Center for Tropical and Subtropical Aquaculture

2010 Accomplishment Report

Center for Tropical and Subtropical Aquaculture

Waimanalo and Honolulu, Hawaii

Center for Tropical and Subtropical Aquaculture

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Introduction

Mission

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

Background

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

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

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

The CTSA region includes 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.

Objectives

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

1. Promote aquaculture research, development, and demonstration for the enhancement of viable and profitable commercial aquaculture production in the United States for the benefit of producers, consumers, and the American economy;

2. Utilize the Regional Aquaculture Centers in a national program of cooperative and collaborative research, extension, and development activities among public and private institutions having demonstrated capabilities in support of commercial aquaculture in the United States.

Organizational Structure

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

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

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 the Marshall Islands, and the Republic of Palau.

Administrative Center

CTSA is jointly administered by Oceanic Institute and the University of Hawaii. Oceanic Institute has fiscal and administrative responsibilities for CTSA operations. CTSA's Administrative Center is located at Oceanic Institute on the island of Oahu in Hawaii. A second office is located at the Manoa campus of the University of Hawaii, also on the island of Oahu. CTSA staff provide all necessary support services for the Board of Directors, the Executive Committee, the Industry Advisory Council, the Technical Committee, project review panels, and project work groups. Executive Director Cheng-Sheng Lee, Ph.D., supervises operation of the Center.

Board of Directors

The Board of Directors is responsible for oversight of CTSA's industry development plans, policies, and programs, including concurrence on the allocation of the available annual budget. 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:

- provides oversight for regional program development, execution, and management;
- appoints and removes members of the IAC and the TC;
- approves the proposed duties and membership of the IAC and the TC;
- approves the proposed strategy for project selection;
- approves the annual Plan of Work, including budget allocations;
- approves the annual Accomplishment Report for consistency with CTSA goals and objectives and the authorizing legislation; and
- directs the Executive Director to respond to its information needs.

The CTSA Board of Directors includes:

Harry Ako, Ph.D. (Chair)
University of Hawaii at Manoa

Anthony Ostrowski, Ph.D.
Oceanic Institute

Sylvia Yuen, Ph. D.
College of Tropical Agriculture and Human Resources,
University of Hawaii at Manoa

Jo-Ann Leong, Ph.D.
Hawaii Institute of Marine Biology, University of Hawaii at Manoa

Todd E. Low, M.B.A.
Hawaii Department of Agriculture Aquaculture Development Program

Singeru Singeo, Ph.D.
Land Grant Programs, College of Micronesia

Lee S. Yudin, Ph.D.
College of Natural and Applied Sciences, University of Guam

Executive Committee

The Executive Committee of the Board of Directors is composed of the two members appointed by the presidents of the University of Hawaii and Oceanic Institute. The Committee is responsible for making final decisions on administrative policy, budget, and procedures, as well as appointing the Executive Director of CTSA. These two directors make up the Executive Committee: Anthony C. Ostrowski (Executive Committee Chair) and Jo-Ann Leong (Board of Directors Chair).

Industry Advisory Council

Members of the IAC include commercial aquaculture farmers and members of government bodies. Members are appointed by the Board of Directors for three-year, renewable terms. As an advisory body, the IAC's capacity provides an open forum through which those involved in the business of aquaculture can provide comments, suggestions, and advice. With the approval of the Board of Directors, the contributions of the IAC can be incorporated into annual and ongoing plans for CTSA. The IAC has the following duties:

- reports the status and needs of aquaculture development in their represented region or field of interest and expertise;
- recommends and ranks, according to perceived importance to industry, expansion, research, and development needs each year;
- elects annually a chair from its eligible membership to conduct the annual IAC meeting, present recommendations regarding proposals to the Board based on reviewers' comments, and serve as a voting member on the Board of Directors; and
- assigns members to serve as industry liaisons for each project. Liaisons monitor progress through quarterly project updates and other reports from the principal investigators (PIs) of projects. Liaisons collaborate with project PIs to report on the progress of projects at the annual meeting.

The IAC is composed of 15 members:

John Brown, Ph.D.
Guam Aquaculture Development Training Centre, University of Guam

David Cohen
Aquatic Innovations, Hawaii

John Corbin
Aquaculture Planning & Advocacy, LLC

Linda Gusman
Island Aquaculture, Hawaii

Steve Hopkins, Ph.D.
Rain Garden Ornamentals, Hawaii

High Talking Chief Ava Hunkin
Native Resources Developer Inc., American Samoa

Glen Joseph
Marshall Islands Marine Resources Authority, Republic of Marshall Islands

David Kawahigashi
Vannamei 101

Jennica Lowell
Kona Blue Water Farms, Hawaii

Valentin Martin
FSM National Government, Federated States of Micronesia

Ryan Murashige
Hukilau Food Inc. Hawaii

Anthony Pellegrino
Saipan Aquaculture Inc., Saipan, Northern Mariana Islands

Thomas Taro
Palau Community College, Republic of Palau

Ron Weidenbach (Chair)
Hawaii Fish Co., Hawaii

Richard Xie
Hawaiian Sealife, Inc., Hawaii

Technical Committee

Members of the TC represent participating research institutions and state extension services, other state or territorial public agencies as appropriate, and non-profit private institutions. The TC evaluates the scientific merit of preproposals submitted to CTSA. The Board of Directors appoints members for two-year, renewable terms. The TC has the following duties:

- develops problem statements for the priority areas selected and identified by the

IAC. The Request for Pre-Proposals is based on these problem statements;

- reviews and assesses the research approach of pre-proposals as to adequacy in addressing the priority problem areas selected and identified by the IAC;
- ensures that proposed research does not duplicate previous research and that it develops new and novel results for application by the industry;
- submits recommendations to the Executive Director regarding which pre-proposals adequately address the priority areas selected and identified by the IAC;
- evaluates the annual progress of funded projects and submits comments on research direction and results; and
- elects annually a chair from its eligible membership to conduct the annual TC meeting and serve as a member on the Board of Directors.

The TC consists of:

Harry Ako, Ph.D. (Chair)
University of Hawaii at Manoa

Tom Iwai, Jr.
Anuenue Fisheries Research Center, Hawaii

Bruce Mundy
NOAA Pacific Island Fisheries Science Center

Wai-Kit Nip, Ph.D.
University of Hawaii at Manoa

Allen C. Riggs, D.V.M.
Aquaculture Development Program

Tetsuzan {Benny} Ron, Ph.D.
Aquaculture Program Coordinator – University of Hawaii, Manoa

Vernon Sato
Hawaii (retired)

James Szyper, Ph. D.
University of Hawaii, Manoa (retired)

Executive Summary

PROGRAM SCOPE

During 2010, the Center for Tropical and Subtropical Aquaculture completed work on projects funded under its Nineteenth Annual Plan of Work and continued work on projects funded under its Twentieth, Twenty-first, and Twenty-second Annual Plans of Work. Also, CTSA initiated work on projects developed under its Fiscal Year 2009 Plan of Work and began developing its Fiscal Year 2010 Annual Plan of Work.

Eight projects were funded under CTSA's Fiscal Year 2009 program, which was approved by CTSA's Board of Directors on January 21, 2010. Three of these projects address continuing priorities and will build on work begun under the programs of previous years, and five of the FY09 projects address new priorities.

Since the inception of CTSA in 1986, it has funded 222 research, demonstration, development, and extension projects. Twenty-four projects were active during 2010. These projects fall into five categories:

1. Information Dissemination
2. Extension Support to Further Industry Development
3. Marketing and Economics
4. Development of New Technologies
5. Demonstration and Adaptation of Known Technologies

Most projects conduct activities that fall into multiple categories. Therefore, the following outline is based on the main project objectives:

1. This project addresses information dissemination:
   - Pacific Regional Aquaculture Information Service for Education (PRAISE) and Publications, Years 1 - 5

2. These projects address extension support:
   - Shrimp Production Demonstration Project and Aquaculture Training for Industry Stakeholders of the Commonwealth of the Northern Mariana Islands and Guam, Years 1 and 2
   - Improving Outputs in the Commercial-Scale Production of Swordtails in Hawaii,

Years 1-3
- Promoting Health Management of Shrimp Aquaculture on Guam and the Commonwealth of Northern Mariana Islands
- Regional Biosecurity: Operational Biosecurity and Diagnostic Surveillance
- Kahala Broodstock Management
- Analyze and Compile the Nutritional Composition of Potential Feed Ingredient Resources in American Samoa into a Feed Manual for Use in Tilapia Feeds

3. These projects address marketing and economics:
   - Inter-Institutional Coordination and Preparation of a Guam Aquaculture Development Plan
   - Developing Bivalve Culture to Diversify and Position Hawaii as a Supplier of Safe, Premium, Edible Shellfish Products, Years 1 and 2
   - Developing a Value-Added Product "Half-Pearls" from the blacklip pearl oyster *Pinctade margaritifera* in Pohnpei (the Federated States of Micronesia), Years 1 and 2

4. These projects address development of new technologies:
   - Development of Captive Culture Technology for the Yellow Tang, *Zebrasoma flavescens*, Years 1 - 3
   - Culturing the Harlequin Shrimp (*Hymenocera picta*) for the Marine Aquarium Industry, Years 1 and 2
   - Improving the Hatchery Output of the Hawaiian Pink Snapper (*Pristipomoides filamentosus*)
   - Development of DNA Markers for Pacific Threadfin Aquaculture, Years 1 and 2
   - Determining Aquaculture Bottlenecks of Pacific Threadfin (*Polydactylus sexfilis*): Increasing Fry Survival, Growth, and Quality, Years 1 and 2
   - Diversifying Freshwater Aquaculture Products for Hawaii: Two Crossover Species, the Red and Black Pacu (*Piaractus brachypomus* and *Colosomma macropomum*)
   - Value Added Approach for Tuna Fish Roe: Local Ingredient for Shrimp Maturation Diet?
   - Evaluating an Engineered Biological Treatment Process for the Application of Aquaculture Waste and Wastewaters
   - Aquaculture of Opihi
   - DNA-Based Identification and Selection of High-growth Tilapia in Hawaii, Years 1 and 2

5. These projects address demonstration and adaptation of known technologies:
   - Improving Pearl Quality by Grafting Technologies and Husbandry Methods for Development of a Hatchery-Based Black Pearl Industry in Pohnpei, the Federated States of Micronesia, Years 1 - 3

- Sea Cucumber Hatchery Production Technology Transfer in Pohnpei, Federated States of Micronesia, Years 1 and 2
- Adapting Aquaponics Systems for Use in the Pacific Islands
- Collection and Health Certification of Coral grouper Broodstock in the Mariana Islands

On the following pages, we present a summary of the goals, accomplishments, and impacts of these projects. See the Progress Reports section for further details.

Information Dissemination

Pacific Regional Aquaculture Information Service for Education (PRAISE) and Publications, Years 1 - 5

The PRAISE program provides research support services that empower regional aquaculturists to compete in the business world. These services range from development of educational products to direct delivery of research information. For a relatively small investment, the Pacific region has access to information which has enabled users to apply successfully for grants, design better facilities, increase survival rates, produce quality merchandise, and market research results. Regional educators and librarians have also benefited through product development and resource sharing. Every year, we receive the grateful commentaries of our users assuring us that they could not have done it without our assistance or the job would have taken considerably longer.

There is an ongoing need for the services we offer. Hamilton Library is the only research library in the Pacific. Compared to the high costs of transportation, electronic transfer of resources makes this project cost effective. This makes it logical that our focus for new products should be on digitizing resources which will be useful to the aquaculture community. To enhance our digital projects, we are working collaboratively whenever possible with the staff at the Pacific Aquaculture & Coastal Resources Center (PACRC) at the University of Hawaii at Hilo. Coordination with PACRC expands the ability of either group to reach the maximum number of end-users.

The Publications component of this project has two important, interrelated goals: (1) inform industry members and educators in our region of pertinent aquaculture information through various media and (2) inform the aquaculture community and interested parties of the progress of CTSA-funded projects through our own publications and those produced by others. In an effort to make our activities more environmentally conscious, we have restructured the manner in which we release CTSA news and information. In place of our previous quarterly printed publication *Regional Notes,* a monthly e-newsletter has been implemented and is distributed once a month to an audience of over 1,000 that ranges from local farmers and researchers to U.S. senators and other Beltway policymakers. The Publications project provides an invaluable service, creating and disseminating information that is difficult and sometimes impossible to find elsewhere.

Extension Support to Further Industry Development

Shrimp Production Demonstration Project and Aquaculture Training for Industry Stakeholders of the Commonwealth of the Northern Mariana Islands and Guam, Years 1 and 2

The Commonwealth of Northern Mariana Islands (CNMI) and, to a lesser extent, Guam, are looking to stimulate and stabilize their economies through industry diversification, due to declines in the garment manufacturing and tourism industries. The development (or expansion) of aquaculture is attractive because (a) increased aquaculture production can be supported by the domestic market due to the high demand for seafood fueled by tourism, and (b) live or fresh aquaculture products may have a high market value as domestic aquaculture is the only source (e.g., shrimp). Furthermore, farmers can sell directly to hotels and restaurants and receive much higher prices than if selling on the global commodities market.

Several small farms in the CNMI and Guam produce tilapia or marine shrimp (*Litopenaeus vannamei*). Inefficient farming practices and inconsistent seedstock availability, however, along with an unproven track record for shrimp aquaculture in the CNMI, have limited the development of the shrimp farming industry.

This project had two main goals: 1) build a knowledge and technical base for shrimp aquaculture in the CNMI and Guam and 2) stimulate interest (and possibly investment) in aquaculture in the CNMI and Guam. In an effort to achieve these goals, a 2-day aquaculture workshop was conducted in Saipan during Year 1. The workshop was attended by over 80 local farmers, business leaders, and government representatives, among others. In addition, OI scientists visited the Guam Aquaculture Development and Training Center (GADTC) on two separate occasions (November 2007 and July 2008). During these visits, scientists provided training on shrimp artificial insemination (AI) techniques, discussed broodstock feeding, water quality management, and broodstock sourcing protocols with GADTC staff, and gave presentations on regional opportunities in shrimp aquaculture and the effects of inbreeding on hatchery and growout performance of shrimp to industry stakeholders and University of Guam students and faculty. In July 2009 (Year 2), another trip was made, during which Clete Otoshi (OI scientist) gave a presentation on super-intensive shrimp aquaculture.

A shrimp production trial was conducted during Year 2 at SyAqua Saipan (formerly Saipan Aquaculture - the largest shrimp farm in the CNMI). A primary goal of this trial was to evaluate an alternative oxygen delivery system in an effort to reduce electrical usage per kg of shrimp produced. Electrical costs in the CNMI account for a large portion of production

costs (~40%), and this is a major impediment to industry development/expansion. The trial afforded the opportunity for SyAqua Saipan to test the oxygen concentrator equiment and see first-hand the simplicity of the equipment and its immediate impact on dissolved oxygen concentrations (DO). They have since purchased an oxygen concentrator and they have been able to increase stocking densities while maintaining satisfactory DO. This should lead to increased production and lower cost-of-production.

As part of its dissmenition efforts, the project created and distributed a manual on Shrimp Farming in the Commonwealth of the Northern Mariana Islands throughout the CNMI. In addition, as a result of the workshop, a shrimp farm has been established in Tinian.

Improving Outputs in the Commercial-Scale Production of Swordtails in Hawaii, Years 1 - 3

The overall goal of this project was to improve Hawaii's swordtail production output and impact on its product value. The desired outcome of the project is to improve the statewide swordtail production volume by at least 25%, and the contribution by swordtails to the freshwater ornamental product value to approximately 30%. This outcome was to be accomplished via several approaches, such as improving farm management strategies, diversifying product lines, developing technologies for the production of homozygous lyretail strains, and the dissemination of the technologies to appropriate end users. Initially, it was projected that meeting project objectives would require a minimum of two years, but that expectation was revised to three years. The principal reason for the longer timeline was because a single gene marker for the homozygous lyretail genotype was not obtained during Year 1. Development of that particular genotype will require additional time to conduct progeny testing. Although that aspect of the project was disappointing, other accomplishments, such as feminizing technology, were successful in laboratory trials.

A stakeholder meeting convened on June 10, 2006 at Windward Community College indicated that freshwater ornamental fish producers in Hawaii would like to see continued development of new technologies or improved production efficiency of already developed technologies that will help continue the growth of the industry. Developing varieties of homozygous lyretail swordtails is one advance that would boost output from a farm that produces live bearers, because the lyretail variety of swordtails is of much higher value than the common swordtail varieties. To accomplish this goal, the project work group has been utilizing some of the most advanced tools (e.g., artificial insemination, molecular techniques in search of gene markers, sex reversal technology) in the development of a production technology. Major outcomes from the previous work have been the demonstration of the genetic basis for the lyretail trait and the first known production of homozygous lyretail individuals, albeit at laboratory-scale. One major setback was that a potential gene marker that appeared to be associated with the recessive lyretail trait did not

stand up to double blind testing, limiting the ability to easily distinguish between the recessive (e.g., desired) and heterozygous genotype.

The laboratory-scale investigation of feminizing swordtails undertaken during Year 1 proved to be very successful. Although not the first report of successfully producing all female populations of swordtails via estrogen therapy, it is the first to show that using a low dose, longer duration strategy results in feminized swordtails that are fertile and possibly have a higher reproductive output than untreated swordtails. Validation trials of the results were consistent with the laboratory results. Producers of swordtails report that alleviation of the skewed female sex ratios in their production lines of common swordtails is as important as production of the homozygous lyretail varieties.

As a result of the project, lyretail swords have become Rain Garden Ornamentals best crop in terms of the return on expenses and labor. There are three market outlets - a wholesale broker/middleman who ships product to distributors on the mainland, wholesale directly to local pet stores, and direct retail sales to the hobbyist.

Promoting Health Management of Shrimp Aquaculture on Guam and the Commonwealth of Northern Mariana Islands

The goal of this project was to conduct research activities on enhancing health management on Guam and CNMI through raising biosecurity awareness and establishing a surveillance program. The project aimed to provide a timely service to Guam and the CNMI, to determine the current state of biosecurity practices on existing shrimp farms in the region, to evaluate the health condition of shrimp stock through diagnostics, and to provide farm-specific recommendations on health management. The main objectives of the project were to conduct biosecurity audits of current shrimp farm operations, take shrimp samples for health status evaluation, and write reports that combine disease-screening results, farm-specific biosecurity risks and suggestions for improvement for distribution to local farmers.

Due to extreme difficulty in obtaining necessary reagent solutions for analytical purposes at remote farm sites, the project experienced a significant delay and was not able to conduct biosecurity audits until July 2009. A total of seven shrimp facilities in Guam and the CNMI were assessed during biosecurity audit trips. Individual reports were compiled and distributed to the farmers or facility manager of each shrimp aquaculture site after the audits. A comprehensive report evaluating the current health status of shrimp aquaculture in the Mariana Islands region, identifying the key biosecurity risk factor, and prioritizing the issues for improving industry-wide biosecurity measures in the region was generated and distribution regionally.

Regional Biosecurity: Operational Biosecurity and Diagnostic Surveillance

Under the auspices of this project, researchers expect to assist in the continuation and future expansion and diversification of Hawaii and Pacific aquaculture industries. A major constraint in achieving expansion and diversification is the threat posed by various pathogens that can cause catastrophic losses in both vertebrate and invertebrate production systems. Therefore, this project features several objectives that fit in two main categories: operational biosecurity procedures for the prevention of diseases and improved diagnostics and surveillance.

Project working groups have been formed to address specific priority areas and align them with what was obtained through stakeholder input. The project group anticipates that the objectives, rationale and work plan will result in an increased capacity for the state and Pacific region to address aquaculture biosecurity issues, ultimately resulting in achieving the goal of continued regional growth and expansion of the aquaculture industry.

To date, a fully operational laboratory capable of conducting PCR testing has been established at Moana Technologies. Researchers have developed the PCR protocol for testing DNA extracted from fish tissue samples submitted for evaluation, and a workshop entitled, "What is happening with the culture of koi and tilapia in Hawaii?"was organized and attended by over 40 koi and tilapia stakeholders.

Kahala Broodstock Management

Pioneering research conducted by the Oceanic Institute and Kona Blue Water Farms, LLC (both of Hawaii) has led to the successful establishment of captive culture technology for a locally important *Seriolia* species known as kahala, (*Seriola rivoliana*). Previous work conducted at the Oceanic Institute established a captive breeding program and demonstrated the feasibility of hatchery based fingerling production methods for stocking in open ocean cages. Parallel efforts at Kona Blue (KB) led to the development of the first large-scale commercial hatchery and open ocean growout of the species, with current production of 10,000 lbs per week of a high-value, sashimi-quality, ocean-raised fish, trademarked as "Kona Kampachi™" which is targeted for high end sushi bars, white-tablecloth restaurants and high-end retail outlets.

Although kahala broodstock have now been successfully domesticated, egg supplies continue to be variable due to challenges in long-term broodstock maintenance and concern over the effects of environmental and dietary factors on egg quality. Therefore, the goal of this collaborative project between the Oceanic Institute (OI) and KBWF was to establish and optimize broodstock holding conditions toward securing long-term

broodstock health and developing a more reliable year-round supply of viable eggs to facilitate year-round hatchery output. In addition to assisting commercial operations at KBWF, results of these studies will assist other commercial startups in determining the best methods for maintaining valuable broodstock populations.

Project results showed that kahala broodstock can be maintained in either flow-through or recirculating broodstock holding systems following suitable quarantine procedures to eradicate ectoparasites with excellent egg output. A diet evaluation found that commercial high-protein/high-lipid broodstock diet (Vitalis) produced by Skretting successfully supports high-quality egg production, although challenges with egg quality in this study suggests the need to further examine diets for broodstock growout and maturation.

Improving the Hatchery Output of the Hawaiian Pink Snapper (Pristipomoides filamentosus)

Initial efforts in the development of culture technologies for opakapaka have resulted in the only captive spawning broodstock and hatchery techniques that produce modest amounts of opakapaka juveniles. The desired outcomes of the project are to develop hatchery and nursery techniques for the production of opakapaka juveniles that can meet commercial-scale requirements, and transfer those developed technologies to appropriate end users for either public or private use. Successful development of hatchery and juvenile production technologies offers opportunities for expansion and diversification of the emerging off-shore aquaculture activities and forms the basis for the project.

The first rearing trials to investigate first feeding conducted a comparison of larval growth between a fed and unfed group. Additional laboratory-scale rearing trials (n=2) focused on the suitability of a particular live food organism as a first feed. Some interesting trends were observed from these laboratory-scale trials, the first being the significantly lower survival of the larvae when rotifers were presented as a first live food organism. While the result was not unexpected, in both trials, the treatment was significantly ($P<0.05$) lower than when copepod nauplii were used either in combination with rotifers or when copepod nauplii were used alone, leading researchers to conclude that rotifers do not appear to be a suitable transitional live food organism. Researchers did, however, confirm that the first feeding of opakapaka larvae can be achieved using copepod nauplii, resulting in high survival up to 10-14 days posthatching.

High speed filming conducted under the project shows that older copepods are not suitable during the early stages of the larval rearing process because their escape mechanism surpasses the ability of the opakapaka larvae to capture them. This is not the case for copepod nauplii.

Larval rearing trials that were done with the moi larvae as a training exercise did result in data that was useful to the hatchery manager of Hukilau Foods and their hatchery operations. Some thought about incorporating copepods into the rearing protocol of moi larvae has been ongoing for several years; insight into the question about whether there is a definite need was provided as a result of our initial trial. Essentially, there is no benefit to using copepod nauplii in raising moi larvae and, for that reason, further research is not being actively pursued unless the need arises, such as with an alternative fish species.

Analyze and Compile the Nutritional Composition of Potential Feed Ingredient Resources in American Samoa into a Feed Manual for Use in Tilapia Feeds

Under the auspices of this project, researchers expect to conduct and publish research activities on the capability of locally-available animal and plant products or byproducts in American Samoa for use in fish feed. This project has four main goals: 1) Identify, quantify and collect potential local products and byproducts for aquatic feeds development in American Samoa; 2) Analyze the nutrient composition of selected samples; 3) Compile a feed manual containing the following information: a) list of locally available ingredients and byproducts, and their nutritional composition; b) practical finfish feed formulations using local ingredients; and, c) feed processing techniques and quality control tests (mix time for mixers, ingredient particle size determinations, pellet stability test) for use in making aquaculture feeds containing the identified ingredients; 4) Transfer technology through a work shop and feed manual hand-outs to local producers and farmers.

The nutritional composition data of locally-available products and byproducts generated by this project will be a valuable database in formulating sustainable cost-effective feeds for the aquaculture industry in American Samoa. Basal diet formulas for omnivorous and carnivorous finfish feeds will be developed using the local feedstuffs, according to published nutrient requirements for each species. Based on the physical properties of the local feed ingredients, suitable processing procedures and conditions will be set up to guarantee quality control of final feed pellet. The results of the analysis of all potential ingredients from the laboratory will be added to a database of ingredient nutrient profiles and transferred to American Samoa. The nutrient database will be compiled into a manual with suggested diet formulas and feed processing techniques. The manuals will be sent to local agricultural officials, aquaculture farmers, feedstuff producers as well as feed business persons.

The project had a late start but has has to date created a simple, at-home feed manufacturing system in American Samoa, and increased the feed processing speed at the local community college.

Marketing and Economics

Inter-Institutional Coordination and Preparation of a Guam Aquaculture Development Plan

Guam has been developing a commercial aquaculture industry since 1973, when the first research was begun locally. The last full aquaculture development plan was written in approximately 1988, and it has not been comprehensively revisited. Most of the information available to potential local and foreign investors is seriously outdated. The plans for the development of aquaculture on Guam need to be revisited, and new development strategies need to be devised that capitalize on Guam's competitive advantages of United States' laws, its location, air connections, climate, and clean environment.

This project developed a new Aquaculture Development Plan for Guam. The process of developing the Aquaculture Plan was intended to be inclusive, and it began by forming a working group of commercial producers and involved government agency personnel. The workgroup collaborated to compile information and conduct several exercises, including an institutional analysis and a SWOT analysis, which was written and incorporated into the aquaculture development plan. Researchers obtained the historical records of the Guam Division of Aquatic and Wildlife Resources (DAWR), which list aquatic species that have been imported in the past. While it has been determined that these records are incomplete, they are the basis on which import permits are issued on Guam. Clarification has been obtained on the procedures for obtaining import permits for new species, and some sense of which species may be considered possible and which types of species will definitely be prohibited has been elicited from the DAWR. The report on environmental regulations was incorporated into the final draft of the development plan. The institutional analysis is complete and has been incorporated into the plan as well. The review of economic incentives on Guam indicated that most had lapsed into lassitude over the last 30 years, since aquaculture was a new and exciting economic development prospect for Guam.

The core team of the PI and three outside experts in aquaculture and environmental management issues reviewed the output from the working group, and they drafted the revised Aquaculture Development Plan for Guam. Due to project delays, the funds allocated to print the plan were returned to CTSA. The Secretariat of the Pacific has agreed to publish the final draft, at which time it will be presented to the public for comment in an open forum.

Developing Bivalve Culture to Diversify and Position Hawaii as a Supplier of Safe, Premium, Edible Shellfish Products, Years 1 and 2

Effort in this project has focused on resolving issues that have historically impeded growout of edible bivalves in Hawaii, including issues related to appropriate species, sites, lack of a certified laboratory for shellfish growing water analysis, and gaps in State policy. The expected outcomes include the demonstration of the biological and economic feasibility of edible bivalve culture, identification of the steps necessary to certify a laboratory in Hawaii, and capacity building at several levels for culture technology and awareness of shellfish sanitation requirements.

An assessment of the potential for bivalve culture in Hawaii was conducted under the recently completed USDA project, "Bridging Gaps for Ensuring Long-term Viability of Small Tropical Mariculture Ventures in Hawaii and the U.S.-Affiliated Pacific Islands." The final report from the assessment was authored by bivalve experts Dr. John Supan (Louisiana State University Sea Grant Program) and Dr. Maria Haws (Pacific Aquaculture Center, UH-Hilo). This work identified sites and bivalve species with potential for culture and marketing in Hawaii, including those with export potential. The report concluded that significant opportunities exist to grow both standard bivalve species and native Hawaiian species with commercial potential. There are a significant number of native Hawaiian species and established introduced species preferred by consumers of many cultural and ethnic backgrounds. There also exist major constraints that need to be resolved.

To date, the project has demonstrated the biological feasibility of edible bivalve culture, identified steps necessary for the certification of a laboratory in Hawaii, and is addressing these steps. The project has also made significant advances to build capacity for Hawaiian fishpond operators to grow shellfish in coastal areas. As of November 2010, over 10,000 Hawaiian Oyster spat (>1 CM) have been produced. In June 2010, growout trials were begun in Oahu fishponds with good preliminary results.

Developing a Value-Added Product "Half-Pearls" from the blacklip pearl oyster Pinctade margaritifera in Pohnpei (the Federated States of Micronesia), Years 1 and 2

The pearl oyster usually ends its life after producing the round pearls or when it becomes incapable of producing sellable pearls. These so-called "useless" pearl oysters are killed and sold to the shell market as materials for buttons, handicrafts and others. Hemispherical pearls (or "half pearls" or "Mabe pearls" as they are more commonly known) have the value-added opportunities to the pearl oyster shells (mother-of-pearl shells) in the jewelry and handicrafts, particularly to the domestic market opportunities and local cultural carving and handicraft traditions. However, the South Sea half-pearl businesses declined to near extinction before the early 2000s with minor productions from Mabe pearl oyster *P. penguin*.

The blacklip pearl oyster *P. margaritifera* have the unique luster and color from silver or grey to dark green or purple. In Micronesia where there has been a small, niche tourism, the half-pearls have potential to support a sustainable pearl business and rural development particularly for a small family and/or community-based enterprises. One of the advantages of producing half-pearls is the lower capital investment and technical requirements compared to the round-pearls. Most importantly, the College of Micronesia Land Grant Program (COM) has been training Micronesians for half-pearl seeding for the last five years. A preliminary study by COM has shown clearly that high quality half-pearls with unique color and luster can be produced by local labor force to develop a new export market. Production of half-pearls represents not only one form of adding value to the pearl shells but also a low-risk means of generating revenue.

The project activities will focus on demonstration and skill training onsite in Pakin Atoll for grading techniques of the shells and the half-pearls of the blacklip pearl oysters by the grading expert, the half-pearl harvest and subsequent half-pearl accessory making involving all community members, grading of the products by the grading expert, and trial sales in Pohnpei and/or overseas for the half-pearl and shell curving accessories. As the Pakin community commences commercial pearl farming in early 2010 under its integrated aquaculture and marine protected areas development, the proposed project will contribute to strengthen technical and economic bases of sustainability of such activities. The project started in July 2010 and is in its early stages, but researchers have already conducted half-pearl seeding demonstration and skill training for the local youths by the project's Micronesian technicians both in Pohnpei and at the outer islands. In addition, an international pearl quality grading method from the Gemological Institute of America has been applied to grade the half-pearls.

Development of New Technologies

Development of Captive Culture Technology for the Yellow Tang, Zebrasoma flavescens, Years 1 - 3

The development of captive culture technology for yellow tang and other high-value reef species is imperative to protect our increasingly threatened coral reef ecosystem. Not only will captive production technologies help take pressure off wild fish populations, but they also will provide new economic opportunities associated with the nearly billion dollar worldwide trade in marine ornamental species.

Under earlier studies, researchers at Oceanic Institute made preliminary progress in describing the reproductive biology of this species and successfully spawning captive stocks. These studies also helped to identify a number of key challenges that need to be overcome to successfully rear yellow tang in captivity. The first of these challenges is the development of a year-round supply of viable eggs. Although captive stocks can be spawned year-round, they demonstrate a gradual deterioration in condition with the majority of spawns being infertile, and the small numbers of eggs that are fertilized having low viability. Therefore, efforts are focused on improving diet and holding environment for captive adult stocks to generate viable eggs.

This project has thus far established appropriate holding systems and diet for conditioning and maintaining spawning stocks of yellow tang providing a year-round supply of viable eggs and larvae. In addition, researchers have developed a larval rearing system supporting survival and early development of these extremely small and delicate newly hatched larvae, and have succeeded in getting the larvae to start feeding using our copepod-based hatchery methods under development at OI.

The final phase of this project will focus on refining captive production technologies, with an emphasis on production bottlenecks, and the transfer of developed technology to local industry.

Culturing the Harlequin Shrimp (Hymenocera picta) for the Marine Aquarium Industry, Years 1 and 2

The harlequin shrimp, *Hymenocera picta*, is a popular marine ornamental shrimp. However, almost all harlequin shrimp sold in pet shops around the world are collected from shallow tropical waters of the Pacific Ocean, including Hawaii. Collecting harlequin shrimp from the wild is not sustainable, and there may be collateral damage to coral reefs associated with this practice. Unfortunately, there is a paucity of published information about the captive reproduction and husbandry of these valuable aquarium shrimp, so the collection of wild shrimp likely will continue in the future. Based on scant published literature, it appears that harlequin shrimp feed exclusively on live asteroid echinoderms, particularly those of the genera *Linckia* and *Acanthaster*. Anecdotal reports suggest that *H. picta* also may feed on other enchinoderms, and that there may not be an obligatory need for live feed. No published literature, however, is available to support these claims. The restricted diet of *H. picta* represents a considerable obstacle to large-scale production and marketing of this valuable marine ornamental shrimp, as reliance on live asteroid prey presents a major challenge and economic burden to the producer or aquarium hobbyist.

In light of the apparent dietary restriction inhibiting the mass culture and marketing of *H. picta*, one goal of this CTSA-funded project was to evaluate alternative diets for this shrimp

with the expectation that these results ultimately will contribute to the development of a formulated, artificial diet. Preliminary adult feed trials demonstrated that H. picta can use frozen Linckia to a limited extent. Juvenile feeding trial results suggest that by-catch Asteria sp. could serve as a more ecologically sustainable food for rearing these shrimp. Larval-rearing efforts demonstrated that harlequin shrimp can be reared with high (40-70%) survival through the early feeding period, using conventional approaches similar to those used in rearing marine fish larvae.

Obtaining large numbers of juvenile or adult shrimp needed for diet development was difficult. Regular spawning events provided researchers with valuable information about spawning and molting frequency among mated pairs, as well as the opportunity to characterize individual spawns in terms of number of viable larvae, number of fertilized eggs, and number of unfertilized eggs. In addition, spawned larvae were used in numerous larval-rearing trials designed to obtain competent post-settled juveniles.

