-estradiol, florfenicol, formalin (extension), fumagillin, hydrogen peroxide, 17 alpha-methyltestosterone, MS-222, OvaplantÔ, OvaprimÔ, oxytetracycline, pet fish therapeutants, potassium permanganate, Pyceze™, Romet-30™, ReproBoost™, sea lice control agents, strontium chloride, and trichlorfon. In addition, there will be considerable effort on the MUMS legislation, antimicrobial resistance, Aquaculture Effluents Task Force, efficacy initiative for the IAFWA Project, and international harmonization of aquaculture drug approvals and sensitivity tests.

Impacts

Establishment of the National NADA Coordinator position in May 1995 has resulted in coordination, consolidation, and increased involvement in the INAD/NADA process on 18 of the 19 high priority aquaculture drugs and activities on additional new drugs of interest to aquaculture. Twenty-one INAD/NADA sponsors have initiated INADs or confirmed their commitment to gaining approvals of their products for the aquaculture industry. Progress has been made toward unified efforts on existing and new INADs/NADAs for a variety of priority drugs. An original NADA approval was gained for human chorionic gonadotropin in September 1999. A supplemental NADA was obtained for formalin as a fungicide on all fish eggs and as an external parasiticide for all fish in June 1998. A new NADA has been granted to Western Chemical Inc. for its MS-222 product (an anesthetic). Data packages have recently been submitted to CVM for the following drugs: AQUI-S™, chloramine-T,

copper sulfate, formalin (extension), florfenicol, hydrogen peroxide, 17 alpha-methyltestosterone, oxytetracycline, potassium permanganate, and Pyceze™.

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

Efforts to develop the Minor Use/Minor Species document into legislation will encourage more sponsors to support aquaculture drug approvals. Efforts to deal with the antimicrobial resistance issue and EPA's Effluents Standardization Plan will ensure that aquaculture can continue to legally use drugs and chemicals. Efforts on the international scale will result in more international harmonization related to aquaculture drug approvals.

Publications in Print, Manuscripts and Papers Presented

Publications in Print

Schnick, R.A. 2001. International harmonization of antimicrobial sensitivity determination for aquaculture drugs. Aquaculture (3-4):277-288.

Schnick, R.A. 2001. Progress of the Federal-State Aquaculture Drug Approval Partnership Project. American Fisheries Society Fish Health Newsletter 29 (4):6-7, 9.

Manuscripts

Schnick, R.A. In press. Aquaculture chemicals. Chapter *in* Kirk-Othmer Encyclopedia. John Wiley & Sons, Inc., New York, New York.

Papers Presented

Schnick, R.A. 2001. Overview of progress toward aquaculture drug approvals. USFWS – 7th Annual INAD Coordination Workshop, Bozeman, Montana, August 1-3, 2001

Development of Best Management Practices for Hawaiian Aquaculture -

Termination Report

This project was terminated because all objectives were met.

Project Duration

September 1997 – September 2001

Funding Level

Year 1	$10,000
TOTAL	$10,000

Participants

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

Objectives

1. Conduct a comprehensive literature review of current and proposed Best Management Practices for aquaculture systems in the United States (e.g. trout, channel catfish, salmon).

2. Review BMPs developed for other industries nationwide (beef, poultry, dairy, silvicul ture) and in Hawaii (sugar, pineapple) to determine how BMPs support and facilitate compliance with effluent discharge regulations.

3. Evaluate documents generated through other Regional Aquaculture Centers concern ing effluent discharge and best management practices for aquaculture.

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

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

The final result will be the development of a practical manual outlining guidelines, recommendations and defining principles of Best Management Practices for Hawaiian Aquaculture. It is then anticipated that this manual can be used by Hawaii's aquaculture farmers to comply with permit regulations and increase farm efficiency

Principal Accomplishments

Objective 1

A thorough literature search was carried out using the internet and the University of Hawaii Hamilton Library. The CTSA-funded PRAISE (Pacific Region Aquaculture Information Service for Education) project was used to assist in the literature search.

Results at a glance . . .

Manual for *Best Management Practices for Hawaiian Aquaculture* was published and is available through CTSA.

Objective 2

Best management practices from other agriculture industries were reviewed and BMPs that were relevant to aquaculture were incorporated into the publication, Best Management Practices for Hawaiian Aquaculture.

Objective 3

A number of key documents produced through other USDA Regional Aquaculture Centers were especially useful. These publications included: *A White Paper on the Status and Concerns of Aquaculture Effluents in the North Central Region* (NCRAC); *Characterization and Management of Effluents from Aquaculture Ponds in the Southern United States* (SRAC); *Beneficial Utilization of Aquaculture Effluents and Solids* (NCRAC). Numerous other publications that were derived from RAC funded projects were also helpful.

Objective 4

A number of recent and relevant publications were reviewed including *Best Management practices for shrimp culture in Latin and Central America*; *Environmental Code of Conduct for Australian Prawn Farmers*; *Australian Water Quality Guidelines for Fresh and Marine Waters*; *Aquaculture and the Environment*; *Effluent and Solid Waste Management in Pond Aquaculture*; *Development of Strategies for Sustainable Shrimp Farming in Thailand*.

Objective 5

No interaction with USDA Farm Service Agency was carried out.

Objective 6

A thirty-one page manual entitled Best Management Practices for Hawaiian Aquaculture was pub-

lished by CTSA. This manual is available to aquaculture farmers in Hawaii to assist them in compliance with federal and state water quality regulations. Moreover, by following the practices outlined in the manual aquaculture producers may be able to increase farm efficiency and increase profitability.

Impacts

It is very difficult to quantify impacts of this project. The final outcome, a publication of best management practices will be made available to aquaculture producers in Hawaii. Although for there to be any positive impacts farmers should follow many of the recommendations outlined in the manual. By utilizing best management practices producers will be more likely to comply with permit regulations and increase farm productivity and profitability.

Recommended Follow-up Activities

The Best Management Practices manual produced from this activity is, by the very nature of aquaculture in Hawaii, non-specific to species or culture systems. It is recommended that additional documents be produced that are specific to the wide variety of cultured species and culture systems found in Hawaii (e.g. traditional Hawaiian fishponds, re-circulating systems, Chinese catfish).

Publications in Print, Manuscripts and Papers Presented

Best Management Practices for Hawaiian Aquaculture. Center for Tropical and Subtropical Aquaculture. Publication No. 148.

Aquaculture Research, Extension and Training in the U.S. Affiliated Pacific Islands, Year 12

Reporting Period

October 1, 2000 - October 1, 2001

Funding Level

Year 1	$100,000
Year 2	85,870
Year 3	83,600
Year 4	70,000
Year 5	75,000
Year 6	98,000
Year 7	70,000
Year 8	75,000
Year 9	85,000
Year 10	85,000
Year 11	75,000
Year 12	100,520
TOTAL	$1,002,990

Objectives

The goal of this project is to facilitate the development of a sustainable, economically viable aquaculture industry in the U.S. Affiliated Pacific Islands. Specific objectives are:

1. Extension support and technical assistance

2. Institution strengthening and capacity building

3. Technology transfer

4. Information collection and dissemination

5. Applied research

Anticipated Benefits

Workshops, publications and information dissemination will lead to an increased perception of aquaculture and its potential benefit to the region, particularly in species identified as being regionally appropriate.

Currently operating aquaculture facilities will benefit from the constant presence of an extension agent who can advise on problems, provide information on aquaculture topics and make site visits.

Training and regular site visits will help to build capacity and skills among people interested or involved in aquaculture.

Work Progress and Principal Accomplishments

This project began in 1989, when CTSA funded an aquaculture extension specialist for the region. The extension specialist provided technical advice and assistance to establish the FSM National Aquaculture Center in Kosrae and established a demonstration ocean growout farm on the reef outside the National Aquaculture Center. A giant clam demonstration farm was established in Pohnpei, FSM. There are currently several small sponge farms producing commercially in small numbers throughout the FSM. The agent has provided information on aquaculture of various species in response to parties throughout the region. Literature and advice on aquaculture of various species were provided consistently to private and government agencies in the region. Since 1997, the extension agent has held over 70 workshops and training sessions with more than 500 participants. The agent continues to be an extremely valuable resource to the U.S. Affiliated Pacific Islands and has helped to establish a pearl farm in the RMI, which is about to have its fourth successful harvest and is finally experiencing profits.

