CENTER
FOR
TROPICAL
AND
SUBTROPICAL
AQUACULTURE

Center for Tropical and Subtropical Aquaculture

2011 Accomplishment Report

In cooperation with

USDA United States Department of Agriculture
National Institute of Food and Agriculture

Center for Tropical and Subtropical Aquaculture

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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 16 members:

John Brown, Ph.D.
College of Natural and Applied Science, WPTRC, 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
Kampachi 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

Bernard Tsao
UH-Kaua'I Community College, Hawaii

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 (retired)

Alan Everson
NOAA Aquaculture Coordinator

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

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 2011, the Center for Tropical and Subtropical Aquaculture completed work on projects funded under its Twentieth Annual Plan of Work and continued work on projects funded under its Twenty-first, Twenty-second, and Twenty Third Annual Plans of Work. Also, CTSA initiated work on projects developed under its Fiscal Year 2010 Plan of Work and began developing its Fiscal Year 2011 Annual Plan of Work.

Nine projects were funded under CTSA's Fiscal Year 2010 program, which was approved by CTSA's Board of Directors on January 27, 2011. Five of these projects address continuing priorities and will build on work begun under the programs of previous years, and four of the FY10 projects address new priorities.

Since the inception of CTSA in 1986, it has funded 231 research, demonstration, development, and extension projects. Twenty-three projects were active during 2011. 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

2. These projects address extension support:
   - Regional Biosecurity: Operational Biosecurity and Diagnostic Surveillance
   - Analyze and Compile the Nutritional Composition of Potential Feed Ingredient Resources in American Samoa into a Feed Manual for Use in Tilapia Feeds

- Pacific aquaculture development and extension support for the U.S. affiliated Pacific islands of the Federated States of Micronesia, FY2010
- Marine Finfish Aquaculture Development in the Northern Marianas Islands

3. These projects address marketing and economics:
   - 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
   - Assessing Hawaii's Aquaculture Farm and Industry Performance

4. These projects address development of new technologies:
   - Development of Captive Culture Technology for the Yellow Tang, *Zebrasoma flavescens*, Years 1 - 3
   - Improving the Hatchery Output of the Hawaiian Pink Snapper (*Pristipomoides filamentosus*)
   - 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
   - Alternative Methods for Marine Copepod Production in Hawaii, Year 2
   - Culturing Native Species of Macroalgae in Hawai'i and the U.S. Affiliated Pacific Island

5. These projects address demonstration and adaptation of known technologies:
   - Adapting Aquaponics Systems for Use in the Pacific Islands
   - Collection and Health Certification of Coral grouper Broodstock in the Mariana Islands
   - Seed Production Mangrove Crab *Scylla serrata* in Palau
   - Broodstock Management, Seed Production and Grow-out of Rabbitfish, *Siganus lineatus* (Valenciennes, 1835) in Palau, Years 1 and 2

- Aquaponics for Hawaii and the U.S. Pacific Islands: Technology Refinement and Transfer to the Commercial Aquaculture Sector

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

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. 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 that will be useful to the aquaculture community. We are completing bibliographies on alternative feeds, open ocean cages and aquaponic systems, and working with AquaculureHub to provide one access point current aquaculture news available on the internet.

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. To meet both of these goals, a new more user-friendly CTSA website was developed and went "live" in June 2011. Two of the more notable new features are an interactive regional map and a comprehensive resource section for commercial producers. In addition, CTSA's e-newsletter, Regional e-Notes, 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. A video project was also started this year to profile and highlight various aquaculture activities in the CTSA region, for both stakeholder and public audiences. 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

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 at both the University of Hawaii (UH) and Oceanic Institute (OI) 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, the group responsible for the UH component of this project has successfully developed and validated PCR assay through a collaborative private-public partnership. The assay has resulted in a much improved understanding about certain aspects of the life history of FLB that is laying the ground work for future development of a disease management program for this emerging pathogen. Several workshops have been conducted to disseminate the information to commercial operators. The OI research group is still in the relatively early stages of project development; gathering information, collating the available literature on disease and biosecurity issues of relevance to the Hawaii aquaculture industry, and a formulating survey plan which we will be taking to industry in the upcoming months.