Development of DNA Markers for Pacific Threadfin Aquaculture, Years 1 and 2

The goal of this project was to develop DNA-based genetic analysis technology for use in aquaculture and conservation of Pacific threadfin (*Polydactylus sexfilis*), locally known as moi. Under a two year plan, the objectives of this collaborative project between Jinzeng Yang, Ph.D., of the University of Hawaii (UH) and Charles Laidley, Ph.D., of Oceanic Institute (OI), were to establish genomic and skeletal muscle cDNA libraries and identify suitable DNA microsatellite markers of Pacific threadfin (Year 1), and to use these markers to characterize the genetic diversity of captive broodstock populations and natural populations. In addition, Year 2 activities examined parental contributions to group spawning events.

Under Year 1 project activities, the UH molecular biology research team established laboratory methods and procedures for identifying microsatellite DNA markers in Pacific threadfin. They collected more than 1200 Pacific threadfin fin clip samples from captive broodstock and wild populations. Genomic DNA was extracted and digested, and a short insert genomic DNA library was constructed. 52 microsatellite loci were developed, along with PCR protocols for optimal amplification of DNA markers. No significant genetic differentiation was found among all of the populations sampled.

Five highly polymorphic microsatellite loci were selected to develop a PCR panel for a parentage assignment study. A microsatellite DNA-based method of parental assignment was developed. Based on results from a second round of parentage analysis using eight microsatellite markers, 98% of offspring were assigned to their parents.

Based on initial microsatellite DNA results, 10 to 20 microsatellite loci can be used for further genotyping and comparisons of genetic diversity between broodstock and wild populations. This will have practical application to Pacific threadfin commercial aquaculture for better maintenance and management of genetic stocks and selection of seedstock.

Determining Aquaculture Bottlenecks of Pacific Threadfin (Polydactylus sexfilis): Increasing Fry Survival, Growth, and Quality, Years 1 and 2

Despite much progress in moi culture over the past few decades, the moi culture industry still faces major loss during early larval stages. The main limitation to increased production is the relatively low number of juveniles available for grow-out to market size. This can largely be attributed to high larval mortality ranging between 70-99% during the pre-metamorphic and metamorphic stages. Three major problems in the threadfin culture have been identified: 1) extraordinary loss during the pre-metamorphic and metamorphic periods, 2) the dramatic size variation between fish after metamorphosis, and 3) cannibalism during hatchery and nursery phases of operation.

Recent research from our laboratory (in collaboration with the Oceanic Institute) has shown that reduced growth and survival are correlated with low environmental iodide supplied by seawater wells during broodstock and larval rearing. Iodide is an essential component of TH and low iodide levels are directly linked to poor development and survival in all vertebrates. In fish, low iodide impedes the activity of the thyroid gland, thereby reducing maternal transfer of thyroid hormones (THs) to eggs. The specific aims of this project are to characterize the efficacy of using aqueous and dietary iodide to improve survival and growth of larval and juvenile moi up to stocking age. Ultimately, our goals are to improve commercial rearing protocols to increase production of fish for the market, and encourage expansion of moi culture.

Successful completion of the project will establish the method of iodide supplementation that leads to a practical and important increase in the survival and growth of moi larvae and fry. Ultimately these results will be utilized to reduce rearing costs and increase the efficiency of moi production and improve the financial bottom line for moi farmers.

To date, the project has successfully reduced and reversed goiter formation rates in highly valuable moi broodstock populations. Investigators have developed multiple methods to supplement diets and restore thyroid economy, and showed that dietary supplementation procedures are completely safe for broodstock, as well as eggs and larvae generated from treated broodstock. They also developed a pragmatic sausage diet for broodstock facilitating other dietary improvements including vitamin supplementation.

Diversifying Freshwater Aquaculture Products for Hawaii: Two Crossover Species, the Red and Black Pacu (Piaractus brachypomus and Colosomma macropomum)

Under the auspices of this project, researchers expect to establish black pacu, *Colosomma macropomum* and red-bellied pacu, *Piaractus brachypomus,* as freshwater aquaculture products for both the aquarium and food fish markets in Hawaii. It is anticipated that this will take several years, mainly due to the time it takes the target species to reach sexual maturity, which is reported to be three and four years for the male and female, respectively, for the black pacu, *Colosomma macropomum*, (Campos-Baca and Kohler, 2005).

There is an opportunity to increase food fish and ornamental fish production, particularly in the freshwater portion of aquaculture, in Hawaii by importing, spawning and culturing two crossover species that are native to South America, commonly known as pacu, *Colosomma macropomum,* (Black pacu or Black-finned pacu, Tambaqui) and *Piaractus brachypomus.* Both species have ideal characteristics that would make them excellent candidates for commercial aquaculture. These characteristics include; feeding low on the food chain; rapid growth; amenable to high density; hardy and resistant to disease and marginal water quality; ability to utilize high carbohydrate diets; high marketability as a food fish and as an ornamental species; and commanding a high market price (Campos-Baca and Kohler, 2005). Moreover, pacu can be co-cultured with other food fish and invertebrate species (Teichert-Coddington, 1996; Campos-Baca and Kohler, 2005). Culture trials currently being conducted in the private sector with black pacu being reared in cool water (20-23 ^{o}C) sources on the island of Hawaii (Takata, pers. comm. 2008) have shown some success and have led to increased interest among commercial farmers. Commercial producers are very interested in expanding opportunities to diversify Hawaii's freshwater aquaculture sector, and pacu appears to be very promising. Activities of the project will result in the establishment of populations of both pacu species on three islands (e.g., Maui, Oahu and Hawaii). This is being done to take advantage of the various niches/micro climates each island possesses. In addition, the project working group will be conducting feeding trials utilizing locally available commercial diets in order to assess performance and identification of the most cost-effective feed type. Pacu have been reported to be cultured using low protein-high carbohydrate feeds (e.g., chicken feed 20% crude protein), and the feed trials in the proposed project will investigate their utility in the production of these species under conditions encountered in Hawaii. Likewise, these species reportedly do well when polycultured with other fish (e.g., tilapia and carp) and crustaceans (e.g., *Macrobrachium rosenbergii*), and growth and survival under similar conditions experienced in the islands are to be investigated. Lastly, as freshwater is one of the islands most precious natural resources, the performance of both species in closed recirculating systems (including aquaponics systems) are to be determined.

The first shipment of 400 Red Pacu arrived on August 30, 2010 and was distributed into 5 quarantine tanks located at WCC. During the next week an outbreak of *Ichthyophthirius multifiliis*, and a flagellated protozoan, *Ichthyobodo* sp. took place with mortalities being observed in one tank on September 5. Notification of the event and a treatment plan was submitted to the UH Veterinarian and approval for a low dose (25 ppm) formalin treatment every other day for a minimum of two more dosages but no more than five treatments total. Mortalities spread to all tanks and only six survivors remain as of the submission of this report. A second shipment of 200 pacu from the same source was received on October 6, 2010 and once again divided up into five holding tanks and placed under quarantine. A preliminary survey conducted the same day of receiving the pacu revealed no signs of parasites. However, two days later, a second survey revealed a small number of *Ichthyophthirius multifiliis* in just one tank and was treated. A total of 150 individuals remain and are being used to initiate the polyculture experiment with Chinese catfish. Fish were under quarantine until November 11, 2010, when the polyculture of pacu and Chinese catfish experiment was initiated with the stocking of ten 600 gallon tanks with multiple stocking densitites.

Value Added Approach for Tuna Fish Roe: Local Ingredient for Shrimp Maturation Diet?

The over-arching goal of this project is to develop new knowledge to sustainably increase the value of underutilized tuna fish roe by exploring its potential usage as an aquaculture feed ingredient in a shrimp maturation diet. To achieve the proposed goal, studies within four consecutive research thrusts will be carried out to obtain the baseline information, to develop a practical method for utilizing tuna roe in a shrimp maturation diet, to evaluate such diet, and to transfer the knowledge. The successful application of tuna roe will not only produce cost-savings for shrimp hatchery operations, but it also will turn a waste by-product into a value-added product, providing additional environmental benefits by reducing the amount of organic wastes. Preliminary feeding trials with young shrimp and tilapia showed good acceptance of raw tuna roes. However, it is unknown how effectively tuna roe can boost the maturation process as compared to the conventional fresh-frozen maturation feeding regimes from both health and nutrition points of view. It is worthwhile to assess the nutritional and health status of fish roe based on analytical results, and to develop practical ways to utilize them in maturation diets. Instead of direct usage of fish roe in its raw form, the pelagic fish roe can be homogenized and incorporated into a semi-moist diet to achieve the suitable density, stability and palatability. The proper processing procedures need to be developed not only to preserve the nutritional values of fish roes, but also to ensure the feed stability and consistency in general, and to provide convenience in feed storage and handling.

To date, 23 sample batches of tuna roe have been collected. The preliminary trials for various freezing dry processes to standardize the fish roe samples have been completed, and the samples have been sent for nutritional analysis.

Evaluating an Engineered Biological Treatment Process for the Application of Aquaculture Waste and Wastewaters

In the Pacific Islands and other land limited regions, there is growing demand to manage water resources efficiently. In Hawai'i, most water is consumed for agricultural and domesticated purposes, which generally contains carbon and nutrient (nitrogen and phosphorus) pollution; and excreted pharmaceuticals and personal care products (PPCPs). Subsequent discharge and reuse of treated wastewater necessitates the use of secondary (e.g. biological) treatment in order to (1) reduce biological/chemical oxygen demand and (2) reduce nutrients for disposal. These constituents pose a direct threat to aquatic and marine environments as well as public health. The introduction of hormones in aquaculture systems may pose an additional inherent risk to environmental stability. The EPA is considering that hazardous pharmaceutical wastes to be included in the universal waste rule, which will enable a system for the proper disposal of hazardous and non-hazardous pharmaceutical wastes (EPA, 2009).

The use of biological treatment processes were developed for this purpose. Biological treatment processes use microorganisms to metabolize and treat wastewater. In general, biological treatment processes are classified under two categories: anaerobic or aerobic process. An aerobic treatment process has been developed for water/wastewater treatment at the University of Hawai'i at Mānoa for years, which can optimally treat water at an operational condition of either intermittent aeration or continuous aeration. On the other hand, physicochemical treatment processes (PTP) rely upon a combination of physical and chemical methods to treat wastewater (Metcalf & Eddy, 2003). Disadvantages of utilizing PTPs include larger unit-space required, requires energy, and the use of chemicals and machinery to achieve treatment objectives at high capital cost.

There has been extensive research on EMMC (Entrapped Mixed Microbial Cell) in treating wastewater from domestic and agriculture; consequently, EMMC is a feasible candidate to be applied for aquaculture wastewater. The proposed work is intended to contribute in demonstrating the utility of EMMC at the University of Hawaii at Manoa in treating wastewaters from aquaculture systems. Additionally, the process performance of the EMMC to another biological treatment process will be compared. Additionally, the possible integration of both systems will be explored in order to determine which process design option will optimally achieve desired treatment/reuse objectives.

The project, which started in June 2010, has begun characterizing the aquacultural waste and wastewaters. This information is necessary to better develop a treatment system process to achieve remediation and re-use goals. In addition, researchers have developed a wastewater characteristic study for use in the project.

Aquaculture of Opihi

Opihi are a high value product in Hawaii, with prices averaging $10-15 per pound with shells on (Bird, 2006). An established niche market exists bolstered by the need for opihi at Hawaii gatherings. Demand for opihi exceeds the level that the wild caught fishery can supply because of low natural abundances due to overfishing. While some highly academic marine biology studies have been conducted, a concerted aquaculture research effort has never been attempted and research is needed to develop cost effective means of opihi culture. The previous work on opihi focused on the yellow foot (*Cellana sanwicensis*) and black foot (*C. exerata*), which live above the waterline on rocks splashed by waves. However, the giant opihi (*C. talcosa*) holds the greatest aquaculture potential as it lives below the waterline, and therefore eliminates the expense associated with trying to mimic waves in the aquaculture enclosure. The giant opihi also grows faster than other species (Kay et al., 2006), and people like its flavor (Bird, 2006).

Juvenile opihi have been raised in a laboratory but grew slower than wild opihi. They were fed only the algae and biofilm that grew on the walls of aquaria. The development and use of an accessible, nutritious, and palatable artificial feed is essential to commercial opihi culture. While no work has been done on artificial opihi feeds, work has been done for abalone and sea urchins, two other herbivorous benthic invertebrates. An artificial feed that is consumed by opihi needs to be found, and then formulations need to be adjusted to improve growth rates. In addition, life cycles need to be closed. Previous work with other opihi species suggests that natural spawning can be induced by heavy aeration when the animals were in season, but other methods exist to induce spawning. These will provide alternatives to the natural spawning method. Standard methods for raising opihi larvae must be developed with an emphasis on successful settlement. Once opihi can be spawned and cultured, the flavor of these farmed opihi taste must be tested and adjusted to match wild opihi.

The project, which started in August 2010, was in its early stages at the time of this report. To date, the worksite has been prepared and broodstock opihi have been collected. In addition, artificial feeds have been made and are ready to begin feeding trials.

DNA-Based Identification and Selection of High-growth Tilapia in Hawaii, Years 1 and 2

Lack of genetically suitable seedstock of tilapia has been a limiting factor for tilapia aquaculture in Hawaii. Importation of tilapia strains to Hawaii has been challenged by environmental concerns and field-testing experiments. Strains of tilapia such as O. *ossambicus* and Blackchin tilapia *(Sarotherodon melanotheron)* were introduced to Hawaii to control aquatic weeds in reservoirs, ditches, and canals several decades ago. Over the years, aquarists also released a stunning number of tilapias into Hawaii's streams and reservoirs. Some have entered the marine environment and established saltwater-tolerant populations. In addition to the "wild" tilapia in Hawaii, various strains or hybrids of tilapia are also present in local tilapia farms. These tilapias existing in the wild and farms can be used as genetic resources for developing high-growth tilapia without importing new strains.

Advances in molecular genetics have been applied for species identifications and genetic improvement of economically important traits. The sequence of a single mitochondrial protein-coding gene, namely cytochrome c oxidase subunit I (COI), is used as a platform for DNA barcoding of all living species. The tilapia strains or hybrids currently existing in Hawaii can be classified by COI sequencing. Then, DNA-based genetic selections can be applied for building up high-growth tilapia broodstock. The differences in the DNA and gene expression level of an animal can indicate their genetic differences in production performances. Microsatellite genotyping and gene expression analysis have been proved as effective tools for genetic selection of superior animals with desirable production traits. Application of these techniques to tilapia broostock selection will significantly speed up its genetic progress.

The project, which started in August 2010, was in its early stages at the time of this report. To date, genomic DNA isolation from Tilapia fin clip sampling has been conducted, and testing and establishment of the COI PCR and DNA sequencing protocol has begun. In addition, the principal investigator has initiated discussions with local Tilapia farmers.

Demonstration and Adaptation of Known Technologies

Improving Pearl Quality by Grafting Technologies and Husbandry Methods for Development of a Hatchery-Based Black Pearl Industry in Pohnpei, the Federated States of Micronesia, Years 1 - 3

Improving the quality of a pearl has always been a primary objective in pearl farming. The price of a cultured pearl is determined by a combination of factors representing pearl quality, including roundness and the absence of flaws. Irregular shapes and flaws, however, occur more or less during the natural process of layering of nacre by the oyster, and are thought to be inevitable. Post-grafting survivorship has also been a major concern in the black pearl industry. The number of producers using chemically treated nuclei, particularly treated with antibiotics or other substances such as fibronectine, has increased with little scientific evidence of *in situ* trials showing their effectiveness in improving post-grafting survivorship and/or pearl quality.

This project aimed to find simpler and more economical ways of grafting and husbandry methods to improve pearl quality and post-grafting survivorship. The project is also an integral part of the College of Micronesia (COM)/COM-FSM Pearl Project for developing a sustainable hatchery-based pearl industry in the State of Pohnpei, and for improving the profitability of existing black pearl farms in the Pacific.

Understanding the mechanisms of flaw formation is key to this project, particularly, the formation of so-called "circles" and/or "spots", which are commonly found in a large proportion (i.e., 60%-95%) of the total production in black pearl farming. During Year 1, grafting and re-grafting experiments were conducted, resulting in the fist scientific confirmation in this field of study of pearl quality improvements in shapes and flaws from re-grafting. Trials also indicated that the use of chemical-coated nuclei may not be necessary in terms of profitability of pearl farming, knowledge that will reduce production costs.

A major component of the project was the transfer of pearl farming technology to local Micronesian technicians using hands-on skill training methods at both Pakin Atoll farm and at Nett Point demonstration farm in Pohnpei. In total, over 30 Micronesians were trained in various skills including pearl grafting, husbandry, farming, and accessory making, resulting in the establishment of a pearl industry in Micronesia.

Sea Cucumber Hatchery Production Technology Transfer in Pohnpei, Federated States of Micronesia, Years 1 and 2

The ultimate purpose of this project, which began in July 2008, was to address the "boom and bust" pattern of the current sea cucumber industry by introducing hatchery production of the organism, from spawning and larval rearing to grow-out. The goal of this project is to provide greater supplies of sea cucumbers on a regular basis through hatchery technology, affording the needed bio-mass for a sustainable industry. If the hatchery technology proves successful in achieving the production objectives, then additional

activities relative to conservation and stock management programs may be developed in collaboration with different conservation groups, coastal communities, and in "marine protected area" programs. Collaborations with the Pohnpei State Government, local municipal governments and communities, and other Land Grant institutions in the Marshall Islands and College of Micronesia-FSM, such as in Yap State, will provide the framework for this sustainable industry in the future. While the ultimate goal is a sustainable sea cucumber industry, the immediate objective was to search for and secure broodstock of the sandfish, and rapidly transfer the necessary hatchery technology to Micronesian technicians.

In Pohnpei State of the Federated States of Micronesia (FSM), the once-prosperous sea cucumber fishery has been chronically over-fished, to the level of extinction of almost all high-valued species, despite a State Government-imposed export ban in effect since 1995. Rebuilding the industry and enhancing commercially important sea cucumber resources is an urgent issue. Hatchery-based juvenile production could help to rehabilitate wild stocks of high-value sea cucumber species, such as the sandfish (*Holothuria scabra*) in the lagoon of Pohnpei. The ability to rebuild depleted sea cucumber populations through restocking would be a valuable tool for future management planning, which could be achieved by the development of cost-effective methods for mass production of juveniles. Hatchery production and release of cultured juveniles would help to increase the number of potential spawners in the wild as well.

In the past, Micronesian technicians lacked the opportunity to acquire the necessary hatchery skills and subsequent grow-out technologies. However, another CTSA funded project (Black-Pearl) has afforded hatchery training for Micronesians, who are now able to utilize and transfer the knowledge amongst their community. Currently, the College of Micronesia has a functional hatchery at Nett Point in Pohnpei being operated by Micronesians for production of the blacklip pearl oyster *Pinctada margaritifera* and the sandfish. The local hatchery technicians who attained the required skill-set during the pearl hatchery project adapted quickly for this project and aided in collecting broodstock, inducing spawning and rearing larvae during Year 1. Larval development and larval rearing protocols were developed and refined under this project. A total of 24 spawning induction trials and 12 larval runs were conducted during the project and resulted in several hundred juveniles, which are awaiting the next phase of work on marking and/or tagging experiments for a restocking project.

One significant accomplishment of this project was the development of a semi-closed system with a false-bottom tank for long term holding work. This system simulates the natural habitat of the sandfish with seagrass (eel grass) and seaweed (i.e. *Caulerpa, Gracilaria*, *Sargassum* and other species) on the top layer, supplying naturally occurring

food. In addition, differences in abundances of sandfish during the day and night observed in this project urge that all existing survey reports on the sandfish be reviewed by researchers and policy makers to re-construct present status of resource management strategies.

Adapting Aquaponics Systems for Use in the American Pacific Islands

There is an interest in the local production of fish and produce in the Pacific Islands. One solution is integrating plant culture with fish farming, commonly called aquaponics. Extension of an existing aquaponics system has been attempted before in the Saipan, but failed due to its reliance on expensive, breakage prone, high maintenance mechanical components and lack of basic scientific knowledge to modify the system. Preliminary studies suggest that retro-engineering of complex recirculating aquaponics systems may be possible by achieving an understanding of the nutrient flow in the system and developing an integrated recirculation/anti-denitrification system. Promising results have been obtained with a small scale (50 m^2), modular tilapia and lettuce co-culture system. The system is suitable for a group of families and can be readily expanded for commercial purposes. It does not use much electricity nor does it use more than one piece of equipment.

Research is necessary to finalize this simplified aquaponics system. The efficacy of using the air breathers Chinese catfish *Clarias fuscus* or Asian snakehead *Channa sp.* for aquaponics will be tested. This could obviate the need for aeration and make the system entirely electricity free. The effect of removing electrical aeration on denitrification requires further investigation. Commercial feeds are expensive to ship to Pacific Islands, but low cost, high quality fish meal is available locally in American Samoa due to the presence of a tuna canning mill. A locally produced feed should be utilized, and the resultant nutrient profile generated by fish fed this feed must be determined. Once the necessary research is complete, the project work group will extend the technology to interested Pacific Island clients. This includes the completion of a manual which explains, in detail, system construction and contains numerous photos, water quality monitoring to avoid problems at the start of operations, daily operations, and harvesting. This manual has been started. A harvesting regime must be established which ensures a constant supply of products for consumers.

Hands-on extension is planned throughout the life of the project, starting with an extended site visit at the end of the first year of the proposed project. The system will be constructed and run alongside farmers and a local project work group member, who will work with farmers to ensure that the system remains productive. This will be conducted with the assistance of the Agricultural Development in the American Pacific (ADAP) program, and will utilize local Sea grant and Land grant extension networks. The project, which started

in August 2010, was in its early stages at the time of this report. To date, a graduate student is currently being trained, and aquaponics systems are being built in American Samoa based on the project's a low cost, mechanically simple, lettuce and tilapia aquaponics system.

Collection and Health Certification of Coral grouper Broodstock in the Mariana Islands

This project is being conducted to begin the process of establishing a domesticated, high health population of two species of coralgroupers, *Plectopomus leopardus* and *P. leavis*, commonly known as the Leopard and the Giant coralgrouper respectively, at the Guam Aquaculture Development and Training Center. The project has three objectives: 1. to capture sufficient numbers of each species to establish a breeding population, 2. to test all fish for viral infections and maintain the fish in a secure, high heath environment and, 3. to raise the fish to the point where we have sufficient numbers of mature fish both sexes to be ready to begin reproduction trials.

The coralgroupers are among the most sought after and valuable food fish of the coral reef habitat worldwide. They are some of the highest priced fish in the Live Food Fish markets in Hong Kong, where they are imported from across vast regions of Asia and Oceania. As such, many are listed as vulnerable on the IUCN Red list, including the two target species for this project. *P. leopardus* and *P. laevis* are suffering from over fishing on Guam to the point where their natural recruitment may be threatened. The Division of Aquatic and Wildlife Resources of the Guam Department of Agriculture is willing to support a program geared towards restoration of the natural stocks to these two species. They have included a request for funding for the construction of the coralgrouper broodstock facility in their current year proposal for Sport Fishing restoration funds.

The aquaculture industry of Guam has a strong desire to develop a local, high end product that can be marketed as a live, in-restaurant product to the tourist trade. The bright red coloration of the Leopard coralgrouper and the distinctive markings of the saddleback phase of the Giant coralgrouper make them ideal products for a premium live fish dish for a prestigious dinner in any white table cloth Asian cuisine restaurant.

The project will begin in January, 2011.

A Look Ahead

Development

The development of the Fiscal Year 2010 program began in March 2010 with a Call for Pre-Proposals that was based on the priorities identified by farmers and researchers in the region. The IAC and TC reviewed and selected the submitted pre-proposals that would aid industry development. Out of the 22 pre-proposals received in response to this call, CTSA requested that applicants submit nine full proposals. Of the nine requested, eight were received. These proposals will be forwarded to the Board of Directors for approval in January 2011 as the FY 2010 Plan of Work.

Proposals

1. Assessing Hawaii's Aquaculture Farm And Industry Performance
2. Culturing Native Species of Macroalgae in Hawai`i and the U.S. Affiliated Pacific Islands
3. Marine Finfish Aquaculture Development in the Northern Marianas Islands
4. Seed Production of Mangrove Crab (Scylla serrata Forskal) in Palau
5. Broodstock Management, Seed Production and Grow-out of Rabbitfish, *Siganus lineatus* (Valenciennes, 1835) in Palau
6. Pacific Aquaculture Development and Extension Support for the U.S. Affiliated Pacific Islands of the Federated States of Micronesia, FY 2010
7. Refinements to Aquaponics Systems and Technology Transfer to Professional Farmers
8. Pacific Regional Aquaculture Information Service for Education (PRAISE) & Publications, Year Six

Review

In July 2010, CTSA began its two-month review process. All full proposals were subjected to peer review by three or more experts in the project topic area and then reviewed at the IAC-TC annual meeting. The CTSA administrative center summarized reviewer comments and forwarded them to each P.I. for revision. The IAC chair will present the proposals to CTSA's Board of Directors for approval on January 20, 2011. CTSA staff will incorporate these proposals into the Fiscal Year 2010 Plan of Work and will submit this FY2010 Plan of Work to the U.S. Department of Agriculture National Institute of Food & Agriculture (NIFA) for final approval.

Progress Reports

Individual accounts of the principal accomplishments of the active projects during 2010 are presented on the following pages. These reports detail each project's funding, participants, objectives, progress, work planned, impacts, and publications. Additionally, reports from ongoing projects include anticipated benefits, and reports from terminated projects include recommended follow-up activities. Additional appendices, figures, tables, and graphs are included in the appendix to this report and referenced individually within the reports. Information and results from projects in previous years are given in the CTSA Annual Accomplishment Report for that correlating year. Annual reports are also available at the CTSA Web site at http://www.ctsa.org/ProjectAnnual.aspx.

1. Culturing the Harlequin Shrimp, *Hymenocera picta*, for the Marine Aquarium Industry, Years 1 and 2

General Information

Reporting Period	August 1, 2007 to July 31, 2008; 1st no-cost extension through January 31, 2009; 2nd no-cost extension through April 30, 2009 (Year 1) April 16, 2009 to April 15, 2010; no-cost extension through July 31, 2010 (Year 2) (Project Termination Report)
Funding Level	Year — Amount **1 — $38,340** **2 — $35,400** Total — $73,740
Participants	**Shaun Moss,** Ph.D., Vice President Research & Development, Oceanic Institute Charles Laidley, Ph.D., Director Finfish Department, Oceanic Institute Chad Callan, Ph.D., Research Scientist Finfish Department, Oceanic Institute Clyde Tamaru, Ph.D.,

RESULTS AT A GLANCE…

- Larval-rearing efforts demonstrated that harlequin shrimp can be reared with high (40-70%) survival through the early feeding period, using conventional approaches similar to those used in rearing marine fish larvae. The greatest survival was achieved using a combination of Artemia nauplii and rotifers from day 1 post hatch.

- Juvenile feeding trial results suggest that by-catch Asteria sp. could serve as a more ecologically sustainable food for rearing these shrimp.

- Preliminary adult feed trials demonstrated that H. picta can use frozen Linckia to a limited extent.

- Two Hawaii Pacific University graduate students participated in the project.

College of Tropical Agriculture & Human Resources,
University of Hawaii

Dustin Moss, Ph.D., Research Associate
Shrimp Department, Oceanic Institute

Clete Otoshi, Research Associate
Shrimp Department, Oceanic Institute

Danielle McKay, graduate student, Hawaii Pacific University

Melissa Carr, graduate student, Hawaii Pacific University

Karen Brittain, Hawaii Institute of Marine Biology,
University of Hawaii

Goals & Objectives

Year 1

1. Collect and disseminate information about the culture of *H. picta*.

   Objective 1.1: Conduct a workshop to identify and document techniques used for the captive reproduction and culture of H. picta

2. Improve culture techniques for H. picta.

   Objective 2.1: Broodstock maturation and reproduction

   Objective 2.2: Larval rearing

3. Evaluate alternative diets for H. picta.

   Objective 3.1: Live echinoderm feeding trial

   Objective 3.2: Prepared feeds trial

4. Explore potential genetic and environmental effects on post-settlement dietary preference for H. picta.

   Objective 4.1: Evaluate *alternative diets for F_2 generation shrimp*

Year 2

1. Collect and disseminate information about the culture of *H. picta*.

Objective 1.2: Conduct a workshop to share research results about *H. picta* to interested stakeholders

Objective 1.3: Disseminate information about *H. picta* to interested stakeholders

2. Improve culture techniques for *H. picta*.

   Objective 2.1: Broodstock maturation and reproduction

   Objective 2.2: Larval rearing

4. Explore potential genetic and environmental effects on post-settlement dietary preference for *H. picta*.

   Objective 4.1: Evaluate alternative diets for F_2 generation shrimp

   Objiective 4.2: Evaluate alternative diets for shrimp reared under different larval-rearing conditions

5. Characterize biochemical and mineral composition of prey items.

   Objective 5.1: Characterize biochemical and mineral composition of prey items

6. Production and evaluation of a formulated, artificial diet for adult *H. picta*.

   Objective 6.1: Artificial feed production

   Objective 6.2: Feeding trial

Principle Accomplishments

Objective 1.1: Conduct a workshop to identify and document techniques used for the captive reproduction and culture of H. picta.

A workshop was held on April 15, 2008 at Oceanic Institute's Learning Center. Workshop participants included Dr. Shaun Moss (Oceanic Institute), Dr. Clyde Tamaru (University of Hawaii), Mr. Dustin Moss (Oceanic Institute), Ms. Danielle McKay (HPU graduate student), and Ms. Karen Brittain (Hawaii Institute of Marine Biology, University of Hawaii). Another local harlequin shrimp expert, Mr. Frank Baensch, was unable to attend, but agreed to provide assistance when needed. Danielle McKay was a graduate student at Hawaii Pacific University with ornamental shrimp culture experience and she was hired to assist with this project. Karen Brittain provided valuable information on harlequin shrimp reproduction and larval rearing and this contributed to the project.

Objective 2.1: Broodstock maturation and reproduction.

Seven pairs of harlequin shrimp were purchased from local pet stores over the 2-year project. Data on spawning and molting frequency were recorded (see Appendix A –

Spawning and Molting Frequency), and the effects of mate-switching on female reproductive performance were observed (see Appendix B – Mate Switching Experiment). In both captive and wild populations, H. picta are found primarily in socially monogamous, heterosexual pairs. Social monogamy (partner fidelity without sexual fidelity) most likely is enforced by male mate-guarding and could be reproductively advantageous in the wild given the species low population density, high intra-specific aggression, and limited female sexual receptivity. Work from this project showed that wild-caught pairs in captivity readily accepted new partners as evidenced by immediate pair-sitting and regular and predictable copulation thereafter. Females suffered no reproductive cost to losing their long-term mates and immediately produced large clutches of eggs where high percentages were fertilized by novel male mates. Overall, mean number of larvae produced, % hatch, and total clutch size increased slightly after the mate switch. Results suggest that although H. picta are biologically driven to form long-term mating pairs, they also adapt their strategy to increase reproductive opportunities when established partners disappear by readily accepting any potential mate.

Objective 2.2: Larval rearing.

During Year 1 and most of Year 2, larvae from multiple spawns were collected and attempts to produce post-settled shrimp were unsuccessful (see Appendix C – Larval rearing Trials). During this time period, we evaluated different larval rearing systems, different feeds, and water type. Using a combination of 1-L beakers with OI well water and feeding the larvae with enriched artemia nauplii along with newly hatched artemia nauplii, we were able to rear some larvae to 30 days post-spawn. This was a significant improvement over previous efforts but we were still unsuccessful in getting post-settled juveniles.

During the No Cost Extension period in Year 2, and with assistance of OI's Finfish Department, we were able to produce post-settled harlequin shrimp juveniles (see Appendix D – Production of Post-Settled Juveniles). Settlement of harlequin shrimp postlarvae began at day 34 post-hatch and continued through day 57. Peak settlement occurred between days 40 and 44. Newly settled shrimp were translucent yellow and pink and did not appear to feed for 3-4 days post settlement. After one or two molts (4-6 days), postlarval shrimp began to assume coloration very similar to adults (white with purple and pink spots) and readily fed on live Linckia. seastars. These larval-rearing efforts demonstrate that harlequin shrimp can be reared with high (40-70%) survival through the early feeding period, using conventional approaches similar to those used in rearing marine fish larvae. The greatest survival was achieved using a combination of Artemia nauplii and rotifers from day 1 post hatch.

Objective 3.1: Live echinoderm feeding trial.

Oceanic Institute (OI) applied for State of Hawaii import permits for live harlequin shrimp and live Linckia spp. seastars. The permit for Linckia spp. seastars was rejected, so all seastars were purchased from local pet stores. During the No Cost Extension period in Year 2 when naïve juvenile shrimp were produced, we were able to conduct preliminary feeding trials (see Appendix E – Feeding Trials). These preliminary trials examined the use of frozen Linckia and Asteria diet combinations and yielded survival rates comparable to those for individuals fed pieces of live seastars. However, shrimp fed frozen pieces of Linckia exhibited greatly reduced growth and delayed pigmentation development compared to shrimp reared on the live pieces. It was also determined that shrimp which had settled and began consuming live whole Linckia could be transitioned to frozen seastar pieces from either Linckia or Asteria sp. Somewhat surprisingly, harlequin shrimp transitioned from live Linckia to frozen Linckia exhibited much lower survival than those transitioned from live Linckia to frozen Asteria. Although this difference needs to be confirmed, these results suggest that by-catch Asteria sp. could serve as a more ecologically sustainable food for rearing these shrimp in captivity.

In addition to feeding trials with naïve, newly settled juveniles, feeding trials also were conducted on adult H. picta. Although adults were observed feeding and apparently consumed frozen Linckia, they fed less frequently and consumed a smaller amount when compared to H. picta fed live Linckia. Mortalities were not observed during the trial period although frozen treatment shrimp were likely malnourished. A longer duration study would be required to determine how long H. picta could survive on a frozen-only diet and whether they suffer any sub-lethal effects. Prior to the start of the feeding trial, it was observed that frozen pieces of Linckia consistently developed fungal growth two days after being added to the tanks. It was therefore necessary to remove and add new Linckia daily for the frozen treatment. If used as a food source, frozen Linckia may need frequent replacement which would be wasteful. It is conceivable that frozen Linckia may be used as a supplement to live Linckia although it would also be important to determine whether switching between live and frozen would further dissuade H. picta from accepting frozen Linckia. This preliminary study demonstrated that H. picta can use frozen Linckia to a limited extent although further research would be required to determine the efficacy of long-term use.