Objective 1

The extension agent continues to provide constant extension services to the region on all topics of aquaculture. Some of the major areas he is working in are listed below:

a. Continued extension assistance for Nukuoro pearl farm including securing them a seeding technician, sending staff for training, assistance with proposals for funding and technical advice.
b. Continued assistance to Robert Reimers Enterprises, Inc.'s pearl, clam and coral farms. Workshops, materials sourcing and continued training through site visits to assist this company in improving their product line and increasing production.
c. Closer links with Black Pearls of Micronesia

(BPOM). Now the largest pearl farm in the RMI, the agent has been working more closely with BPOM to address problems of snail and fish predation on their farms. Technical assistance has also been provided for their hatchery operation.

d. Continued work with Marine and Environmental Research Institute of Pohnpei. He continues to have close links with this educational establishment, providing them with technical advice, hands on training and involvement with their giant clam, sponge and coral demonstration projects and also their coral research project. Recently assistance has been given in setting up a demonstration pearl oyster hatchery.

e. New involvement with Land Grant hatchery. The Land Grant office is installing a pearl oyster hatchery in Pohnpei. While they have hired a qualified manager for this project he is working closely with this person to provide assistance in ordering and on how best to approach problems in Pohnpei.

f. New assistance to PAFTP. PAFTP is a newly formed aquaculture venture in Chuuk. Aside from providing hands on training the extension agent has assisted with input for their business plan and in sourcing materials and livestock.

g. Collaboration on a sponge marketing study with the Pacific Business Center. The extension agent facilitated a sponge marketing study to open new markets for farmed sponges.

h. Provided planning and technical assistance to new CMI Land Grant aquaculture facility on Majuro, RMI.

Results at a glance . . .

Distributed over 145 literature packets

Completed research on culture of eight hard and soft corals

Conducted 11 workshops or training sessions

Objective 2

a. Three one day training sessions for staff and students of MERIP on spawning, larval rearing, lagoon culture, cage and trestle construction and general husbandry for giant clams.

b. A four-day workshop held on Mili atoll providing training to staff of Wau mariculture farm on spawning and larval rearing of *T. gigas*, lagoon based clam farming and cage manufacture, coral collection and culture techniques and farm scheduling and organization.

c. Half day training on shore collection of marine invertebrates with staff of RRE clam farm in Majuro.

d. Half-day workshop for MERIP staff and students on plumbing and seawater systems.

e. Three 1-day workshops to provide microalgae culture and transfer techniques.

f. Five-day training course in Chuuk on sponges, corals and giant clams for residents of Polle. This workshop replaced the one scheduled for CNMI due to a lack of support and interest by his counterparts there.

g. One-day workshop for MERIP staff on collection and care of pearl oysters from the wild.

In order to build capacity in the region the Regional Aquaculture Extension Agent will focus training efforts on local extension agents in government and private organizations.

a. Pohnpei information dissemination and planning meeting. On September 27 and 28 CTSA sponsored a 2-day aquaculture conference in Pohnpei. The extension agent organized and conducted this meeting and also presented talks on pearls, clams, corals and sponges. This meeting was an excellent way to garner input on the future direction of aquaculture in the FSM and also to disseminate information.

b. Information dissemination and outreach meeting in Majuro, RMI during November 28 and 29 during which the agent gave talks on marine ornamentals and sponge farming.
c. Inshore fisheries and aquaculture planning meeting for the FSM in Pohnpei, December 5-7 at which the agent gave a talk on the role of CTSA in aquaculture development in the FSM and participated in planning discussions on aquaculture for the FSM.
d. Member of a team developing aquaculture training curricula and demonstration facilities for College of the Marshall Islands.

Objective 3

A simplified manual based on research conducted by the extension agent on optimum farming practices of eight species of hard and soft coral is approximately 95% completed and is expected to be printed by February 2002.

Objective 4

A total of 145 literature packets, including mass mailings of recently completed CTSA publications, have been sent out during the reporting period. In addition, CTSA extension publications and videos are always given out in limited numbers whenever the agent gives information dissemination talks. The library is constantly being expanded with relevant literature both from journals and periodicals and also literature requested through PRAISE or other organizations. Two new videos were also added to the collection, some rough sponge farming footage and a pearl oyster grafting video. To provide information on regional aquaculture activities, events and work in progress, the extension agent is now publishing a quarterly newsletter. This is sent via email to save mailing costs and improve delivery time.

Objective 5

All objectives for this study have now been met with the completion of shipping trials to the USA and the aquarium trials in April/May 2001. Growth trials were completed in March 2001.

Work Planned

While next year's work will continue to focus on expanding and strengthening culture of the four specific groups of aquaculture species identified in Year 9 of this project: giant clams, sponges, black pearl oysters and marine ornamentals, there will be a greater specificity on particular areas that have been identified as bottlenecks in the industry. Although all bottlenecks cannot be addressed in one year, next year's work will start to methodically address some of these identified critical areas. As time progresses, training and technology transfer efforts are expected to effectively transition institutional personnel in the region to address these, and evolving, problem independently. In the meantime, the resources, institutional knowledge and skills provided by the CTSA sponsored development program in the US Affiliated Pacific Islands remain critical to the future development of aquaculture in the region as it provides a stable platform of aquaculture resources on which other institutions can grow.

Publications in Print, Manuscripts and Papers Presented

Lee, C.-S., S. Ellis and K. Awaya. 2001. Giant clam farming in the U.S. affiliated Pacific Islands. *World Aquaculture*. Vol. 32, No. 3. September 2001. Pages 21-27, 62-63.

Transitioning Hawaii's Freshwater Ornamental Industry, Year 2

Reporting Period

October 1, 2000 - October 1, 2001

Funding Level

Year 1 $100,000

Participants

Clyde Tamaru, Extension Agent, Sea Grant Extension Service

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

Jennifer Olson, Windward Community College (WCC)

Matt Lyum, Sea Grant Extension Service

Objectives

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 (HUFA's) in the culture of fresh water ornamentals.

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

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 maintaining an ornamental fish specialist to provide 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.

Work Progress and Principal Accomplishments

Last year in the first year of the project, work group members were able to demonstrate that sex ratio is clearly linked with the variety of swordtail being cultured. The freshwater rotifer variety, *Brachionus calciflorus*, has been made available to farmers as a live food for freshwater ornamental fish larvae in Hawaii. The procedure of artificially inseminating the swordtail as well as other live bearers was documented. As a result of this project, freshwater rotifers are now available for use by Hawaii's aquafarmers.

Objective 1

Trial #2: Repeat of Experiment with Different Swordtail Strain

Nine 10-gallon aquaria were filled with 28 L of water and all tanks were equipped with biofilters and with an aquarium heater to keep water temperatures near 27-28° C. The tanks were stocked with one-week-old pineapple swordtails that were at a mean weight of 21 mg. Stocking densities were 1 fish/L, 3 fish/L, and 6 fish/L with each density being triplicated per group. The fish were fed measured amounts of commercial feed, progressing from Lansy to Silver Cup size 0 and finally to Silver Cup size 1. The fish were fed three times daily at 8:00 a.m., 12:00 p.m., and 4:00 p.m. At the conclusion of the experiment, (150 days) all fish from each tank were sexed, weighed, and total body length measured. Males were identified by the presence of a gonapodium. There were males with the gonapodium and no sword and these were classified as "mahu" males. Females were characterized by a lack of gonapodium and a round body.

Average survival appeared to be negatively correlated with the increase stocking density. However, only the tanks that were stocked at a density of 6

fish per liter 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 as all 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 were 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.

Results at a glance . . .

26 site visits were made

10 workshops were conducted

Based on the results of these experiments it would appear that stocking densities between 1-3 fish/liter would be the highest one would use for the culture of swordtails in a recirculating system. To confirm the results large-scale (2000-l) rearing trial conducted outdoors were conducted using a stocking density of 3 fish/L. The red velvet strain of swordtails and 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 feeding regimen overcame the capacity to control water quality parameters in the tanks with no biofiler (control) and those with the submerged biofilters. Of the surviving fish, sex ratios were consistent with that of reported for the aquaria where the sex ratio apparently is related to 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 be those that maximize growth to result in suitable quantities of males.

Objective 2

Previous trials using fresh Chlorella were found to produce the highest densities yet recorded. However, the prohibitive costs of importing the algae makes in impractical for use unless at a large enough scale yet to be seen in Hawaii. An alternative feed that produces similar results would obviously have practical application. Small-scale trials (8-liter) trials using a variety of "green water" (tilapia, guppy, recirculating systems) were tested and a very encouraging result has been obtained. Very high densities were achieved using the resulting water present in tanks that are being recirculated.

The trials were confirmed during the reporting period and it is clear that the "green water" present in the recirculating systems provides for a good food source for culturing Moina. Larger-scale growout systems (300 gallon/1200 L) have been examined and it appears that if the recirculating water is combined with yeast a stable source of moina can be achieved for approximately a month.