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 project 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. The nutritional composition data of locally-available products and byproducts generated by this project has provided a valuable database in formulating sustainable cost-effective feeds for the aquaculture industry in American Samoa. A simple diet for tilapia was created. A children's feed manual (English & Samoan) containing simple ingredient proportions for batching and feed making was created and published, along with an adult feed manual. A training workshop was conducted where both children and adults learned about fish farming and making tilapia feed on the farm site.

Pacific Aquaculture Development and Extension Support for the U.S. Affiliated Pacific Islands of the Federated States of Micronesia, FY 2010

The goal of this project is to re-establish a CTSA Pacific Island extension agent position. For the last five years, the CTSA extension agent's work has been unavailable in this region. Now, such an extension service is urgently needed to cope with changing circumstances in aquaculture development as the model of effectiveness and impact. Under the auspices of this project, the extension agent will 1) assist the development of an economically sustainable aquaculture industry in the U.S.-affiliated Pacific islands of FSM; 2) transfer hatchery-based aquaculture technologies and specialized pearl culture skills; and 3) coordinate and administrate active CTSA projects in the region.

The project commenced on July 1. A significant amount of work has been conducted during this reporting period in Pohnpei for the blacklip pearl oyster (Pinctada margaritifera), including harvesting of the round-pearls and half-pearls, skill training in nucleus implantations, half-pearl accessory making and grading, and promotional sale of the round-pearls and half-pearl products produced from the skill training programs. Pearl farming training also continued on the outer islands, and new juveniles were transferred from Pohnpei to two outer islands. Each outer island received 5,000 animals 8 – 10 months old produced from selective breeding work for blue-peacock and golden green nacre. The sea cucumber work in Pohnpei continued to grow juvenile sandfish (Holothuria scabra) in the raceway tanks, and preliminary work on the black teatfish (H. whitmaei) commenced with a search for broodstock for spawning-runs. While in Yap, the PI conduced the first meeting with stakeholders including private sea cucumber operators, fishermen, Yap State Government, the COM-Yap aquaculture extension agent, community elders and local business owners. All parties expressed their interests and support to develop a hatchery-based sea cucumber grow-out and restocking project. The COM-Yap aquaculture agent began implementing a hatchery-building plan with collaboration from the State Government's MRMD (Marine

Resources Management Division), EPA (Environmental Protection Agency), R&D (Resources and Development) and local communities.

Marine Finfish Aquaculture Development in the Northern Marianas Islands

The primary objective of this project is building marine finfish aquaculture capacity in the CNMI to help address food security and poverty reduction in the face of socioeconomic change in the region. This will be achieved through a series of workshops and training sessions. The project will initially focus on working with CNMI stakeholders to identify the most appropriate local species for initial development, followed by training in broodstock and hatchery technologies. Although there is strong support for high-value species such as snapper and amberjack, complications in ocean law make these a low priority for now. Therefore we will likely focus on developing fingerling supplies for species more appropriate for land-based growout such as locally popular Siganus and Mugil species.

The project commenced on September 1, 2011 and has not yet reported progress.

Marketing and Economics

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." 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.

Under the auspices of this project, the work group demonstrated the biological feasibility of edible bivalve culture, identified steps necessary for the certification of a laboratory in Hawaii, and is still addressing these steps. The project also made significant advances to build capacity for Hawaiian fishpond operators to grow shellfish in coastal areas.

Researchers identified multiple species as potential culture candidates. There are potentially at least 2-dozen Hawaiian bivalve species that could be good aquaculture candidates, foremost among them the clams. In addition, the Hawaiian oyster, Dendrostrea sandvichensis, was successfully spawned fifteen times with larvae numbers ranging from 10,000 to 50,000. Grow-out trials proved the viability of the species in aquaculture. 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.

Despite cooperation between DOH and FDA to certify a laboratory in Hawaii, due to the current fiscal challenges for the State of Hawaii, this continues to be the largest bottleneck to the development of a viable commercial bivalve industry for the State. The project work group is facilitating further discussion with the DOH and FDA to move the certification forward.

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) provide value-added opportunities to use the pearl oyster shells (mother-of-pearl shells) in the jewelry and handicrafts industries, particularly the local cultural carving and handicraft industry.