No progress on objectives 1.2, 1.3, 3.2, 4.1, 4.2, 5.1, 6.1, 6.2.

Impacts

Results from this project will contribute to a greater understanding of the larval biology and captive reproduction of harlequin shrimp. Important information on the reproductive performance and larval rearing of harlequin shrimp has been obtained. Specifically, project research has generated unique information about spawning and molting frequency for these marine ornamental shrimp, as well as a characterization of spawn quality. In addition, larval rearing protocols have been developed for the successful settlement of competent juveniles. All of the information generated under this project will be of interest to industry stakeholders. In addition, data generated from this project will be of interest to crustacean biologists who study mating behaviors of socially monogamous crustaceans.

Although information necessary to develop formulated, artificial diets for harlequin shrimp was not generated, useful preliminary data on the ability of *H. picta* to use frozen feeds were obtained. If an artificial diet can be produced to wholly or partially replace live starfish, then the economic burden of maintaining live prey would be reduced for the producer or aquarium hobbyist, and the environmental and ecological impacts of collecting live starfish would be eliminated.

Recommended Follow-up Activities

Because naïve juvenile shrimp were produced at the end of the funded project, a rigorous series of feeding trials could not be conducted. However, in light of documented protocols for the production of juvenile *H. picta*, naïve shrimp can now be produced on a regular basis and proper scientific studies should be conducted to develop artificial diets for this unique ornamental shrimp.

Publications and Manuscripts Written and Papers Presented

McKay, D.N. 2009. Effects of mate switching on reproductive performance of female harlequin shrimp, *Hymenocera picta*. Master's of Science degree in Marine Science thesis, Hawaii Pacific University.

2. Development of DNA Markers for Pacific Threadfin Aquaculture, Years 1 and 2

General Information

Reporting Period: December 1, 2006 to November 30, 2008; 1st no-cost extension through July 31 2009; 2nd no-cost extension through December 31, 2009 (Project Termination report)

Funding Level:

Year	Amount
1	**$97,500** ($72,500 for UH, $25,000 for OI)
2	**$98,384** ($73,384 for UH, $25,000 for OI)
Total	$195,884

Participants: **Jinzeng Yang,** Ph.D., University of Hawaii at Manoa

Heng Wang, Ph.D., University of Hawaii at Manoa

Gang Pan, Ph.D., University of Hawaii at Manoa

Baoping Zhao, M.S., University of Hawaii at Manoa

RESULTS AT A GLANCE…

- A short insert genomic DNA library was constructed and stored

- 52 microsatellite loci were developed, along with PCR protocols for optimal amplification of the markers

- A microsatellite DNA-based method of parental assignment was developed. Based on results from a second round of parentage analysis using eight microsatellite markers, 98% of offspring were assigned to their parents.

- Based on initial microsatellite DNA results, 10 to 20 microsatellite loci can be used for further genotyping and comparisons of genetic diversity between broodstock and wild populations.

- Two University of Hawaii graduate students participated in the project.

Gavin Iwai, Graduate Student, University of Hawaii at Manoa

Shizu Watanabe, Graduate Student, University of Hawaii at Manoa

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

Ken Liu, M.S. Oceanic Institute

Thomas Iwai Jr, Dept of Land and Natural Resources,
State of Hawaii

Objectives

Year 1

1. To establish a genomic and skeletal muscle cDNA library of Pacific threadfin;
2. To identify at least 50 microsatellite loci and develop PCR protocols for optimal amplification of the markers;
3. To collect fin clip samples from the OI and AFRC broodstock populations and analyze polymorphic DNA markers in these samples.

Year 2

1. To develop a microsatellite DNA-based method of parental assignment;
2. To collect fin clip samples from the wild populations (Oahu and Hawaiian Islands) and characterize their genetic diversities using established DNA microsatellite markers;
3. To develop DNA-based testing protocols for monitoring Pacific threadfin broodstock and wild populations.

Work Progress and Principal Accomplishments

Year 1

Objective 1: To establish a genomic and skeletal muscle cDNA library of Pacific threadfin.

Samples from ten Pacific threadfin fingerlings generated through the Oceanic Institute hatchery were sampled for genomic DNA extraction, and skeletal muscle tissue samples

were taken for muscle cDNA library construction. A pilot sequencing project from 181 clones identified 129 useful ESTs, of which 90 ESTs exhibited significant homology to known genes and 39 ESTs have low homologies to unknown genes. Fourteen novel genes were retrieved from the sequenced clones and subjected to gene ontology annotation. Four mRNA sequences were identified as significant regulators of transcription. These results indicated that a quality muscle cDNA library was constructed.

Objective 2: to identify at least 50 microsatellite loci and develop PCR protocols for optimal amplification of the markers

A summary of sequence information is listed in Table 1 (appendix). A total of 142 microsatellite loci were identified. A great number of microsatellites were too close to the linker to allow the primer design and were discarded. Of the suitable colonies, we first designed 73 PCR pairs of primers. After initial PCR assays, 52 microsatellite loci could be successfully amplified, and their PCR products were observed with a clear and strong band after electrophoresis (see Figure 2).

Objective 3: to collect fin clip samples from the OI and AFRC broodstock populations and analyze polymorphic DNA markers in these samples.

Both F1 and F2 populations of growth-selected and control threadfin lines OI have been maintained for use in genetic analyses. The F1 population included 23 growth-selected and 18 control fish reared from 2001 hatchery runs, and a second tank containing 111 broockstock fish selected for growth performance from 2007 hatchery runs. We also have 54 growth selected F2 spawned from the growth-selected F1 population in 2004. We plan to compare genetic differences and similarities of OI and AFRC broodstock populations with wild and captured populations through DNA marker analysis. Both the F1 and F2 populations began spawning on a monthly basis following each full moon, with the older F1 population generating significantly more eggs per spawning cycle (Figure 1) during the first year of the project. Fertility rates in the selected population may be negatively affected by the low percentage of remaining males (the species is a protandrous hermaphrodite) prompting us to generate a second tank of F1 selects as a source of male for introduction into this older female-dominated broodstock population.

In collaboration with the state of Hawaii Moi stock enhancement program at Anuenue Fisheries Research Center (AFRC), we have also collected fin clip samples of more than 500 fish samples from several locations in Oahu Island, including Diamond head, Kaneohe, Bellows, and Kapapa Island. The genomic DNA was extracted from most of the collected samples and kept for planned PCR amplification and DNA fragment analysis.

Year 2

Objective 1: To develop a microsatellite DNA-based method of parental assignment.

In designing a cost-effective protocol for parental assignment, we selected loci on the basis of their polymorphic information content, number of alleles and the cost of genotyping. In Pacific threadfin hatchery operations, a population of dams and sires are normally maintained in the broodstock tank during the reproduction season. The high-efficiency of spawning is highly appreciable. The main purpose of the parentage analysis was to identify spawning broodstock and non-spawning broodstock fish. Then spawning animals will be kept to maximize the spawning possibilities. After parental assignment, it is expected to remove the dams that are not spawning or those rare spawning parents from the tank and to see what will happen in the next spawning event.

A core set of 5 Pacific threadfin microsatellite loci was further refined to produce a set of multiplexed markers suitable for routine parentage testing. Based on these results from five microsatellite markers, 90% of the offspring were assigned to their parents. Another 10% could be traced back to two or more parental couples. The information is enough for the small-scale selection applications mentioned above. In consideration of a large population with more precise tracings in the future, more loci will be added in the genotyping panel. With the same broodstock and offspring samples from the second and third spawning events, we tested three additional microsatellite loci, namely Pse24, Pse34 and Pse82. The percentage of offspring assigned to their parents increases to 93% compared with the original 5 microsatellite loci in this broodstock population. The result with 8 microsatellite loci also further confirmed the main spawning sire (S2) and non-spawning broodstock dams (D7 and D8). Although the accuracy increased, the cost also increased.

Parents that showed mismatches at only one locus or even one allele were considered as potential parents to allow for the possibility of either a mutation or a genotyping error. The potential parents option in the software was applied to re-test the offspring against all the original parents. After the second round of parentage analysis, 98% (82/84) of the offspring were unambiguously assigned parentage to the correct sire and dam. After inspecting the mating patterns from the first and second cycle of parentage analysis, the correct dam was assigned with >99% confidence in each case where the sire was unavailable.

Objective 2: To collect fin clip samples from the wild populations and characterize their genetic diversities using established DNA microsatellite markers.

In collaboration with the state of Hawaii Moi stock enhancement program at Anuenue Fisheries Research Center (AFRC), we have collected fin clip samples of more than 500 fish samples from several locations in Oahu Island, including Diamond head, Kaneohe,

Bellows, and Kapapa Island. The genomic DNA was extracted successfully and kept for PCR amplification and DNA fragment analysis. Characterization of ten microsatellite loci in the wild and broodstock populations was completed. All the ten microsatellite loci were found to be highly polymorphic in the wild and captive populations. The estimated genetic variability for each population is shown in Table 2.

The analysis of each pair of loci in each of the populations indicates that none of the loci exhibit noticeable linkage ($p>0.05$). However, significant LD ($p<0.01$) was observed between the loci pairs in the sibling progenies used in the parentage assignment studies. Hence, it seems very likely that the presence of related individuals in the offspring, rather than the physical linkage of the loci in the genome, contributes to the LD in the tanks with only a few breeders. Results indicated that the broodstock and wild populations are closely related. Therefore, it is difficult to monitor the migration of alleles (i.e. introgression) in physically close regions along the Oahu island coastline. Breeding with domesticated Pacific threadfin in broodstock population without monitoring genetic characteristics of the wild population may significantly change the genetic structure of the wild population (see Tables 2 and 3).

Objective 3: To develop DNA-based testing protocols for monitoring Pacific threadfin broodstock and wild populations.

To get a further evaluation of the genetic diversities of Pacific threadfin in different populations, more polymorphic microsatellite markers were explored. An initial screening was carried out in 14 unrelated individuals randomly selected from the wild population. Ten microsatellite loci with high polymorphisms were identified for further analysis. Primers of the nine selected loci were further labeled with fluorescence-dye and detected in 30 individuals from a wild population. The detailed characteristics are shown in detail in Table 4. Based on the analysis of the wild population, it was determined that these microsatellite loci are highly polymorphic and are independently inherited and can be used separately as meaningful genetic markers.

In year two of the project, OI continued to maintain both F1 and F2 populations of growth-selected and control threadfin lines. Both the F1 and F2 populations continued to spawn on a monthly basis following each full moon (Figure 1). Fertility rates in these populations were negatively affected by the low percentage of remaining males (the species is a protandrous hermaphrodite). We also recruited approximately 100 new F1 juveniles from the October 2007 hatchery run which were grown-out to supplement males in both the F1 or F2 selected populations.

The established protocols of fin clip sampling, DNA isolations genotyping microsatellite loci have been validated in the experiments of parental assignment and population analysis. For application of the DNA-based technology for parental assignment, fin clips will be collected from 60 offspring of three consecutive spawning events of fixed group of spawning broodsock (male and female parents). DNA isolations will be needed for the offspring and possible parents. Then, 5-10 microsatellite loci will be selected on the basis of the results of the parental testing results of microsatellite DNA analysis. Less microsatellite DNA loci will be needed for high diversity of the parental population. Parental pair identifications will be analyzed by the PAPA program or other computer software for parental assignment of the all offspring based on the microsatellite DNA testing results. For monitoring the captive and wild broockstock populations, we currently already have fin clip samples in the OI broodstock population and collected fin clip samples from the coastline along Oahu island. Based on initial microsatellite DNA results, 10 to 20 microsatellite loci can used for further genotyping and comparisons of genetic diversity between these populations.

Impacts

The completion of this project provides the first series of DNA markers for Pacific threadfin and these markers will have practical application to Pacific threadfin commercial aquaculture for better maintenance and management of genetic stocks and selection of seedstock. The microsatellite DNA marker for Pacific threadfin can be used for establishment of analytical methods of discriminating Pacific threadfin from other fish groups. It will also be useful for ecological studies of Pacific threadfin by identifying, tracing and analyzing this species in the nature. In Hawaii, the current founder broodstock populations maintained in Oceanic Institute were collected from the wild and have undergone generations of domestication. The genetic tools developed in this project can be used in the evaluation of genetic diversity and determination of pedigree in the broodstock. The findings from this project will be used to ensure genetic diversity in Pacific threadfin broodstock populations and to assist industry in developing more advanced breeding programs for this species. For example, the first phase of this project resulted in a 15 to 25% increase on growth rates with one round of selection. Microsatellite marker technology will further enable these efforts and facilitate more effective breeding programs for this and other species of marine finfish of importance to Hawaii and the Pacific Region. This will allow us to determine whether significant loss of genetic variation and increase of inbreeding have already occurred in the broodstocks. Then, the broodstock managements can be conceived and carried out to maintain allelic variations and to decrease inbreeding depression through microsatellite DNA testing. In addition, the newly identified DNA markers will help us to integrate molecular genetic technologies into an organized selective

breeding program aimed at the genetic improvement of Pacific threadfin for aquaculture production efficiency. Therefore, results from current project will greatly benefit Pacific threadfin aquaculture and technology development for a sustainable and profitable seafood industry, as well as the conservation of wild marine species in Hawaii islands.

Recommended follow-up activities

1. Pacific threadfin has become an important aquaculture food fish in local market since open ocean cage culture was established in Oahu island. At the same time, wild population of pacific threadfin is decreasing. It is important to identify the genetic diversity and identities of the captive broodstock and wild populations by using the established microsatellite DNA analysis. Therefore, we will have genetic information of the wild population for continued conservation efforts such as stock enhancement or other measurements

2. Effective management of broodstock population for better growth and hatchery operations is critical for sustainable and successful Pacific threadfin aquaculture due to their communal spawning behavior. By DNA-based parental assignment developed from this project, we will be able to identify spawning patterns of the captive broodstock. Furthermore, with growth data and records of the offspring, we can rearrange or re-group broodstock fish for efficient hatchery management. Therefore, it is recommended that close collaborations between Dr. Yang's DNA laboratory and the hatchery operations of Pacific threadfin (Dr. Laidley's finfish group) should be continued. Hopefully, the DNA-based technology can be adopted to broodstock management and hatchery operations.

Publications, Papers Issued, Approved, or Presented

Wang H, Iwai TJ, Zhao B, Lee CS, Yang J. 2009. Identification of microsatellite DNA markers for Pacific threadfin parentage assignment. *J. World Aquaculture Society*, Accepted in May 2009.

Yang J, Wang H, Iwai, T Jr., Zhao B, Lee CS . 2008. Development of DNA-Based Testing for Pacific Threadfin. CTSA Regional Notes. Vol. 19 No. 3 Page 4-5

Watanabe S, Zhao B, Ako H, Yang J. 2009. Identifications of expressed sequence tags from Pacific threadfin skeletal muscle cDNA library. *Aquaculture Research*, Published online September 2009.

Callan, C.K., and C.W. Laidley. Opportunities for culturing coral reef species for the marine ornamental industry and food-fish production in the Pacific Islands. Saipan Workshop on Aquaculture Opportunities. Saipan College, July 17, 2008.

Laidley, C.W. Saving the Reefs: Aquaculture of coral reef species as an alternative to wild collection. Hawaii Sea Grant Program, Hanauma Bay Seminar Series, June 12, 2008.

Laidley, C.W., C.K. Callan, and K.Liu.. Saving the Reefs: Aquaculture of coral reef species as an alternative to wild collection. International Symposium in Honor of Professor Yoshitaka Nagahama Sex Determination and Gametogenesis in Fish: Current Status and Future Directions. University of Hawaii at Manoa, June 1, 2008.

Laidley, C.W. Saving the Reef: Culturing coral reef species, Ocean Networks Celebration of the Year of the Reef, Waikiki Aquarium, April 12, 2008.

Laidley, C.W. Saving the Reef … Development of aquaculture technology as an alternative to wild-collection of coral reef species, Hawaii Pacific University, February 13, 2008.

Laidley, C.W., and Liu, K.K.M. 2005. Selective breeding for improved growth performance in the Pacific threadfin, *Polydactylus sexfilis*. CTSA Regional Notes, 16(1): 4-7.

Laidley, C.W. 2007. Development of Captive Culture Technology for Marine Fishes of Hawaii, Invited speaker on Hawaii's transition to the New Global Economy, The One that Didn't get Away: Hawaii's emerging fish farms, Meeting of Hawaii Society of Corporate Planners, September 30, 2007.

Laidley, C.W. 2007. Tropical and Subtropical Aquaculture of High-value Marine Finfish species in Hawaii and the Pacific Region, presented at The 4th National Aquaculture Extension Conference, Cincinnati, OH, May 2007.

3. Inter-Institutional Coordination and Preparation of a Guam Aquaculture Development Plan

General Information

Project Period: October 1, 2007 to September 30, 2008; 1st no-cost extension through March 31, 2009; 2nd no-cost extension through September 30, 2009 (Project Termination Report)

Funding Level: **$25,000.00**

Participants: **John W. Brown,** Ph.D., University of Guam

Maria C. Haws, Ph.D., University of Hawii at Hilo

John Gourley
Randy Sablan
Doungho Jiang
Hui Gong
Brent Tibbats
Manny Duenas
Judy Baza
Angelina Wang
John Bernardo
David Crisostomo
Arnie Moreno
Janet San Nicholas
Bennie San Nicolas
Peter San Nicolas
Norma Smith
Martina S.N. Strong
Josephine Williams
Barbra Zimmerman
Antoinette C. Cruz
Bill McDonald
Frank Eclavea
L. Robert Barber
Ilene Irarte
Craig Smith
Tom Camacho

RESULTS AT A GLANCE…

- Promising aspects and critical inhibitors to the aquaculture industry in Guam were reviewed and compiled to create the 82-page Guam Aquaculture Development Plan.

- Included in the Plan are a list of current and potential species available for aquaculture on Guam, a review of local and federal policies, regulations and permitting processes effecting on-going and potential aquaculture development, an institutional analysis for public and private sector entities, a review of currently available economic incentives for aquaculture development on Guam, and suggested changes to the current regulatory and economic environment.

Objectives

1. Form an Aquaculture Development Working Group to execute the work and improve institutional coordination and cooperation for future implementation activities.
2. Review current status and potential areas for development including a SWOT analysis (Strengths, Weaknesses, Opportunities, and Threats).
3. Review current and potential list of species available for aquaculture on Guam.
4. Review local and federal policies, regulations and permitting processes effecting on-going and potential aquaculture development on Guam.
5. Conduct an institutional analysis for public and private sector entities on Guam and potential partners located elsewhere.
6. Review currently available economic incentives for aquaculture development on Guam.
7. Suggest changes to the current regulatory and economic environment that could be locally adopted.
8. Publish the results of objectives 1 to 7 as a CTSA/GADTC joint publication with distribution to all concerned stakeholders.
9. Make oral presentations of finding to concerned stakeholders and at aquaculture meetings/conferences.

Principal Accomplishments

Objective 1: Establish a Guam Aquaculture Development Working Group

A formally commissioned Aquaculture Development Working Group was not established. An initial work session was conducted to gage the interest in a formal working group and little was found. There seemed little enthusiasm for participating in an on- going series of formal meetings by either the commercial aquaculture producers or the government agency members contacted. Instead an ad-hoc process of communicating directly with individual farmers and agency employees was used.

Objective 2: Review current status

The available historical literature on aquaculture development on Guam was assembled.

Some of this literature has been lost over the years due to a lack of proper archiving, the discontinuous personnel history in the lead aquaculture development position and changes in the lead agency for aquaculture development. The last formal production survey for aquaculture production and value on Guam was done in 1996. The scope of this project did not include the collection of new aquaculture production and value statistics nor was funding provide for such work by other sources. Thus, the current status was reviewed informally through interaction with local aquaculture producers, market visits and routing extension efforts with new entrants. The SWOT analysis is included as the first section of the final Chapter, Implementation Strategy and Recommendations.

Objective 3: Review current and potential list of species

We obtained from the Guam Division of Aquatic and Wildlife Resources (GDAWR) their historical list of the non-indigenous species that have been approved and imported into Guam. From published reports and interviews with key informants, it was determined that these records are not complete. However, some of the species that have been imported in the past and which are not in the lists are no longer desired. Thus, no effort was made to fully correct these records. GDAMR did provide guidance as to which species have been imported in the past and which are considered possible for future approval. Permits were issued for the first formal effort at importing pure line *Oreochromis aureus*, the blue tilapia as an indirect result of these discussions. Traditionally the tilapia seed fry imported to Guam from Taiwan have been *aureus x niloticus* hybrids.

Objective 4: Review of local and federal policies

Local and federal regulatory policies were reviewed by two of the work group. Mr. Randy Sablan reviewed the Government of Guam regulatory environment and Mr. John Gourley reviewed the federal side. These have been included the Development Plan as Chapter VI, Regulatory Environment.

Objective 5: Conduct an institutional analysis

The institutions that play or could play a supporting role for aquaculture development on Guam were reviewed by John Brown and Maria Haws. They are listed and discussed in Chapter V, Support Infrastructure, in the Development Plan.

Objective 6: Review current economic incentives

It was determined that there are almost no active economic incentives for development of private sector aquaculture on Guam. There are limited federal programs that can be used to start or expand an aquacultural enterprise on Guam. These have been included in the discussion in Chapter V, Support Infrastructure, in the Development Plan.

Objective 7: Suggest Changes

The second section of Chapter VIII, Implementation Strategy and Recommendations, divides the suggested changes into five headings: 1. reinforce current successes, 2. take advantage of opportunities for new aquaculture development, 3. address institutional gaps, 4.work to mitigate problems with feeds, seeds and energy, and 5. focus species selection and development.

Objective 8: Publish results

Funds for publication were returned to CTSA due to the expiration of the period of the grant. However the Aquaculture Program of the South Pacific Community (SPC) has agreed to provide editorial assistance and to publish the Development Plan as a part of its current year work effort.

Objective 9: Oral presentations of results

Formal oral presentations to the community have not been done. They are waiting for the peer reviews of the development plan and further refinement of the current draft.

Impacts

There have been no impacts to date as the Aquaculture Development Plan has not been released to the public or to local governmental policy makers.

Recommended Follow-up Activities

The efforts in the original grant proposal under Objective 9, make oral presentations of finding to concerned stakeholders and at aquaculture meetings/conferences, still needs to happen if the development plan is to have a chance at having an impact.

Publications and Manuscripts Written and Papers Presented

John W. Brown, Maria Haws, John Gourley and Randal Sablan. 2010. A Development Plan For Aquaculture on Guam. Unpublished manuscript. 75 pp.

4. Improving Pearl Quality by Grafting Technologies and Husbandry Methods for a Hatchery-based Black Pearl Industry Development in Pohnpei, the Federated States of Micronesia (Years 1 -3)

General Information

Reporting Period: July 1, 2007 to June 30, 2010 (Project Termination Report)

Funding Level:

Year	Amount
1	**$47,100**
2	**$50,000**
3	**$49,200**
Total	$146,300

Participants: **Masahiro Ito,** Ph.D., Director and Chief Scientist, Aquaculture Development, College of Micronesia Land Grant Program

Fumio Ike
Master Pearl Grafting Technician

Yuko Kibe
GIA Certified Pearl Grading Expert

RESULTS AT A GLANCE…

- Pearl quality improvements in shapes and flaws from re-grafting were confirmed scientifically for the first time in this field of study.

- Pearl farming technology was transferred to local Micronesian technicians using hands-on skill training methods at both Pakin Atoll farm and at Nett Point demonstration farm in Pohnpei. In total, over 30 Micronesians were trained in various skills including pearl grafting, husbandry, farming, and accessory making, resulting in the establishment of a pearl industry in Micronesia.

- Trials indicated that the use of chemical-coated nuclei may not be necessary in terms of profitability of pearl farming; this knowledge will reduce production costs.

Objectives

1. To improve roundness rate of pearls by oyster grafting techniques.
2. To reduce flaws in pearls by oyster grafting techniques and husbandry methods.
3. To improve host survivorship by husbandry methods.
4. To transfer pearl aquaculture technology immediately to the Micronesian and other regions.

Principal Accomplishments

All the oysters were produced from the COM/COM-FSM pearl hatchery project with known date of fertilization, grow-out location and husbandry history (Years 1 – 3). The donor oysters were selected from younger groups at around 20 – 30 months old such as S13 group (in Years 2 and 3) for supplying mantle piece (graft). Post-grafting husbandry culture was conducted by a chaplet or "ear-hanging" culture method, by hanging 10 oysters each to a drop-rope in 1 m intervals suspended from the surface line system, except for a part of the Year 2 experiments using lantern nets during the first two months of the post-grafting period. Pre-grafting conditioning of hosts and donors employed lantern-net culture (3 mm square mesh, 4 oysters per net, 3 nets per drop-rope) for two months prior to the grafting operation.

Year 1

The Year 1 work of monitoring grafted oysters over a ten month period and harvesting the pearls was completed in June 2008. Preliminary results of the pearl quality assessments of the pearls and the post-grafting survivorships were described in the Year 1's final report as well as summarized in the publication from the CTSA AquaTip (Ito, 2009). The project was successful to employ a master grafting technician who is a one of key players of this research and had retired from commercial pearl grafting work after 30 years of service in the industry. Technology transfer program of the Year 1 also accomplished the initial phase of grafting preparations and post-grafting monitoring by involving approximately 20 local people and four project staff.

Year 2

Right after the Year 1's pearl harvest work, the re-grafting and new grafting were conducted for the Year 2's experiments on circle reduction tests, FNC tests and post-husbandry methods. The Year 2's circle reduction tests involved re-grafting for "the seconds" and for the "thirds" or the pearls from three-year consecutive grafting. During the Year 2, data analyses of the post-grafting survivorships, pearl quality assessments and pearl success rates from the Year 1's experiments were completed. The results were given

in the Year 2 final report, in which the pearl quality improvements of the shapes and flaws from the re-grafting were confirmed scientifically for the first time in this field of study. It was a significant achievement that the pearl harvest and grafting experiments were also conducted at Pakin Atoll with participations of the members from this remote island community. Pearl farming technology transfer work continued by hands-on skill training both at Pakin Atoll farm and at Nett Point demonstration farm in Pohnpei, which involved similar number of trainees in the Year 1 project.

Year 3

Pearl quality assessments of the Year 2 experiments were completed. A major breakthrough in this field of research was that the project successfully employed a certified pearl grading expert enabling the quality assessments reliable in the international standard. The harvesting work was conducted between May and June 2009 for the Year 2 experiments which used 2240 oysters for the FNC tests and 581 oysters for the circle reduction tests from June 2008. The Year 3 final report included the results on the pearl quality improvement, post-grafting survivorships and pearl success rates. It was one of the rare accomplishments in pearl research that pearl quality changes in shape, flaw, luster and color were studied for consecutive re-grafting experiments from 2007 to 2009, where the host oysters were traced after they produced virgin pearls by re-grafting for the seconds and by re-grafted again for the thirds. Furthermore, a GIA (Gemological Institute of America) certified pearl grading expert was hired to introduce GIA's standard of pearl grading knowledge. Post-harvest pearl washing, sorting and grading were also taught by the master grafting technician and the grading expert as a part of pearl culture technology transfer work. During the harvest and grafting work in the Year 3, two of the project staff and two Micronesian trainees were chosen to receive basic grafting skill on the round pearls by the master grafting technician as well as advanced skill training on the half-pearls.

Impacts

Pearl production has been dependent heavily on a performance of grafting technicians who usually do not reveal their skills or provide clear explanations to the farmers or managers. So far, so many oysters have been wasted in the present day pearl farming overseas. Scientists tended to be kept away from the research work involving the grafting because they knew that they required so many pearl oysters to be tested and, in many cases, sacrificed without generating money but exploiting precious natural resources. Hatchery technology is a key to conduct this sort of research work and willing support from a

grafting technician and farmhands as well as hatchery technicians. Fortunately, this project possessed all except for an established pearl industry and researchers. The findings from this project could contribute immediately to the Micronesians and existing pearl industries overseas. The assessment of pearl quality experiments on the circle tests by re-grafting and the FNC tests by grafting operations could directly benefit the existing pearl industry by providing a scientific ground in farming management. The results of the Year 1 and Year 2 indicated that significant quality improvements in roundness increase and flaw (circle-marks) reduction, which can be adopted by on-farm approaches. Pearl farm's productivity could be improved greatly by strategy change in grafting operations, that is, re-considering using not only those oysters that produced superior quality virgin pearls for re-grafting but also those produced circled pearls and irregular shaped non-round pearls.

The uses of chemical-coated nuclei, such as FNC fibronectine-coated or so called "bio-coated" nuclei, did not indicate significant difference in the post-grafting survival rates over a 10 month period for all experimental treatments among the circle-tests and FNC-tests. Similarly, no clear effect was obtained on the pearl quality. The post-grafting survivorships could depend more on a degree of grafting technician's skill and farmhand's maintenance work. Therefore, the use of chemical-coated nuclei may not be necessary in terms of profitability of the farm business, particularly in Micronesian situations like Pohnpei and remote outer islands, as the cost of such nuclei are more expensive at about $0.4 – $0.5 per monme (or 3.69 g) than those of normal nuclei. For example, several million blacklip pearl oysters are used for grafting work each year in the French Polynesia, which uses several million nuclei. So, the amount of spending on those chemical-coated nuclei cannot be ignored including cool storage cost and shelf-life of about a year. More studies may require particularly on the economic side of farm performances and environmental impact assessment for using such nuclei.

As described in the final reports of Years 1 and 2, the results indicated significant improvements in roundness and reduction in circle flaws among "the seconds" compared to the matching virgin circled pearls. In contrast, spot marks did not change or improve very much among the seconds or the pearls from re-grafting, suggesting that the majority of spots are passed along to subsequent pearls produced by the host oysters (Ito, 2009). The results of three-year consecutive grafting experiments were consistent to prove quality improvements in the shape and circle flaws by the re-grafting work, which could shed light on the century-old myth of the circled-pearl formation mechanisms. Color and luster are also important quality elements in pearl business. Appendix 7-3 shows the results of color and luster changes from 2007 – 2008 and 2008 – 2009 pearl quality improvement experiments. It was apparent that color and luster tended to fade (i.e. lighter color and less lustrous) in "the seconds" and/or "the thirds" by the re-grafting compared the virgin pearls. The three-year consecutive grafting experiments also confirmed this tendency as

well as roundness, circles and spots (Appendix 7-4). The quality changes either by improvement or by deterioration appeared to be complex because of combinations of shape, flaw, color and luster elements. As shown in Appendix 8, some improved greatly their shapes by retaining color and luster and others deteriorated their shapes but flaw disappeared, and so on. It was concluded that the re-grafting contributed greatly to producing larger sizes, rounder and less circle but it might have adverse effect on reducing luster and fading color. The spot was not largely derived from the re-grafting but it could be originated from the first (virgin) grafting operation.

This project proved scientifically quality improvements and/or changes by grafting work, including roundness and flaws. Although circles and spots are simply categorized in the flaw or blemish, this series of studies suggested that there was functional features of the outer mantle epithelial cells and the pearl's flaw formation mechanisms and the circles are highly likely formed during the first/virgin pearl formation because of the improvement results of "the seconds" (the re-grafted) had less circled pearls and tended to disappear, compared to those matched virgin pearls ("the firsts") from the corresponding hosts. This project's results would shed a light on a century old myth of the circled pearl formation mechanisms and contribute to unify current studies on mechanisms of the mantle epithelium cell proliferation in cell biology and gene-controlled nacre crystallization processes in bio-mineralization. This project's other findings included differences in formations between circle marks and spot marks before and after the re-grafting operations: the majority of the circle flaws were apparently formed during the first/virgin pearl formation, but the spots tended to recur in the pearls from the re-grafting (the seconds and the thirds), indicating a different formation mechanism.

As a result of this project, many pearls with high and unique quality were produced. They were used for development of a new export market overseas and the efforts have been in progress to promote the Micronesian brand pearls, "Micronesian Blue Pearls" (Appendix 11-4). A display and sale was conducted in Pohnpei in April this year and another display sale will be planned later this year. Some of the overseas jewelry makers and traders have shown their interests and began promoting the Micronesian brand pearls, which also collaborates to ecological fair-trade and traceable gem marketing groups. This project became also a good example of a case study of micro-economic and community-based farming development through the USDA-funded research and technology transfer project.

Recommended Follow-Up Activities

Further studies may be necessary to collaborate with group of researchers overseas in mantle cell propagation physiology and genetic level of nacre crystal formation. It was

difficult to conclude how to reduce such spot marks at this early stage, but it could be related to malfunction of mantle's outer epithelial cells. The cell(s) might have been damaged permanently or temporary and partially because of exogenous (e.g. bacterial infections) or endogenous (e.g. physiological abnormality). If it was originated from bacterial infection, we can control such a factor by relatively simple modifications of grafting conditions including sanitary of grafting tools and preparation of mantle piece. To clarify these issues, further research funding and industry's support will be necessary.

Publications and Manuscripts and Papers Presented

Publications

Ito, M. 2009. Improving pearl quality by grafting and husbandry methods. Aqua Tips, Regional Notes, Center for Tropical and Subtropical Aquaculture, Hawaii, USA. 8 Pages.

Oral and Poster Presentations

Ito, M. 2008. A community-based pearl farming development in Pohnpei, Federated States of Micronesia. 3rd Australasian Aquaculture Conference, August 3-6, 2008, Brisbane, Australia.

Ito, M., M. Hagilmai, B. Harvorsen, C. Mulwelgyie and J. Smith. 2008. Practical techniques for mass spat production and ocean grow-out culture of the blacklip pearl oyster *Pinctada margaritifera*. 3rd Australasian Aquaculture Conference, August 3-6, 2008, Brisbane,Australia.

Ito, M. 2009. A revival of " half-pearls" as a value-added product of the shells from the blacklip pearl oysters *Pinctada margaritifera* in Pohnpei, Federated States of Micronesia. Abstract, Asian-Pacific Aquaculture Conference, Kuala Lumpur, Malaysia.

Manuscripts

Ito, M. Flaw formation mechanisms of the blacklip pearl oyster *Pinctada margaritifera* Linnaeus. I. circle formation. (*in ms*)

Literature Cited

Acosta-Salmon, H., Martinez-Fernandez, E., and Southgate, P.C. 2004. A new approach to pearl oyster broodstock selection: Can saibo donors be used as future broodstock? Aquaculture, 231: 205–214.