Objective 3

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

Kathleen McGovern-Hopkins was hired in September of 2000 and has taken up the extension work. During the reporting period she made 26 site visits, had 21 personal meetings with clientele at WCC, and has provided 4 group tours of WCC.

There are three manuals that are in various stages of preparation. The commercial production of swordtail is expected to be completed by the end of the project and a no cost extension of the project has been obtained to carry out this activity. An artificial insemination of livebearer manual and a live feeds production manual are in the planning stages of development.

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

Fancy Livebearer Workshop, Cooperative Extension Service, Komohana Complex, Hilo. October 21, 2000. 46 people in attendance.

Fancy Livebearer Workshop, Windward Community College, November 25, 2000. 21 people in attendance.

Fancy Livebearer Workshop, Maui Community College, December 16, 2000. 26 people in attendance.

Pacific Tropical Ornamental Fish Project, Windward Community College, January 6, 2001. 23 people in attendance.

Pacific Tropical Ornamental Fish Project, January 10, 2001. Maui Community College 30 people in attendance.

Pacific Tropical Ornamental Fish Project, January 11, 2001 Natural Energy Laboratory of Hawaii. 6 people in attendance.

Pacific Tropical Ornamental Fish Project, January 12, 2001, Cooperative Extension Service, Komohana Complex. 10 people in attendance.

Ornamental Fish Industry Stakeholders Input Seminar , April 28, 2001, Windward Community College.

Ornamental Fish Industry Stakeholders Input Seminar, June 16, 2001, Cooperative Extension Service, Komohana Complex, UH Hilo.

Work Planned

The printing of the swordtail manual is a major activity that is to be completed by the end of the project. Continued work will be conducted to document large-scale production of live feeds (rotifers and moina) and a draft manual for live feeds production is planned for the next reporting period. The work with recirculating systems is being replicated using different species where insight will be obtained on species specific responses to recirculating systems will be obtained as well as refining the biofilters and tank systems. It is a goal that we will be able to recommend the most cost effective method of water reuse that is based on empirical results. Lastly, the project is to be used as a forum in which discussions can begin on how the industry as a whole might begin to collaborate as a unified entity.

Publications in Print, Manuscripts and Papers Presented

Ornamental Fish Industry Stakeholders Input Seminar, August 4, 2001, Maui Community College.

Presentations

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., 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.

Tamaru, C.S., H. Ako, L. Asano, L. 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.

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).

Articles and Manuscripts

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.

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.

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.

Manuscripts Submitted

Tamaru, C.S, H. Ako, H., 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).

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

Library Aquaculture Workstation (PRAISE), Years 12 & 13

Reporting Period

June 1, 1999 – November 30, 2000
October 1, 2000 – October 1, 2001

Participants

Kristen Anderson, Reference Librarian, Hamilton Library, University of Hawaii
Lois Kiehl-Cain, Project Assistant, University of Hawaii

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
TOTAL	$268,650

Objectives

Year 12

1. Continue to provide established services.
2. Develop programs for user education.
3. Promote communication between the US Affiliated Pacific Islands
4. Technology Transfer

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.

Work Progress and Principal Accomplishments

A breakthrough in the Pacific Islands' ability to access scientific information came in August 1993, when two remote workstations were established on Guam. Users at the site at the offices of the University of Guam's Cooperative Extension Service and at the Guam Department of Commerce gained access to PRAISE through a toll-free telephone line.

PRAISE entered a cooperative agreement with PEACESAT, a federally funded communications satellite, whereby residents at five Pacific Island sites can directly access the Aquatic Sciences and Fisheries Abstracts (ASFA) database through an Internet connection between the local PEACESAT station and the mainland vendor. In addition, PRAISE established a home page on the World Wide Web. Search assistance and document delivery requests can be sent to PRAISE personnel via the webpage.

The Pacific Islands Gray Literature project was established to address the inaccessibility of gray literature in the Pacific, where libraries and other organizations that collect and disseminate information are few. This project began with a printed bibliography which has been expanded and is now also available on the website.

Year 12

Objective 1

Service has been greatly enhanced for PRAISE customers due to the advances in electronic technology. There are three major changes which are improving our service: 1) increased use and acceptance of the Ariel document delivery software which allows us to send articles as an email attachment, 2) the increasing number of people accessing the PRAISE Web site and utilizing the online request forms, and 3) the growing reliability of electronic access from Pacific Islands.

During the period of the grant there were 31,236 queries posted to the ASFA and Oceanic Abstracts databases. The PRAISE staff responded to 461 queries by returning to our users 5,514 journal citations, delivering 1,184 documents totaling 13,553 pages, and answering 166 miscellaneous queries. Turn-around time on research and article requests is usually 24 hours and email users may get articles the same day via our Ariel document delivery software.

Objective 2

The Principle Investigator participated in the "Fish Health Seminar" in Hilo on the Big Island and discussed services offered by PRAISE as well as how individuals might do searching on their own. She met with individuals to discuss searching and distributed brochures in the Kona area, specifically at businesses near the Energy Lab. Instruction sessions were also given on Oahu, including ongoing participation with the Micronesia and American Samoa Student Internship Program (MASSIP). The Principal Investigator and the Project Assistant continue to develop instructional enhancements for the website. The Principal Investigator attended the annual conference of the International Association of Marine Science Libraries and Information Centers and focused on sessions dealing with instruction, both classroom and web-based, as well as digitizing projects.

Objective 3

The Principal Investigator was an invited speaker at the Pacific Islands Association of Libraries and Archives (PIALA) Annual Conference in Koror, Palau, in November 1999. PIALA is a wonderful venue for reaching all levels of the Pacific Island communities as librarians and information specialists from throughout the Pacific gathered for this conference. The Principal Investigator presented a talk on regional interlibrary-loan development and the benefits of an established information dissemination service utilizing the Ariel software for a geographically diverse region to PIALA attendees. She also passed out brochures, and was able to address a number of Pacific researchers gathered at a meeting on coral reefs which took place on the campus of Palau Community College.

Objective 4

PRAISE staff delivered 78 article reference submissions to Cambridge Scientific Abstracts (CSA) for inclusion to the ASFA database. The focus was to ensure inclusion of all CTSA publications and area interest with proceedings of the PACON conferences. The PRAISE Web site and the Pacific Grey Literature Database are continually being updated and enhanced by the Principal Investigator and the Project Assistant. The Education page now features separate categories for degree programs (both national and international), short course and workshops in aquaculture, current postings for grants, fellowships, scholarships and other funding resources. This ongoing work allows us to provide the best possible service to our users.

Year 13

Objective 1

PRAISE continues to enhance its value to aquaculturists through the application of electronic technology. Examples of these applications are: 1) recent arrival of the latest and reportedly color-capable version of the increasingly popular Ariel docu-

ment delivery service which allows us to send articles as email attachments; 2) creation of the "Find It Yourself " webpage highlighting free aquaculture related databases and websites; 3) development of online request forms; 4) the arrival of the new NT server at the latter part of the reporting period. We are currently in discussion with Kapiolani Community College to migrate their files to this server.

During this reporting period there were a total of 18,435 queries to the ASFA database. The PRAISE staff responded to an average of 33 research or journal requests per month by forwarding to our users 6,277 journal citations, delivering 1,048 documents totaling 13,022 pages, and answering 198 miscellaneous queries. Turnaround time on research and article requests is usually 24 hours and email users often get articles the same day via our Ariel document delivery software.

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:

31,236 queries averaging 3 minutes or

1,562 hours online @ $60/hr = $93,720

1,184 articles @ $15.50/ea. = $18,352

Total $112,072 (Year 12)

Approx. 18,435 queries* averaging 3 minutes or

922 hours online @ $60/hr = $55,320

1,048 articles @ $18.00/ea. = $18,864

Total $74,184 (Year 13)

*ASFA and Oceanic Abstracts only, the figure does not include additional PRAISE queries to Biological or Zoological Abstracts

work on the grey literature digitization project. During exhibit periods the Principal Investigator also presented demonstrations of the services PRAISE offers.

The Principal Investigator and the Assistant attended the Hawaii Aquaculture Association Conference at Windward Community College on Oahu, distributed brochures and gave a PRAISE PowerPoint presentation. Other instruction sessions were also given on Oahu. A workshop was given for Pacific Islands students participating in the MASSIP project administered at the University of Hawaii - Hilo. The Principal Investigator and the Project Assistant continue to develop instructional enhancements for the website.