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 exists a small, niche tourism industry, the half-pearls have potential to support a sustainable pearl business and rural development, particularly for small family and/or community-based enterprises. 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. One of the advantages of producing half-pearls compared to round pearls is the lower capital investment and

technical requirements. 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 the local labor force is capable of producing high quality half-pearls with unique color and luster, thereby creating an opportunity to develop a new export market. The project activities are focusing on demonstration and skill training for grading techniques of the shells and the half-pearls by a grading expert, as well as the half-pearl harvest and subsequent half-pearl accessory making involving all community members.

During Year 1, approximately 700 oysters, 1,400 oysters, 200 oysters and 500 oysters were implanted with half-pearl nuclei at Nett Point, Pakin, Pingelap and Pweniou, respectively. The project's Micronesian technicians and 43 trainees, who participated in training sessions at each farming location, did all of the grafting operations. The project's core technicians also conducted half-pearl pendant and pearl shell accessory making training sessions on-site. The total number of participants in the jewelry making training sessions was 24 at Nett Point (including 3 trainees from Pingelap), 110 (including 30 school children) at Pakin, and 20 at Pweniou.

During Year 2 of the project, Micronesian technicians were capable of transferring half-pearl seeding skills to 12 trainees without the supervision of a foreign master technician while they grafted 3,000 oysters. Although a small quantity of 24 items (totaling $525) was sold in a day during the August promotional sale in Pohnpei, high luster and unique colors with simple designs of Micronesian branded half-pearl accessories are gaining good reputations and positive responses from both domestic customers and overseas pearl traders and the jewelry industry.

Assessing Hawaii's Aquaculture Farm and Industry Performance

The overall goal of this 18-month project is to make use of confidential individual farm level information from the Census of Agriculture to evaluate performance of the aquaculture industry and its various subsectors during the past decade. The findings of this proposed work will serve to provide recommendations for increasing efficiency of the various subsectors, and provide industry leaders and policy makers an assessment of the strengths and weaknesses of the industry mapping out suggestions for future opportunities of growth.

The project commenced on September 1, 2011 and has not yet reported progress.

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 a reliable year-round supply of high quality yellow tang eggs, and developed a larval rearing system and protocols yielding excellent survival of yellow tang larvae through the highly challenging pre-feeding larval period. Researchers have identified eggs and early nauplii stages of Parvocalanus copepods as a suitable first-feed for yellow tang larvae, and developed highly efficient algae and copepod production systems with a mean output approaching 100 million copepod nauplii per day. They also documented the development of yellow tang larvae out to two weeks of age.

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.

Some interesting trends were observed from laboratory-scale rearing trials to investigate first feeding, 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.

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.

Unfortunately there were no major impacts with regard to an overall improvement of the hatchery technologies being developed for the opakapaka. However, an impact of another nature is being realized as James Jackson, the graduate student from the department of Zoology successfully defended his thesis on May 18, 2011 and graduated in the summer. The project helped to provide the necessary training and experiences needed to produce the next generation of researchers.

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

Pacific threadfin broodstock maintained on saltwater well systems develop goiters over a period of several years, leading to the loss of highly valuable broodstock animals. This project is working to develop a pragmatic solution that improves broodstock performance while insuring or possibly increasing the survival and growth of moi larvae and fry.

Thyroid goiters are the major cause of moi broodstock mortality; the broodstock are also producing eggs with low levels of key thyroid hormones. Under the auspices of this project, researchers discovered that iodine supplementation through either the water or diet helped reverse goiter formation, restore egg thyroid hormone levels, and increased overall broodstock survival. Broodstock currently maintained on a practical

sausage diet supplemented with iodide and vitamins are showing far superior reproductive output and egg quality than stocks maintained on previous diets.

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). Both species have ideal characteristics that would make them excellent candidates for commercial aquaculture. Moreover, pacu can be co-cultured with other food fish and invertebrate species (Teichert-Coddington, 1996; Campos-Baca and Kohler, 2005). 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). In addition, the project work 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. 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 project to date has found that, while it is clear that growout of pacu in an aquaculture setting is feasible, its consumption of water alone is unacceptable unless there is a resource available that will be cost effective. The growout in an aquaponic setting was only briefly addressed but the preliminary results look very encouraging. Likewise, the preliminary results for the marketability as a food fish appear to be very encouraging.