Akiyama, N., A. Itoh, H. Morohoshi, K. Asahina, H. Hirose, N. Mano, N. Fukushima, J. Taniyama and J. Itoh. 1997. Effect of tetracycline hydrochloride (TC-HCl) coating to nucleus on pearl oyster (*Pinctada fucata martensii*) survival after nucleus insertion. Aquaculture Science, 45(4): 457-466.

Fang, Zi., Q. Feng, Y. Chi, L. Xie and R. Zhang. 2008. Investigation of cell proliferation and differentiation in the mantle of *Pinctada fucata* (Bivalve, Mollusca). Marine Biology, 153: 745–754.

Ito, M. 2009. Improving pearl quality by grafting and husbandry methods. Aqua Tips, Regional Notes, Center for Tropical and Subtropical Aquaculture, Hawaii, USA. 8 Pages.

5. Kahala Broodstock Management

General Information

Reporting Period: October 1, 2008 to September 30, 2009; 1st no-cost extension through March 30, 2010; 2nd no-cost extension through October 31, 2010 (Project Termination Report)

Funding Level: **$92,500**

Participants: **Charles Laidley,** Ph.D., Director
Finfish Department, Oceanic Institute

Chad Callan, Ph.D.,
Oceanic Institute

Mr. Ken Liu,
Oceanic Institute

Mr. Neil Sims,
Kona Blue Water Farms

Ms. Jennica Lowell,
Kona Blue Water Farms

RESULTS AT A GLANCE…

- This study has shown that Kahala broodstock can be maintained in either flow-through or recirculating water supplies following suitable quarantine procedures to eradicate ectoparasites.

- A diet evaluation found that commercial high-protein/high-lipid broodstock diet (Vitalis) produced by Skretting successfully supports high-quality egg production, although challenges with egg quality in this study suggests the need to further examine diets for broodstock growout and maturation.

Objectives

1. Design and commission a two-tank recirculating aquaculture system (RAS) for holding kahala broodstock.
2. Establish an expanded kahala broodstock population (eight tanks, four at OI and four at KB) for study of broodstock holding systems.

3. Compare broodstock health and performance in flow-through versus water reuse broodstock holding systems using either water derived from saltwater wells (OI) or ocean water (KB).
4. Evaluate long-term effects of a formulated commercial diet on broodstock health and reproductive performance at both OI and KB.
5. Disseminate project findings on kahala broodstock holding conditions through presentation of results at the annual Hawaii Aquaculture Association meeting and through CTSA Regional Notes.

Principal Accomplishments

Objective 1: Design and commission a two-tank recirculating aquaculture system (RAS) for holding kahala broodstock.

In preparation for planned broodstock holding trials we commissioned two 2-tank broodstock holding systems at OI for comparison of flow through and recirculating water treatment systems for holding kahala broodstock (Figure 1). The flow-through water treatment system was upgraded with a vacuum degassing column to help reduce gas bubble-induced trauma to kahala broodstock and pressurized glass filters to remove particulates entering from the OI/SLP saltwater well system (Figure 2). A recirculating water treatment system was also commissioned to examine the use of recirculating aquaculture system (RAS) technologies to supply water for broodstock holding (Figure 3). In a parallel effort at KBWF, kahala broodstock were maintained in 8 deep by 50′ diameter ($45m^3$) tanks constructed using an HDPE liner surrounded by a steel structure with a center drain (Figure 4). The KBWF flow-through holding system utilized surface seawater, provided by the Natural Energy Laboratory. The KBWF recirculation system consisted of passing tank water through a Triton II sand filter utilizing a crushed glass media with the capabilities to filter water to 20μm, a RK2 protein fractionators, 450L biofilter tank containing bioballs, and UV sterilizer (Emperor Aquatics) before return to the tank.

Objective 2: Establish an expanded kahala broodstock population for study of broodstock holding systems.

At OI, a total of 40 kahala broodstock (approximately 6 to 12 kg) were taken through a four-week quarantine period and randomly stocked into each of the four broodstock tank holding systems. Although broodstock were ready for stocking in early January (at project commencement), delays in tank and over tank lighting system repairs postponed stocking of broodstock into the new holding systems until May 2009. In addition, we supplemented

the broodstock populations with four additional (~2 kg) kahala per tank from a recent growout trial.

Objective 3: Compare broodstock health and performance in flow-through versus water-reuse broodstock holding systems, using either water derived from saltwater wells (OI) or ocean water (KB).

OI Broodstock. With the commissioning of flow-through and recirculating systems at OI complete, we began monitoring the effects of broodstock holding systems on kahala reproductive performance beginning in June 2009. The kahala broodstock quickly adapted to the new holding systems with almost immediate resumption of spawning activity in both flow through and recirculating water holding systems (Figure 5) with similar performance seen in the 12' diameter x 6' deep tanks as was seen in the previously used 20' diameter x 4' deep tanks. However, over time it became apparent that fish in the RAS treatment group were losing condition with a decline in egg output and declining fertility and egg viability rates (Table 1). Over the project period, kahala maintained in the flow-through water treatment generated over 15.3 million eggs with a mean spawn output of 1.4M eggs/month, while the broodstock maintained in the RAS system generated 8.6 million eggs with a mean spawn output of 787,534 eggs/month. In addition to lowered egg output, there was also a decline in egg quality with slightly lowered fertility rates (73% vs. 63%) and egg viability rates (42% vs. 20%) in fish maintained under RAS compared with flow-through treatment. In part, this decline in egg output in the RAS group may be associated with poorer water quality in this group as the water had relatively high levels of suspended solids giving the water a more cloudy appearance. To address this issue, we installed an additional pressurized crushed glass filter prior to the protein skimmer in the RAS system which improved water clarity. Spawn frequency did appear to improve along with some increase in the number of viable eggs, although overall egg production, fertility and egg viability rates remained substantially below those of stocks in the flow-through tanks.

KBWF Broodstock. The KBWF kahala broodstock also appeared to adapt well to either flow-through or recirculating water treatment systems (Table 2). The wild-caught group kept in flow-through tanks produced large numbers of eggs, averaging over 7 million eggs per month over the project period and continues to generate large number of eggs to this day with more recent egg output around 3 million eggs per week. The F1 group maintained in the RAS were stocked somewhat later than the flow-through group and began spawning in April 2007, generating almost 4 million eggs per month. Due to differences in fish origin, age, size, and diet (see objective 4) it is not possible to directly compare treatment groups. However, both broodstock systems supported broodstock egg production.

Overall egg viability rates remained extremely low throughout the study period under in both broodstock systems. The wild-flow through group averaged only 25% viability, with monthly means typically ranging from 15 to 40%, while the F1- RAS group averaged only 11% viability over the project period, ranging from 2% to a high of 20%. The larger number of eggs obtained from these extremely large broodstock holding systems likely afforded sufficient egg supplies to meet hatchery requirements, but clearly work is required to improve the supply of high-quality eggs for hatchery operations.

KBWF has experienced some significant improvement in broodstock output over the last year. From August, 2009 to July, 2010, viable egg production has averaged around 15 million viable eggs per month (around double what we were previously obtaining, from around the same number of broodstock). They are now consistently obtaining 1 – 2 spawns per week of 1 million eggs each, and 2 – 4 smaller spawns of 400 – 500,000 viable eggs each. These are all on flow-through; we have discontinued the broodstock recirculating systems. We have no understanding of the factors have led to this increase.

Objective 4: Evaluate long-term effects of a formulated commercial diet on broodstock health and reproductive performance at both OI and KB.

In addition to examining the effects of water treatment system, the project also examined the long-term performance of utilizing a formulated commercial salmon broodstock diet (Vitalis SA, Skretting) on broodstock health and reproductive performance. Experience at both OI and KBWF has shown that wild-caught kahala do not take readily to pellets and therefore they are provided with raw diets. The raw diet used at OI included alternate feedings of smelt, squid and shrimp, while raw-fed stocks at KBWF were provided with squid and sardines.

For the purposes of this study, the OI experimental stocks were all cage grown F1 kahala broodstock and therefore were fed only pelleted diets. In contrast, the KBWF broodstock population included wild-caught stocks fed sardines and squid maintained in the flow-through tank systems, and F1stocks fed solely on pelleted Vitalis broodstock diet from Skretting.

Although the formulated diet appeared to support year-round egg production throughout the duration of the project, egg quality in terms of viability, hatch and early larval development clearly was insufficient to meet the egg stocking requirements of a production hatchery (Table 3). Therefore, after six months on the test diet the OI broodstock populations was introduced to a calamari supplemented broodstock feed (Vitalis CAL, Skretting) for two of the four test broodstock populations at OI. Initially the calamari

supplemented formulation appeared to improve egg production and egg viability rates, but by the end of the trial broodstock maintained on the control (i.e., Vitalis) diet did not appear to differ in performance from stocks maintained on the calamari supplemented diet. Although hatch rates appeared somewhat higher on the Vitalis CAL diet (22%) than the control Vitalis diet (3%) neither rate was acceptable for hatchery production (Figure 6).

In discussions with our industry partners at Kona Blue Water Farms, who also have experienced reproductive performance and egg quality issues in their captive reared (i.e., domesticated) broodstock populations, suggest that sourcing broodstock from their growout population reared on high-fat growout diets may be a factor. The high-fat growout diets lead to extremely fatty livers which may compromise hepatic egg yolk protein (vitellogenin) synthesis and consequently affect egg quality. Therefore, we sampled the remaining broodstock population to examine fish size, liver and gonad size at the end of the trial (Figure 7). On average, female broodstock weight was slightly less than found for the males. Female broodstock also had relatively larger livers and gonads than seen for the males. The increased liver size, expressed as hepatosomatic index (Figure 7), reflects the additional role of the female liver in producing the egg yolk precursor protein vitellogenin. The increased gonad weight reflects the accumulation of liver generated egg yolk protein in developing female gametes prior to ovulation and spawning. Water treatment did not seem to affect overall fish weight, liver weight (hepatosomatic index), or gonad weight. However, the calamari supplemented diet did appear to support larger ovaries in the females likely associated with female fecundity under the calamari supplemented diet. In writing this final report our project partners at KBWF relayed that the continuing poor spawning output and egg viability issues associated with their F1 and F2 stocks has led them to eliminate the use of these domesticated stocks from their hatchery program. They also believe that this captive-reared broodstock constraint is most likely diet-related.

Objective 5: Disseminate project findings on kahala broodstock holding conditions through presentation of results at the annual Hawaii Aquaculture Association meeting and through CTSA newsletter Regional Notes.

An article on kahala culture for interested stakeholders was featured in the November issue of *Regional e-Notes*.

Impacts

With the startup of an open-ocean cage culture industry for kahala (amberjacks) in Hawaii, there was a critical need to establish captive broodstock holding technologies to facilitate long-term stock holding and ensure an adequate year-round supply of eggs for hatchery-

based fingerling production. Early experience in working with local *Seriola rivoliana* and other local amberjack species has shown that broodstock operations can be challenging. This study, along with our earlier work has shown that the broodstock can be maintained in either flow-through or recirculating water supplies following suitable quarantine procedures to eradicate ectoparasites, including *Cryptocaryon irritans* to which the amberjacks are particularly prone. The successful long-term maintenance of captive populations is critical in developing broodstock programs and ensuring year-round egg supplies for hatchery operations. To date, both wild-collected and captive reared broodstock can be used for stocking broodstock populations, although the wild-collected stocks tend to express higher fertility rates and improved egg quality over captive generated stocks. This trend likely reflects differences in nutrition between wild and captive reared stocks, and requires further research. A commercial high-protein/high-lipid broodstock diet (Vitalis) produced by Skretting successfully supports high-quality egg production, although challenges with egg quality in this study suggests the need to further examine diets for broodstock growout and maturation. Under ambient conditions in Hawaii, kahala broodstock held in either indoor or outdoor systems generated eggs on a year-round basis. This allows for the establishment of year-round hatchery production of kahala juveniles going toopen ocean cage growout systems. Although more work is obviously required to refine holding and rearing procedures, this research clearly leads the way to the establishment of a viable marine finfish production sector farming kahala and other warm-water marine fishes and helping reduce pressures on over-exploited ocean fisheries.

Recommended Follow-Up Activities

With the establishment of successful long-term holding procedures for amberjack (kahala) broodstock, there is now a need to explore the development of specific diet formulations supporting growout, maturation and high-quality egg production for domesticated stocks. At this time industry partners have reverted to the use of wild-collected broodstock and the use of mixed raw diets. Although pragmatic at present, this approach presents significant biosecurity risks to hatchery operations with potential import of pathogens through both broodstock and their diets, along with higher costs of operation, and an inability to initiate meaningful selective breeding program.

Publications and Manuscripts Written and Papers Presented

We plan to write a CTSA Regional Notes article on kahala culture upon project closeout.

6. Shrimp Production Demonstration Project and Aquaculture Training for Industry Stakeholders of the Commonwealth of the Northern Mariana Islands and Guam, Year 1 and 2

General Information

Reporting Period	October 1, 2007 to December 14, 2009; no-cost extension through June 14, 2010 (Project Termination Report)	
Funding Level	Year	Amount
	1	**$34,571**
	2	**$34,530**
	Total	$69.101
Participants	**Dustin Moss,** Research Associate Shrimp Department, Oceanic Institute	
	Shaun Moss, Ph.D., Director Shrimp Department, Oceanic Institute	
	Steve Arce, Research Associate Shrimp Department, Oceanic Institute	
	Clete Otoshi, Research Associate	

RESULTS AT A GLANCE…

- A workshop on shrimp farming in the CNMI was attended by over 80 local farmers, business leaders, government representatives, and media. It resulted in first shrimp farm on the island of Tinian.

- OI scientists provided training on shrimp AI techniques to GADTC staff, discussed broodstock feeding, water quality management, and broodstock sourcing protocols with GADTC staff, and gave presentations on regional opportunities in shrimp aquaculture, the effects of inbreeding on hatchery and growout performance of shrimp, and super-intensive shrimp aquaculture to industry stakeholders and University of Guam students and faculty.

- A fact sheet on shrimp farming in the CNMI was developed and distributed throughout the region.

Shrimp Department, Oceanic Institute

Dr. Chatham Callan, Research Scientist,
Finfish Department, Oceanic Institute

Dr. Clyde Tamaru, University of Hawaii Sea Grant

Dr. Hui Gong, University of Guam

Michael Ogo, Aquaculture Extension Specialist
Northern Marianas College

Rommel Catalma, Operations Manager
Saipan SyAqua (formerly Saipan Aquaculture)

Objectives

1. Build a knowledge and technical base for shrimp aquaculture in the CNMI and Guam.

   Objective 1.1. Improve efficiency of local shrimp production by educating industry stakeholders on current shrimp production methodologies.

   Objective 1.2. Address technical constraints or issues/interests specific to the Guam shrimp farming industry.

2. Stimulate interest and investment in shrimp aquaculture in the CNMI and Guam by demonstrating profitable and environmentally friendly shrimp production using existing SyAqua Saipan facilities and culture techniques developed by OI.

   Objective 2.1. Shrimp production demonstration.

   Objective 2.2. Publishing and distribution of a shrimp farming guide.

Principle Accomplishments

Objective 1.1: Improve efficiency of local shrimp production by educating industry stakeholders on current shrimp production methodologies.

An aquaculture workshop covering various topics relevant to Guam and the CNMI was held at the World Resort in Saipan on July 10-11, 2008. Four scientists from OI (Dustin Moss, Dr. Shaun Moss – co-principle investigator, Clete Otoshi, and Dr. Chatham Callan)

gave presentations at the workshop, along with Dr. Clyde Tamaru from the University of Hawaii Sea Grant and Dr. Hui Gong from GADTC. In addition, Mike Ogo, Dr. Ignacio de la Cruz (Director of CNMI DLNR), and representatives from the CNMI Departments of Environmental Protection and Coastal Resource Management also participated. Presentation topics for the workshop included: shrimp maturation and hatchery techniques, high-intensity shrimp growout using technologies appropriate for Pacific islands, opportunities for shrimp aquaculture in the CNMI and Guam, aquaculture biosecurity, propagation of marine finfish, culture of marine ornamental fish, off-shore cage culture of marine finfish, aquaculture development and extension activities in Hawaii, aquaculture extension activities in the CNMI, and CNMI import and export regulations for marine species (see Appendix 1 for complete workshop program). The workshop was well received, as there were over 80 attendees including local farmers, business leaders, government representatives, and media (see Appendix 2 for examples of media coverage). Compact discs containing presentations (with accompanying audio files) were distributed to attendees.

Objective 1.2: Address technical constraints or issues/interests specific to the Guam shrimp farming industry.

OI scientists visited the GADTC three times and (1) provided training on shrimp AI techniques to GADTC staff, (2) discussed broodstock feeding, water quality management, and broodstock sourcing protocols with GADTC staff, and (3) gave presentations on regional opportunities in shrimp aquaculture, the effects of inbreeding on hatchery and growout performance of shrimp, and super-intensive shrimp aquaculture to industry stakeholders and University of Guam students and faculty. All training and presentation topics were chosen by Drs. John Brown and Hui Gong of GADTC with the aim of improving shrimp reproduction/culture at GADTC and/or educating local stakeholders. GADTC is the sole supplier of shrimp seedstock to most shrimp farms in the region and, unfortunately, limitations and inefficiencies at GADTC often result in seedstock shortages. Thus, any improvements in GADTC operations should benefit the regional shrimp farming industry.

Objective 2.1: Shrimp production demonstration.

A shrimp growout trial was conducted at SyAqua Saipan. The goals for this trial were to (1) demonstrate intensive shrimp culture under local conditions and (2) to evaluate an alternative oxygen delivery system in an effort to reduce electrical usage per kg of shrimp produced. Electrical costs in the CNMI account for a large portion of production costs (~40%), and this is a major impediment to industry development/expansion. The alternative oxygen delivery system tested was similar to that used at OI for super-intensive shrimp culture and could be easily installed at any intensive farm in the region. Oxygen

concentrators are easily maintained and don't use large amounts of electricity, so they can be very useful to intensive farms where high levels of aeration are needed.

To test the effectiveness of the alternative oxygen delivery system, two identical growout tanks at SyAqua Saipan were used. One tank had standard farm aeration (cyclonic diffusers and paddlewheels) and the other had the alternative oxygen delivery system. Demonstration of the effectiveness of the alternative oxygen delivery system was dependent on the trial being conducted in a rigorous manner so that clear conclusions could be derived. Numerous problems occurred that compromised the experiment to the extent that meaningful conclusions were not possible. At stocking, poor PL survival during quarantine and inadquate estimation of numbers during stocking resulted in an unclear estimation of stocking density in the two tanks. Tank densities were not equalized until two months into the trial and the estimation of the standing crop was made indirectly based on feed consumption, which is far less accurate than direct estimation. After day-28, severe spikes in ammonia and nitrite concentration resulted in shrimp mortality and sublethal effects on growth. This further compromised the ability to make comparisons of growth and survival between tanks. Furthermore, SyAqua Saipan began harvesting the control tank (standard aeration) on day-69 without notification. The day-69 harvest was completed for sales and did not result from a need to reduce biomass due to low DO (as was agreed upon in the trial plan). The reduced biomass in this tank resulted in increased DO and further confounded the abilitiy to make meaningful comparisions between the control and test tank (and to evaluate the oxygen delivery system), resulting in unusable data. However, this trial did afford the opportunity for SyAqua Saipan to test the oxygen concentrator equiment and see first-hand the simplicity of the unit and its immediate impact on DO. They have since purchased an oxygen concentrator and they have been able to increase stocking densities while maintaining satisfactory DO. This should lead to increased production.

Objective 2.2: Publishing and distribution of shrimp farming manual.

Thirty copies of OI's *Intensive Shrimp Production Manual* and 100 copies a shrimp farming fact sheet were sent to GADTC and NMC for local distribution to extension agents, relevant university/college staff involved in aquaculture, current farmers, and individuals/parties interested in starting an aquaculture farm. The manual covers all areas of intensive shrimp culture from maturation/hatchery through growout and broodstock production and should serve as an important reference document for aquaculture scientists and farmers in the region. The fact sheet contains basic farming information that will be of interest to farmers and those interested in starting a farm, including contact information for local experts and government agencies involved with permitting.

Impacts

The aquaculture workshop held in Year 1 (Objective 1.1) had over 80 attendees including local farmers, business leaders, government representatives, and interested people from the general public. After the workshop, NMC reported that several residents/local companies were interested in starting aquaculture farms. However, the most positive impact of the workshop to date is the establishment of the first shrimp farm on the Tinian (CNMI island south of Saipan). Melvin Crisostomo (a workshop attendee) contacted NMC shortly after the workshop about starting a family-run shrimp farm. With the help of NMC, the newly established farm stocked its first juvenile shrimp in late 2008. Importantly, the farm is currently still in operation.

OI scientists visited the GADTC and (1) provided training on shrimp AI techniques to GADTC staff, (2) discussed broodstock feeding, water quality management, and broodstock sourcing protocols with GADTC staff, and (3) gave presentations on regional opportunities in shrimp aquaculture, the effects of inbreeding on hatchery and growout performance of shrimp, and super-intensive shrimp aquaculture to industry stakeholders and University of Guam students and faculty. GADTC is the sole supplier of shrimp seedstock to most shrimp farms in the region. Thus, any improvements in GADTC operations should have a positive benefit on the regional shrimp farming industry.

Two documents on shrimp aquaculture have been provided to stakeholders (and potential stakeholders) in the region. These should serve as important reference documents for extension agents, relevant university/college staff involved in aquaculture, current farmers, and individuals/parties interested in starting an aquaculture farm.

Publications, Manuscripts, or Papers Issued, Approved, or Presented

Shrimp Farming in the USAPI (fact sheet); provided to GADTC and NMC for local distribution to industry stakeholders.

7. Sea Cucumber Hatchery Production Technology Transfer in Pohnpei, the Federated States of Micronesia, Years 1 and 2

General Information

Reporting Period	July 1, 2008 – June 30, 2010 (Project Termination Report)	
Funding Level	Year	Amount
	1	**$34,868**
	2	**$34,968**
	Total	$69,836
Participants	**Masahiro Ito**, Ph. D., Director and Chief Scientist	
	Aquaculture Development, College of Micronesia Land Grant Program	

RESULTS AT A GLANCE…

- This project developed a semi-closed system with a false-bottom tank for long term holding work. This system simulates the natural habitat of the sandfish with seagrass (eel grass) and seaweed (i.e. *Caulerpa, Gracilaria*, *Sargassum* and other species) on the top layer, supplying naturally occurring food.

- Larval development and larval rearing protocols were developed. A total of 24 spawning induction trials and 12 larval runs were conducted during the project and resulted in several hundred juveniles, which are awaiting the next phase of work on marking and/or tagging experiments for a restocking project.

- Differences in abundances of sandfish during the day and night observed in this project urge that all existing survey reports on the sandfish be reviewed by researchers and policy makers to re-construct present status of resource management strategies.

Objectives

1. Search and collect sandfish (H. Scabra) broodstock in the Pohnpei lagoon.

2. Conduct broodstock conditioning, spawning induction and larval rearing.

3. Develop a method for the sandfish juvenile production in Pohnpei.

4. Train Micronesians for the sea cucumber hatchery operation.

Principal Accomplishments

All the four objectives were accomplished within a limited time frame of the proposed period as following details:

Objective 1: Search and collect sandfish (*H. scabra*) broodstock in the Pohnpei.

Before commencing broodstock search, the project personnel began collecting information about the sandfish, "Lahngon", from local people particularly senior villagers in the municipalities of Nett, Sokehs, Madolenihmw, Kitti and U. A preliminary survey was conducted at Nett Point in the vicinity of the hatchery. The project accomplished proposed work by continuing searching and colleting sandfish broodstock where the survey sites were extended to the southern part of Pohnpei lagoon as well as the vicinity of the hatchery prior to the spawning runs. Among the Holothurian sea cucumbers, total of 10 species were found from various habitats in the Nett Point area (Appendix 3-1). The habitat of the sandfish was restricted within the tidal flat along the mangrove shoreline with very soft sand or muddy areas of knee-high deep, where the sea grasses (eel grass) grow densely. A standard survey method by using a scuba gear was not applicable for this particular species, so the work was done by snorkeling and by walking. Between November 2008 and June 2010 (Years 1 and 2), total of 480 sandfish were found in the wild from which 461 were actually collected for the hatchery work. Among them, 253 broodstock were collected in the vicinity of the hatchery (Appendix 4-1). As described in the Year 1 report, this figure gave us significantly higher abundance of the sandfish compared to those reported stock surveys by others in 1996-98 (Pohnpei State Marine Resources and Japan Overseas Fishery Foundation, unpublished data) and in 2004 (Bourgoin and Edward, 2005). The results of the broodstock search and collection revealed a unique pattern of abundances between daytime (CPUE 2.2) and night-time (4.3) at Nett Point, encountering more sandfish at night during high tide period. This confirmed local elder's judgments on timing and areas of searching "Langohn", a local name of the sandfish, who advised us on the broodstock collection. Wolkenhauer et al. (2009) reported daily and seasonal behavioral pattern, which was closely associated their feeding activities. Nocturnal behavior of the sandfish, in which they bury in the muddy/soft sand during the day and come out from the sand after sunset, was also observed and confirmed from the project's broodstock holding tanks with a natural habitat simulated sub-sand filter system.

Objective 2: Conduct broodstock conditioning, spawning induction and larval rearing.

Indoor and semi-outdoor hatchery systems were modified from pearl oyster hatchery system for a trial spawning-run: spawning induction tanks, incubators, larval rearing tanks, settlement tanks and juvenile grow-out tanks. The sub-sand filter system allows the sandfish to bury in the soft sand layer and the seawater re-circulates by an air-lift method through the layers of fine sand, coral gravels and coral rocks within the tank. For feeding purposes, the seaweed (*Graciralia* and *Sargassum* spp.) and other sea grasses (eel-grass) were collected from the vicinity of the hatchery and they were kept alive in the tanks. As the sandfish is a deposit-feeder feeding on detritus and anything on the substrate, muddy sand was also collected occasionally from the mangrove tidal flat areas as additional food for the sandfish. The land-based broodstock holding tank system was installed during Year 1 of this project, making it possible to keep approximately 200 broodstock in two separate raceway tanks for long term broodstock conditioning (Appendix 5-1), maintaining approximately 20 animals per square meter. Agudo (2007) described in her hatchery manual that it was difficult and labor-intensive to keep the sandfish broodstock in the land-based tank unless they were kept in a larger outdoor facility, such as an earth-pond or sea-pen.

In this project, the PI aimed to develop a simple and economical method unlike a large-scale earth-pond or sea-pen culture. A land-based recirculating seawater system seems to be one option, but it requires higher running costs than pond or pen cultures. To compensate disadvantages of a recirculating seawater method using a simple bottom tank with expensive filter machinery equipment, this project developed a semi-closed system with a false-bottom tank for long term holding work. The tank featured three-layer sub-sand filtering and airlift piping to create a closed recirculating seawater system, and introduced a partial flow-through from a water inlet and overflow drain pipes to maintain water quality by minimize salinity fluctuation (Appendix 5-2). Furthermore, this system had advantages to simulate natural habitat of the sandfish by planting seagrass (eel grass) and seaweed (i.e. *Caulerpa, Gracilaria*, *Sargassum* and other species) on the top layer, so that the broodstock were supplyed with naturally occurring food in the tank system. Since the installation in 2008, tank change or cleaning has never been done except for brushing off epiphytes on the walls and airlifting pipes as a part of feeding work weekly or bi-weekly. It was also easier and more accurate to observe the nocturnal nature of animal behavior on a long term continuous basis. Basic system design was adopted from the PI's past work on the broodstock conditioning of the squat lobsters which also burrow in the sand. The indoor hatchery facility was the same as the pearl oyster hatchery (Appendix 6-1) for microalgae culture, spawning induction and larval rearing work.

As described in the Year 1 final report, a trial spawning induction was conducted in November – December, 2008 during the absence of one of the senior technicians who participated to the sandfish hatchery training workshop in Fiji. This very first trial resulted in a couple of hundred thousands eggs to work with for the larval runs that were conducted using the existing sandfish hatchery manuals. Then the methods for the spawning induction, micro-algae feeding combinations, larval sampling and rearing water quality maintenance were gradually modified to suit the project's training work schedule and hands-on practice. In the second hatchery run in January 2009, approximately several thousand larvae settled to the pentactula or post-larval stage and subsequently became juveniles for the first time in Micronesia. The spawning induction and larval rearing methods are shown in Appendix 6.

Objective 3: Develop a method for the sandfish juvenile production in Pohnpei.

Larval development and larval rearing protocol were summarized in Appendix 6-4 and the microalgae feeding schedule was shown in Appendix 6-5. Naturally occurring algae or epiphytes were cultivated in about two weeks. On around day 10 when they become Doliolaria stage, a mixture of seawater and the eel grass (Appendix 6-4) was prepared through 80 micron sieve and given to the settlement tanks every other day. To encourage the growth of diatoms, nutrient media with approximately $1/10^{th}$ strength of the indoor mass cultures were added to the settlement tanks. An additional tank system was constructed under-cover and outdoor areas next to the indoor hatchery for settlement and juvenile grow-out work (Appendices 7-1 and 7-2). Eel grass, seaweed (*Gracilaria* and *Sargassum* spp.) and other epiphytes were obtained within the tank or collected together with silt from the sandfish habitat in the vicinity of the hatchery to feed juveniles. Soft sand and silt on the tank surface, which contained decaying eel grass fragments and other detritus, were main food sources for the juveniles during grow-out of this project. Excess plants were kept alive in the tank system, but eel grass extract was also added weekly or bi-weekly.

The sandfish is a deposit-feeder feeding on detritus and anything on the substrate. The grow-out tank system developed for this project served well for a long term and continuous feeding work with minimal maintenance. Decaying part of live sea grass, seaweed and other epiphytes fallen and deposited on the tank surface became mixture of food with muddy sand. A settlement method followed basically a hatchery manual by Agudo (2007), however, growing epiphytes such as *Navicula jefferii* and *Skeletonema costatum* required time consuming maintenance from a stock culture stage to 500 L mass cultures. After several hatchery trials, a method for introducing pure cultured phytoplankton into the juvenile grow-out was switched to culture naturally occurring phytoplankton and other epiphytes as described here in the objectives 2 and 3. A wide

range of body sizes during the early phase of juvenile grow-out is shown in Appendices 7-2d and 8-3.

Objective 4: Train Micronesians for the sea cucumber hatchery operation.

This project accomplished technology transfer of the sandfish hatchery work and continued on-the-job training during the hatchery juvenile production. A total of 24 spawning induction trials and 12 larval runs were conducted during the project. In some cases, larval runs were successful but in other cases unsuccessful. This might be attributed to unavailability of a commercially established methods for the larval feeding for a large scale juvenile production, so that the project needed to rely on the published hatchery training manuals of the sandfish or other species or continuous modifications of the feeding strategy during the course of skill training. However, this project was able to transfer hatchery technology, which became at least a basis for the subsequent improvements. Four Micronesian technicians kept working on all features of the sandfish hatchery operation including broodstock search and collection, a long-term broodstock conditioning, spawning induction, larval rearing and juvenile grow-out. As of this reporting date, several hundred juveniles of 12 and 18 months old juveniles are being kept in two raceway tanks awaiting the next phase of work on marking and/or tagging experiments for a restocking project. Four additional local youths, who have been learning pearl farming skills, also participated in the wild stock survey and broodstock collection work as well as supporting work on the spawning induction. The recent spawning event supplied 3 million fertilized eggs and larval rearing began with approximately one million Auricularia larvae. In order to catch up with a commercial scale juvenile production, the present work requires further improvement of feeding protocols of the larvae and juveniles for better survival and growth. Currently, all the hatchery operations are maintained by the Micronesian technicians with minimum supervisions by the PI. A training manual of the sandfish hatchery work for the Micronesians will be submitted after this report.

Impact

An urgent issue of this project was the transfer of hatchery technology. Acquiring skills on the sandfish hatchery work has been considered to be a time consuming process for someone who does not have experience in the sea cucumber biology and resource work or basic hatchery operation in mollusks and crustaceans. This project offered opportunities to learn all features of sandfish aquaculture skills to the local Micronesians within a limited time frame of less than two years. A key technical feature of the project's grow-out system is a combination of a closed recirculating and a partial flow-through methods, which does not require frequent tank change or cleaning but enables the juveniles to grow for a long

term, hopefully to maturity. Furthermore, feeding can be done without a renewal of sandy and muddy substrates because of the system contains live and dead sea grasses, sea weeds and other epiphytes and detritus. This type of recycling system is also called an "eco-system culture method" or self-sustainable system, which allows attaching epiphytes, seaweeds and sea grasses growing and majority of waste from the sea cucumbers being recycled within the system by utilizing the sunlight. The water re-circulates through the surface fine sand and muddy layer to coarse coral gravels and coral rocks by air-lifted pump system which also helps maintaining aerobic condition of the tank bottom substrates. The system developed by this project could be clarified for its economical effectiveness in growth and the survivorship compared to conventional grow-out methods widely used with a simple flat bottom tank, which requires frequent cleaning and changes of sandy and muddy substrates in long term grow-out work.

The sandfish does not inhabit hard coral rubble areas nor grass areas close to mangrove shore if not provided sufficient depth (i.e. at least 4 – 5 inches deep) of soft sandy/muddy substrates. No sandfish were found from reef flats and reef slopes on patchy reef areas or barrier reef areas. Therefore, a conventional line transact method using scuba or snorkeling does not seem to be appropriate for the wild stock survey for this particular species in Pohnpei lagoons. As reported in the previous year, differences in abundances during the day and night urges that all the existing survey reports on the sandfish published worldwide should be reviewed by the researchers, and that policy makers re-construct the present status of resource management strategies. This is because few studies have conducted a night time survey of the sandfish, even though some researchers reported distinct nocturnal nature of behavior (e.g. Mercier, et al., 2000). This apparently did not reflect in any real-world stock surveys. It is highly likely that abundances could have been underestimated or erroneously estimated.

Recommended Follow-Up activities

The proposed project could be more practical if further research activities are carried on: 1) to improve a land-based culture method for the broodstock conditioning and juvenile grow-out such as feeding frequency, quantity and quality of food being available from the wild in particular; 2) to improve larval rearing techniques to obtain better survivorship of the late Auricularia, Doliolaria, Pentactula to the settlement of the juveniles. This is because the feeding regime is still in trial in terms of constant large scale hatchery operation; 3) conduct tagging experiments both in the tank and ocean enclosure for the hatchery produced juveniles at around 6-month-old and/or 12-month-old for restocking purposes; and 4) to continue hands-on training for the Micronesian technicians in a commercial scale hatchery work. A sandfish hatchery production manual will be a site specific to Pohnpei as

naturally occurring foods are the main source to grow and a layman's manual will be appropriate for the local people without much scientific background.