Objective 2

The Principle Investigator attended the Pacific Islands Association of Libraries and Archives Conference on Guam and participated in the pre-conference workshop on digitization in preparation for

Objective 3

We have created a page with links to marketing information for aquaculture products. The current education page has been expanded to include eight different categories of educational resources. A

"Find It Yourself Online" page for aquaculturists has been developed and features links to free databases, online publications and full text materials for aquaculture and marine science. The availability of full text literature will be significantly enhanced by the additional storage capability of the NT server.

Objective 4

Thanks to the generous contribution of publications from PACON International, PRAISE staffers are scanning the abstracts from the proceedings of the PACON conferences for submission toCSA.

The PRAISE Web site is continually updated and enhanced by the Principal Investigator and the Project Assistant. We are currently investigating the possibility of enhancing the Grey Literature Database through a collaborative project with a marine biologist from the Coastal Resource Management Office-CNMI who has compiled an extensive bibliography of marine resources. The goal is to mutually integrate and expand our respective projects.

Another researcher with the Division of Fish and Wildlife - CNMI has sought our collaboration in compiling a similar bibliography on reef fish species. This ongoing cooperation allows us to expand our knowledge management base and make it accessible online, thereby increasing the value of PRAISE to the industry.

Results at a glance . . .

Educated researchers, farmers, students and librarians regarding locating resources and acquisition of materials

Updated and expanded the PRAISE website providing access to the most current aquaculture information.

Updated and expanded the Gray Literature Bibliography of otherwise unpublished research.

Impacts

The PRAISE Web site is a bonus. It allows users to make requests online, publicizes otherwise unpublished research in the Pacific islands via the Gray Literature Bibliography and gives local aquaculture ventures access to the most current information in their industry and a presence on the World Wide Web.

Work Planned

- Provide Grey Literature documents in full text PDF format.
- Present workshops for aquaculturists and extension agents on effective use of acquired information, database management and use of CD-ROM technology.
- Migration of the PRAISE web page from KCC Library to the new NT server.
- Provide PACON International Conference Proceedings to CSA for inclusion in the ASFA database.

Aquaculture of Marine Ornamentals, Years 1 & 2

Reporting Period

October 1, 2000 - October 1, 2001 (Year 1)
April 1, 2001 - October 1, 2001 (Year 2)

Funding Level

Year 1	$128,735
Year 2	$135,000
TOTAL	$263,735

Participants

Year 1

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

Frank Alig, Guam Aquaculture Development and Training Center

Dorothy Harris, Guam Department of Commerce

John W. Brown, Ph.D., University of Guam (replacing Dorothy Harris)

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

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

Clyde S. Tamaru, Ph.D., University of Hawaii Sea Grant Extension Service

Richard Bailey, University of Hawaii Sea Grant Extension Service (resigned)

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

Objectives

The solicitation for this research effort called for a multi-institutional approach. Members of the Project Work Group were part of Teams established to complement each other's efforts. The Teams for Year 1 were: The Oceanic Institute, The University of Hawaii and UH Sea Grant Extension Service – Finfish, The University of Hawaii Sea Grant Extension – Invertebrates, and the Guam Aquaculture Development and Training Center. Year 2 Teams were The Oceanic Institute and the Waikiki Aquarium.

Year 1

The Oceanic Institute

1. Establish broodstock populations of yellow tang (Zebrasoma flavescens) and flame angelfish (Centropyge loriculus), or other angelfish for project use.
2. Compare various live food organisms as first-feeds for larvae.
3. Conduct semi-intensive, "brown" water mesocosm trials and determine live food preferences of larvae.

The University of Hawaii and UH Sea Grant – Finfish

4. Compare rotifer and semi-intensive, "green" water mesocosm systems for culture of larvae.
5. Identify other fish species adaptable to "green" water systems.
6. Perform mass culture trials with identified species using, initially, a "green" water mesocosm, with transition onto nutritionally enhanced rotifers.

The Guam Aquaculture Development and Training Center

7. Establish broodstock populations of Clown coris (Coris gaimard) or other wrasse.
8. Develop methods for wild zooplankton collection.
9. Conduct larval rearing trials if larvae and zooplankton are available.

The University of Hawaii and UH Sea Grant – Invertebrates

10. Establish broodstock colonies of feather-duster worms (Sabellastarte sanctijosephi) and trochophore collection techniques at chosen farm facilities.
11. Develop methods to settle and culture metamorphosed worms.
12. Describe growth rates and stages of development.

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.

Anticipated Benefits

The commercial production of marine ornamental fish and invertebrate species represents a key economic opportunity for Hawaii aquafarmers. Presently, aquaculture production of marine ornamentals is limited to a few modestly successful operations, most of which concentrate on anemone fishes and neon gobies as their products. There is opportunity to expand this effort and develop new technologies for species that have a market, but are presently unexploited because of bottlenecks in the technology. This project is designed to take a multi-institutional, multi-pronged approach toward resolution of the key first-feeding bottleneck of marine ornamental fish using a variety of mono-, mixed, and natural zooplankton culture methods to begin to address the issue of first-feeding. Year 1 marks the initiation of these research efforts.

The focus on high-value species complements an ongoing Sea Grant project (headed by Project Work Group member, Dr. Clyde Tamaru) to develop techniques for so-called “bread and butter” species. These are species that are amenable to standard rotifer and Artemia culture methods. While development of ‘bread and butter” species creates initial interest and impact, caution must be taken to avoid market saturation by a potential explosion of supply from hobbyists. Rather than duplicate efforts, CTSA will focus efforts on resolving technological bottlenecks for difficult-to-raise marine species.

This coordinated approach was deemed the best way to rapidly advance technology for the commercial sector.

Successful completion of this project will immediately affect the aquarium industry by providing hatchery techniques to culture several species of marine ornamental, thereby offering a more environmentally sustainable alternative to wild collection practices. Identification of the most appropriate food item would lead to development of methods to mass-produce them for larvae. 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. For instance, larger species of wrasses are a valuable food commodity in Guam and elsewhere in the Pacific. It may be possible to apply techniques developed for Clown coris to food species. If so, production of juvenile wrasses for both aquaculture and reef stock enhancement may be possible. Further, development of reliable methods to mass culture live feeds would benefit the entire spectrum of marine ornamental culture. As expressed in the June 1997 newsletter of the American Marinelife 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

The culture of marine ornamentals represents a significant opportunity for commercial fish farmers in Hawaii and the Pacific. This first year of a multiyear, multi-institutional project combined talents from the Oceanic Institute (OI), the University of Hawaii Sea Grant Extension Services (UHSGES), and Guam Aquaculture Development and Training Center (GADTC) into a three-pronged research approach toward identification and resolution of bottlenecks in the culture of difficult-to-rear marine ornamental finfish and invertebrates. Success at OI over the past year was obtained in establishing several colonies of yellow tang (*Zebrasoma flavescens*) and multiple spawning pairs of flame angelfish (*Centropyge loriculus*). Both groups of animals yielded viable spawns without hormone inducement under natural environmental conditions. Husbandry protocols established for flame angelfish resulted in improved fertilization and fecundity. Several widely available feed items were tested on resulting angelfish larvae without success wild application of a classical semi-intensive rearing approach using pond extracts (i.e., mixed plankton assemblages) led to the successful generation of 19 day-old fame angelfish post-larvae. All OI objectives were met, and research has already been initiated on Year 2 of this project. A no-cost extension beyond the terms of this contract is being requested by the UHSGES group to conduct the targeted work on finfish. Research with feather-duster worms (*Sabellastarte sanctijosephi*) by UHSGES also was not initiated due to the resignation of the key investigator. It is recommended that the funding for this effort be reprogrammed back into the CTSA for future efforts. Research at GADTC met several administrative bottlenecks, which hampered progress, including the transfer of the GADTC from the Guam Department of Commerce to the University of Guam. GADTC was successful in establishing some pairs of clown coris (*Coris gaimard*), and it is expected that identification of collected zooplankton samples will be com-

plete by the final report. Dr. John W. Brown of the University of Guam has assumed responsibility of the GADTC portion of this project and will oversee its completion. Year 2 of this project combines talents of researchers from OI and the Waikiki Aquarium, Honolulu.

Year 1

Objective 1

At the end of the first year of funding by CTSA for marine ornamental research, we have successfully developed broodstock populations and achieved fertile spawns from both flame angelfish and yellow tang. Results from Year 1 studies were presented at the Hawaiian Aquaculture Association meetings at Windward Community College in February, 2001.