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, tuna fish roe samples have been collected during both dry and wet seasons through a Guam tuna fish loining company. The research group has established the baseline nutritional information of tuna fish roe, including proximate analysis (protein, total lipids, carbohydrates and ash) and fatty acids analysis, etc., and verified that the tuna roe samples were free of major shrimp viruses using PCR method. The group has also formulated and developed the semi-moist diets using tuna fish roe samples for the acceptable texture, storage and nutritional composition through a series of trials, and established the protocols for evaluating the reproductive traits through preliminary experimentation.

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.

Under the auspices of this project, aquacultural waste and wastewaters were characterized to better develop a treatment system process to achieve remediation and re-use goals. In addition, researchers developed a wastewater characteristic study for use in the project. Final results are forthcoming.

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/taste of these farmed opihi must be tested and adjusted to match wild opihi.

To date, the project work group has learned how to capture opihi with the lowest mortality possible and hold them without killing them when moving them from tank to tank. They have determined methods to grow and feed opihi their natural food, and have also generated an artificial diet than opihi can be kept on indefinitely.

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.

The project has thus far established the MtDNA CR PCR and DNA sequencing protocol, concluding that mtRNA CR sequencing provides better DNA testing for identification

of the local feral and captive tilapia strains and hybrids. Discussion and collaboration with local tilapia farmers has been initiated; testing and DNA-based classification of ten local tilapia populations was conducted, resulting in the identification of seven distinct groups of tilapia.

Alternative Methods for Marine Copepod Production in Hawaii, Year 2

The main goal of this project is to develop and transfer extensive copepod production technology to farms so they are able to create feeds on-site. The objectives are to 1) Conduct short-term controlled experiments to assess the adaptation of the copepod cultures to artificial seawater and artificial feed particles; 2) Conduct short-term controlled experiments aimed at intensification of copepod cultures (greater animal density, target: 20 animals/mL, expansion to 300–500 liter volumes, more intensive support and maintenance) in artificial seawater with artificial feeds: 3) Assess and document results of long-term culture comparisons; and 4) Transfer technology to farmers.

Results to date are that all of the algal species (e.g., Isochrysis galbana, Chaetoceros sp. and Tetraselmis sp) examined grew similarly irrespective of whether the source of seawater was Instant Ocean® or natural seawater from Kaneohe Bay. While there may be subtle differences between the results obtained using artificial seawater and natural seawater for culturing P. crassirostris, overall the data indicate that there are no major differences in the use of one or the other in culturing this species of copepod. The harpatacoid copepod, E. acutifrons was also investigated and also found to perform similarly irrespective of whether the culture was done in artificial or natural seawater. No difference in performance of producing E. acutifrons was found when using either Tetraselmis or Chaetoceros and artificial seawater. Consistent with previous results when using natural seawater, Chaetoceros sp. does result in growth of the P. crassirostris but it does not perform as well as when using I. galbana. Both nauplii and adult production of P. crassirostris grown at various salinities indicates that the optimal range for this copepod species will be restricted to salinities of 30 ppt or higher. The culture of P. crassirostris can be done using only artificial seawater and for extended periods of time. Only live phytoplankton can be used to culture both copepod species investigated while using only artificial seawater for culturing both phytoplankton and copepod.

Future prospects for the project are to complete the extension and outreach components of the project that will have to be covered by the PI as part of his extension duties with CTAHR.

Culturing Native Species of Macroalgae in Hawai'i and the U.S. Affiliated Pacific Islands

This two-year project will aim to determine environmental and culture requirements for two native species of algae: Asparagopsis taxiformis (limu kohu) and Codium edule (limu wawae'iole). These species were chosen as model species due to their high value, export potential, input from industry members, value by managers of traditional Hawaiian fish ponds, and initial successful culture trials at PACRC. If commercially feasible culture methods can be developed for these species, this will lay the foundation for future work with additional species and for technology transfer within the State and to the U.S. Affiliated Pacific Islands.

The project commenced on September 1, 2011 and has not yet reported progress.