Publications and Manuscripts Written and Papers Presented

Ito, M. 2009. Resource Enhancement Project of the Sandfish *Holothuria scabra* in Pohnpei, Federated States of Micronesia. Abstract, ID263, Asian Pacific Aquaculture 2009. November 4-6, 2009, Kuala Lumpur, Malaysia.

Ito, M. 2010. Status of wild stock and hatchery production of the sandfish *Holothuria scabra* in Pohnpei, the Federated States of Micronesia. Abstract, PIN521, Australasian Aquaculture 2010. May 23-26, 2010, Hobart, Australia.

Ito, M. A layman's manual of hatchery juvenile production for the sandfish *Holothuria scabra* in Pohnpei, the Federated States of Micronesia. (*in ms*)

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Ito, S. 1995. Studies on the technological development of the mass production for sea cucumber juvenile, *Stichopus japonicas*. Saga Prefectural Sea Farming Center, Japan, 87 pp.

Mercier, A., S. C. Battaglene and J-F. Hamel. 2000. Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands. Hydrobiologia, 440: 81-100.

Wolkenhauer, S., T. Skewes, M. Browne and D. Chetwynd. 2009. Daily and seasonal pattern in behaviour of the commercially important sea cucumber *Holothuria scabra*. Abstract, the 13th Echinoderm International Conference, Hobart, Tasmania, Australia.

8. Promoting Health Management of Shrimp Aquaculture on Guam and the Commonwealth of the Northern Mariana Islands (CNMI)

General Information

Reporting Period	September 1, 2008 to August 31, 2009; 1st no-cost extension through February 28, 2010, 2nd no-cost extension through August 31, 2010 (Project Termination Report)
Funding Level	**$35,000**
Participants	**Hui Gong,** Ph.D. University of Guam Kathy Tang, Ph.D. University of Arizona, Shrimp Pathology Lab John W. Brown, Ph.D. University of Guam

RESULTS AT A GLANCE…

- Biosecurity audits of shrimp farms in Guam and the CNMI were conducted, and a site-specific executive report was generated for each facility.

- Audits found that the greatest risk for some facilities is the seed sources imported from the Asian countries, and that there is possible presence of IHHNV in a couple of locations.

- A comprehensive report evaluating the current health status of shrimp aquaculture in the Mariana Islands region, identifying the key biosecurity risk factor, and prioritizing the issues for improving industry-wide biosecurity measures in the region was generated and distribution regionally.

Objectives

1. To evaluate current shrimp health management practice in the region by conducting biosecurity audits of all existing shrimp farms and identify the key risk factors.
2. To set up farm-specific bimonthly surveillance program in two major shrimp farming facilities, one will be selected on Guam and the other on Saipan, as models for the other operations.

3. To promote the awareness of biosecurity in the region via various means of education, and to prepare and distribute a comprehensive summary report to aquaculture stakeholders and the corresponding government agencies.

Principal Accomplishments

Due to the unexpected challenges encountered, such as difficulties in the process of acquiring some chemicals and unexpected long time for receiving the biohazard reagents and shipping from Guam to CNMI, two no cost extensions with total of 12 month were therefore requested and granted in order to accomplish all the tasks outlined. Details of the timeline for completing the specific task were summarized in a table listed under appendix A.

Objective 1. To evaluate current shrimp health management practice in the region by conducting biosecurity audits of all existing shrimp farms and identify the key risk factors.

In addition to the University of Guam (UOG) hatchery, also known as Guam Aquaculture Development and Training Center (GADTC), there are three shrimp farms in Guam, two in Saipan, and one in Tinian. Contacts were made with the managing personnel in all of shrimp farms with regards to the health management project and arrange for the biosecurity audits.

The biosecurity audits were first performed for the shrimp facilities in Guam in November 2008, and then in July 2009 for CNMI due to unexpected difficulties in shipping the chemical reagents, such as Davidson's solution to CNMI. A site-specific executive report was generated for each individual facility. The strength and risk of each shrimp facility was evaluated and suggestions were also provided. A brief summary of key components in biosecurity audits is listed in Appendix B. The farm locations were also pinpointed in Google map attached in Appendix C.

In addition, based on the information gathered from the initial round of the biosecurity audits, key risk factors were identified for the site specific facility and recommendations were also outlined for improvement. Overall, the greatest risk for some facilities is the seed sources imported from the Asian countries, and no standardized quarantine procedure is in place to minimize such risk. Fortunately, there has not been any major shrimp disease outbreak in the region in the past decade even though there are indications of the possible presence of IHHNV in a couple of locations. If such health issues are not being scrutinized

and proper solutions being sought at both the facility and regional levels, the adverse effects may magnify and could evolve into much more serious situation.

Objective 2. To set up farm-specific bimonthly surveillance program in two major shrimp farming facilities, one was selected on Guam and the other on Saipan, as models for the other operations.

Based on the geographic location and the levels of impact of the facilities on the aquaculture development in the region, two facilities were selected for the health surveillance: one is in Guam and one is in Saipan. Sampling schemes have been tailored to fit the sources, size and numbers of shrimp stock in the facility. On-site training for sampling the shrimp tissues for specific diagnostics was also provided during the health surveillance visits. The two visits and related tasks were carried out in December 2009 and March 2010, respectively. A few pictures taken during the sample collection are included in the appendix of this report.

Objective 3. To promote the awareness of biosecurity in the region via various means of education, and to prepare and distribute a comprehensive summary report to aquaculture stakeholders and the corresponding government agencies.
A comprehensive report was generated to evaluate the current health status of shrimp aquaculture in the Mariana Islands region and identify the key biosecurity risk factors after careful examination, and prioritize the issues for improving industry-wide biosecurity measures in the region.

Distribution of this publication to the aquaculture stakeholders and corresponding government agency, such as territorial veterinarian's office, etc., were completed.

Impacts

Information generated from this health project fills up the blank of baseline information of health status of shrimp farming in Guam and CNMI. In addition, the project also serves as a useful tool in increasing the biosecurity awareness among the regional shrimp aquaculture society and improving the health management of shrimp aquaculture in Guam and CNMI.

In the end, the establishment of a systematic health management requires the collaborative efforts from all the stakeholders in order to improve the health status of the regional shrimp aquaculture and eventually lead to long-term sustainable shrimp aquaculture development in Guam and CNMI.

In summary, all the research efforts from this project help to increase the awareness of biosecurity, and serve as a useful tool in promoting the health management of shrimp aquaculture in Guam and CNMI, eventually promote long-term sustainable shrimp aquaculture development on Guam and CNMI.

Recommended Follow-Up Activities

An annual biosecurity audit is recommended to be conducted for each shrimp aquaculture facility by the shrimp health management expert.

Publications and Manuscripts Written and Papers Presented

Papers Presented in workshop and conference

Present "Aquaculture Biosecurity in CNMI and Guam" in the workshop "Opportunities in Aquaculture" held in Saipan from July 10-11, 2008.

The on-going project "Promoting Health Management of Shrimp Aquaculture on Guam and Commonwealth of Northern Mariana Islands" was presented by Hui Gong in "Integrated Technologies for Advanced Shrimp Production" held in the Honolulu, HI from Oct. 13-15, 2009.

Publications in Print

Gong, H. and J. W. Brown. 2010 Is Shrimp Aquaculture in Guam and the Commonwealth of Northern Mariana Islands Biosecure? - A Review of the Industry's Health Management. Brochure.

Manuscripts

Gong, H. (Accepted). Promoting Health Management of Shrimp Aquaculture on Guam and Commonwealth of Northern Mariana Islands. Asian Fisheries Science.

9. Improving Outputs in the Commercial-Scale Production of Swordtails in Hawaii, Years 1 – 3

General Information

Reporting Period: September 1, 2006 to October 31, 2007, no-cost extension through December 31, 2007 (Year 1);
March 1, 2008 to February 28, 2009 (Year 2);
March 1, 2009 to February 29, 2010 (Year 3)
(Project Termination Report)

Funding Level

Year	Amount
1	**$70,000**
2	**$35,000**
3	**$35,000**
Total	$140,000

Participants

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Jim Szyper Ph. D., CTAHR

RESULTS AT A GLANCE…

- Results from this project showed that a dose of 400 mg kg/feed 17β-estradiol results in 91-100% feminization but also produces fertile fish.

- Work conducted in Year 1 had significant impacts in the awarding of two graduate degrees from the University of Hawaii.

- Lyretail swords have become Rain Garden Ornamentals best crop in terms of the return on expenses and labor. There are three market outlets - a wholesale broker/middleman who ships product to distributors on the mainland, wholesale directly to local pet stores, and direct retail sales to the hobbyist.

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Lei Yamasaki, Graduate student, HNFAS,
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Private Sector Collaborators: Steve Hopkins, Rain Garden Ornamentals
Bob Kern, Tropical Ponds Hawaii (TPH)
Randy Weir, Tropical Ponds Hawaii (TPH)
Mark Bornheimer, Tropical Ponds Hawaii (TPH)
Pat Maloney, Ohana Flowers of Hawaii
Pat Vahey, Aquatics Hawaii
Jason Akamine

Objectives

Year 1

1. Improve overall production of swordtails statewide.
2. Test and validate production techniques for all female swordtail individuals
3. Establish two additional varieties of homozygous lyretail strains

Year 2

1. Completion of a field tested technique that results in the production of all female homozygous lyretail swordtails.

2. Increase lyretail swordtail production statewide to account for 25% (e.g., 2,000 lyretails per month) of the current total swordtail production at project's end.
3. Technology transfer in the form of workshops, technical handouts and newsletter articles.

Year 3

1. Establish two varieties of homozygous lyretail strains.
2. Demonstrate that feminized females can alleviate the highly skewed female sex ratio on farm site.
3. Transfer technology to appropriate end users.

Principal Accomplishments

Year 1

Objective 1: Improve overall production of swordtails statewide.

This objective was to be achieved through several avenues, the first being to investigate the impact of improving water circulation in the production ponds at Tropical Ponds Hawaii (TPH) on the island of Hawaii. Unfortunately, not enough electricity could be generated to sustain continuous operation of the blower and further testing was not pursued. Upgrading to appropriate sized solar panels were cost prohibitive during that reporting period and further work would need to be pursued in future projects.

A second mechanism to improve overall swordtail production is to improve diversity of available swordtails. Working with Dave Cohen, Aquatic Innovations, a founder stock (n=100) of calico swordtails was purchased and quarantined at the Windward Community College (WCC) aquaculture complex. Swordtails were grown out till sexual maturity and excess fry were distributed to three farms (e.g., TPH, Rain Garden Ornamentals and Ohana Flowers of Hawaii) during the Year 1 activities The calico swords at the time were valued at a whole sale price of US$ 3.00/individual.

A request to improve the existing swordtail broodstock at TPH was partially addressed by providing the best red swordtail strain available at the WCC aquaculture complex. Approximately 300 broodstock individuals of the red variety were shipped to TPH during the reporting period. Likewise, approximately 1,000 juveniles of a highly productive common black swordtail variety was sent to TPH to establish broodstock for commercial production of that strain. The red variety was used to improve that particular product line at TPH and a standing request for additional varieties when available had been established with TPH. As mentioned previously, the downturn in the economy has resulted in major

changes in ownership and operation currently being undertaken at TPH.

Objective 2: Test and validate production techniques for all female swordtail individuals

The sex ratio of five varieties of swordtails that are found in the WCC Aquaculture Complex was ascertained and summarized in Table 1. The data clearly indicates the extent of the skewed sex ratio as all five varieties were found to have a female:male sex ratio that significantly deviated from a 1:1 ratio. The data also validates the claims by commercial farmers that this remains one of their greatest challenges, as it takes additional labor to sort through inventory to find enough males to complete an order, in addition to taking up space and consuming feed (Table 1).

The size frequency distribution of the various strains sampled during the reporting period were also obtained and have been summarized graphically. One example is the redwag variety that is presented in Figure 3. The sex ratio is the same as the redwag swordtail reported in Table 1 (3:1 Female:Male). It is not clear as to the mechanism(s) that result in these highly variable sex ratios, and review of the literature indicates it is probably linked to the fact that the swordtails do not have discrete sex chromosomes (Gordon, 1957; references cited in Yamamoto, 1969). Instead the sex determining genes are spread across throughout their chromosomes and apparently if the sum of male genes outweighs the sum of female genes the male phenotype is expressed. In any event, to alleviate this challenge a proposal was submitted during the Year 1 reporting period to CTSA soliciting funding for an additional year to address this constraint. The feminizing treatment that was suppose to be used for producing all female homozygous individuals would focus on the common swordtail broodstock in an attempt to alleviate the highly skewed sex ratio towards males. During the extension period feminizing two strains of swordtail fry had begun. The feminized fry were to be part of a field test as to the ability to produce a higher percentage of males.

Four doses of 17β-estradiol were administered to 3-day old fry for 30 days. Treatment with 400 mg kg/feed was determined to be the optimum dose resulting in a range of 91-100% feminization (Figure 4). No indication of any adverse impacts were detected on growth and survival. No detectable presence of intersex individuals were observed with fish examined histologically. Phenotypic females from this treatment underwent progeny testing to determine if the feminized males were reproductively functional. The results showed that a dose of 400 mg kg/feed 17β-estradiol results in feminization but also produces fertile fish. This work was done in partial fulfillment of an M.A. Degree from the University of Hawaii College of Tropical Agriculture and Human Resources, Department of Human Nutrition and Animal Sciences for Lei Yamasaki (Major Advisor, Spencer

Malecha).

Objective 3: Establish two additional varieties of homozygous lyretail strains

This objective was done in collaboration with another USDA project (U.S. DOA/CSREES 2004-34167-14801 and 2005-34167-16244) and the research results were used in partial fulfillment of a M.S. degree by Masaki Nasu (Major Advisor Dulal Borthakur) from the University of Hawaii, College of Tropical Agriculture and Human Resources, Department of Molecular Biosciences and Bioengineering. Three main approaches were used to identify DNA markers to distinguish a homozygous dominant lyretail individual from a heterozygous lyretail individual and these were: 1) Microsatellite primers, 2) Random amplified polymorphic DNA (RAPD), and 3) Suppression subtractive hybridization (SSH) cDNA library. Unfortunately, the ratio of homozygous to heterozygous individuals resulting from the micro-satellite analysis suggested a 1:1 ratio and the expected ratio from a heterozygous x heterozygous cross is 1:4 (Tamaru et al., 2003). Lastly, two homozygous individuals identified by progeny testing were misidentified by using the micro-satellite primer (Figure 5). The conclusion from the double blind test was that the marker is apparently for an allele that is unrelated to the recessive trait and therefore cannot be used to identify the homozygous individual.

Lyretails already available at WCC were amplified during the reporting period and were distributed to interested end users in order to begin establishing the lyretail product line on various farms. Approximately 500 female lyretails of the black and 300 marigold variety were sent to TPH to become the founder stock of lyretails at TPH. Approximately 250 of the black and marigold lyretails have also been distributed to Rain Garden Ornamentals and Ohana Flowers during the reporting period.

Year 2

Objective 1: Completion of a field tested technique that results in the production of all female homozygous lyretail swordtails.

Fry were separated into a control group and an experimental group receiving estrogen treatment. Feminized groups resulting from the treated group were stocked with males where mating was allowed to take place at random. Fry collected from each of these tanks were then collected and stocked into 40 gallon tanks (in replicate tanks) and were grown out to the point where the sex ratios can be verified. On January 9, 2009 the resulting sex ratios of the progeny tests were evaluated and the results are summarized in Table 2. Survival ranged between 48.4 – 92.4% but the most disturbing results were the highly skewed female biased sex ratios for all of the treatments.

This was not an expected result, as a sex ratio closer to a 1:1 F:M ratio was the desired outcome. The results indicate that the collection of fry from a production tank and grading them using a scoop net may not be sufficient in collecting fry that are of the appropriate stage/age.

Between March – May of 2008 four varieties of swordtail fry at approximately 1000 individuals each were received from Tropical Ponds Hawaii and underwent feminizing using the 400 ppm dosage as described previously. Fry were treated for one month and returned to TPH for growout and later production of broodstock that would be mated with males. Unfortunately, the results from the latter production ponds (n=4) were consistent with the results obtained at WCC and "a higher than normal" female production was observed although no exact numbers were available.

A new trial was initiated where individual clutches from five individuals from three varieties of swordtails were collected, separated into untreated and treated groups. The untreated group received Nelson Silver cup feed that has only been treated with ethanol and the feminizing groups were fed the same feed that has been impregnated with 400 ppm E2. In essence the experiment is being repeated but will be following the progeny from individuals and assessing the impacts of the feminizing treatment on male swordtail production after screening of progeny via progeny testing. While much more time consuming the results will be definitive as to whether the hypothesis being tested is correct and arguably should have been done earlier. Results of the feminizing stage of the overall treatment would become available by the end of the third year of the project due to the time necessary to carry out the progeny testing.

Objective 2: Increase lyretail swordtail production statewide to account for 25% (e.g., 2,000 lyretails per month) of the current total swordtail production at project's end.

Lyretail swordtail varieties were made available to outside farms in partial fulfillment of this objective. The intent was to provide a larger number of varieties of lyretails to farmers to diversity their product lines. TPH took the most advantage of this activity and utilized a different approach with the production of lyretails and places them in single production ponds. The lyretail varieties were not kept in discrete lyretails strains as is done for the common swordtails. The mixed lyre tail product approach was apparently more cost effective for their market demands and manpower requirements.

Artificial insemination of lyretails commenced during the Year 2 project in an effort to produce homozygous lyretail populations and had begun to undergo the various progeny testing to isolate homozygous individuals. Work is severely hampered because of the lack of a gene marker and the focus was reduced to the production of a single lyretail strain.

Objective 3: Technology transfer in the form of workshops, technical handouts and newsletter articles.

A total of three presentations were made. Two of these were at the Honolulu Aquarium Society's monthly meetings and provided updates on the search for the gene marker for the lyretail trait. The second centered on the feminizing work being done on swordtails. The third presentation was made at the international symposium held in Hawaii that honored the retirement of Dr. Yoshitaka Nagahama.

One newsletter article providing an update on the search for a gene marker was submitted to the Honolulu Aquarium Society for publication in their monthly newsletter. Lastly, the data obtained from the Masters work conducted by Lei Yamasaki was summarized into a manuscript and was submitted to the Journal of the World Aquaculture Society for review and publication.

Year 3

Objective 1: Establish two varieties of homozygous lyretail strains.

14 trials using artificial insemination (AI) to cross lyretail males and females were conducted. A total of only three crosses resulted in fry that have been grown out during the current reporting period. Of the three crosses that have resulted in progeny two of them had sufficient numbers of fry where both males and females (e.g., brothers and sisters) can be crossed with each other. Tese were artificially inseminated to produce the next generation of progeny, which should increase the probability of having homozygous lyretail individuals. As mentioned previously, however, the lack of a gene marker will hamper the progress of this effort as the identification of the homozygous individuals will rely on progeny testing. Such activities are planned with the availability of progeny that have been grown out to sexual maturity.

Objective 2: Demonstrate that feminized females can alleviate the highly skewed female sex ratio on farm site.

A separate feminizing trial was initiated during the previous reporting period where three varieties of swordtails would be investigated simultaneously. They are red, marigold and pineapple swordtails and 5 females of each variety were the source of fry for this trial. In this case the fry from the same female (n=20 or more) would be the basis for controls and treatment groups. During the reporting period the following fry were distributed and treated in 5 gallon buckets.

Feminized and control fry were grown to sexual maturity where the sex of each individual

could be confidently scored and the feminizing treatment could be assessed. As reported previously the untreated controls result in a sex ratio that is predominately female and the basis for the research being conducted. The results from this experiment was encouraging as unlike the treatment of fry taken from the production tanks where the majority of fry resulted in being feminized (e.g., 96% - 100%), only females resulted in all of the feminizing treatments indicating the appropriate ages for feminization were selected (Figure 5). Likewise, the parents from which the fry were produced are known and we have a better perspective of the lineage of the fry being tested.

The next phase females were placed into individual buckets and allowed to mate with a single male over the course of one month. If no fry are detected after a month another male is reintroduced. When fry are detected the female is removed and the fry are allowed to grow out in the same bucket until their sex is capable of being scored (duration of 4-5 months). Because of the duration of the grow-out periods during feminizing and then progeny testing, the work is still ongoing with some preliminary data being obtained. A graph summarizing the results (Figure 7) is provided in the Appendix. A breakdown of the results as of March 10, 2010 is provided in the Appendix.

While the work is still in progress there already is the same trend in that the untreated progeny results in an overall skewed sex ratio towards females as has been previously reported. However, it is also becoming clearer that the hypothesis that expression of the male sex is controlled by a threshold mechanism is correct and can be manipulated to our advantage. It is the best explanation of the results being obtained when using the strategy of first feminizing swordtail fry followed by mating with normal male swordtails. In this manner individuals accumulate male genes increasing the overall number of phenotypic males in the population. When the number of male genes exceeds a particular threshold the phenotypic sex of the individual will be male. The laboratory-scale trials, however, indicate that care must be taken to insure that all of the fry being feminized are of the same age group for the strategy to work. Demonstrating that this is the case at pilot-scale is the logical next step necessary to validate the working hypothesis.

Objective 3: Transfer technology to appropriate end users.

Swordtails and technical assistance were provided to Adam Baker, a graduate student with the Department of Molecular Biosciences and Bioengineering CTAHR. Under the supervision of Dr. Harry Ako, the fish and info was used in a project that was summarized at the 2009 CTAHR student symposium in a poster presentation.

In addition to the poster presentation one oral presentation was also made during the reporting period summarizing the Year 2 project. That presentation was made at the

Annual Progress Report to the Public, May 14, 2009, at the Oceanic Institute Learning Center. Lastly, a brief article summarizing the feminizing work was published in CTSA's Aquatips during the reporting period.

References

Gordon, M. 1957. Physiological genetics of fishes. In: M.E. Brown (Editor) The Physiology of Fishes. Academic Press, Inc., New York, New York. Volume II, Chapter X pp 481 – 501.

Tamaru, C.S., K. McGover-Hopkins, G. Takeshita, and M, Yamamoto. 2003. Creating the homozygous genotype for lyretail swordtails. Tropical Fish Hobbyist, #566, Volume LI(9):66-70.

Yamamoto, T. 1969, Sex Differentiation. In: W. S. Hoar and D.J. Randall (eds.). Volume **3**. Academic Press, New York. p. 117-175.

Impacts

Year 1

The Year 1 project had some significant indirect impacts in the awarding of two graduate degrees. Since completion of his MS dissertation, Masaki Nasu was accepted into the Ph.D. program with the John A Burn's School of Medicine and is currently working on obtaining his Ph.D. Lei Yamasaki was accepted into The College of Veterinary Medicine at Western University of Health Sciences and is currently in her fourth year and enrolled in the "Ornamental Aquaculture Production & Medicine Externship Rotation" at the University of Florida.

Despite not having the homozygous lyretails available at this time the impacts of the heterozygous lyretails distributed under the auspices of the project had started to have some modest impacts. TPH had experienced some initial setbacks in the overall production of the lyretail broodstock but had started to produce enough to begin marketing in Hilo at approximately 200 pieces/month. Farm gate prices that were being obtained were $1.00/piece.

An interview with Dave Cohen (Aquatic Innovations, Wholesaler), confirms that the lyretails had started to show up for wholesalers but at levels that were still too early to determine the size of the market. A quote from Dave Cohen, October 19, 2007. *"So far most of the lyretails have been quite large. I am finally getting something closer to the standard 2" swordtail. The production numbers are too low and too erratic to give me a good feel for what the market (or more accurately, my market) will do. Sales prices are +75% over non-lyretails (nonlyretails farm-gate are $0.35/piece for 2 inch) at this point. These production and sales numbers*

cannot give me an accurate reading on what the larger wholesale market will pay or what will happen at larger volumes. I hope to see that this winter if TPH can get their numbers up".
Probably the most progress that was made with the lyretails was with Rain Garden Ornamentals (e.g., Steve Hopkins). Large lyretail swords coming out of the continuous production system had opened a new market for Rain Garden Ornamentals and that is sales to local pet stores and direct sales to the consumer. The farm gate price to local pet stores is $0.75 per piece and in direct retail sales to consumers Rain Garden Ornmamentals receives $2.00 to $3.00 per piece depending on quantity. The quantities available for the large sizes are limited and adult swordtails do not seem to become as attractive in appearance unless the other adults are continually cropped out of the system to control competition.

Year 2

In correspondence dated March 9, 2009 from Mark Bornheimer of Tropical Ponds Hawaii he states that "we are selling more Lyretails" and that has been a direct result of the various strains provided under the auspices of the project. While production of those varieties at farm site has increased the news has to be tempered with the overall downturn in the freshwater ornamental business and economy in general as sales has slowed considerably. At present, the economic downturn in the economy has forced major changes in operations at all three facilities in addition to the primary wholesaler who was to market the swordtails.

Year 3

At present there are no major impacts at the level of industry as the results are still being determined experimentally and at laboratory-scale. Currently the use of estradiol 17B as the feminizing agent is only approved for use in a research setting. Use at the commercial level will require formulation of an INAD that a feed company would be willing to invest in and distribute feminizing feed. Initial inquiry with Rozalyn Schnick about whether this would be feasible indicates that there would need to be a relatively large demand for the use of a feminizing feed for a commercial entity to be interested in pursuing such an endeavor. For the results to be taken to commercial scale will be a challenge as the only major interest at this point in time is for masculinizing feeds rather than feminizing ones.

Recommended Follow-Up Activities

The work initiated in demonstrating that the strategy of first feminizing swordtails can be used as a means to improve the sex ratio will be completed in spite of the termination of the project. Fry from the progeny testing are at various stages of being grown out and the

remaining individuals will be grown till their sex can be confidently scored. That result will be summarized and the manuscript that was withdrawn will be revised and resubmitted. Likewise, with the production of a homozygous lyretail population as at the moment we have progeny that are a mixture of homozygous and heterozygous individuals that are being grown out. The next generation of progeny is suppose to have a ratio of 1:1 homozygous:heterozygous and the progeny testing is ongoing to identify the individuals that are homozygous, albeit with only one strain.

Publications and Manuscripts Written and Papers Presented

Publications:

Nasu, Masaki, 2006. Department of Molecular Biosciences and Biosystems Engineering, College of Tropical Agriculture and Human Resources, M.S. Dissertation. Identification of DNA markers for the homozygous lyretail genotype in swordtail fish. December 2006.

Yamasaki, L., S. Malecha, D. Vincent and C.S. Tamaru. 2007. Department of Human Nutrition and Animal Sciences. MA Dissertation. Feminization of *Xiphophorus helleri* by dietary administration of 17ß-estradiol.

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Tamaru, C.S., L. S. Yamasaki, K. McGovern-Hopkins, S. Malecha, and D. Vincent. 2009. Feminization of Commercial Swordtails, *Xiphophorus helleri,* by Dietary Administration of 17ß-Estradiol. Aquatips, Center for Tropical and Subtropical Aquaculture. http://www.ctsa.org/upload/note/Feminizing_Swordtails633806780645168749.pdf

Presentations:

Nasu, Masaki, 2006. Department of Molecular Biosciences and Biosystems Engineering, College of Tropical Agriculture and Human Resources, M.S. Defense.Identification of DNA markers for the homozygous lyretail genotype in swordtail fish. December 2006.

Tamaru, C.S., Lei S. Yamasaki, Spencer Malecha, Kathleen McGovern-Hopkins and Douglas Vincent. Submitted. FEMINIZATION OF *Xiphophorus helleri* BY DIETARY ADMINISTRATION OF 17ß-ESTRADIOL. Aquaculture America 2008 February 9 -12 2008, Lake Buena Vista, Florida USA.

Tamaru, C.S., M. Nasu, D. Borthakur and K. McGovern-Hopkins. 2008. Update on the

search for a gene marker for the lyretail trait in swordtails, *Xiphoporus helleri.* July 4, 2008 Honolulu Aquarium Society.

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Tamaru, C. S. and K. McGovern-Hopkins. 2009. Improving Outputs in the Commercial-Scale Production of Swordtails in Hawaii, Year 2 Contract No. 2006-189. CTSA's Annual Progress Report to the Public, May 14, 2009, Oceanic Institute Learning Center

Baker, A., H. Ako and C.S. Tamaru. 2009. Manipulation of the arachidonate to eicosapentaenoate ratio and its effect on fry production in freshwater ornamental maturation feeds. CTAHR Student Research Symposium, April 3-4, 2009. *NOTE: Poster presentation by Baker et al., 2009 would receive the Gamma Sigma Delta Award of Merit for a CTAHR graduate student poster presentation*

10. Pacific Regional Aquaculture Information Service for Education (PRAISE) and Publications, Year 8

General Information

Reporting Period October 1, 2009 to September 30, 2010 (PRAISE Termination Report; Publications Progress Report)

Funding Level	PRASIE:	$20,000
	Publications:	$64,079
	Total:	**$84,079**

Participants

Kristen Anderson, Reference Librarian
University of Hawaii at Manoa

Meredith Brooks, Information Specialist, CTSA

Sarah Myhre, Assistant
University of Hawaii at Manoa

RESULTS AT A GLANCE…

- The dollar value for PRAISE's services is caluculated as follows: 10,407 queries averaging 3 minutes each or:

520.35 hours online @ $80/hr =	$ 41,628
825 articles @ $35.00 ea. =	$ 28,875
Total =	$ 70,503

-PRAISE scanned over 385 documents for digitization projects with Hawaiian fishpond and MOP documents.

- The Publications project began an e-newsletter *Regional e-Notes*, which is developed and distributed to approximately 1,000 subsribers monthly.

- The Publications project manager helped facilitate and organize CTSA's FY2010 Development process, the Annual Accomplishment Report, and the Plan of Work.

Objectives

PRAISE

1. Contribute Hawaiian names for marine fauna to www.ubio.org
2. Collect and disseminate aquaculture technical information or news related to CTSA region in PRAISE and CTSA web sites four times per year.
3. Promote Pacific Region Information infrastructure.

4. Technology transfer.

Publications

1. Inform industry members, educators, and other key individuals of pertinent aquaculture information, and update them on the status of regional aquaculture through various media.

2. Inform the aquaculture community and interested parties of the progress of CTSA and other Regional Aquaculture Center (RAC) projects in relation to our mission through the dissemination of our own and other publications.

Principal Accomplishments

PRAISE

Objective 1: Contribute Hawaiian names for marine fauna to www.ubio.org

There are currently thousands of species listed in ubio and most of those now include not only the Hawaiian name but also the local names used in many Pacific nations/languages. PRAISE staff verified over 700 entries and added an additional 81. This project will be ongoing as staff have time.

Objective 2: Collect and disseminate aquaculture technical information or news related to CTSA region in PRAISE and CTSA web sites four times per year.

PRAISE staff will continue to regularly submit news items of interest to aquaculture in Hawaii and the Pacific to CTSA. The PRAISE website links to AquacultureHub and posts new publication announcements as they are found. This project has proved to be quite popular and has been incorporated into standard operating procedure.

Objective 3: Promote Pacific Region information infrastructure.

The PI and Assistant continue to promote and work to expand the online digital resources related to aquaculture. We are attempting to get copyright permission to add more documents to some of our bibliographies, particularly the Hawaiian Fishpond Bibliography.

Objective 4: Technology transfer.

PRAISE staff have delivered research support as documented in the Impacts section below. We continue to assist students, faculty, researchers, and farmers on a daily basis.

We have completed scanning the non-copyrighted documents on Hawaiian fishponds that are listed in the fishpond bibliography. PACRC staff report that their site has been updated and have asked us to scan some more materials. We are nearly done scanning the existing papers of the University of Hawaii's Marine Option Program (MOP). We will need to complete the scanning, compile all the documents, and index them before loading in to the UH institutional repository, ScholarSpace. We will then establish a procedure for submitting all new MOP papers to the database as they are produced.

Publications
The Center switched from traditional printed dissemination to an online format by establishing an e-newsletter in December 2009 titled *Regional e-Notes*. This newsletter is sent to nearly 1,000 subscribers each month, and includes dissemination of CTSA project results, as well as regional aquaculture news and event announcements. The "Pacific Spotlight" section highlights aquaculture news from CTSA regional locations outside of Hawaii. The project continuously works with P.I.'s to help compile and disseminate publications resulting from CTSA-funded projects. In addition, the Center has become active on the aquaculture networking website *aquaculturehub.org*, and has begun developing a new CTSA website scheduled for completion by the end of 2010.

This project is also responsible for the preparation of CTSA's Annual Plan of Work and Annual Accomplishment Report. The P.I. has worked extensively on CTSA's FY2010 development process and the preparation of the Plan of Work, and has compiled this Annual Accomplishment Report from project progress and termination reports.

Impacts

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

10,407 queries averaging 3 minutes each or:
520.35 hours online @ $80/hr = $ 41,628

825 articles @ $35.00 ea. =	$ 28,875
Total	$ 70,503

In replying to 543 requests for direct assistance, 3,466 of those queries were emailed to PRAISE patrons. The 825 articles represent 16,019 pages delivered exclusively by email. In addition, the staff responded to 39 miscellaneous requests. The PRAISE Web site is a bonus. It allows users to make requests online, provides links to resources for students in the region, and gives local vendors a venue to advertise themselves to the world.

For our digitization projects with Hawaiian fishpond and MOP documents, PRAISE staff scanned over 385 documents totaling some 28,000 pages so far. Most will be freely available on the University of Hawaii at Manoa's institutional repository, ScholarSpace.

The information dissemination activities under the Publication project have helped extend CTSA and other RAC research to industry stakeholders and interested parties throughout the region. The new newsletter format has allowed the Center to reduce its environmental impact by distributing pertinent news in an electronic format.

Recommended Follow-Up Activities

It would be smart of us to try and get copyright permission for all of the documents we scan. Contacting the authors and publishers is a time consuming and disheartening process, but I believe we could get enough permissions to make the process worthwhile. We continue to develop new informational and educational products and look forward to collaborating with the directors of UH System aquaculture programs.

Publications and Manuscripts Written and Papers Presented

None to report.

11. Developing Bivalve Culture to Diversify and Position Hawaii as a Supplier of Safe, Premium Edible Shellfish Products, Years 1 and 2

General Information

Reporting Period: July 1, 2009 to October 31, 2010; no-cost extension through December 31, 2010 (Year 2 Progress Report)

Funding Level

Year	Amount
1	$40,342
2	**$32,992**
Total	$73,334

Participants

Robert Howerton, Ph. D.
Aquaculture Extension Specialist
University of Hawaii Sea Grant

Walter Ritte, Loko i`a

Brian Goldstein, Kona Bay Marine

Lynn Nakasone, DOH

Noelani Lee, KMH

Dave Nesbit, Goose Point Oysters

Paul Bienfang, Analytical Services Inc.