Flame Angelfish Reproductive Research. The flame angelfish research has provided the first study of captive spawning for the species. Broodstock populations were successfully developed in October 1999, with natural spawns obtained within one month of broodstock establishment. Angelfish have continued to spawn daily, without interruption, for a period over 17 months. Fish routinely spawn once per day around dusk. Spawning output has increased substantially over time, from spawns of several hundred eggs with fertility rates below 50% early on, to present spawns of over 3,000 eggs/tank and mean monthly fertility rates above 70%.

Year 2 activities examining the effect of tank size in attempt to maximize fecundity have been initiated ahead of schedule with the purchase and primary quarantine of 15 pairs of flame angelfish in late March and experiment initiation in early April. To date, we have already had two pairs in the larger (700-L) tanks initiate spawning with several days of stocking.

Yellow Tang Reproductive Research. The yellow tang research project has also seen major recent success, with broodstock populations producing numerous natural spawns just prior to dusk throughout February and March. In March 2001, exciting developments lead to the captive production of the first fertile eggs ever recorded for this species. The resulting eggs are extremely small (approximately 708 μm) with a small (163 μm), yellow-pigmented lipid droplet. Embryonic development is somewhat slower than for the Centropyge angelfish, with hatching between 24 and 36 hours post-fertilization.

Objective 2

A series of trials was conducted during this reporting period to examine the survival rate, growth rate and feed uptake of flame angelfish larvae offered different prey-types. These small-scale, replicated experiments were carried out in 25L polycarbonate tanks. All larvae were reared in filtered oceanic seawater, which was "greened" using cultured microalgae (Tetraselmis sp). The test diets comprised ss-type rotifers, oyster trochophores (cryopreserved source) and mixed plankton (dinoflagellates and ciliated protozoa) harvested from a milkfish broodstock pond.

In an experiment comparing rotifers versus harvested plankton, first-feeding angelfish larvae were observed to ingest dinoflagellates and rotifer eggs, in preference to adult rotifers. In a separate study, thawed trochophore larvae of the Pacific oyster (Crassostrea gigas) were not ingested by larvae.

Objective 3

The increased egg production from OI's captive angelfish broodstocks enabled larger scale rearing trials to be undertaken during this reporting period in 1,500L fiberglass tanks. A semi-intensive rearing approach was applied, whereby inoculated prey (dinoflagellates, ciliated protozoa and rotifers) were reproduced within the rearing tank and were only supplemented when stocking densities fell below a specified target. Higher survival rates were attained through yolk resorption than in previous trials, by maintaining static conditions to day 3 post-hatch.

Water exchange rate was subsequently set to 100%/day. Using this approach, it was possible to rear flame angelfish larvae up to 19 days post-hatch. Future studies will be targeted at increasing larval growth rate.

Objective 4

Gregory damselfish eggs were obtained from spawning nests that had been laid on tiles placed along the shoreline at Anuenue Fisheries Research Center (AFRC) in September of 2000. The first treatment used tilapia green water obtained from the green water production system at AFRC and the second treatment used the s-type rotifer stocked at 20 rotifers/ml with both treatments being replicated. *N. oculata* was used as a background algae. A single no feed control was also included. Complete mortality occurred in all of the treatments and the no feed control, which indicates that the two treatments tested were not sufficient to support larval survival. From the size of the larval mouth (145 µm) that has been reported previously it would appear that the larvae may be too small for the conventional rotifer (s-type) rearing technology and a more focused effort is needed utilizing the varied "green water" mesocosm methods. The experiment was not repeated due to lack of spawning as it is already at the tail end of the season.

Facilities are being established and a close working relationship has been formed with the bottom fish project at HIMB as much of the facilities are jointly used. A green water mesocosm was established in a single 10,000-liter high-density polyethylene tank that received Kaneohe Bay water that was not filtered. Squid extract is prepared by blending approximately a pound of squid and squeezed through a cloth mesh. The resulting extract is mixed directly into the tank and a bloom occurs in approximately 3 days. Copepod densities were not quantified but visually the population can be observed to increase dramatically in approximately a week to the point that copepod nauplii can be harvested in significant numbers to stock small (30-100 liter) larval rearing trials.

Spawned kahala eggs were obtained from Uwajima Fisheries in Kailua-Kona on May 10, 2001 that was

Results at a glance . . .

- Successfully maintained centralized broodstock populations of yellow tang and flame angelfish for over 18 months.
- Obtained the first recorded spawning of yellow tang in captivity, including demonstration of a lunar rhythm in spawning activity.
- Optimized flame angelfish broodstock husbandry protocols with completion of studies demonstrating a strong relationship between tank size and both fecundity and spawn fertility.
- Initiated live feed trials toward rearing yellow tang larvae
- Established new protocols for handling eggs and maintaining yolk-sac larvae of flame angelfish, which have increased early (4 days post-hatch) survival rates to nearly 70%.

to be used as an initial trial of the rearing facilities. Rearing was conducted in 100-l conical fiberglass tanks and three treatments were tested. Eggs were stocked at a density of 30 eggs/l and allowed to hatch. Hatching was approximately 80% resulting in a larval density of approximately 20 larvae per liter. Copepods collected from the bay that were screened through a 100-µm nytex mesh at a density of 5 individuals/ml (treatment 1), ss-type rotifers stocked at 20 individuals/ml (treatment 2) and a combination of rotifers + copepods (treatment 3) at ½ the density when used alone. All treatments were duplicated. A single no feed control was also set and all larvae in the control group perished on the 5th day post hatching. The rearing trial was allowed to go for 12 days posthatching at which time the surviving larvae were counted. Larval survival overall was very poor with the best result in the copepod only treatment averaging 1%. The combination of rotifers and rotifers only were 0.6% and 0.2% respectively. Although the copepod only treatment resulted in the best survival, a few larvae fed rotifers either alone or in combination with copepods also managed to survive indicating that the kahala may be considered a candidate for the conventional rotifer rearing technology.

A tank field that contains 20 2000-l fiberglass tanks has been cleared from the plant. This facility is to be used to produce the tilapia green water for use in producing rotifers and testing as a marine infusoria with eggs when available. Indoor laboratory space is being modified to insert a water table to place 12 30-liter polycarbonate tanks that can be used to conduct the mesocosm type experiments in proper replication.

Objective 5

No progress made during the reporting period on testing larval culture aspects, as the small-scale larval rearing system still needs to be completed.

Objective 6

No progress made during the reporting period on the larval culture aspects of this objective. However, the fatty acid profiles of both rotifers and artemia enriched with the newest developed boosting media was obtained. It was felt that this work should be conducted prior to conducting the larval culture activities in order to have a full understanding of what the nutritionally enhanced rotifer profiles actually are.

Objective 7

In October 2001 the GADTC was transferred from the Guam Department of Commerce to the University of Guam. Dr. John W. Brown will be assisting in the completion of this project, replacing Dorothy Harris. We have collected six animals from the wild. Currently, they are being held in two 3-ton fiberglass tanks. Due to a lack of flowing saltwater at the GADTC and our consequent hesitancy about overfeeding the tanks, the animals have not reached sexual maturity.

Objective 8

Since the mid-term project report, we have received supplies for the plankton tows and collecting barge. The barge was assembled using floatation support and holds a marine deep cycle battery, bilge pumps, collecting light and netting. The bilge pumps and netting sizes are interchangeable. We have experimented with different combinations of 150 micron and 60 micron nets with a 1,500 gph bilge pump as well as a smaller 360 gph pump.

The barge is designed to operate as follows. First, the barge is secured in a stationary position before sundown. Then after the sun sets, an underwater light (Starfire II) below the barge is turned on for 10 minutes. At the end of this period, water is pumped from the vicinity of the light into the pre-screening unit. The pre-screening unit contains both 500 micron and 250 micron mesh barriers, and it is designed to prevent clogging of the collection device and exclude larger organisms. Finally the water is passed trough the fine mesh plankton net.

Preliminary collections were made at a man-made canal to test the barge. The site, an intake canal for a local power plant, was chosen for accessibility and safety. The canal is deep enough for barge operations but easily accessible, and water is pulled into the channel beyond the reef.

Nathan Martin, a biologist hired under the grant, made seven evening collections at the intake canal using combinations of different mesh sizes and pumps. The samples were collected after sundown, each with sample representing a 10-minute collection period. During the trials, we used the pre-screening device to exclude larger plankton and avoid clogging the finer meshed collection nets. However, several large organisms were captured in each sample, and we are refining the design of the pre-screening device to address the problem.

In addition to the barge collections, we conducted plankton tows from a boat using the same size nets. Two plankton tows were done in Cocos Lagoon. Both the 150 micron and 60 micron nets clogged immediately on entry into the water.