Demonstration and Adaptation of Known Technologies

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 m2), modular tilapia and lettuce co-culture system. The system is suitable for a group or family, and can be readily expanded for commercial purposes. It does not use much electricity nor does it use more than one piece of equipment, however further research is essential to finalize this simplified aquaponics system. That research includes the utilization of different species to obviate the need for electrical aeration. Once the necessary research is complete, the project work group will extend the technology to interested Pacific Island clients.

One of the most significant accomplishments of the project to date is the extension work completed in American Samoa. Three systems were constructed and run alongside farmers and a local project work group member, who is working with the farmers to ensure that their systems remain productive. In addition to extension, beginnings were

made in the understanding of the biochemistry of aquaponics systems. This led to affordable designs and management schemes that were transferrable to farmers.

Chinese catfish were successfully tested as alternative fish for aquaponics and were used to improve upon denitrification problems suffered by some farmers; these problems were causing decreased yield and lengthened growout times. Planting and harvesting schemes were also developed to cut growout time in half.

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.

Unfortunately, the inability to obtain the live broodstock from the wild thus far has left the research group to anticipate that there will be a significant delay in completing this project.

Seed Production Mangrove Crab Scylla serrata in Palau

The overall goal of this project is to verify and package a simple, reliable and practical technology on the seed production of mangrove crabs (Scylla serrata Forskal) in Palau. Seed production of mangrove crabs in existing hatcheries need to be established in the country so that crab farmers can have a reliable source of crablets for grow-out.

Allowing these crabs to spawn, produce the crablets in captivity and growing them in ponds and pens using improved aquaculture techniques may provide a lucrative solution to have a steady supply of this high valued commodity and to meet the market demand.

The project has not yet commenced.

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Broodstock Management, Seed Production and Grow-out of Rabbitfish, Siganus lineatus (Valenciennes, 1835) in Palau, Years 1 and 2

The main goal of this project is to develop and package a simple and reliable technology tailored to conditions in Palau on broodstock management, seed production, nursery and grow-out of economically important rabbitfish Siganus lineatus (Valenciennes, 1835). At present, there is an increasing interest in producing this species of rabbitfish commercially. Refinement of technology on broodstock management, larval rearing, nursery and grow-out of S. lineatus is, therefore, essential to ensure sustainability in future commercial grow-out operations.

The project has not yet commenced.

Aquaponics for Hawaii and the U.S. Pacific Islands: Technology Refinement and Transfer to the Commercial Aquaculture Sector

The overall goal of this project is to assist commercial aquaponics farmers and backyard aquaponics farmers engaging in food security and to extend knowledge generated to the American Pacific Islands. While there are some technologies that need to be refined, the main effort of this project will be technology transfer through hands-on, one-on-one assistance that will be provided via numerous site visits. In addition, it is anticipated that solutions to problems will be farmer and site specific.

The project has not yet commenced.

A Look Ahead

Development

The development of the Fiscal Year 2011 program began in March 2011 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 18 pre-proposals received in response to this call, CTSA requested that applicants submit five full proposals. These proposals will be forwarded to the Board of Directors for approval in January 2012 as the FY 2011 Plan of Work.

Proposals

1. Establishing Bivalve Farming in Hawaii
2. Pacific Aquaculture Development and Extension Support in the U.S. Affiliated Pacific Islands
3. Developing diets for Hawaii cultured abalone with normal shell color and high growth performance using local algae and their co-products
4. Economic Analyses of Aquaponic Systems in Hawaii and Guam
5. Mitigating the Diseases of Freshwater Cultured Fish Species in Hawaii

Review

In July 2011, 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 24, 2012. CTSA staff will incorporate these proposals into the Fiscal Year 2011 Plan of Work and will submit this FY2011 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 2011 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/index.php/projects/category/annual_reports

1. 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: October 1, 2007 to September 30, 2008; no-cost extension thru March 31, 2009 (Year 1) July 1, 2009 to June 30, 2010; no-cost extension thru December 31, 2010 (Year 2) **(Project Termination Report)**

Funding Level

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

Participants: **Robert Howerton, UH Sea Grant**

Brian Goldstein, Kona Bay Marine

Lynn Nakasone, DOH

Noelani Lee, KMH

Dave Nesbit, Goose Point Oysters

Paul Bienfang, Analytical Services Inc.