RESULTS AT A GLANCE…

- The project has demonstrated the biological feasibility of edible bivalve culture, identified steps necessary for the certification of a laboratory in Hawaii, and is addressing these steps.

- The project has also made significant advances to build capacity for Hawaiian fishpond operators to grow shellfish in coastal areas.

- As of November 2010, over 10,000 Hawaiian Oyster spat (>1 CM) have been produced. In June 2010, growout trials were begun in Oahu fishponds with good preliminary results.

Bruce Anderson, Hawaii Pacific University

David Cohen, Aquatic Innovations

Lori Nagatoshi, DOH

Hi'ilei Kawelo, Paepae o He`eia, He`eia Fishpond

Keli'i Kotubetey, Paepae o He`eia, He`eia Fishpond

Alan Riggs, ADP

Maria Haws, UH Sea Grant

Leonard Young, ADP

Steve Chaiken, Moloka`i Sea Farms

Objectives

1. Determine which Hawaiian bivalve species represents the best potential for culture. Conduct preliminary spawning, hatchery, nursery and grow-out trials in a laboratory setting for three Hawaiian species determined as having the most potential.
2. Determine whether permits can be obtained to culture established, non-native bivalves in open waters, and if successful, conduct grow out trials with these species in fishponds.
3. Building on preliminary efforts, conduct a study to collect economic and market data for bivalves for mainland United States, Asia and Europe.
4. Develop two pilot bivalve grow out sites on Moloka`i for use in demonstration growth trials and as possible future commercial grow out sites.
5. Technology transfer, including publication of hatchery and grow out manuals on Hawaiian bivalve culture.

Principal Accomplishments

Objective 1: Determine which Hawaiian bivalve species represents the best potential for culture. Conduct preliminary spawning, hatchery, nursery and grow-out trials in a laboratory setting for three Hawaiian species determined as having the most potential.

Field surveys were conducted on the islands of Moloka`i, Hawaii and Oahu to find sources of broodstock for the hatchery trials. A summer intern sponsored by the UHH PIPES internship program spent two months on Moloka`i during June and July 2007 conducting surveys in Keawanui fishpond, other traditional Hawaiian fishponds and coastal areas. Very few live specimens were found although Crassostrea gigas shells were found in some areas. Live Dendostrea sandvichensis (Hawaiian oyster, previously Ostrea sandvichensis) were found at several sites. Oral histories on bivalve presence, abundance and traditional use were also collected by the intern. It appears that the abundance and distribution of all bivalves on Moloka`i have decreased significantly over the past generation to the point where very few can be found today.

Multiple species have been identified as potential culture candidates; the main barrier is finding sufficient numbers of broodstock. Most species have become very rare over the last thirty years and are hard to find.

There are potentially at least 2 dozen Hawaiian bivalve species that could be good aquaculture candidates, foremost among them the clams. It should be noted that despite the generally small size of the Hawaiian oyster, which grows up to 2.5 inches, its taste is excellent and its smaller size is not a barrier. It could be developed as a substitute for the Kumamoto oyster, which is the most popular and highest priced half shell oyster species in the NW. Surveys were then conducted at the He`eia fishpond in Kaneohe and adjacent areas within the bay. Hawaiian clam broodstock has been found on Oahu in sufficient numbers to transfer to PACRC and begin spawning trials. Abundant numbers of D. sandvichensis were also found and transferred to PACRC.

The Hawaiian oyster, Dendrostrea sandvichensis, was successfully spawned fifteen times with larvae numbers ranging from 10,000 to 50,000. Spat were raised at PACRC and have been distributed to ponds and farms for grow-out trials. Several attempts at spawning and conditioning have been executed but it appears that lower temperatures are needed for conditioning. This is now being tested. Pearl oysters have also been successfully spawned. Several other species are planned for trials, but have had trouble finding sufficient numbers of brood stock.

As of November 2010, over 10,000 Hawaiian Oyster spat (>1 CM) have been produced. In June 2010, growout trials were begun in Oahu fishponds with good preliminary results.

They have also been stocked into the Hale `O lono fish pond in Hilo where similar growth studies are being conducted in partnership with the teachers and students of a Hawaiian immersion school. A second trial will be started next month in another Big Island fish pond.

Taste tests were also conducted with the Hawaiian oyster using specimens which had reached a size of approximately 4-5 cm. All testers stated that the taste was excellent and had commercial possibilities. Several opined that even rather small oysters could have a market as "cocktail oysters" given their excellent flavor. Tasters variously classified the taste as "sweet", "nutty" and "salty". One mentioned a cucumber like flavor, which is one of the flavor characteristics of Ostrea lurida, the native West Coast oyster (considered endangered) which is now the focus of restoration and culture efforts in Washington state. Clam spawning will resume in January, which appears to be the natural spawning season. Meanwhile, broodstock of two species (Pinna bicolor and Streptopinna saccata) of pen shell has finally been obtained after two years of searching in areas where beds of these were historically reported. There is some concern about the scarcity of these species since apparently beds no longer exist, although single specimens are still rarely found. The specimens being held at PACRC are being conditioned and spawning will be attempted in December.

Objective 2: Determine whether permits can be obtained to culture established, non-native bivalves in open waters, and if successful, conduct grow out trials with these species in fishponds.

There is considerable cooperation from the DOH in assisting with the revival in the bivalve industry. It was stated in the Shellfish Working Group meeting that the FDA is willing to help with the certification of a state laboratory to the specifications of the Interstate Shellfish Sanitation Committee (ISSC) guidelines. A member of the DOH staff has gone through a preliminary training session with FDA on the shoreline certification process. Follow up training with the FDA is to be continued in the near future.

Import permits were approved for Crassostrea gigas, C. virginicus and manila clams for four sites; Keawanui fishpond, Keawanui Farms, and Heei`a fishpond as well as Moli`i fishpond (Figure 1). Results of initial growth trials were variable and juvenile oysters have recently been distributed for additional growth trials

All identified grow-out sites, including PACRC, have received DOA PQ-7 permits (Permit Application for Restricted Commodities into Hawaii). The species included on the application are Crassostrea gigas, C. sikamea, Tapes semidecussata and the Hawaiian oyster, Dendostrea sandvichenis.

Objective 3: Building on preliminary efforts, conduct a study to collect economic and market data for bivalves for Mainland U.S., Asia and Europe.

In preparation.

Objective 4: Develop two pilot bivalve grow out sites on Moloka`i for use in demonstration growth trials and as possible future commercial grow out sites.

Preliminary site surveys were conducted on Moloka`i by the PI and a representative from Goose Point Oysters, Inc. Four Hawaiian fishponds were identified as potential sites for use in growth trials. In addition, He`eia and Moli`i fishponds on Oahu were found to be suitable sites and are being used for growth trials. The sites on Moloka`i that are being used for bivalve growth trials include Keawanui fishpond (Hawaiian Learning Center), Moloka`i Sea Farms and Keawanui Shrimp Farm. Additional possible sites include Ualapu`e fishpond and Honouliwai fishpond on Moloka`i and Sunrise Capital on Kauai. There is also interest from mainland groups in starting bivalve hatcheries in Hawaii if suitable sites can be found. The co-PIs are working with these groups on that initiative as well.

Objective 5: Technology transfer, including publication of hatchery and grow out manuals on Hawaiian bivalve culture.

Training fishpond operators in grow-out technology began in December 2008 after the transfer of oyster and clam spat to sites on Moloka`i and Oahu. Training is occurring at the PACRC hatchery for students, including two Pacific Island students and four UH-Hilo students, who all plan to work in aquaculture in either Hawaii or the Pacific region. Requirements to comply with shellfish sanitation guidelines was a topic of discussion in the third bivalve working group meeting held September 9, 2009 on Moloka`i. There have been additional workshops at He`eia fishpond on Oahu and Keawanui fishpond on Oahu on alternative grow-out techniques in May, 2010.

A manual on hatchery and growout methods is currently in draft.

Impacts

Arrangements were made to transfer spat to the four demonstration sites in early December 2008. A workshop was held with all cooperators participating. Spat were put in mesh bags and animals are being monitored for growth and mortalities. Water quality

parameters are also being measured. If successful, a large sector of the commercial aquaculture landscape currently missing; bivalves could develop into a significant part of the industry. Numerous existing aquaculture producers could supplement income and increase production and many more aquaculture operations could be developed. Hatchery trails were successful with the Hawaiian oysters.

Recommended Follow-Up Activities

We have asked for and received a no-cost extension for year two of this project. Year two activities have consisted of distributing more spat and monitoring growth and mortalities at the four demonstration sites. Hatchery work has continued on indigenous bivalve species. If sufficient numbers of spat are available they will be made available to interested fishpond operators for grow-out.

Publications and Manuscripts Written and Papers Presented

"Developing a Community-Based Shellfish Industry for Hawaii". Presentation at Int. Conference on Shellfish Restoration. Nov. 22, 2008. Charleston, S.C.

"Developing a Bivalve Industry for Hawaii". Presentation at Hawaii Aquaculture Association Conference. July 9, 2009. Kapiolani Community College, Honolulu, HI

12. Development of Captive Culture Technology for the Yellow Tang, Years 1 - 3

General Information

Reporting Period: January 1, 2008 to September 30, 2010; no-cost extension through December 31, 2010 (Year 1 Final Report, Year 2 Progress Report)

Funding Level

Year	Amount
1	**$92,500**
2	**$100,000**
3	$100,000
Total	$292,500

Participants

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RESULTS AT A GLANCE…

- Established appropriate holding systems and diet for conditioning and maintaining spawning stocks of yellow tang providing a year-round supply of viable eggs and larvae.

- Developed a larval rearing system supporting survival and early development of these extremely small and delicate newly hatched larvae.

- Successfully got the larvae to start feeding using our copepod-based hatchery methods under development at OI.

- The research group includes two Hawaii Pacific University graduate students.

Objectives

Year 1

1. Establish appropriate holding system/conditions for maintaining spawning stocks of yellow tang.
2. Develop appropriate diet to maintain broodstock condition and produce high quality eggs.
3. Establish early larval rearing system to maximize larval hatch and early (pre-feeding) survival.

Year 2

1. Identify suitable first feed for yellow tang larvae.
2. Scale-up culture of identified first feed to level required for conducting replicated larviculture trials.
3. Develop larviculture feeding regimen suitable for rearing yellow tang larvae through metamorphosis.

Year 3

1. Develop suitable methods to transition yellow tang into juvenile settlement phase.
2. Establish suitable feeds and holding system to ensure juvenile quality suitable for marine ornamental wholesale market.
3. Transfer technology to industry through workshops, conference presentations, and publication in CTSA Regional Notes.

Anticipated Benefits

The development of captive culture technology for yellow tang and other high-value reef species is imperative to protect our increasingly threatened coral reef ecosystem. Not only will captive production technologies help take pressure off wild fish populations, they will also provide new economic opportunities associated with the nearly billion dollar worldwide trade in marine ornamental species. Clearly the yellow tang has proven to be a very difficult species to culture and will require significant progress to overcome current bottlenecks to captive culture. However, OI researchers have made significant progress and are well positioned to address apparent challenges in (1) securing a year-round supply of viable eggs, (2) identifying an appropriate first feed, and (3) scaling up egg production, live feeds culture and hatchery production methodologies. The establishment of the proposed captive production technologies will lead to the immediate emergence/expansion of the

new commercial marine ornamental industry in Hawaii. Captive production of yellow tang will provide a sensible alternative to current wild collection practices, helping to reduce pressures on wild stocks and allowing us to preserve our coral reef ecosystem.

Work Progress and Principle Accomplishments

Year two project activities (in progress) are focusing on a continuation of broodstock trials examining the effects of diet and water source on reproductive performance of yellow tang broodstock along with efforts to optimize the early larval rearing environment, describe early larval development and identify a suitable first feed for the very small mouthed larvae.

Objective 1: Establish appropriate holding system/conditions for maintaining spawning stocks of yellow tang.

Broodstock recruitment: Yellow tang broodstock were collected with the assistance of Richard Xie of Hawaii Sea Life, Inc. and brought back to the Oceanic Institute (OI) where they underwent an initial assessment followed by a one-month quarantine period prior to stocking in broodstock holding systems. In our earlier work we found that using larger, more mature fish improves egg production and egg quality. However, due to the sexually dimorphic nature of the species (males being larger than females), selecting older/larger fish created a problematic bias in the sex ratio toward males. Working with UH graduate student Megan Bushnell, we were able to demonstrate that mature egg-producing females can be identified by the development of an enlarged ovipositor (Fig. 1). This now allows us to select older females for stocking broodstock tanks.

Broodstock holding systems: Fish were stocked in either larger 25m^3 outdoor fiberglass tanks maintained on OI/SLP well water or smaller 5m^3 fiberglass tanks maintained under OI/SLP well water, treated OI/SLP well water, or recirculating ocean water as described later under this objective.

Under year one activities we established working broodstock populations in a large (25m^3) tank and twelve small (5m^3) tanks. Thirty-three recruits were stocked in the 25m^3 outdoor fiberglass tank, and another 96 fish were allocated into twelve 5m^3 experimental tanks (8 fish/tank). These smaller tanks were used to conduct replicated trials examining the effects of diet and water source on reproductive performance and egg quality.

Broodstock in the 25m^3 tank provided the largest and most reliable supply of eggs throughout the project period (Fig. 3). Mean spawn size over this period was

approximately 30,000 eggs/spawn with relatively good rates of fertility (~73%), but low rates of viability (~31%) with a large proportion of the fertilized eggs failing to complete embryonic development prior to egg collection and evaluation. Despite the low number of usable eggs (mean 177,365/month), this output is much improved over our historic production, therefore allowing us to begin developing larviculture procedures for this species.

Water Treatment Trial: The water treatment/source trial initiated in year one continued into the second project year with two males and six females allocated into each of the twelve 5,000L fiberglass broodstock holding tanks. Four "control" tanks were maintained in outdoor tanks. Stocks in all treatment groups initiated spawning activity relatively quickly (Fig. 4) with relatively even egg output throughout the project period in all treatment groups. The RAS treatment group tended to have slightly higher egg output (635 eggs/spawn) relative to control (442 eggs/spawn) and degassed (399 eggs/spawn) treatments. However, spawn sizes remained remarkably small (generally less than 1,000 eggs/spawn) in all of the treatment groups throughout the study period. Fertility rates were somewhat variable over the early part of the project, but increased to remarkably consistent mean rates of over 70% by the last six months of the project. Again, egg viability rates remained low throughout the project period (mean 21%) with slightly improved rates in the degassed water (28%) compared with either control (16%) or RAS treatments (20%).

Despite the lack of improvement in reproductive output or egg quality through changes in either water source or treatment, we did see a remarkable change in fish condition in the ocean water RAS treatment group. Yellow tang (and flame angelfish) tend to lose condition over extended period of captive maintenance in standard broodstock holding systems with the gradual appearance of head, lateral line, and fin erosion (Fig. 5a). These effects appear particularly severe in the fish maintained in our shared OI/Sea Life Park well water system with high levels of total gas (~110% saturation) and lowered pH (pH 7.4) due to increased levels of CO_2. Although the degassing water treatment effectively resolved gas supersaturation issues, it did not appear to affect fin erosion. However, the maintenance of yellow tang in the RAS system yielded a remarkable improvement in fish condition over the study period (Fig. 5b). Consequently yellow tang stocks will be converted to the RAS water treatment using ocean water for the remainder of the project.

Objective 2: Develop appropriate diet to maintain broodstock condition and produce high quality eggs.

Previously, yellow tang broodstock have been maintained on a mixed diet, consisting of various commercial marine ornamental pellet and flake foods, in addition to a "raw" diet consisting of blended squid, shrimp, peas, spinach, Nori seaweed, and spawned fish eggs.

This "shotgun approach" appears to have at least partially met the basic nutritional requirements for stock maintenance and year-round spawning. However, our research with flame angelfish has demonstrated that species-specific broodstock diets can be formulated which improve reproductive performance and egg quality over less-defined mixed diets and offer greater levels of stock biosecurity. Given similar problems with yellow tang egg quality, and based on our recent research with flame angelfish, we have formulated a high protein (60%), low lipid (16%) diet that is high in DHA (27mg/g DW) and arachidonic acid (2.2 mg/g DW) for testing on yellow tang broodstock. Total n-3 HUFA levels are 3.8% of diet, with a DHA:EPA ratio of 2.7, and EPA:ARA ratio of 4.6 (Table 1).

Diet trials testing this formulated diet against our standard feeding protocols were initiated in mid-May on two of the four replicates under each water treatment protocol generating six tanks under control diet against six tanks on the test broodstock diet. The new diet was relatively well accepted by yellow tang stocks (some acclimation was required) with stocks showing a strong feeding response to this formulation. Although the formulated diet did not significantly improve egg production or tested egg quality parameters, the formulated diet did work as well as the complex mixed raw diet, thus providing a simpler and more biosecure, and possibly consistent dietary source for yellow tang maintenance (Fig. 6).

Post-trial egg production: With the completion of the CTSA yellow tang broodstock trials last spring, we sorted through the remaining stocks and established three small ($5m^3$) broodstock holding tanks with 12 yellow tang/tank held under recirculating ocean water (the best of the tested protocols under the CTSA project) and retained two sets of ~30 yellow tang held in the larger ($25m^3$) broodstock tank under OI flow-through water supply. Unfortunately we do not have sufficient resources to convert the larger tank systems to ocean recirculation. All of the captive broodstock continue to show highly variable egg output with a clear lunar pattern in egg production centered around each full moon (Fig. 7).

The resorted broodstock from the $5m^3$ tank trials rapidly adjusted to the reorganization and have demonstrated dramatic improvements in egg output from May to September this year (Fig. 8). The combined improvements in egg output and egg quality from both sets of broodstock has greatly improved egg supplies for larval rearing.

Objective 3: Establish early larval rearing system to maximize larval hatch and early (pre-feeding) survival.

With improving egg supplies we have initiated efforts to hatch and rear yellow tang larvae in a range of hatchery systems. Results have quickly revealed that larvae behave quite differently from those of flame angelfish and that our early larval rearing systems and

protocols are not adequate to get large number of larvae through the pre-feeding period and onto first feeds.

With increasing egg supplies, small numbers of yellow tang larvae were examined through embryonic and the early stages of larval development (Fig. 7). Careful examination of hatch and early survival rates under static and low aerations levels showed slightly improved hatch rates with aeration followed by slightly lower survival rates (relative to static conditions) through the first five days after hatch (Fig. 7). However, survival under both approaches is lower than acceptable. Microscope examination of developing larvae through this early larval rearing period revealed rather rapid utilization of yolk reserves (Fig. 8) which may have left larvae nutritionally deficient before they have an attempt to start exogenous feeding. An initial trial examining the effect of lowering the culture temperature from 27ºC to 22ºC slowed the rate of yolk utilization (Fig. 9).

However, subsequent trials conducted under alternate funding demonstrated that although lowering rearing temperature effectively lowers larval developmental rates, the lowered yolk utilization rate did not translate into improved survival. Instead larval survival appears highest at 76 to 80°F which is the natural environmental temperature range for the species. Future hatchery work with this species will target a rearing temperature around 78°F (25.6°C).

As egg supplies increased dramatically over the last few months we were able to run larger trials examining early (pre-feeding) yellow tang larval development. Early behavior differences between flame angelfish (upon which we are basing our methods) and yellow tang larvae appear important in the initiation of feeding. Newly hatched larvae of both species lack developed eyes or mouths and spend the first day on the tank water surface. At day one the flame angelfish larvae transition to the tank bottom while completing mouth and eye development followed by transition into the water column on day 3 as first-feeding larvae. The yellow tang transition from the tank water surface directly into the water column on day two where they complete eye and mouth development and also begin feeding on day three. In contrast to the flame angelfish, yellow tang larvae do not appear to be affected by tank size, stocking density, or water exchange rates making them a much hardier species at this early developmental stage.

Year 2

Objective 1: Identify suitable first feed for yellow tang larvae

Initial trials using methods successful for the flame angelfish resulted in the yellow tang larvae filling their guts with microalgae, but they did not appear to consume harvested *Parvocalanus* nauplii, resulting in no survival through the early feeding period.

In subsequent trials we increased both the algae and *Parvocalanus* copepod nauplii densities and despite most of the ingested material appearing unidentifiable (likely microalgae), we did see a small number of yellow tang larvae that were clearly consuming copepod eggs at this first feeding stage (Fig. 9). The feeding larvae survived to day 9 and clearly showed signs of development, including a reorganization of tissue at the site of dorsal spine development (Fig. 8).

We also explored using eggs/nauplii from a second species of local calanoid copepods, *Bestiolina similis* obtained courtesy of the University of Hawaii. Based on our findings, and on information from the literature, it does not appear that *Bestiolina* is particularly suited for culture given challenges in generating significant numbers of copepod nauplii, at least with current production approaches.

Over recent months with increasing availability of viable yellow tang eggs and completion of algae room renovations, we were able to increase the scale of larval rearing efforts. Given that the larvae of many marine species do not perform well in small tank systems, we conducted a trial comparing pre-feeding larval survival in tanks ranging from 200L to 1000L. Upon initiation of feeding there was a large difference in feeding rates, with essentially no feeding in the smaller (18L) tanks and excellent feeding by day four in the larger 200L and 1000L tanks. A notable difference between the small (18L) tanks and larger (200L and 1000L) tanks was that of tank color, with the larger tanks having black surfaces and the smaller tank having a grey color. Indeed a follow-up trial discovered a large effect of tank color with yellow tang larvae feeding well in both small and large black tanks, while feeding poorly in smaller grey, green, and blue tanks.

At present we can repeatedly get large number of yellow tang eggs hatching into viable larvae and have resolved a number challenges in getting larvae through the exogenous feeding stage. These larvae appear to feed very well on our *Isochrysis*/*Parvocalanus* copepod feeding regimen with more than 80% of the examined larvae having full guts (Fig. 10). Through this early feeding stage the larvae show dramatic changes in appearance as they begin developing. However, despite strong feeding and early signs of development in both small and large scale black tanks, the larvae begin to disappear by day five and are almost completely gone by day six. As we near the end of year two project activities we are currently examining effects of background algae, water source, and copepod egg/nauplii stage on larval performance as they transition through the first feeding period and begin to grow and develop.

Objective 2: Scale-up culture of identified first feed to level required for conducting replicated larviculture trials.

Given the small size of these first feeding larvae and clear evidence that they can consume copepod eggs, and possibly first-stage nauplii, we are currently focusing on scaling up our microalgae and *Parvocalanus* cultures and developing methods to maximize collection of eggs and early nauplii.

Microalgae production: The first step has been to scale up production of *Isochrysis* and *Chaetoceros* microalgae using an array of indoor photobioreactors under controlled/optimized environmental parameters. Using alternate funding we renovated our algae production laboratory (See Fig. 11) to facilitate generating sufficient quantities of high-quality *Chaetoceros* and *Isochrysis* microalgae to meet copepod and larval rearing requirements.

Copepod Production: Copepod maturation and nauplii production systems were established in separate laboratories for scaling up copepod nauplii production. The maturation system utilizes a series of 1,000L tanks to mature copepod nauplii into egg producing adults. Tanks are stocked with newly hatched nauplii on a daily basis and harvested after one week for maintaining adult densities in the nauplii production systems. The nauplii production system utilizes 1,500L tanks for daily harvest of eggs and nauplii (Fig. 12). Adults are returned to the production system, with female densities maintained through regular adult supplementation as needed.

Objective 3: Develop larviculture feeding regimen suitable for rearing yellow tang larvae through metamorphosis.

With increased supply of viable larvae and development of suitable early larval rearing systems we have had some exciting success in getting newly hatched yellow tang larvae to initiate feeding. The live feeds system is being scaled up to generate sufficient numbers of early stage copepod nauplii to support continued larval development and get fish to the later larval stages. As these methods progress we will hopefully begin getting early feeding yellow tang larvae to the later larval stages and begin establishing suitable feeding regimens to get these larvae to metamorphosis.

Year 3

Objective 1: Develop suitable methods to transition yellow tang into juvenile settlement phase.

Planned studies on methods to settle yellow tang post-larvae is scheduled for year three project activities (we are currently part way through year two), and requires the successful

rearing of yellow tang larvae and generation of sufficient numbers of post-larvae to conduct planned trials.

Objective 2: Establish suitable feeds and holding system to ensure juvenile quality suitable for marine ornamental wholesale market.

Planned studies on juvenile feed and holding systems for yellow tang is scheduled for the end of year three project activities (we are currently part way through year two), and requires the successful rearing of yellow tang larvae and generation of sufficient numbers of juveniles to conduct planned trials.

Objective 3: Transfer technology to industry through workshops, conference presentations, and publication in CTSA Regional Notes.

Since project initiation we have been active in transferring technology through conference proceedings and workshops as listed under the publications and presentations section below.

Impacts

Project activities are still in the early stages and therefore it is too soon to assess impacts of this project. However, the commercial trade in wild-collected yellow tang collected from Hawaii reefs (estimated at 300,000 to 1 million fish per year) is coming under increasing pressure from dive operators and other stakeholders in coral reef ecosystem. This makes the development of captive culture technology for yellow tang (and other high-value reef species) urgent. In addition to helping take pressures off the wild fish populations, yellow tang aquaculture technology will provide new economic opportunities with an estimated value of three to ten million $US per year.

Publications in print, or papers presented:

Laidley, C.W. C. Bradley, C. Callan, E. Martinson, M. Kline. Development of copepod-based hatchery technology for marine fishes with extremely small-mouthed larvae. World Aquaculture Society Meetings, Veracruz, Mexico, September 26, 2009.

Callan, C.K., and C.W. Laidley. Opportunities for culturing coral reef species for the marine ornamental industry and food-fish production in the Pacific Islands. Saipan Workshop on Aquaculture Opportunities. Saipan College, July 17, 2008.

Laidley, C.W. Saving the Reefs: Aquaculture of coral reef species as an alternative to wild collection. Hawaii Sea Grant Program, Hanauma Bay Seminar Series, June 12, 2008.

Laidley, C.W., C.K. Callan, and K. Liu.. Saving the Reefs: Aquaculture of coral reef species as an alternative to wild collection. International Symposium in Honor of Professor Yoshitaka Nagahama Sex Determination and Gametogenesis in Fish: Current Status and Future Directions. University of Hawaii at Manoa, June 1, 2008.

Laidley, C.W. Saving the Reef: Culturing coral reef species, Ocean Networks Celebration of the Year of the Reef, Waikiki Aquarium, April 12, 2008.

Laidley, C.W. Copepod-based hatchery technology development, Marine Finfish culture symposium, Hawaii Institute of Marine Biology, March 20, 2008.

Laidley, C.W. Saving the Reef … Development of aquaculture technology as an alternative to wild-collection of coral reef species, Hawaii Pacific University, February 13, 2008.

13. Improving the Hatchery Output of the Hawaiian Pink Snapper, *Pristipomoides filamentosus* to Meet Stock Enhancement and Open Ocean Aquaculture Expectations, Years 1 and Year 2

General Information

Reporting Period: March 1, 2010 to October 31, 2010 (Year 1 Final Report; Year 2 Progress Report)

Funding Level:

Year	Amount
1	$24,000
2	**$24,000**
Total	$48,000

Participants: **Clyde Tamaru,** Ph.D., University of Hawaii College of Tropical Agriculture and Human Resources, (CTAHR) Department of Molecular Biosciences and Bioengineering (MBBE)

Petra Lenz, Ph.D.,
Pacific Biomedical Research Center (PBRC)

Karen Brittain
Hawaii Institute of Marine Biology.

James Jackson, Graduate Student

RESULTS AT A GLANCE…

- Researchers confirmated that the first feeding of opakapaka larvae can be achieved resulting in high survival up to 10-14 days posthatching.

- Researchers have observed that rotifers do not appear to be a suitable transitional live food organism.

- High speed filming conducted under the project shows that older copepods are not suitable during the early stages of the larval rearing process because their escape mechanism surpasses the ability of the opakapaka larvae to capture them. This is not the case for copepod nauplii.

Department of Zoology

Objectives

1. Improve survivorship and growth of opakapaka larvae through improvements in live feeds through controlled feeding trials, and complementary observations on foraging behavior.
2. Increase hatchery output by improving other tank conditions.
3. Transfer developed technology to appropriate end users.

Anticipated Benefits

By conducting small scale feeding trials and also viewing larval feeding behavior was hoped to be a viable means of overcoming some of the constraints being experienced in developing a rearing technology for the opakapaka. The work to date confirms results obtained using a large rearing tank (3,000-L) and provides at least some understanding of the results being obtained. Overall, the successful development of a rearing protocol would present an invaluable opportunity for continued expansion and diversification of another marine species.

Work Progress and Principal Accomplishments

Objective 1: Improve survivorship and growth of opakapaka larvae through improvements in live feeds through controlled feeding trials, and complementary observations on foraging behavior.

During Year 1, the following was completed: 1) establishment of cultures for two species of calanoid copepods: *Bestiolina similis* and *Parvocalanus crassirostris*, and 2) testing and timing of nauplius production in batch cultures and in a bioreactor. A focused effort of developing a protocol for the provision of *Parvocalanus crassirostris* was investigated. Work done by Dr. Petra Lenz with *Bestiolina similis* demonstrated that female reproductive rate has an inverse relationship with stocking densities and with the age of the culture. During work completed early in the project report period *P. crassirostris* cultures were shown to behave in a very similar manner.

A second method for efficient production of copepod nauplii was developed under the direction of Dr. Lenz and results from this work are currently in review for publication.

This method involved the use of an airlift bioreactor device coupled to a nauplii collection chamber. The prototype device effectively separates the nauplii from the reproductive adults and allows for the harvesting of the nauplii (Figure 1, in Appendix) while maintaining good growth conditions for the adults. The design consists of an upright clear acrylic cylinder which contains the stock population of reproducing adults and a cooled collection jar that stores the eggs and early stage nauplii produced. In the center of the main cylinder, another smaller clear acrylic cylinder is suspended around an air stone that rests in its center gently aerating the culture while minimizing turbulence. Beneath this upper cylinder, a removable collection jar is attached and surrounded by cooled copper tubing while a knife-gate valve separates the two chambers. This valve is left open to allow the negatively buoyant eggs to fall into the collection jar and closed only when the jar is to be removed for harvesting of the nauplii that accumulate inside. A 210μm Nitex mesh barrier prevents adult copepods from becoming trapped in the cooled collection jar but allows the eggs to pass through unharmed. The construction of this device and the development of a protocol for its use contributed significantly to the subsequent development of the nauplii production protocol currently in use at PBRC. In addition, the bioreactor prototype is currently undergoing slight design improvements and may soon be available for use in upcoming *P. filamentosus* feeding trials slated for the spring of this year.

The first rearing trials to investigate first feeding were initiated. One of the most basic of investigations was conducted and this was a comparison of larval growth between a fed and unfed group. A summary of the temporal changes in total length of opakapaka larvae that took place between the two treatment groups is summarized in Figure 2. A significant change in total length of the non fed larval group is clearly evident just after 80 hours (3.3 days) post-hatching. The data indicates that introduction or having the optimal numbers of the appropriate live food organism(s) must be in place no later than the third day post-hatch. The data was summarized during the current reporting period and the temporal changes in mouth gape for fed and unfed larvae is presented in Figure 3. At 108 hours post-hatching is the first time the differences in observed mouth gapes from both treatment groups is statistically different ($P<0.05$) Figure 4. Interestingly, this is much longer (e.g., an additional 40 hours) than when the impacts of starvation (change in total length) are first detected (e.g., 80 hours) underscoring the need to find an appropriately sized live food organism as the gape size apparently does not change for an additional 40 hours after the onset of first feeding.

Additional laboratory-scale rearing trials (n=2) focused on the suitability of a particular live food organism as a first feed. These trials were conducted in 7.5 L rearing vessels provided with a continuous source of seawater and stocked with various feeding treatments. Duration of the trial was conducted for 7 days post-hatching which is the time when total

mortality is observed in the no feed control. Two separate experiments were conducted during the previous reporting period and the results are summarized in Figure 4. Some interesting trends were observed from these laboratory-scale trials the first being the significantly lower survival of the larvae when rotifers are presented as a first live food organism. While the result was not unexpected, in both trials, the treatment was significantly ($P<0.05$) lower than when copepod nauplii were used either in combination with rotifers or when copepod nauplii are used alone. What is of particular interest is the significantly higher survival and growth of the first feeding opakapaka larvae when it is presented only with copepod nauplii. The introduction of rotifers at first feeding provides no apparent benefit and may potentially be detrimental. Gape size of larvae presented the different feeding regimens were particularly revealing as there was no statistical differences in gape size between larvae fed rotifers only and rotifers combined with copepod nauplii. Larvae presented only copepod nauplii resulted in larvae possessing significantly larger gape sizes. Figure 5. The results are consistent with the challenges that have been encountered with transitioning the opakapaka larvae on to rotifers although the rationale for that response remains elusive.

During the current reporting period, high speed filming of first feeding opakapaka larvae were made in preparation to assess changes in behavior in response to various stimuli or tank conditions. One of the most striking and obvious behavioral observations is that of the transition to a vertical orientation (Figure 6) of the opakapaka larvae during the first two days after hatching. Unfortunately, these trials were conducted towards the ending of the spawning season for opakapaka and additional trials would have to wait for the arrival of the next spawning period sometime this spring (e.g. April or May).

During the current reporting period we have attempted to rear 10 separate batches of *P. filamentosus* eggs in the Bekesy laboratory located on the University of Hawaii at Manoa campus. The batches have ranged from 300-3,000 eggs each and have experimented with various rearing conditions and feed sources.

Conclusion:

Several challenges were encountered this spawning season that hindered efforts to resolve the "second feeding" issue. However, we did have recurring success with first feeding and at this point all indications point to early stage *P. crassirostris* nauplii as the most suitable prey for first feeding *P. filamentosus* larvae. Day 7-8 post-hatch is clearly a critical time in which the larvae most likely require a novel prey source that we have been unable to provide.

From the 17 hours of video footage that have been captured and reviewed, preliminary observations have focused on (I) predominant behaviors, (II) attack strategy, (III) success rate of attacks and (IV) effects of various live food combinations.

(I) The *P. filamentosus* larvae alternate between passive and active behaviors. Passive behavior is characterized by virtually motionless "floating" in the water column or resting ventral side downward on the bottom of the holding container with some movement. Active behavior is characterized by more typical searching for prey and attacking prey items.