Objective 9

Plankton samples collected under the second deliverable were microscopically examined and although we could distinguish a variety of crustacean larvae, hydroids, and other potentially useful food organisms, attempts to identify the plankton beyond very general terms were unsuccessful. There is currently no one with the required expertise available on Guam. On the advice of Dr. Anthony Ostrowski of the Oceanic Institute, we have contacted Dr. Cindy Hunter of the Waikiki Aquarium. Dr. Hunter has agreed to supervise her student helpers in the identification of the collected organisms and to host a brief training for Mr. Frank Alig of the GADTC once the process of sample collection and identification is well underway.

Objectives 10-12

No progress was made due to the inability to find an alternative work group member to carry out the planned farm trials. It is recommended that money allocated for this effort be either deleted or reprogrammed toward other activities.

Year 2

Objective 1

We continue to successfully maintain centralized broodstock populations of both yellow tangs and flame angelfish as a source of seedstock for larval rearing research. Yellow tang holdings currently include eight tanks, each containing 8 to 12 fish in approximately equal ratio of males to females, with plans to increase holdings by another 8 tanks to complete hormonal induction studies. Flame angelfish holdings currently include two tanks of production broodstock that continue to spawn daily and 15 experimental tanks for studies on broodstock husbandry.

Objective 2

The yellow tang research project has continued to present major successes, with the continued demonstration and documentation of daily spawning activity that began in January 2001 with relatively infrequent infertile spawns, with peak spawning activity in April and again in August. Although overall fertility generally remains low, fertility rates have slowly increased over time with isolated spawns demonstrating fertility rates as high as 88% and an overall mean fertility rate of 22% for July. In the original work plan we had anticipated that this species would not spawn without supplemental hormonal induction and thus had planned a variety of hormonal-induction experiments using these animals. However, the relative importance of obtaining and continuing to track natural spawning activity, a species first, has led us to delay the hormone induction work to follow the natural spawning throughout the year. We continue to believe that hormone induction will be a critical tool in stimulating reproductive maturation and increasing spawn fertility, and thus are in the process of

setting up additional tanks and collecting new broodstock to complete the trials as proposed.

Objective 3

Year two investigations into tank size optimization toward increasing 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 one of 15 broodstock tanks ranging in volume from 40-L up to 750-L. Fish have been maintained and monitored for egg production and spawn fertility on a daily basis beginning in early April, with experiment termination in early October. Preliminary analysis of data demonstrates a significant effect of tank size on the rate of broodstock maturation and reproductive output in terms of both fecundity and spawn quality.

Objective 4

The recent attainment of natural spawning by yellow tang at OI provided small amounts of larvae for an initial diet trial during this reporting period. One-thousand yellow tang eggs were stocked into a 200L 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 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 larval development, before ascertaining the larvae's dietary requirements.

Objective 5

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 scheduled surface-disinfection trials. Experimental treatments were evaluated in terms of hatch rate and survival rate of larvae to day 4 post-hatch. The range of variables tested was as follows:

- Resilience of embryos to mechanical handling in relation to develop mental stage
- Survival rate in experimental containers stocked with embryos ver sus newly-hatched larvae
- Effect of stocking density
- Effect of presence/absence of aeration
- Effect of microalgae (*Nannochloropsis* sp) density
- Effect of different water sources (UV-sterilized 'well' water, 'aged' seawater, artificial seawater)
- Effect of container-type

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 have been established enabling survival rates of circa 70% to day 4 post-hatch. These protocols will be applied in egg disinfection studies to be carried out

during the next reporting period.

Objectives 6-7

Although this research project focuses on early-stage larvae of fish, the University of Hawaii's Institutional Animal Care and Use Committee (UHIACUC) has determined that formal certification in vertebrate care is required for all UH staff involved in the project. Therefore, Waikiki Aquarium staff have been required to complete on-line training in order to comply with the UH IACUC regulations. Certification is expected to be completed by 19 October 2001, at which time Waikiki Aquarium will be formally authorized to begin the project and will begin hiring procedures for a student assistant.

Work Planned

For Year 1, **UHSGES** will continue work on all objectives. Small-scale (30-l) testing facility needs to be completed at the Hawaii Institute of Marine Biology (HIMB) to conduct the varied larval rearing screening process that will identify fish species that can be grown on the Infusoria based green water technique. A continued search for sources of marine ornamental fish eggs will be conducted in order to provide an opportunity to conduct the screening processes that will identify species that can be grown using the rotifer based culture technology or those that need to be tested using the "green water" techniques. Initial collaborative partnerships with a private producer, Frank Baensch, through the Pacific Topical Ornamental Fish Project, has resulted in availability of larvae from *Centropyge potteri* (Potter's angelfish), *C. loriculus* (flame angel) and *C. fisheri*. Because of the delays the screening portion of the project has suffered the most. Only two species the damselfish trial conducted at AFRC and that of the kahala conducted at HIMB have been examined so far. The purpose of the kahala work was actually to only test the available system for bottom fish project. It is expected that the project group will need to be expanded to include other participants that are supported through the Pacific Tropical Ornamental project for additional support in both facilities and eggs in order to continue the process. A no cost request has been made to extend the project until February of 2002 to be consistent with that of the Sea Grant project and allow testing of the angelfish larvae.

GADTC will also be continuing with Year 1 objectives. Shipment of plankton samples to Dr. Cindy Hunter's lab at the Waikiki Aquarium for identification will begin in the near future. Testing of the barge will resume as soon as minor modifications can be made. We will continue to hold the six Clown coris and heavier feeding will be implemented once the saltwater pumps at the GADTC are repaired. More will be captured in there seems to be any chance that will be able to induce spawning and have first feeds for the larvae.

The **Oceanic Institute** will continue with Year 2 objectives to maintain centralized broodstock populations of pygmy angelfish and yellow tang with presentation of this work at the upcoming Marine Ornamentals 2001 conference in Lake Buena Vista, Florida this November/December. The proposed experimental work determining the appropriate husbandry conditions for optimum natural spawning of pygmy angelfish has been completed, allowing OI researchers to examine the usefulness of hormones to induce captive spawning of yellow tang. In addition, research from projects years one and two will be written in the form of two to three publications in international scientific journals.

An egg disinfection protocol for small marine ornamental eggs will be developed during the next reporting period. Experiments will be undertaken to compare the survival rates of larvae receiving different water sources (UV-sterilized, "microbially-matured," antibiotic-treated), in rela-

tion to the numbers and types of bacteria present. The effects of system volume on microbial communities within the rearing tanks and on larval rearing success will also be investigated.

Having completed certification in vertebrate care, staff at the **Waikiki Aquarium** will be formally authorized to begin the Year 2 project and will begin hiring procedures for a student assistant.

Impacts

The ultimate goal of this project is to assist in the development of a marine ornamental industry in Hawaii and the Pacific. This represents a key economic opportunity for farmers in the sate of Hawaii and Pacific Island affiliates such as Guam for several reasons. First, there is a worldwide void in aquaculture production of marine ornamental species. It is estimated that less that 5% of all marine ornamental species traded on the open market are cultured, and that the actual numbers of cultured fish treaded is miniscule compared to that 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. Second, it is well known that the health of coral reef ecosystems around the world is being severely degraded, ant that wild collection practices are likely unsustainable unless alternative are sought. The Hawaiian islands are home to over 85% of the coral reefs in the United States, well-positioning the region to develop and aquaculture-based industry. Success of this project will not only provide new economic opportunities to farmers, but will also help ensure the long-term sustainability of the marine ornamental trade by providing alternative to wild collection practices, and a means to practice resource conservation.

The work conducted by this project to date has provided a base from which research on the culture of difficult to rear, marine ornamental species can proceed. Transfer of technologies to hold broodstock in conditions that promote good health and natural spawning is an important first step for commercialization. Identification of organisms that promote good first feeding allows the research to move to the next phase of identification of appropriate larval rearing technologies. Work targeted for next year should provide further advancements in larval rearing and broodstock conditioning in several species that will be critical to industry development.

Publications in Print, Manuscripts and Papers Presented

Publications in Print

Ostrowski, A. C., C. W. Laidley. 2001. Application of marine foodfish techniques in marine ornamental aquaculture: Reproduction and laval first feeding. Aquarium Sciences and Conservation 3: 191-204.

Papers Presented

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 12

Reporting Period

October 1, 2000 - October 1, 2001

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
TOTAL	$242,200

Participants

Cheng-Sheng Lee, 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. Publish a quarterly newsletter to communicate information about the activities of the Center for Tropical and Subtropical Aquaculture and its funded projects and the latest information about aquaculture from the nation and the region.
2. Produce and publish informational reports of selected CTSA-funded projects, which will be

distributed at no charge to commercial producers, aquaculture researchers and other interested parties throughout the Pacific region, with limited distribution in the continental United States.