Bruce Anderson, Hawaii Pacific University

David Cohen, Aquatic Innovations

RESULTS AT A GLANCE…

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

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

- The Hawaiian oyster, Dendrostrea sandvichensis, was successfully spawned fifteen times with larvae numbers ranging from 10,000 to 50,000. Grow-out trials proved the viability of the species in aquaculture. It should be noted that the taste of this species is excellent and its smaller size is not a barrier to its marketability.

- The project work group is facilitating further discussion with the DOH and FDA to move the bivalve certification process forward.

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, Aquaculture Development Program

Steve Chaiken, Moloka`i Sea Farms

Jim Sweeney, Sunrise Capitol 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

A second meeting of the bivalve working group was held on April 16 2008 at the Department of Health building. In attendance were representatives from State of Hawaii Department of Health (DOH), private industry, the principal investigators, CTSA and the aquaculture development programs' aquatic veterinarians. In addition, there were two representatives from Hawaiian fishpond groups. The DOH molluscan shellfish regulatory program has been limited in the past. The state does not have commercial production of edible bivalves, so the program has been responsible for only

regulatory inspections of local shellfish distributors. With the growing interest in reviving the local industry, DOH has two staff members being trained in shellfish sanitation issues. This meeting addressed working with state agencies responsible for shellfish sanitation to move towards a system for shellfish sanitation. There is considerable cooperation from DOH in assisting with the revival in the bivalve industry. It was stated that FDA is willing to help with the certification of a state laboratory to the specifications of the Interstate Shellfish Sanitation Committee guidelines by training DOH staff members. Legal issues were also discussed, including inter-island transport of bivalve species and introduction of non-indigenous species. One need which emerged is the development of bio-security guidelines before the industry is revived to prevent problems experienced for other species cultured in Hawaii.

A third meeting of the bivalve working group was held September 9, 2008 at the Hawaiian Learning Center at Keawanui fishpond on the island of Moloka`i. Attending this meeting were a representative from State of Hawaii Department of Health (DOH), private industry, the principal investigators, the CTSA Director and the aquaculture development programs' aquatic technician. Also in attendance were representatives from Hawaiian fishpond groups and a Moloka`i shrimp farmer. Relevant issues addressed were shoreline certification, specifically four traditional Hawaiian fishponds, Keawanui, Kupeke, Kalokoeli and One` Ali`i. These ponds were all visited by the meeting attendees to familiarize them with these potential shellfish grow-out areas. Keawanui (shrimp) Farms was also toured as they plan on culturing shellfish as well. The DOH representative had recently been trained by EPA in the shoreline certification process but plans on additional training in the near future. Other issues addressed including recent changes in state law forbidding the importation of non-indigenous algae species. These changes have stalled new hatchery trials at PACRC.

Despite cooperation between DOH and FDA to certify a laboratory in Hawaii, due to the current fiscal challenges for the State of Hawaii, this continues to be the largest bottleneck to the development of a viable commercial bivalve industry for the State. While the DOH is responsible for bivalve sanitation, there are attempts to get a private laboratory certified. Although FDA appears to be open to proceeding in this direction there is some reluctance to working outside of DOH. Discussion between DOH, FDA and the industry continues so that a short-term solution may arise.

In most recent discussions, DOH has agreed to try and work towards having one of their personnel certified as a Laboratory Evaluation Officer (LEO) and to have their own laboratory certified for shellfish sanitation requirements. This is the most positive

route towards classification of shellfish growing areas, although further progress will most likely be delayed until funding can be found to bring FDA personnel over from the mainland to complete these tasks.

Meanwhile, under separate funding, UHH and Sea Grant will work with industry and pond operators to begin water quality sampling during the winter rainy season. While these results are not legally acceptable for classification purposes, it will help define which areas should be included or excluded in the eventual DOH water quality scheme, thus expediting this process.

We have demonstrated the biological feasibility of edible bivalve culture, have identified steps necessary for the certification of a laboratory in Hawaii and are addressing these steps. We have also made significant advances to build capacity for Hawaiian fishpond operators and other aquaculture facilities to grow shellfish in coastal areas and inland sites.