(II) *P. filamentosus* larvae exhibit two chief attack behaviors. The first (see figure 1) is a typical c-start attack where once the larvae target a prey item they curl their body into a "C" or "S" shape. This "spring-loading" of the muscles is then followed by a rapid lunge forward in an effort to capture the prey. The second attack behavior is a more sudden lunge at prey that is not preceded by an observable period of "targeting." This second type of attack seems to manifest haphazardly and is much less successful.

(III) Success rates have been tabulated by reviewing each 60minute block of filming in its entirety and tabulating successful vs. failed attacks. Attacks with unknown outcomes are noted but only attacks with definitive outcomes are used to estimate success rates.

(IV) Adults (C6), copepodites (C1-C5), late stage nauplii (N4-N6), early stage nauplii (N1-N3) and eggs of the calanoid copepod *P. crassirostris* as well as *B. rotundiformis* rotifers were presented to *P. filamentosus larvae* throughout first feeding. (Insert Figure 1 here)

Feeding successes with various live feeds:

Adult & copepodite *P. crassirostris* copepods (C1-C6): too large and escape response too rapid for larvae to be able to capture. Larvae do not appear to commonly target adults but instances in which adult escape responses "startled" larvae have been observed.

Late stage *P. crassirostris* nauplii (N4-N6): too large during first few days of feeding, unclear if escape response is also too rapid. Larvae have been observed to target late stage nauplii and failed attacks result.

Early stage *P. crassirostris* nauplii(N1-N3): able to capture with increasing success as the larvae develop.

P. crassirostris eggs: able to capture, difficult to film as they are negatively buoyant and often challenging to identify clearly

B. rotundiformis rotifers: the smallest rotifers can be captured and consumed. A significant proportion of rotifers added from culture appear to be too large for the first feeding *P. filamentosus* larvae to ingest. Also, feeding rates appear to be depressed when larvae are presented with rotifers. Video analysis supports prior data that rotifers are not a suitable first feed.

Objective 2: Increase hatchery output by improving other tank conditions.

Two attempts were made to test various live food stocking densities beginning with 7 day post-hatched larvae both of which were conducted at PBRC. This required the rearing of larvae to the appropriate age and then transferring them to treatment tanks where they would be exposed to various rotifer stocking densities in an attempt to assess whether high (e.g., 50 – 100 individuals/ml) stocking densities of rotifers can overcome their limited acceptance as a transitional live food item. In short both attempts failed as larval mortality was unacceptably high during the initial 7 days causing the experiment to be abandoned. It is felt that egg quality was a contributing factor as these trials were being undertaken in October and represents that ending of the spawning season. These trials will have to be attempted again in the spring.

No major progress has been made on improving the overall survival of the larger rearing trials being conducted under the auspices of a NOAA supported project. This objective is reliant on the defining of a suitable feeding regimen and that is an outcome of Objective 1. While it is apparent that the fist feeding challenges can be overcome with the use of copepod nauplii the transitioning to the next live food organisms remains to be determined. Additional trials are being undertaken to address this remaining roadblock and to be attempted during the upcoming spawning season.

Several changes in the rearing environment were introduced and fluorescent lights used to light the rearing container were all changed to the same standard daylight spectrum and were placed at a 14cm distance above the water's surface and distributed more evenly. The photoperiod was also shifted to better mimic increased day length in summer and to allow more time for feeding. In an attempt to maintain higher and more steady water temps (at 25C) in the laboratory an aquarium heater was coupled with an air stone and placed inside of a plastic column capped by mesh on the bottom. However, these modifications resulted in no significant improvement in rearing success.

Objective 3: Transfer developed technology to appropriate end users.

First feeding larval rearing trials that were done with the moi larvae as a training exercise did result in data that was useful to the hatchery manager of Hukilau Foods and their hatchery operations. Some thought about incorporating copepods into the rearing protocol of moi larvae has been ongoing for several years. The question about whether there was a definite need and insight was provided as a result of that initial trial. Essentially there is no benefit for using copepod nauplii for raising moi larvae and for that reason is not being actively pursued unless the need arises such as with an alternative fish species.

The first feeding data has been summarized and is to be presented at the 35th Annual Albert Tester symposium sponsored by the Department of Zoology and is to take place between March 17-19, 2010.

Information being generated directly benefits a larger NOAA supported project as it confirms the information to date and that being the utility of the copepod nauplii as the first feeding organisms for opakapaka larvae. The result showing rotifers not being as important as has been found in other marine species has resulted in a rethinking of its use as a transitional feed and resulted in investigating other live food organisms as the transitional food item. Work at HIMB has refocused on use of the "greenwater" mesocosm approach in order to find alternative live food organisms the work which will undoubtedly have to require another year of effort. Work is ongoing to secure funding for that effort.

Work Planned

Remaining work to be completed:
Six hours of footage remains to be reviewed and at least 4 more hours is planned to be recorded over the next few days. The remaining video footage as well as all of the clips of interest sequestered from previously reviewed footage will undergo more detailed analysis.

Successful attacks will be compared with unsuccessful attacks in an effort to identify any patterns or trends in the predator-prey interactions that may help to elucidate the driving factors behind the generally poor performance of *P. filamentosus* larvae beyond first feeding.

Specific copepodite life stages will be taken into account in the analysis so that the points in development for which *P. filamentosus* larvae are able to capture late stage nauplii and possibly copepodites may be identified.

Impacts

Unfortunately there are no major impacts with regard to an overall improvement of the hatchery technologies being developed for the opakapaka. Impact of another nature is being realized as a graduate student continues to work towards fulfillment of his graduate degree in the Department of Zoology. The project provides the necessary training needed to produce the next generation of researchers.

Publications, Manuscripts, or Papers Issued, Approved, or Presented

Two presentations and one newsletter article were made and they were:

Jackson, J., P. Lenz, C. S. Tamaru and J. Brock. 2010. Paracalanid copepod (*Parvocalanus crassirostris*) as a first feed for the rearing of larval Hawaiian pink snapper (*Pristipomoides filamentosus*). Tester's Symposium. March 17-19, 2010[1].

Tamaru, C.S., P. Lenz, J. Jackson and K. Brittain. 2010. Improving the Hatchery Output of the Hawaiian Pink Snapper, *Pristipomoides filamentosus* to Meet Stock Enhancement and Open Ocean Aquaculture Expectations – Year 1. Center for Tropical and Subtropical Aquaculture Annual Progress Report to the Public, May 28, 2010, Oceanic Institute Learning Center.

Tamaru, C.S., J. Jackson, P. Lentz. 2010. Update on the hatchery production of the opakapaka, *Pritipomoides filamentosus*. Aquatips, Center for Tropical and Subtropical Aquaculture. Volume 2: Issue 6. June 2010.

[1] http://www.hawaii.edu/zoology/tester/tester_webprogram.pdf

14. Determining Aquaculture Bottlenecks of Pacific Threadfin (*Polydactylus sexfilis*): Increasing Fry Survival, Growth and Quality, Years 1 and 2

General Information

Reporting Period: January 1, 2009 to September 30, 2010 (Year 1 Final Report, Year 2 Progress Report)

Funding Level:

Year	Amount
1	**$75,000**
2	**$87.000**
Total	$162,000

Participants: **Charles Laidley,** Ph.D., Primary Investigator
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John R. Cates, Jr., Ryan Murashige, and Aaron Moriwake

RESULTS AT A GLANCE…

- Successfully reduced and reversed goiter formation rates in highly valuable moi broodstock populations.

- Developed multiple methods to supplement diets and restore thyroid economy.

- Developed a pragmatic sausage diet for broodstock facilitating other dietary improvements including vitamin supplementation.

- Showed dietary supplementation procedures are completely safe for broodstock, as well as eggs and larvae generated from treated broodstock.

- A University of Hawaii graduate student completed his Masters degree using early stages of this research.

Hukilau Foods, Grove Farms

Objectives

1. To assess the capacity to increase TH deposition into fertilized moi eggs by exposing moi broodstock to iodide through dietary and/or rearing water supplementation.
2. To assess the efficacy of increased TH deposition in moi eggs to improve the survival and growth of moi larvae and fry to stocking size.
3. To determine the efficacy of adding iodide to larval/fry rearing water to increase their survival, and growth to stocking size.
4. To prepare reports, and publications for dissemination and transfer of technology and methodologies to the public.

Anticipated Benefits

The long-term investment in moi culture R&D by CTSA has helped create core hatchery technologies that lead to the successful development of commercial moi production operations in Hawaii, and the first commercial open ocean fish farm in the United States. However, in order to survive in a highly competitive global economy, it is important that we continue to invest in improving fingerling production technologies and address emerging challenges. Although current production methods are now successfully generating year-round egg supplies supporting large scale fingerling production (~300,000 per hatchery run), the species appears particularly prone to thyroid deficiency issues causing high rates of broodstock turnover and possible impacts on egg quality and larval performance. Therefore this project will provide practical solutions to improve broodstock health and impacts on fingerling production. In addition, the project will yield critical new information on the role of thyroid hormones in reproduction and early developmental processes. The overall benefit of this project will be an improved broodstock holding protocol that will greatly improve retention of highly valuable broodstock and lower operational costs. Further, this project may also improve egg quality and larval performance through the hatchery period again helping improve overall production output and lower operational costs.

Work Progress and Principle Accomplishments

Objective 1: To assess the capacity to increase TH deposition into fertilized moi eggs by exposing moi broodstock to iodide through dietary and/or rearing water supplementation.

Goiter formation. One of the issues that have arisen with the development of open ocean farming of moi is the high rates of mortality, and especially goiter associated mortality, in captive broodstock populations maintained at the Oceanic Institute for moi fingerling production. Earlier analytical studies examining saltwater derived from the OI/SLP well system showed relatively normal total iodine levels, but most of the iodine was in an oxidized form (IO_3), with relatively low availability of iodide (I^-) (Crow et al., 1998). Despite the apparent availability of iodine, these conditions (possibly in combination with relatively high NO_3^- levels) is clearly impacting the ability of moi to synthesize sufficient quantities of thyroid hormones leading to goiter formation (Fig. 1).

Although we have made some progress in reducing the overall mortality rates over the last six years, we are experiencing an increase in the appearance of goiters which account for the majority of broodstock losses in recent years (Fig. 2).

Examination of broodstock records show that the goiter problem is relatively slow to develop, with goiter associated mortalities taking several years to manifest (Fig. 3), with no apparent problems during growout and early broodstock conditioning. Given the age/timing of goiter formation it was hypothesized that goiters were in part associated with increased demands for thyroid hormone production for deposition into eggs produced year-round by our captive stocks. However, more careful review of the mortality data reveals that the rates of goiter formation are relatively similar between male and females (Fig. 4), suggesting that the disease is likely a function of time (years) of maintenance in low iodine waters associated with bore-hole water supplies which appear to oxidize available iodine supplies.

Effects of iodine supplementation. In collaboration with Dr. Grau's research team at the Hawaii Institute of Marine Biology we began examining the effects of iodine levels in the water and diet on broodstock health and egg thyroid hormone deposition. Using samples from stocks used in our earlier work we demonstrated a clear difference in egg thyroxine (T4) and triiodothyroidine (T3) levels in spawned eggs and larvae (Fig. 5). This data showed extremely low levels of both thyroid hormones in OI broodstock maintained on high-quality frozen diets of smelt, squid and shrimp compared with similar stocks maintained at the state fisheries facilities (Anuenue Fisheries Research Center) on Sand Island using normal ocean water. In addition, demonstrated that additional iodine

supplementation of the OI broodstock through either the water or diet successfully increased egg thyroid hormone content suggesting that either approach is appropriate for mitigating thyroid deficiencies.

Associated with improved thyroid status (based on egg thyroid hormone measurements) we also showed a significant decline in broodstock mortality associated with goiters in both iodine supplementation groups (Fig. 6). The considerable improvement in both goiter formation rates and egg thyroid hormone certainly suggests that iodine supplementation protocols are moving us in the right direction. The dietary route, in addition to being less expensive and easier to administer, also appeared to be the most effective method in terms of reduction in goiter appearance. Supplementation of water iodine levels to levels at or slightly above levels in natural ocean water was both less effective and more challenging than the dietary approach. However, despite the supplementation, even the treated stocks continued to develop goiters.

Although the use of iodine supplemented formulated feeds (both in-house and commercial Vitalis™ feed formulations) clearly helped reduce goiter formation and increased egg thyroid hormone content, overall stock fecundity was inferior relative to stocks maintained on raw smelt, squid and shrimp diets (Fig 7).

In December 2009 during our annual weighing, measuring and sexing of production we sorted through the broodstock population and moved ten obviously goitered fish (6 males and 4 females) into a separate tank. These stocks were then provided with a raw mash of smelt, squid and shrimp to which we provided supplemental iodine at a level of 3.6 mg/kg dry weight (similar to levels used in the previously effective OI diet). However, after stocking we continued to lose goitered fish at a rate of approximately two fish per month. Therefore with only three fish (one female and two males) remaining we further increased the iodine dosing to one hundred-fold (360 mg/kg dry weight) which prevented further mortality and has partially reduced the apparent size of the goiters. Unfortunately this did not leave sufficient numbers of goitered fish to continued planned trials comparing egg T3 and T4 comparisons.

Over the first year of the project we also conducted a trial using juvenile moi in an effort to develop a simplified model to examine the onset and prevention of goiter formation. As reviewed earlier, goiters form over a period of 3 to 5 years in adult moi maintained mainly on raw (smelt/squid/shrimp) diets. The extraordinarily slow rates of goiter formation in broodstock make them a slow and expensive model for studying protocols to improve thyroid economy. Therefore we ran a preliminary trial rearing juvenile moi, undergoing rapid growth from less than 20g mean body weight to over 150g body weight, on both raw- and iodine-supplemented formulated growout diets to see if we could elicit a faster

manifestation of the goiter process. It was presumed that the more than seven-fold increase in body mass would quickly manifest the effects of iodine deficiency on growth or appearance of thyroid hyperplasia. The trial, run over five months, showed excellent growth under both dietary treatments with similar growth performance amongst the two diets (Fig. 8) with no indication of slowed growth or goiter formation on the raw diet (as is seen for moi broodstock). These results suggest that goiter formation may also be a relatively slow process in juveniles, despite their rapid rates of growth and presumed exhaustion of available iodine.

As we approached the end of the first project year (of this two year project) we developed a new iodine supplementation strategy for maintenance of moi broodstock populations. Our work to date has shown that (1) supplementation tank water with iodide reduced goiter formation and broodstock attrition. However, supplementing the water supply is both expensive and only partially effective. We also have shown that (2) a variety of formulated marine finfish diets also slows goiter formation and broodstock attrition. However, currently available formulated diets yield inferior reproductive performance.

Therefore in July 2010 (with the end of our commercial moi fingerling production contract with Grove Farms) we randomly re-sorted the remaining production broodstock into two production tanks (~55 fish per tank) and initiated a new broodstock feeding regimen. Under this new feeding regimen, half of the moi broodstock population (i.e., one tank) are being maintained on alternate feedings of smelt, squid and shrimp (i.e., optimal broodstock diet to date) while the other half of the broodstock population was switched to a vitamin and iodine supplemented diet using the same proportions of smelt, squid and shrimp using a food grinder and sausage maker as a vehicle for dietary supplementation.

This trial will be continued into the second year of this project (as planned) to allow sufficient time to track changes in feed consumption, goiter formation and mortalities, reproductive performance, egg quality and hatchery performance of eggs derived from the new (iodine & vitamin supplemented) sausage diet and our standard protocol providing alternating feedings of frozen smelt, squid, and shrimp. To date the broodstock appear to have adapted to the new broodstock diet providing a more practical method to provide optimal broodstock diet along with a practical methods for dietary supplementation with vitamins and iodine.

Objective 2: To assess the efficacy of increased TH deposition in moi eggs to improve the survival and growth of moi larvae and fry to stocking size.

Despite the dramatic changes in egg T3 and T4 levels, evaluation of eggs from the water and dietary iodine supplemented groups failed to show any significant change in hatchery

performance. However, each of these treatment groups likely contained fish at various stages of goiter formation, and therefore we carefully screened our broodstock populations for the appearance of goiters and established three test broodstock populations. The raw (smelt/squid/shrimp) fed group from which all goitered fish were removed, a second group of raw fed fish all displaying outwardly visible goiters, and third treatment group which has been receiving KI in the water. Eggs from all three treatment groups were then tested for effects on larval growth and survival in replicated 200L larval rearing trial system (Fig. 9). These results again confirmed that neither low iodine levels in eggs from goitered broodstock, nor eggs from broodstock provided water iodine supplementation affected hatchery survival or growth of early larvae.

Objective 3: To determine the efficacy of adding iodide to larval/fry rearing water to increase their survival, and growth to stocking size.

In our earlier work examining the effects of larval rearing water on moi growth (Fig. 10) and survival (Witt et al. 2009) we demonstrated that larvae raised in ocean water grew slightly faster and exhibited improved survival relative to larvae raised in water from the OI/SLP seawater well system with reduced iodine availability due to iodide oxidation. This difference in growth rate becomes apparent at approximately two weeks into larval rearing when approximately 50% of the larvae raised in ocean water reached flexion-stage, compared to 15% in well seawater.

The analysis of thyroid hormone concentrations of larvae sampled from this trial by Dr. Grau's group (see Witt et al., 2009) showed a notably different whole body thyroxine (T4) profile between treatment groups (Fig. 10). The ocean water reared larvae exposed to presumably normal iodine levels had relatively lower T4 levels throughout much of the run, with exception of a peak from days 15 to 21, while the iodine deficient well water reared larvae tended to have a gradual increase in levels throughout the run. Profiles of triiodothyronine (T_3) were similar between the two groups, decreasing from just before hatch to day 7, followed by a gradual increase out to the end of the larval period. Unfortunately we do not have access to juvenile moi from the wild, so it is difficult to know what is the "normal" pattern for T4 through development.

Given multiple potential differences between ocean and well water (in addition to available iodine for thyroid hormone synthesis) future trials under year two of this project will examine the effect of direct iodine supplementation to OI/SLP well water during larval rearing to help determine the importance of available iodide on larval performance.

Objective 4: To prepare reports, and publications for dissemination and transfer of technology and methodologies to the public.

Technology transfer components of the project to date have included semiannual CTSA reports and the May 2010 industry stakeholder workshop. More complete technology transfer will occur toward the end of the project as planned objectives come to fruition and we have a better handle on optimized broodstock and larval rearing protocols.

Work Planned

Planned activities under year two of this project include:

1. Continued comparison of moi broodstock health, reproductive performance, egg quality on our iodine and vitamin-supplemented raw sausage diet formulation.

2. Completion of egg thyroid hormone analyses in response to the various broodstock diet treatments.

3. Hatchery performance trials comparing egg and larval performance in response to our previous and new improved broodstock diet preparation (based on year one findings) with and without additional hatchery iodine supplementation of larval feeds and water.

Impacts

CTSA investment in moi culture created enabling technologies upon which has led to the successful development of commercial moi production operations in Hawaii, and the first commercial open ocean fish farm in the United States. This project is focused on improving broodstock holding protocols to improve retention of highly valuable broodstock, increase egg supplies, and lower operational costs. The ability to culture moi is allowing stores and restaurants to provide this popular and healthy product to local consumers while reducing pressure on heavily depleted wild populations and providing new economic opportunities for the local community. Even though the industry is still very much in the early stages of development, commercial operations are stocking over 1 million moi fingerlings annually in open ocean growout cages for delivery in to local markets where whole fish are retailing for $7 to $9 per lb.

Publications in print, or papers presented:

Laidley, C.W., Callan, C.K., Martinson, E. 2010. Determining aquaculture bottlenecks of Pacific threadfin (*Polydactylus sexfilis*): Increasing fry survival, growth, and quality. CTSA Progress Report to the Public, Oceanic Institute Learning Center, May 28, 2010.

Witt, E.M., Laidley, C.W., Liu, K.K.M., Hirano, T., Grau, E.C., (2009) Correlation between environmental iodide concentrations and larval growth, survival, and whole body concentrations of thyroid hormones and cortisol in Pacific threadfin *(Polydactylus sexfilis).* Aquaculture 289:357-364.

Witt, E.M. (2008) Effects of broodstock diet and environmental iodide concentrations on larval growth, survival, egg and whole body concentration of thyroid hormones and cortisol in Pacific threadfin, *Polydactylus sexfilis*. M.S. Thesis, University of Hawaii, 83pp.

15. Evaluating an Engineered Biological Treatment Processes for the Application of Aquaculture Waste and Wastewaters

General Information

Reporting Period: June 15, 2010 to October 1, 2010 (Progress Report)

Funding Level: $20,000

Participants:

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University of Hawaii at Manoa

Joshua L. Irvine (graduate student)
University of Hawaii

Clete Otoshi (collaborator)
Oceanic Institute

RESULTS AT A GLANCE…

- This project is developing and analyzing the best system to treat waste and wastewater resulting from the production of shrimp. The system may contribute to a cost-savings for the industry while preserving the integrity and fitness of the environment.

- The project has begun characterizing the aquacultural waste and wastewaters. This is information is necessary to better develop a treatment system process to achieve remediation and re-use goals.

-A wastewater characteristic study has been developed.

- A University of Hawaii graduate student will use this research to complete his Masters degree.

Objectives

1. Write a literature review on the best available technologies for aquaculture wastewater treatment and reuse.
2. Wastewater Characteristic Study.
3. Evaluate the process performance of biological treatment technologies for organic and nitrogen removal using aquaculture wastewater.
4. Develop a design and operational criteria to meet treated wastewater discharge and reuse goals (to be determined)

Anticipated Benefits

The project has clear benefits in the aquaculture industry for shrimp production because the research project is developing the best system that could treat waste and wastewater resulting from the production of shrimp. The treatment system may contribute to a cost-savings for the industry while preserving the integrity and fitness of the environment.

Work Progress and Principal Accomplishments

Objective 1: Write a literature review on the best available technologies for aquaculture wastewater treatment and reuse

A literature review of publishable quality will be written to evaluate the current and cutting edge technologies/practices used to treat and renovate aquaculture wastewater/wastes.

Objective 2: Wastewater Characteristic Study

The first step is to define the waste/wastewater composition. This investigation will determine the wastewater characteristic parameters needed to develop and assess possible remedial designs. The main goal in the WCS is to provide a quantitative assessment of the waste/wastewater composition.

A wastewater characteristic study including biodegradability of the used substrate will be conducted. The following will be assessed: COD (total, soluble), Total Kjeldahl Nitrogen (TKN), Total Phosphorus, pH, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), and Alkalinity. Biodegradability based upon COD removed biologically will be determined.

During July to August of 2010, a preliminary wastewater characteristic study was conducted to determine a baseline value for chemical oxygen demand and other parameters. In November 2010, an aquaculture shrimp study will begin at Oceanic Institute. Throughout the testing period a detailed wastewater characteristic study will be monitored (see Appendix).

The TCOD an SCOD values are considerably high. This did not reflect values from similar research on shrimp aquaculture wastewater ranging from 610 to 2,430 mg/L in a 7 week

trial run (Cohen et al, 2005). Another research group had values of ~1,300 mg/L (Fontenot et al, 2006)

The high COD values were a direct result of sodium chloride interference. The chloride ions readily react with dichromate thereby giving a false read on organic matter consumption of the oxidant (Standard Methods, 1985). The Standard Methods for Wastewater Book suggested adding silver nitrate ($AgNO_3$) to solution to precipitate chlorine.

As suggested in table 2, chloride plays an important role in affecting COD measurement. For the next month, our focused shifted to determine how to minimize the effects of chlorine on the COD measurement. The options explored were: dilution, direct precipitation, and direct precipitation with fluxing (Cohen, 2005; Vyrides and Stuckey, 2008).

The best method that minimized chlorine interference was Cohen et al (2005). The results in Graph 1 are the final soluble chemical oxygen demand results of the tank water. Table 4 shows lab results sent ADS of the tank water sampled on Wednesday, August 25, 2010.

Objective 3 / 4: Evaluate the process performance of biological treatment technologies for organic and nitrogen removal using aquaculture wastewater. Develop a design and operational criteria to meet treated wastewater discharge and reuse goals (to be determined)

Anaerobic BIONEST and aerobic EMMC reactor

From the lab-scale study, a design and operational criteria can be developed tailored to our desired output goals. Preparation of BIONEST reactor will follow Dong (2003) procedures. The EMMC system design and reactor will follow procedures developed in UH Mānoa studies: Yang et al.(1988); Yang et al.(1994); Yang et al. (1997); Yang et al. (2002); Yang et al. (2003); Kongsil et al. (2009); and Lin et al. (2009). It is desired to meet target effluent standards and reuse criteria (yet to be determined) from aquaculture waste/wastewater; thus, the process performance will evaluate organic and nitrogen removal. From this information, an economic evaluation will be determined to assess the cost required for the application of a scaled-up system.

Work Planned

For the detailed Work Plan, see the table in the Appendix.

(1) Detailed literature review: will determine the best/appropriate method/treatment to be implemented
(2) Detail the procedures/steps for the process treatment to be implemented
(3) Design/operating criteria to be developed
(4) Observed achievement and compare to current practices
(5) Economic analysis

Impacts

The shrimp and prawn industry account for 4% of world production. Waste assimilation and disposal is a major area of concern in contributing to the sustainability of the aquacultural industry. Recirculating aquaculture systems are design to provide a cost-effective means to conserve and recycle water supplies; however, water treatment is required before recirculating water in order to maintain optimal and adequate water quality and quantity for aquacultural production. The research and development of a wastewater treatment system for recirculating aquacultural systems confronts three major challenges addressed aquacultural industry faces: sustainable economic growth, environmental stewardship, and equitable distribution of benefits (World Bank, 2007).

Sustainable economic growth could be achieved because recirculating systems provide a substantial water savings. The treatment of recirculating prior to re-use will ensure (1) stable water quality, (2) safeguard from unexpected and frequent algal blooms, and (3) provide and added barrier in disease control and prevention.

Recirculating water reduces the need for continued consumption of water which could put a strain on the water supply availability to society. More importantly, proper disposal of aquacultural wastewaters is necessary to be stewards of the environment. Aquacultural wastewaters and wastes contain organic loads and nutrients, if not properly disposed will negatively impact terrestrial and aquatic life forms. Proper disposal and treatment of wastewaters facilitates in achieving equitable distribution of benefits because it reduces the environmental and societal burdens resulting from improper management of aquacultural waste and wastewaters.

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16. Diversifying Freshwater Aquaculture Products for Hawaii: Two Crossover Species, the Red and Black Pacu (*Piractus brachypomus* and *Colosomma macropomum)*, Years 1 and 2

General Information

Reporting Period: September 1, 2009 to October 31, 2010; no-cost extension through December 31, 2010 (Year 1 Progress Report)

Funding Level:

Year	Amount
1	**$50,000**
2	$50,000
Total	$100,000

Participants:

Robert Howerton, Ph. D.
Aquaculture Extension Specialist
University of Hawaii Sea Grant

Clyde Tamaru, Ph.D., CTAHR
Department of Molecular Biosciences and Bioengineering
University of Hawaii

Kai Fox, CTAHR
Department of Molecular Biosciences and Bioengineering
University of Hawaii

RuthEllen Klinger-Bowen, CTAHR
Department of Molecular Biosciences and Bioengineering

RESULTS AT A GLANCE…

- The purpose of this project is to establish black pacu, *Colosomma macropomum* and red-bellied pacu, *Piractus brachypomus,* as freshwater aquaculture products for both the aquarium and food fish markets in Hawaii.

- A total of 150 individuals are being used to initiate the polyculture experiment with Chinese catfish, which was initiated with the stocking of ten 600 gallon tanks with various stocking densitites.

University of Hawaii

Ron Weidenbach
Hawaiian Fish Company

Jeff Koch
Mokuleia Farms

Objectives

1. Characterize growth of juvenile red pacu under monoculture conditions encountered on Maui utilizing locally available commercial feeds
2. Compare growth and survival in monoculture and polyculture (e.g., Chinese catfish and red pacu) growout trials on Oahu.
3. Initiate the establishment of broodstock populations for red pacu on three islands.
4. Obtain and collate technical information available on pacu including those written in foreign languages.
5. Locate sources of the black pacu that meet import requirements for the state of Hawaii.
6. Conduct technology transfer to appropriate end users.

Principle Accomplishments

Year 1:

Objective 1: Characterize growth of juvenile red pacu under monoculture conditions encountered on Maui utilizing locally available commercial feeds.

To initiate this objective required Institutional Animal Care and Use Committee (IACUC) approval as the activity is being conducted under the auspices of the University of Hawaii. Recent budget shortfalls in UH funding support has resulted in the required biannual inspection of the project to be covered by funding other than the IACUC and was not anticipated when the project was developed. The amount was estimated to be $3500 which could not be covered by the current project funds. This required a change in venue and this objective is now being conducted at the Windward Community College Aquaculture Complex located on the WCC campus and covered under IACUC protocol 09-820.

Objective 2: Compare growth and survival in monoculture and polyculture (e.g., Chinese catfish and red pacu) growout trials on Oahu.

The first shipment of Red Pacu arrived on August 30, 2010 and was distributed into 5 quarantine tanks located at WCC (Figure 1). A total of 400 individuals were received from Aqua Nautic Specialist, PTE, Ltd., located in Inglewood California and only two mortalities were recorded after arrival. The company deals with transshipping a host of freshwater ornamental species and works with hatcheries located primarily in Indonesia. During the next week an outbreak of *Ichthyophthirius multifiliis*, and a flagellated protozoan, *Ichthyobodo* sp. took place with mortalities being observed in one tank on September 5. Notification of the event and a treatment plan was submitted to the UH Veterinarian and approval for a low dose (25 ppm) formalin treatment every other day for a minimum of two more dosages but no more than five treatments total. Mortalities spread to all tanks and only six survivors remain as of the submission of this report.

A second shipment of 200 pacu from the same source was received on October 6, 2010 and once again divided up into five holding tanks and placed under quarantine. A preliminary survey conducted the same day of receiving the pacu revealed no signs of parasites. However, two days later, a second survey revealed a small number of *Ichthyophthirius multifiliis* in just one tank and a treatment of 100 ppm hydrogen peroxide for one hour were used for all fish. The rationale was that the low dose formalin treatment was apparently ineffective and a different treatment was attempted. The treatment, however, did stress the fish and also the infestation continued with approximately 25% mortalities being experienced within the next three days. A high dose (100ppm formalin) repeated every other day was initiated in combination with raising the salinity to 2 ppt. This particular treatment halted the mortalities and with bi weekly inspections has also resulted in removal of the parasites. A total of 150 individuals remain and being used to initiate the polyculture experiment with Chinese catfish. Fish have been under quarantine until November 11, 2010.

On November 11, 2010 the polyculture of pacu and Chinese catfish experiment was initiated with the stocking of ten 600 gallon tanks with multiple stocking densitites. Fish are being fed a diet of Nelson's Silver Cup trout feed, (ad libitum) and their growth and survival will be monitored at monthly intervals and over the course of the project.

Objective 3: Initiate the establishment of broodstock populations for red pacu on three islands.

No Progress made. This objective will take place after the growout trials have been completed.

Five adult pacu (1 kg body weight) were received at the WCC facility on November 5, 2010 and placed under quarantine. They will undergo monthly monitoring for temporal changes in their reproductive characteristics over the course of the project. This is to determine the natural spawning season of red pacu under conditions found in Hawaii.

Objective 4: Obtain and collate technical information available on pacu including those written in foreign languages.

A preliminary literature search has been conducted. A significant amount of the relevant literature is written in Portuguese and Spanish and a translator will be identified and will attempt to translate these articles into English.

Objective 5: Locate sources of the black pacu that meet import requirements for the state of Hawaii.

The same resource that allowed for the importation of the red pacu has indicated that they will also be able to send the black pacu when appropriate. Additional sources, however, are also being investigated.

Objective 6: Conduct technology transfer to appropriate end users.

Publications and Manuscripts Written and Papers Presented

Publications:

Stakeholder input from the aquaponics community has been extremely high regarding alternative species for use in this type of systems. For that reason aquaponics activities have now become part of this particular project. There are 2 publications and 2 presentations associated with this aspect.

Workshops:

During the reporting period a number of workshops were held by project personnel covering aquaponics.

17. Regional Biosecurity: Operational Biosecurity and Diagnostic Surveillance, Years 1 and 2 (OI component)

General Information

Reporting Period	September 1, 2009 to September 30, 2010; no-cost extension through February 28, 2011 (Year 1 Progress Report)
Funding Level	Year 1 **$50,000**; 2 $49,997; Total $99,997
Participants	**Charles Laidley,** Ph.D., Director, Finfish Department, Oceanic Institute Kim Pinkerton, Research Associate, Finfish Department, Oceanic Institute

RESULTS AT A GLANCE…

- The project is in the early stage of collating available literature on disease and biosecurity issues of relevance to the Hawaii aquaculture industry.

- This project will review and assess operational biosecurity for aquaculture operations in the Pacific Region (particularly Hawaii) and develop specific biosecurity plans for major culture species and specific farm operations.

- The information gathered under this project will be used to provide a comprehensive review of existing practices, help identify key weaknesses, and provide insights facilitating the development of effective pathogen control strategies for the region.

Objectives

Year 1:

Develop a General Aquaculture Biosecurity Plan for Hawaii and the Pacific Region

1. Develop a survey plan for reviewing operational biosecurity for aquaculture operations in the Pacific Region.
2. Complete a general biosecurity assessment of aquaculture operations in Hawaii.

Year 2:

Develop Specific Biosecurity Plans for Major Culture Species and Specific Farm Operations

1. Complete specific biosecurity assessments of specific farm operations for each of the major culture species.
2. Write a project report reviewing species-specific operational biosecurity for specific farm operations in Hawaii and the Pacific Region.
3. Convene biosecurity workshops reviewing the biosecurity evaluations and facilitating further discussion on aquaculture biosecurity and future needs in the Islands.

Anticipated Benefits

The information gathered under this project will be used to provide a comprehensive review of existing biosecurity practices, help identify key weaknesses, and provide insights facilitating the development of effective pathogen control strategies for the region.

Work Progress and Accomplishments

Year 1

Objective 1: Develop a survey plan for reviewing operational biosecurity for aquaculture operations in the Pacific Region.

As the first step in this project we have recently initiated our literature review process and begun collating available literature on disease and aquaculture biosecurity issues of relevance to the Hawaii aquaculture industry. Due to staffing reassignments while waiting for project startup we have been somewhat delayed in completing this initial review process. However, we are now in the process of making a new hire to help free up time for our microbiology research associate to increase focus on this effort.