3. Improve and maintain an information website. The site will contain information on CTSA, its activities, projects, and publications. It will also contain copies of CTSA's newsletter, and various publications suitable for download and printing at no cost to the user.
4. Duplicate and distribute CTSA's and other Regional Centers' publications and videos to information networks throughout the Pacific. This is coordinated with the Pacific Regional Aquaculture Information Service for Education (PRAISE), Hamilton Library, University of Hawaii and the University of Hawaii Sea Grant College Program.
5. Produce and publish the Year 2001 CTSA Accomplishments Report, which compiles information on the activities of all CTSA projects that were active during 2001 and distribute to USDA, the CTSA Board of Directors, and the other Regional Aquaculture Centers.
6. Provide editing, layout and production assistance as needed on all publications produced by CTSA-funded projects to ensure that the standard of quality is kept at an exceptional level.

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.

By 2001, the CTSA staff had produced (entirely or cooperatively) and published 46 progress reports, manuals, or fact sheets; duplicated and distributed 18 videos produced by the other Regional Aquaculture Centers and distributed them to extension agents, libraries and aquaculturists throughout the region; and developed and maintained the CTSA webpage.

Objective 1

A special double issue was published in March 2001 and regular issues were produced in June, September, and December 2001, and disseminated thereafter.

Objective 2

One informational report was published and disseminated: *Economic results of Pacific threadfin culture.*

Objective 3

The homepage was updated and maintained as needed.

Objective 4

Three CTSA publications, as well as two videos from the Southern Regional Aquaculture Center were distributed to the Board of Directors and other information networks.

Objective 5

The Year 2001 Accomplishment report was completed.

Objective 6

The Center assisted with the creation, production and distribution of three publications: CTSA Publication #145, *Growout techniques for the Pacific threadfin (*Polydactylus sexfilis), CTSA Publication #146, *Economics of a Pacific threadfin hatchery in Hawaii*, and CTSA Publication #148, *Best management practices for Hawaiian aquaculture.* Editing and layout assistance has also been provided for three other CTSA publications due for publication in mid-2002.

Work Planned

The staff will continue to produce and publish the quarterly newsletter, initiate development of a new 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. Four manuals are due to be published in 2002.

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 2 & 3

Reporting Period

October 1, 2000 - October 1, 2001 (Year 2)
April 1, 2001 - October 1, 2001 (Year 3)

Funding Level

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

Participants

Year 2

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

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

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

Objectives

Year 2

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 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 bottomfish species as potential aquaculture candidates and develop broodstock capabilities as money and fish become available.

Year 3

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.

Anticipated Benefits

The project is well poised to meet its goal to assist in the development of a sustainable marine food fish industry. Clearly, an emerging marine food fish industry is present in Hawaii. Threadfin and milkfish farms have been established as a result of research and technology transfer conducted under the previous CTSA-funded hatchery and growout projects, and the demand for fingerlings continues to rise rapidly. In fact, requests by farmers for both fingerling threadfin (344,500) and milkfish (443,000) in 1998 were beyond the targets established for the final year of Phase II (CTSA Year 11). A track record of commercial sales and commitment by farmers also has been established. Sales of both species in 2000 alone could total $1.0–1.5 million. A hatchery manual for threadfin has been published (Ostrowski and Molnar, 1998) to provide a basis for commercial hatcheries. An unrelated project to train local farmers in general marine finfish hatchery and growout techniques at The Oceanic Institute will be leveraged to assist industry development.

This final phase of work will also provide the basis of a breeding program to ensure long-term sustainability of the threadfin industry. Successful completion of the project will produce a domesticated, select line of Pacific threadfin that is better suited to the stresses of aquaculture. They will survive and grow better in ponds or tanks than the fingerlings currently produced from wild-caught broodstock. These select lines themselves or fingerlings produced from the lines could be made available to farmers to increase their profitability.

Work Progress and Principal Accomplishments

The Pacific threadfin (*Polydactylus sexfilis*) and milkfish (*Chanos chanos*) represent enormous opportunities for fish farmers in Hawaii and affiliated Pacific Island nations to diversify their crops and expand their income potential. Year 2 of this multiyear project targeted to continue support and development of these opportunities and fostering transition of the commercial sector to self-sufficiency, while exploring new opportunities to expand the marine finfish industry though examination of new species development. This project was successful in distributing fingerlings, eggs, and providing technical advice to over a dozen farmers on the islands of Oahu, Maui, Molokai, and Hawaii to support the budding Pacific threadfin and milkfish industries in Hawaii. These efforts lead to establishment

of the first commercial Pacific threadfin hatchery in the state, on the island of Hawaii. The first selected line of Pacific threadfin was also established for future research efforts towards domestication and selective breeding of this species. This effort is targeted to consolidate the production gains made by industry in Pacific threadfin culture and to provide the industry with the tools necessary to respond to increased competition both domestically and abroad. Efforts to develop bottom fish species such as the yellowstriped snapper (*Etelis carbunculus*) and gray snapper (*Aprion virescens*) did not progress well as research on these species is presently only in rudimentary phases. The outlook for successful completion of this multi-year project and its overall goals as it enters its final, and third year is very good. Production of Pacific threadfin in particular has steadily risen since 1997, contributing to over $1 million in farm revenues, and there is ever indication that the industry will remain a vital component of diversified agriculture in Hawaii, with incentive and room for further expansion.

Year 2

Objective 1

By the time this phase of the project was initiated, there were only four broodstock 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 National Marine Fisheries Service in Honolulu. Dr. Kelley will continue to oversee the snapper project at HIMB and assist in distribution of eggs to OI for CTSA.

OI continues to maintain Pacific threadfin and milkfish broodstock for the project. Pacific threadfin have spawned on regular monthly intervals throughout the report period. Milkfish began spawning in May and have continued to spawn as of this writing. Eggs are collected routinely and many have been distributed to local farmers to attempt mass culture.

Objective 2

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.

Preliminary results of trials in 200-liter tanks have 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. Results of these trials are currently being analyzed.

Only 3,600 milkfish fry have been distributed during the project period. A drop in algal production at the OI facility that resulted in low rotifer output has hampered production efforts. Efforts have been directed to correct the situation, and additional runs have been conducted as of this writing. We expect to continue milkfish production runs through October, and until broodstock stop spawning to reach the targeted production goal.

Results of the Aquamat™ trials have been inconclusive to date.

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 the report period, 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.

Year 2 results at a glance . . .

- Produced and delivered 215,896 Pacific threadfin and 3,600 milkfish fingerlings to farmers on the islands of Oahu, Maui, Molokai, and Hawaii.
- Produced and delivered over 1.7 million Pacific threadfin and 1.34 million milkfish eggs to three growout and research operations on the islands of Oahu and Hawaii.
- Developed first select line of Pacific threadfin for future domestication and selective breeding research.
- Developed a preliminary estimate of costs of milkfish growout in Hawaii based on farmer input and current production practices.
- Provided technical advice that lead to the establishment of the first commercial Pacific threadfin hatchery in the state of Hawaii, on the island of Hawaii.

Objective 3

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. The health monitoring program will assure farmers a quality product as well as help maintain a healthy industry. Documented results of these findings are in Appendix 1.

Within the past six months, Dr. Brock has resigned his position as the State of Hawaii's Aquatic Veterinarian. Dr. Robert Bullis from OI has assumed temporary responsibility for this task and is in the process of reading out the remaining cases in collaboration with Dr. Brock. Results will be forwarded to the Project Work Group in a timely fashion.

Objective 4

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. It is anticipated that eggs derived from spawns in January will be used for selection purposes. As indicated in the proposal, the resulting progeny will be raised to market size (0.34 to 0.45 kg), after which 50 randomly selected fish (controls) and the 50 largest fish (select line) will be tagged. Blood samples will be taken for genetic analysis and development of a genome library with micro-satellite markers. These fish will then be grown to sexual maturity to continue the selective breeding program.

Dr. Warren has withdrawn from the effort. We are currently seeking a new partnership to develop the micro-satellite markers. Dr. Charles W. Laidley was recently hired by OI as a reproductive physiologist and has joined this effort.

Spawns from semi-domesticated broodstock populations were reared through the larval hatchery in February producing 4000 fingerlings that are currently being transferred from nursery to 30-ton growout tanks. These fish will be grown to market size at which time control and selected top growers will be PIT-tagged and stocked into broodstock tanks. Preliminary tissue samples have been sent to Kent Sea Tech, Biotechnology division for preliminary microsatellite marker development. Fish are growing well and we expect to begin selection efforts next year as planned.