This review will be followed up with a series of interviews to facilitate comprehensive stakeholder input during the early design phase of this project to ensure their biosecurity concerns (hazards) are not overlooked in the planned operational biosecurity assessments under objectives two and three.

Objective 2: Complete a general biosecurity assessment of aquaculture operations in Hawaii.

After development of the survey plan (in progress) we will initiate site evaluations at a wide variety of aquaculture, research, and other animal holding or processing facilities to develop a comprehensive review/overview of existing biosecurity concerns and practices for use in developing best management strategies and practices for the region.

Year 2 objectives will be completed in the second year of the project.

Impacts

This project will review and assess operational biosecurity for aquaculture operations in the Pacific Region (particularly Hawaii) and develop specific biosecurity plans for major culture species and specific farm operations. Biosecurity workshops will provide an opportunity for project investigators to review project findings with the many stakeholders in the aquatic animal health sector including policy makers, regulators, scientists and farmers. The information gathered under this project will be used to provide a comprehensive review of existing practices, help identify key weaknesses, and provide insights facilitating the development of effective pathogen control strategies for the region. The overall goal to develop a series of recommended biosecurity operating protocols for each of the sectors and to identify future research needs to further operational biosecurity as the industry grows and intensifies operations in the region.

Publications in print, or papers presented

None to report.

18. Regional Biosecurity: Operational Biosecurity and Diagnostic Surveillance, Years 1 and 2 (UH component)

General Information

Reporting Period	February 1, 2010 to October 31, 2010 (Year 1 Progress Report)	
Funding Level	Year	Amount
	1	**$50,000**
	2	$50,000
	Total	$100,000
Participants	**Clyde Tamaru,** Ph.D., CTAHR Department of Molecular Biosciences and Bioengineering. University of Hawaii	

RESULTS AT A GLANCE…

- A fully operational laboratory capable of conducting PCR testing has been established at Moana Technologies.

- Researchers developed PCR protocol for testing DNA extracted from fish tissue samples submitted for evaluation.

- A workshop entitled, "What is happening with the culture of koi and tilapia in Hawaii?"was organized and attended by over 40 koi and tilapia stakeholders.

Objectives

1. Establish a local diagnostic laboratory with PCR technology able to provide rapid turn around time for providing test results.
2. Hold workshop with Koi producers to solicit participation and input in establishing health status of Hawaii's koi with regard to koi herpes virus disease (KHVD)
3. Establish pro-active screening methods for KHVD
4. Conduct initial survey of KHVD in farmed and feral koi populations statewide
5. Produce technical handouts of resulting information. Information obtained is to also be included in the Operational Biosecurity handouts.

6. Conduct follow up workshop to disseminate information and begin discussions on the challenges and opportunities of establishing a health certificate program for farmed koi.

Principle Accomplishments

Objective 1: Establish a local diagnostic laboratory with PCR technology able to provide rapid turn around time for providing test results.

During the reporting period, negotiations with Moana Technologies and Dr. James Brock, DVM have resulted in the use of their laboratory and technician to conduct the PCR testing that was proposed. A working agreement had been finalized and was submitted during the previous reporting period. Despite the fiscal and personnel challenges faced by the project personnel, the major deliverable of this objective which is "a fully operational laboratory capable of conducting PCR testing" has been achieved. However, because of the changes in laboratory and personnel some changes in the project activities under this objective have also been implemented. Specifically, rather than conduct testing on extraction methods using various commercial kits the project will utilize standard extraction protocols already in place and routinely used by Moana Technologies. The rationale being that the procedures being used are OIE approved procedures. Moana Technologies has developed a PCR test protocol principally following the protocol listed in the OIE Aquatic Animal Manual. The details of the KHV PCR Test Protocol that we propose to apply to test fish tissue samples for KHV is available upon request.

Tilapia Tissue

On 16 March 2010 Moana Technologies received a frozen sample of 16 subadult to adult (12 – 17 cm total length) tilapia (*Sarotherodon melanotheron*) that had been collected as a random sample from the Nuuanu Reservoir by State of Hawaii, Division of Aquatic Resources (DAR) staff. Dr Brock had requested assistance in collection of tilapia to serve as tissue donors for the the current project and the DAR staff were kind enough to provide the material. These fish were logged into the Moana case record system as case 10-22 and deposited in the REVCO freezer for later dissection and tissue collection. Eventually, the tilapia were thawed and organ tissues were collected including spleen, kidney and muscle from each fish, the tissue samples were grouped together by organ into three whirl-top bags which were labeled for identification and the bags were stored frozen in the -80°C REVCO freezer in the Halawa Laboratory.

Fish Tissue Sample DNA Quality Control PCR Test

Moana Technology staff conducted a scan of the literature on line and a suitable candidate primer of DNA base pair sequences was found which reflect a conserved area in the genome in fish and would be expected to be reactive to extracted "fish DNA" from tissue samples derived from fish across a wide spectrum of teleost fish. The PCR protocol was adapted from the publication and this procedure which will be used when testing DNA extracted from fish tissue samples submitted for evaluation for the CTSA project. We will refer to fish DNA control primer set as CO-I and the methods used in its preparation are presented in a separate attachment in the Appendix.

Objective 2: Hold workshop with Koi producers to solicit participation and input in establishing health status of Hawaii's koi with regard to koi herpes virus disease (KHVD)

A workshop to inform both koi and tilapia stakeholders entitled, "What is happening with the culture of koi and tilapia in Hawaii?"was held on July 17, 2010 at Windward Community College. A copy of the workshop announcement, sign in sheet and evaluation is provided in the appendices. Approximately 40 people were in attendance (Figure 1).

While completing the objective, it should be noted that the participants were dominated by tilapia stakeholders and another workshop specifically for koi producers is being planned for the latter stages of the project. Probably the most appropriate time would be with the completion of the survey for KHV using PCR technology. Likewise, stakeholder input was obtained regarding work with tilapia that will be incorporated during the Year 2 phase of the current project as it deals with tilapia.

Objective 3: Establish pro-active screening methods for KHVD

A requirement of the PCR testing is a positive control that was obtained from one of the OIE laboratories. We received a positive KHV control sample using gill tissue and preserved in absolute ethanol on October 8, 2010.

In addition, a positive control sample for a Francisella positive population that consist of 5 fish composite (spleens) confirmed by PCR at University of Arizona with ADP documentation was also received on October 14, 2010 from Dr. Allen Riggs, DVM. Both positive controls have been transferred to Moana Technologies and are to be used during the remainder of the project period. On 1-November 2010 you provided us with a electronic copy of a pre-publication manuscript, "Identification and isolation of *Francisella*-like bacteria (FLB) from tilapia (*Oreochromis mossambicus*) for the first time in Hawaii"

authored by M. Misumi, et al. As the manuscript is currently under review a copy of the manuscript is only available upon request. The manuscript reports PCR primers suitable for the PCR detection of extracted TRLO DNA. In the coming weeks Moana Technologies will submit a request to have the FLB primers synthesized in the primer laboratory at the University of Hawaii.

Since receipt of the KHVD control sample, DNA was extracted from the KHV positive carp gill tissue that resulted in a positive PCR test result with the OIE KHV PCR primer.

PCR Tests and Outcomes for KHV positive Control and CO-I

Photo documentation and explanations for PCR tests conducted for the KHVF/R and the CO-I primers are presented in the Appendix. The results confirm that the positive control material for KHVD is working. The DNA from the KHV positive control tissue sample also gave a positive PCR test result with the "fish" DNA sequence primer (e.g., primer CO-I) that was obtained from the University of Hawaii primer laboratory and to be used for work that will be done at MOANA Technologies with fish tissue samples. It is a quality control primer that will be used for every fish tissue sample that is processed and run for fish pathogens with DNA. A positive PCR test reaction with CO-I indicates that 1) the DNA in the submitted tissue sample has not been degraded, 2) the extracted DNA that was obtained from the extraction processes was also not degraded and 3) the extracted DNA was suitable for use as a substrate to test for the fish pathogen's DNA in the tissue sample.

A document with the PCR protocols that will be used for KHV and for CO-I for the remainder of the project has been prepared and is also available upon request. Material collected from gels resulting from the completed KHV DNA and CO-I DNA trials are to be submitted to the PCR sequencing laboratory at the UH. Funds from the current project will be used to obtain sequencing data.

In addition to establishing the methods for detection of KHVD in the laboratory, protocols on the collection of samples, their storage and documentation of the chain of custody is being established in collaboration with Dr. Allen Riggs, DVM. The same kind of transparency already being practiced with the Shrimp SPF program is to be adopted with the KHVD program.

Objective 4: Conduct initial survey of KHVD in farmed and feral koi populations statewide

While the procedure from collection to reporting is being finalized, 10 juvenile koi have been purchased from Koolau Pets and 10 juvenile long fin koi have been purchased from

Modern Pet Center. They will be the first of the koi samples to be sampled using a non-lethal (e.g., gill clip) sampling protocol.

Sites where feral koi have been located (e.g., Manoa Stream adjacent to the East West Center, Lake Wilson) have been located and upon collection they will be sacrificed and a terminal sampling protocol will be used. In this case, gill, spleen and kidney will be sampled and tested for KHVD using PCR technology. As many feral koi individuals that can be obtained will be tested in this manner and will be conducted during the remainder of the project.

Objective 5: Produce technical handouts of resulting information. Information obtained is to also be included in the Operational Biosecurity handouts.

No progress made as the survey has yet to be initiated.

Objective 6: Conduct follow up workshop to disseminate information and begin discussions on the challenges and opportunities of establishing a health certificate program for farmed koi.

No progress made as the survey and testing has yet to be completed.

Challenges encountered

As noted during the previous reporting period the major challenge of budget shortfalls has resulted in several changes in the overall project. Likewise, a glitch in the finalization of the contract between UH and CTSA resulted in a delay in the start of the project. However, these major challenges have now been addressed and the project is back on track and should be able to complete the terms of reference. A no cost extension will need to be requested due to the late start date.

Publications and Manuscripts Written and Papers Presented

None to report.

19. Value Added Approach for Tuna Fish Roe: Local Ingredient for Shrimp Maturation Diet?

General Information

Reporting Period	December 1, 2009 to September 30, 2010; no-cost extension through August 31, 2011 (Progress Report)
Funding Level	**$50,000**
Participants	**Hui Gong,** Ph.D., Research Scientist University of Guam John W. Brown, Ph. D. University of Guam

RESULTS AT A GLANCE…

- 23 sample batches of tuna roe have been collected.

- The preliminary trials for various freezing dry processes to standardize the fish roe samples have been completed.

- Samples have been sent to the lab for nutritional analyses.

- One undergraduate student is assisting with this project.

Objectives

To achieve the proposed goal, studies within the four consecutive research thrusts will be carried out to obtain the baseline information, to develop a practical method for utilizing tuna roe in a shrimp maturation diet, to evaluate such diet, and to transfer the knowledge (see Four Thrusts flow chart).

In each thrust, a specific objective is further identified:

1. Analyze the nutritional values of tuna fish roe, and verify its specific-pathogen-free status. (Thrust 1)
2. Develop a semi-moist maturation diet with the tuna fish roe as the major ingredient. (Thrust 2)

3. Evaluate the reproductive performance of shrimp fed with the tuna roe based maturation diet, in comparison with conventional fresh-frozen maturation feeding regimes. (Thrust 3)
4. Publish the findings from the project and conduct one workshop on utilization of tuna fish roe as the technology transfer effort (Thrust 4)

Anticipated Benefits

The successful application of tuna roe will not only produce cost-savings for the shrimp hatchery operations, but it also will turn a waste by-product into a value-added product providing additional environmental benefits by reducing the amount of organic wastes.

Work Progress and Principal Accomplishments

Objective 1: Analyze the nutritional values of tuna fish roe, and verify its specific-pathogen-free status.

Samples of tuna fish roe from the different loining operations on Guam (dry and wet seasons) have been collected. Analaysis of the samples, for proximate analysis (protein, total lipids, carbohydrates and ash), phospholipids, cholesterol, fatty acids profiles, etc., is ongoing. The samples are also being checked for the C-1 viruses of the US Marine Shrimp Farming Program SPF list, including WSSV, IHHNV using PCR, and TSV, YHV, IMNV using RT-PCR.

Objective 2: Develop a semi-moist maturation diet with tuna fish roe as the major ingredient.

Pending upon the nutritional baseline information. Once determined, a maturation diet will be formulated by incorporating tuna fish roe as the major ingredient and supplementing with other ingredients as needed, for examples, flour, soy meal, mineral and vitamin mix, binders (gelatin or carboxymethylcellulose) etc.

Objective 3: Evaluate the reproductive performance of shrimp fed with the tuna roe based maturation diet, in comparison with conventional fresh-frozen maturation feeding regimes.

Shrimp have reached approximately 30 grams. Evaluation has not yet begun.

Objective 4: Publish the findings from the project and conduct one workshop on utilization of tuna fish roe as the technology transfer effort.

Not available for this report

Work Planned

We plan to complete the tasks in objective one in one month or so and move forward with making the diets and start with the experiment. Because of the unexpected long time to obtain the Tuna roe samples with consistence and getting some feed ingredients to Guam, as well as the facility space limitation due to the on-going renovation in UOG hatchery, we anticipate a significant delay in completing this project and would like to request a nine month no-cost extension.

Impacts

The results form this research would increase the understanding of shrimp maturation process in response to Tuna fish roe based maturation diets and provide useful information to both academia and industry in shrimp aquaculture.

Publications, Manuscripts, or Papers Issued, Approved, or Presented

None to report.

20. Analyze and Compile the Nutritional Composition of Potential Feed Ingredient Resources in American Samoa into a Feed Manual for Use in Tilapia Feeds

General Information

Reporting Period: August 1, 2009 to October 1, 2010; no-cost extension through January 31, 2011 (Progress Report)

Funding Level: **$36,450**

Participants: **Warren G. Dominy,** Ph.D., Director Aquatic Feeds and Nutrition Department, Oceanic Institute

Dong Fang-Deng, Ph.D., Research Scientist, Aquatic Feeds and Nutrition Department, Oceanic Institute

Zhi Yong Ju, Ph.D., Research Scientist, Aquatic Feeds and Nutrition Department, Oceanic Institute

Collaborators: Darren Okimoto, University of Hawaii, Manoa Campus Sea Grant College Program Extension Leader

Ephraim Ellsworth Temple, the local extension Agent in American Samoa

RESULTS AT A GLANCE…

- This project is developing a manual of local ingredients available for feed, and will aid in improving production efficiency and sustainability for tilapia and other aquatic species in American Samoa.

- Investigators have increased the feed processing speed at the American Samoa community college.

- A simple, at-home feed manufacturing system has been created for application in this project.

- Two undergraduate students are assisting with this project.

Industrial Partners:
Aquaculture finfish farmers in American Samoa

Objectives

1. Identify, quantify and collect potential local products and byproducts for aquatic feeds development in American Samoa and dry samples for shipment to the Oceanic Institute (OI) for compositional analysis.
2. Analyze the nutrient composition of selected samples. Analyses will include: proximate composition (crude protein, crude fat, ash, moisture, and fiber), gross energy, amino acids, and fatty acids, when applicable. Data on ingredients will be compiled in a database.
3. Compile a feed manual containing the following information: a) list of locally available ingredients and byproducts, and their nutritional composition; b) practical finfish feed formulations using local ingredients; and, c) feed processing techniques and quality control tests (mix time for mixers, ingredient particle size determinations, pellet stability test) for use in making aquaculture feeds containing the identified ingredients.
4. Transfer of technology and dissemination of information will be achieved through a work shop and feed manual hand-outs to local producers and farmers.

Anticipated Benefits

This work addresses the CTSA FY08 priorities in the Pacific Island Development Manual for developing feed from local ingredients, and will aid in improving production efficiency for tilapia and other aquatic species. The success of this goal will make a great contribution to sustainability of the aquaculture industry in American Samoa.

Work Progress and Principal Accomplishments

Objective 1: Identify, quantify and collect potential local products and by-products for aquatic feeds development in American Samoa and dry samples for shipment to the Oceanic Institute (OI) for compositional analysis.

Local ingredients and by-products identified as potential candidates for inclusion in tilapia diets have been refined in order to simplify implementation of collection, analysis and feed

formulation. Local ingredients and by-products collection has not been implemented yet because the drying oven that was purchased and sent has not been installed as previously reported. The lab where the drying oven, 2-meat grinders and the hammer mill are to be installed also needs a 3-phase power line, switch box and electrical panel. We now need to run a cable from a 3-phase source to the lab building but we also have to purchase an electrical panel and enough wiring to reach our building, so another re-budget is in progress.

Objective 2: Analyze the nutrient composition of selected samples. Analyses will include: proximate composition (crude protein, crude fat, ash, moisture, and fiber), gross energy, amino acids, and fatty acids, when applicable. Data on ingredients will be compiled in a database.

Local ingredients and by-products identified as potential candidates for inclusion in tilapia diets have been refined in order to simplify implementation of the, collection, analysis and feed formulation, currently is on hold until samples can be processed (dried) .

Objective 3: Compile a feed manual containing the following information: a) modified list of locally available ingredients and byproducts, and their nutritional composition; b) practical simplified finfish feed formulations using local ingredients; and, c) feed processing techniques and quality control tests (mix time for mixers, ingredient particle size determinations, pellet stability test) for use in making aquaculture feeds containing the modified ingredients.

Objective 4: Transfer of technology and dissemination of information will be achieved through a work shop and feed manual hand-outs to local producers and farmers.

A Technology transfer workshop is in progress; however processing equipment needed for technology transfer must first be operational to disseminate information on feed processing. A dryer oven has been purchased; shipped and is waiting to be installed. . A new hammer mill purchased by another project has been approved also waiting for installation; two (2) Hobart meat grinders are on site waiting to be installed. . A mini-workshop for the farmers' children at the same time as the workshop, or an on- farm demonstration is also planned. On-farm feed making, using appropriate "coffee can & wire screen technology" to measure feed ingredients and form feed pellets will be taught.

Work Planned

1. Re-budget to get 3phase line and panel installed
2. Install oven, meat grinders, hammer mill, get operational

3. Collect and oven dry ingredient samples and send to OI for analysis.
4. Formulate a simple tilapia feed easy to manufacture. Implement and test for applicability.
5. Work out workshops and manual for the Adult farmers and the on farm mini workshop for the children

Impacts

Create a small scale local base community feed manufacturing industry which will build up and expand the local production of tilapia culture.

The increase demand for tilapia feeds will eventually create a local entrepreneur to start a local feed manufacturing company, to serve the needs of the local tilapia farmers.

Publications, Manuscripts, or Papers Issued, Approved, or Presented

None to report.

21. Aquaculture of Opihi, Years 1 and 2

General Information

Reporting Period August 1, 2010 to November 1, 2010 (Year 1 Progress Report)

Funding Level	Year	Amount
	1	**$50,000**
	2	$50,000
(pending for approval)		
	Total	$100,000

Participants **Warren G. Dominy,** Ph.D., Director
Aquatic Feeds and Nutrition Department, Oceanic Institute

Nhan Hua, University of Hawaii

Addison Lawrence, Texas A&M University

Chris Bird, University of Hawaii

Vernon Sato, retired

Harry Ako, University of Hawaii

RESULTS AT A GLANCE…

- Opihi, which have been fished out of the main Hawaiian Islands, will be a high value product if aquacultured.

- The worksite has been prepared and broodstock opihi have been collected.

- Artificial feeds have been made.

- One Ph.D. student is participating in this project towards his degree.

Objectives

Year 1

1. Collect wild giant opihi and establish a broodstock holding facility.
2. Develop an artificial feed for opihi starting with natural diets and including artificial feeds made for other benthic grazers. The purposes are to maintain the animals and to identify possibly important feed characteristics.

3. Identify the best method of spawning opihi and develop larval rearing methods to increase survivorship. Current data suggest high mortality during larval settlement and metamorphosis.

Year 2

Not listed as year 2 has not started yet

Anticipated Benefits

The project could generate an alternate crop for salt water aquaculturists.

Work Progress and Principal Accomplishments

Objective 1: Collect wild giant opihi and establish a broodstock holding facility.

Arrangements have been made and equipment has been purchased for collecting opihi. The first trip was been scheduled for 10/30/10 and was to be taken by Chris Bird and Nhan Hua. Bad weather and large waves caused cancellation of the trip. The broodstock holding facility was established at the Hawaii Institute of Marine Biology. Two large outdoor tanks were started and rocks were placed in the tanks. Biofilm developed on the rocks. Indoor aquaria were prepared as well. Opihi will be fed rocks with biofilm and then weaned onto artificial feed.

Objective 2: Develop an artificial feed for opihi starting with natural diets and including artificial feeds made for other benthic grazers. The purposes are to maintain the animals and to identify possibly important feed characteristics.

The six feeds all contain about 25% kelp and several marine protein sources. The feeds work with sea urchins and should work with opihi as well. Initial instructions were prepared and given to the graduate student.

Objective 3: Identify the best method of spawning opihi and develop larval rearing methods to increase survivorship. Current data suggest high mortality during larval settlement and metamorphosis.

This objective has not yet been started.

Work Planned

As indicated above, we are poised to begin Objective 1 and are ready to test feeds described in Objective 2.

Impacts

Hoped for impacts are an alternative crop for Hawaii aquaculture producers.

Publications, Manuscripts, or Papers Issued, Approved, or Presented

None to report.

22. Developing a Value-added Product "Half-Pearls" from the Blacklip Pearl Oyster Pinctada margaritifera in Pohnpei (the Federated States of Micronesia), Years 1 and 2

General Information

Reporting Period: July 1, 2010 to September 1, 2010 (year 1 Progress Report)

Funding Level	Year	Amount
	1	**$47,939**
	2	$45,228
	(pending for approval)	
	Total	$93,167

Participants

Masahiro Ito, Ph.D., Director and Chief Scientist
Aquaculture Development
College of Micronesia Land Grant Program

Ms. Yuko Kibe
GIA (Gemological Institute of America)

Mr. Belenko Halverson
Half-pearl seeding technician and extension aide of COM.

Mr. Clayton Maluwelgiye
Half-pearl seeding technician and extension aide of COM.

Pakin Community Association (PCA)

RESULTS AT A GLANCE…

- Conducted half-pearl seeding demonstration and skill training for the local youths by the project's Micronesian technicians both in Pohnpei and at the outer islands.

- Applied an international pearl quality grading method from the Gemological Institute of America for the half-pearls.

- Three undergraduate students are assisting with this project.

Non Governmental Organization of Pakin Atoll

Objectives

The primary objective of this project is to produce high quality half-pearls or "Mabe" (hemispherical pearls) as a valued-added product from pearl farming activity. Half-pearl production techniques are to be transfer from the COM's Micronesian technicians to people of Pohnpei and the outer islands. For a sustainable pearl industry development, creating numbers of Micronesian seeding technicians is a key to success in the outer islands of Micronesia and such a technology transfer should be dome among the Micronesians.

1. To conduct half-pearl seeding and produce half-pearls.
2. To demonstrate and train half-pearl seeding techniques by COM's Micronesian technicians to select youths from pearl farming communities.
3. To demonstrate half-pearl pendants and accessory making by COM staff.
4. To conduct quality assessment of half-pearls and pearl shell-related accessories.

Anticipated Benefits

The project will conduct half-pearl seeding operation and skill training by the project's Micronesian technicians who will train other local youths. As the technology transfer is the most important output, the proposed project will target to create at least two seeding apprentices at each outer island and local community. On- farm training workshop of the half-pearl pendant and accessory making will be offered to the Pohnpei and outer island communities at least once a year. Half-pearl quality standard will be established by adopting and modifying the international pearl grading method from the gemological Institute of America. From the project's demonstration and skill training work during this quarter, which have used approximately 1500 pearl oysters in total, the half-pearls and pearl-related accessories will be produced and sold in a domestic and/or international market as a promotional display-sale. The amount sold from such sales will also be a part of direct income for the farming communities in Pohnpei.

Work Progress and Principal Accomplishments

Objective 1: To conduct half-pearl seeding and produce half-pearls.

Seeding work by the two technicians completed to conduct experiments using 400 pearl oysters each at Pakin Atoll and Nett Point in Pohnpei.

Objective 2: To demonstrate and train half-pearl seeding techniques by COM's Micronesian technicians to select youths from pearl farming communities

Two of the project's technicians performed demonstration of half-pearl seeding demonstration and nine youths including two teenage girls participated to practice half-pearl seeding on site the Pakin Atoll farm, which was the first of its kind in pearl seeding skill training in this region, as a part of the project's technology transfer among the Micronesians.

Objective 3: To demonstrate half-pearl pendants and accessory making by COM staff.

At Nett Point in Pohnpei, four trainees from nearby village community, three trainees from Pingelap Atoll farm, one trainee from Pakin Atoll and three trainees from the college graduate participated to the half-pearl harvest, seeding and accessory making work (Objectives 1, 2 and 3).

Objective 4: To conduct quality assessment of half-pearls and pearl shell-related accessories.

The number of host oysters which the trainees practiced the seeding varied from 2 to 50 a day based on the judgments by the seeding technicians because each trainee had different talent and capability. Pearl grading expert arrived in August and engaged her demonstration and teaching work for the project staff and local community members, which included basic knowledge on pearls, pearl quality and grading preparations such as washing and polishing. The grading expert also conducted quality assessment of the harvested half-pearls and accessories and demonstrated pricing the products (Objective 4).

Work Planned

(Milestone 4 – 12 months, Year 1): The pearl grading expert completes pearl and accessory grading and half-pearl pricing demonstration during the 4th month; Continue post-seeding oyster care and monitoring in Pohnpei and at Pakin; Conduct half-pearl pendant and accessory making; Conduct pearl quality assessment and pricing the half-pearls and accessories made by the COM's technicians and community members.

(Milestone 7 – 9 months, Year 1): Conduct trial display and promotional sales of the half-pearls and accessories in Pohnpei.

Impacts

As of September 30, the experiments of the Year 1 were completed and the post-seeding monitoring work is ongoing by the project's staff and by Pakin Community Association. A year before the commencement of this project, the pearl oysters had been seeded under the COM's project. Pearl quality grading demonstration and training was completed by used the half-pearls harvested during the first quarter of this project. Half-pearl pendant making and half-pearl seeding training contributed to enhance local communities and islander's awareness and interests in the pearl farming activities as these skills can be readily learned from the project's Micronesian technicians.

Publications, Manuscripts, or Papers Issued, Approved, or Presented

None to report.

23. DNA- Based Identification and Selection of High-growth Tilapia in Hawaii, Years 1 and 2

General Information

Reporting Period: August 15, 2010 TO October 31, 2010 (Year 1 Progress Report)

Funding Level:

Year	Amount
1	**$50,000**
2	$50,000
(pending for approval)	
Total	$100,000

Participants:

Jinzeng Yang, Ph.D., Research Scientist
University of Hawaii at Manoa

Liang Wu, Ph.D.
University of Hawaii at Manoa

Awat Yousif, Graduate Student
University of Hawaii at Manoa

Gavin Iwai, Graduate Student
University of Hawaii at Manoa

RESULTS AT A GLANCE…

- Genomic DNA isolation from Tilapia fin clip sampling has been conducted.

- Testing and establishment of the COI PCR and DNA sequencing protocol has begun.

- Discussions with local Tilapia farmers have been initiated.

Objectives

The long-term goal of the project is to develop fast-growing tilapia by using existing strains and hybrids in Hawaii. We plan to initiate this project through a two-year working plan.

Year 1

1. To identify and classify wild and captive tilapia strains and hybrids in Hawaii by DNA-based methods.

Year 2

1. To develop DNA-based testing tools for selecting high-growth tilapia by using existing strains or hybrids in Hawaii.

Anticipated Benefits

Results from Year 1 research work will provide genetic basis of selecting tilapia for the Year 2 project. Results from Year 2 project will provide useful directions of tilapia broodstock breeding for local aquaculture production. In the long term, this project will have significant impacts on Hawaii's aquaculture and technology development for a sustainable industry.

Work Progress and Principal Accomplishments

Objective 1: To identify and classify wild and captive tilapia strains and hybrids in Hawaii by DNA-based methods.

Tilapia fin clip sampling and DNA extraction: Tilapia fin clip samples will be obtained from ten populations (sites), including five wild populations in Oahu and Hawaii islands and five aquaculture and experimental facilities in both freshwater and brackish water. Fin clip samples from 30-40 fish will be collected from each site, along with the records of the body weight and length and growth performance (if available), and possible strain identifications. Genomic DNA will be isolated from caudal fin clips. We have established the method of genomic and mitochondria DNA isolation. We are in discussion/consultation with with local famers and tilapia specialists on the choices of sampling sites.

DNA barcoding analysis: The sequence of a mitochondrial protein-coding gene, namely cytochrom c oxidase subunit I (COI) has become a standardized reference sequence for DNA barcoding. We have tested more than 20 tilapia samples from amplified COI DNA fragment. We are still working on the sequence validations.

Work Planned

We will continue the discussions with local tilapia farmers and decide the sampling sites. Then we can start our fin clip sample collections form local farmers and "wild" tilapia

populations. By using the COI sequencing, we will classify the fish samples. A multiplex PCR protocol will be developed for two panels of PCR amplifications. PCR will be carried out for 35 cycles using appropriate PCR conditions. The fragment results will be analyzed and allele sizes will be calculated.

Impacts

We expect this research will generate the first inventory and identities of the tilapia strains and hybrids in the wild and captive facilities in Hawaii Islands on the basis of DNA analysis in the first year. Results from DNA-based genetic studies for high-growth trait selection in the second year will provide useful directions for tilapia broodstock genetic improvement of growth performance. Successful completion of this project will have immediate impacts on Hawaii tilapia aquaculture for better genetic stocks and selection of seedstock. An organized selection and breeding program of tilapia for better growth performances in Hawaii would have great economic value to the aquaculture industry because of enhancement in feed conversion and production efficiency. Hopefully, the results from this research project will help to build up a selective genetic program for breeding high-growth tilapia for the state of Hawaii and other Pacific regions.

Publications, Manuscripts, or Papers Issued, Approved, or Presented

None to report.

24. Adapting Aquaponics Systems for Use in the American Pacific, Years 1 and 2

General Information

Reporting Period August 1, 2010 to November 1, 2010 (Year 1 Progress Report)

Funding Level	Year	Amount
	1	**$34,012**
	2	$34,012
	(pending for approval)	
	Total	$64,024

Participants **Kent Kobayashi,** Ph.D., CTAHR
Department of Molecular Biosciences and Bioengineering
University of Hawaii at Manoa

Saipul Rapi
University of Hawaii at Manoa

James Hollyer, CTAHR
Department of Molecular biosciences and Bioengineering
University of Hawaii at Manoa

Harry Ako, CTAHR
Department of Molecular biosciences and Bioengineering
University of Hawaii at Manoa

RESULTS AT A GLANCE…

- This project will generate additional revenue schemes for the Pacific Islands, either commercially or for families, via aquaponics.

- A graduate student is currently being trained in the system's design and management.

- Aquaponics systems are being built in American Samoa based on the project's model.

- One graduate student and one undergraduate student are participating in this project.

Objectives

Year 1

1. Test the efficacy of the air breathers Chinese catfish *Clarias fuscus* and Asian snakehead *Channa sp.* for aquaponics in Hawaii. Use of air breathers could obviate the need for aeration and further simplify our aquaponics system. A downside of low dissolved oxygen could be enhanced denitrification. The issue of denitrification will also be addressed for Pacific Island systems using tilapia.
2. Determine the nutrient profile of fish water generated by metabolism of a locally produced feed and determine the need for supplementation of this feed.
3. Develop a planting and harvest scheme that will allow constant marketing of product. Complete a manual describing the construction, start up, and operation of the new aquaponics system.
4. Take materials to a client in the Pacific and build a system. Train farmers and local extension staff to build and operate the system. Demonstrate on site the operation of the system and work with the farmers to make sales arrangements with a local customer.

Year 2

Not listed as year 2 has not started yet

Anticipated Benefits

The project will generate additional revenue schemes for the Pacific Islands either commercially or for families.

Work Progress and Principal Accomplishments

Objective 1: Test the efficacy of the air breathers Chinese catfish *Clarias fuscus* and Asian snakehead *Channa sp.* for aquaponics in Hawaii. Use of air breathers could obviate the need for aeration and further simplify our aquaponics system. A downside of low dissolved oxygen could be enhanced denitrification. The issue of denitrification will also be addressed for Pacific Island systems using tilapia.

A greenhouse site at the Magoon Research facility has been secured for the project. This site is superior because it has electricity which will make research easier.

Objective 2: Determine the nutrient profile of fish water generated by metabolism of a locally produced feed and determine the need for supplementation of this feed.

This objective has not yet been addressed.

Objective 3: Develop a planting and harvest scheme that will allow constant marketing of product. Complete a manual describing the construction, start up, and operation of the new aquaponics system.

This objective has not yet been addressed. The manual has been completed and has been published. The last of 1,000 copies are being distributed.

Objective 4: Take materials to a client in the Pacific and build a system. Train farmers and local extension staff to build and operate the system. Demonstrate on site the operation of the system and work with the farmers to make sales arrangements with a local customer.

This objective has received the most attention. It is being well set up. A graduate student is being trained. By semester break he will have learned to build a system and run it. Dan Aga, Director of the Community and Natural Resources Program in the Land Grant section of American Samoa Community College has been enlisted for help. Connection has also been made with Horticulturist Larry Hirata who has begun some work using the handout and technology we developed (photos included) and has arranged for housing for the student.

Work Planned

We will begin Objective 1 which will have two purposes. One will be to solve the problem of denitrification. This issue is raised in the literature where massive amounts of nitrate were being theoretically generated and very little was available for growth of the plant crop. In addition, eventually air breathers such as Chinese catfish will be investigated as an alternative for locales where they are already present. The tilapia that are being used are being held at densities (20 kg/m^3) that are close to a fish kill if something goes wrong. The other underlying objective is to train the graduate student.

This preparation will prepare the graduate student well for his first technology transfer activities. Money can be saved and used for more technology transfer activities because the student in question does not need support. He is a Ford Foundation scholar. We hope that the student will be successful.

Impacts

Hoped for impacts are sustainable aquaponics systems set up across the Pacific. The question of economic sustainability has been addressed at the HAAA meeting in Hilo. The presentation can be put on a web site.

Publications, Manuscripts, or Papers Issued, Approved, or Presented

Ako, H. and P.-S. Leung, Economics of Aquaponics. Presentation made to the Hawaii Aquaculture and Aquaponics Association, Hilo, Hawaii, Aug. 16, 2010.