Objective 5

This aspect of the project was delayed last reporting period due to a delay in establishing a subcontract agreement with the University of Hawaii. The issue was resolved and site visits to local milkfish farms were conducted and information compiled overt this reporting period. A preliminary model has been constructed and is currently being refined for the final report.

Objective 6

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. To date, we have recovered only $3,268.24 of the $19,618.26 invoiced to farmers.

Site visits were conducted on the islands of Hawaii (Ben Krause) and Maui (Guy Ting) to assist in hatchery and growout set-up. Ben Krause of Pacific Harvest, Inc. 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.

Objective 7

There have been no spawns from any snapper species under any project.

Year 3

Objective 1

An experiment was conducted comparing the survival and growth rates and morphological characteristics of threadfin larvae receiving two different diet enrichment products (Algamac 2000Ô, Aquafauna Biomarine Inc; AquagrowÔ 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.

Year 3 results at a glance . . .

- Comparison of rotifer and artemia enrichments demonstrated a dietary effect on, but did not eliminate, opercular deformity rates in intensively reared Pacific threadfin.
- Semi-intensively reared Pacific threadfin in outdoor raceway system exhibited lower survival and growth rates as compared with intensively reared fish, with no change in the occurrence of opercular deformities.
- 250,000 milkfish eggs and 10,900 milkfish fingerlings were distributed to farmers
- 2.3 million Pacific threadfin eggs and 130,714 fingerlings were distributed to farmers and state agencies

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.

Objective 2

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 suboptimal 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

No reports received to date.

Objective 4

Approximately 200 F1 Pacific threadfin broodstock are currently being maintained in an outdoor 25 m^3 flow through tank system awaiting sexual maturation. Pair-spawning efforts require access to sexually mature male and female broodfish prior to research initiation. Establishment of sexual maturity will be based on the initiation of viable egg production between the 6th and 12th day following the full moon. Experimental protocols, expected to be conducted over the next 4 months, will entail removal of mature male and female threadfin broodstock from batch broodstock tanks at various points prior to spawning and stocking animals in smaller spawning tanks. This will allow broodstock to undergo normal gonadal cycling as a group prior to separation for spawning. Each trial will consist of one male and one female (identified by PIT tags) stocked together in either a 500L or 5000 L flow-through spawning tank equipped with egg collectors. Broodstock will be randomly exposed to one of the following 3 treatment regimens: 1) Natural spawning (no experimental manipulation); 2) Single LHRHa-injection (10 µg/kg); and 3) LHRHa-implant (200 µg). If the non-treated animals do not spawn as scheduled, they will be

maintained in isolated pairs for subsequent spawning cycles, in an attempt to acclimate them to conditions of pair isolation. The single LHRH injection (dose 10 µg/kg) is only effective for a few days while the LHRHa implant (dose 200 µg/kg) should provide a sustained release lasting several weeks. Tanks will be monitored for spawning behavior and the appearance of eggs on collection nets. All spawns will be collected, number of eggs counted, and the percent fertility determined prior to stocking in larval rearing tanks. Seedstock viability will be evaluated using survival rates at hatch, and through the end of pre-feeding larval development.

Objective 5

Ten female F1 domesticated Pacific threadfin and 10 wild-caught males were stocked together in a 25 m^3 broodstock tank under standard conditions used to maintain Pacific threadfin production broodstock in late summer 2000. F1 females were used to ensure that seed came from animals capable of completing the life cycle under captive conditions and wild-caught males were used to expand seedstock genetic diversity. This broodstock population reached sexual maturity by Fall 2000, with erratic spawns starting in December. Attempts to rear the January 2001 spawn proved unsuccessful, while efforts to rear February spawns resulted in modest success, with the production of approximately 600 fingerlings.

Fingerlings resulting from the above spawns were stocked in a single growout tank in March to initiate the stock selection trial. By late August, fish reached market size of 0.34 – 0.45 kg. At this time fish were weighed, and 50 randomly chosen fish with a mean weight of 377g, and length of 25.6 cm were tagged and stocked in a separate 25 m^3 "control" broodstock tank. From the remaining 554 fish, the largest 50 individuals with a mean weight of 516 g and length of 28.9 cm were chosen, tagged, and stocked in a separate "growth-selected" broodstock tank.

The control and size selected lines of Pacific threadfin are currently being grown to sexual maturity under standard OI broodstock rearing procedures. Tanks are being monitored on a monthly basis for appearance of fertile eggs. Upon attaining sufficient and reliable egg production, control and size-selected broodstock will be removed and spawned utilizing pair spawning procedures developed under objective 4.0, to assure the maximum number of parents contribute to the next generation.

The resulting lines will be reared in separate nursery tanks (5 m^3) until large enough to tag (ca. 20-30 g). During this time, the environmental conditions between the tanks will be kept as constant as possible. Five hundred select and 500 control fish will be stocked together and grown to market size in 25 m^3 tanks. Animals will be monitored on a monthly basis for survival, growth, and reproductive development. Upon reaching market size all animals will be weighed and measured to determine the response to selection, and the difference in growth between the select and control lines. This data will also be used to calculate a realized heritability for growth based on the ratio of selection response to selection differential in the parents. Survival, dressing percentage, and reproductive development will be compared between the two lines to determine if these traits are indirectly affected by selecting Pacific threadfin for growth. Again 50 select and 50 control animals will be separated to continue the selection process while non-selected fish will continue to be monitored for reproductive development.

Objective 6

Two threadfin hatchery runs and 1 milkfish run were carried out during the current reporting period. Qualified farmers were asked to submit requests in advance of each run and the total available fingerlings were then distributed on a proportional basis. Eggs of milkfish and threadfin were also supplied on request.

Work Planned

During the final 6 months of the project the third and final CTSA threadfin hatchery run will be carried out, comprising three 4,000L intensive rearing tanks. The planned HUFA enrichment study (Objective 1) will be replaced by vitamin C-supplementation of live feeds, in view of the limited effect of HUFA-enriched diets on threadfin skeletal characteristics in previous trials. The effect of supplementing iodine levels in the larvae rearing water will also be tested. Distribution of threadfin fingerlings from this final hatchery run will complete the project's technology transfer objective.

It is expected that experimental female threadfin broodstock (objective 4) will come into sexual maturity allowing completion of pair spawning development trials. Trials will consist of single male and female broodstock stocked in smaller 5000L spawning tanks for the purpose of obtaining either natural or hormone-induced egg production from which both parents can be controlled and identified. At the same time growth and monthly egg production will continue to be monitored in control and select broodstock tanks (see objective 5) for anticipated spawning during the summer of 2002. Progeny from these spawns will be monitored for survival, growth, and reproductive development on a monthly basis through 2002 and 2003.

Impacts

The ultimate goal of this project is to support the development of a self-sustaining marine food fish industry in Hawaii. Recent information provided by the State of Hawaii's Aquaculture Development program indicated a steady rise in sales of both threadfin and milkfish over the last several years (Table 1). This is a direct result of the technology, fingerlings, and technical support supplied by the project. There has also been a steady rise in the demand for fingerlings to support expanded industry needs. This has prompted several private farmers to begin trial hatchery runs to not only supplement their own needs, but also to explore the potential to support industry needs. Pacific Harvest, Inc., a threadfin farm in Kona, has taken particular aim at establishing a threadfin hatchery. The company plans to be producing its own fry beginning Spring, 2001. Project staff has assisted in the development of this hatchery. The establishment of private hatcheries is consistent with the goal of supporting self-sufficiency of the industry.

The outlook for completion of the project objectives is very good. The phased payment plan appears to be working, although debts owed have not been collected as quickly as anticipated. Farmers are being better trained to raise threadfin larvae and on-farm survival rates are expected to increase. Completion of the hatchery economic model will help farmers estimate their required scale and costs of production, and provide them the basic equipment and supplies needs with which to develop their hatcheries. OI will attempt larval rearing while HIMB develops broodstock management techniques. The health assurance program will continue, but indications are that Hawaii-based farmers are receiving high-quality fry to provide them with the best chance of success in growout.

Table 1. Farm production statistics for Pacific threadfin and milkfish in Hawaii. Production and estimated values are from the Hawaii Agricultural Statistics Service. Average $/lb is derived from these values

	Pacific threadfin			Milkfish		
	Production (lb)	Value ($)	Average $/lb	Production (lb)	Value ($)	Average $/lb
1999	119,568	459,150	3.84	4,024	13,671	3.39
1998	41,500	214,000	5.16	5,100	13,100	2.57
1997	25,000	114,000	4.56	8,000	18,000	2.25

Publications in Print, Manuscripts and Papers Presented

None at this time