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. The Hawaiian oyster is a larval brooder, similar to Ostrea edulis, the European flat oyster, which is the main culture species in Europe. Males appear to trickle spawn on a regular basis, the female's eggs are fertilized and brooded internally. Larvae are released at the 120-150 micron size, and then set in 10 days. 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.
The viviparous nature of the Hawaiian oyster has both advantages and disadvantages. One advantage is that because larvae are released at a later developmental stage, less time would be spent culturing them in the hatchery. One disadvantage is that viviparous bivalves generally have a lower fecundity rate, i.e., fewer larvae are produced per female, although the final production rate at metamorphosis may prove to be the same.

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 are being held at PACRC were conditioned for spawning. Spawning was attempted in December 2010 and January of this year with no success.

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. Due to funding limitations, follow up training with the FDA is delayed until the fall of 2011. In a meeting with DOH personnel earlier this year they have agreed to certify three State laboratories to ISSC specifications by November, 2011. These labs are located on Oahu, Kauai and the big island of Hawaii. This is encouraging news as existence of a certified lab was identified as one of the major bottlenecks to the development of a commercial bivalve industry for Hawaii.
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. Although, results of initial growth trials were variable (Fig 1), oysters reached market size in five to six months, almost three times faster than growth rates on commercial farms on the mainland U.S. Juvenile oysters have been distributed at the four original sites 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. In addition to the above mentioned sites, growth trials have been ongoing at Sunrise Capitol Farms on Kauai with manila clams.

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.

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. Due to the encouraging results of the growth trials at multiple sites in Hawaii, a new bivalve hatchery funded by private investors, is now under construction on the island of Hawaii. The co-PIs are working have worked closely with this group on site selection and hatchery design. It is expected that this new bivalve hatchery will be completed by early summer 2011.

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. Additional workshops were held at both above mentioned fishponds in November 2011 concerning the FLUPSY or floating upweller system. Utilizing the flupsy technique allows the oyster seed to grow at high densities much more quickly and more uniformly that it does in natural flow conditions.

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 were monitored for growth and mortalities. Water

quality parameters were also measured. Bivalve growth rates at all sites were very encouraging, with oysters reaching market size in less than half the time than is found at commercial bivalve farms on the mainland U.S. All collaborators are very encouraged to continue to expand bivalve culture at their facilities. 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. Due to results of the CTSA funded project the PI's were able to successfully obtain additional extramural funding to address current legal bottlenecks to the development of this industry, the two most important bottlenecks being lab certification and training for DOH personnel in the shoreline certification process.

Recommended Follow-Up Activities

None to report.

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

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

General Information

Reporting Period: October 1, 2008 to September 30, 2009; no-cost extension thru March 31, 2010 (Year 1); March 1, 2010 to February 28, 2011 (Year 2) (**Project Termination Report)**

Funding Level

Year	Amount
1	**$24,000**
2	**$24,000**
Total	**$48.000**

Participants: **Clyde S. Tamaru,** College of Tropical Agriculture and Human Resources (CTAHR)

Dr. Petra Lenz. Pacific Biomedical Research Center

Michael J. Cooney School of Ocean Earth Science and Technology

Karen Brittain: Hawaii Institute of Marine Biology.

Benjamin Alexander, Hawaii Institute of Marine Biology

RESULTS AT A GLANCE…

- Laboratory-scale trials with opakapaka resulted in researchers concluding 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.

- Larval rearing trials that were done with the moi larvae as a training exercise resulted 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.

- The graduate student working on the project earned his degree in Zoology. The project helped to provide the necessary training and experiences needed to produce the next generation of researchers.

James Jackson, Graduate Student, Department of Zoology.

Private Sector Collaborators:
Syd Kraul, Pacific Planktonics
Ryan Murashige, Hukilau Foods
Ed Cichon, Maui Fresh Fish

Objectives

Year 1

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.

Year 2

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

Principle Accomplishments

Year 1

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

Principal accomplishments that were made during Year 1 under this objective includes:

- Hiring a graduate student (James Jackson) in good standing from the Department of Zoology
- Establishment of cultures for two species of calanoid copepods: Bestiolina similis and Parvocalanus crassirostris at Pacific Biomedical Research Center (PBRC)

- Characterizing the size distribution of various live feed organisms (e.g., rotifers, copepods and their various developmental stages).
- Testing and timing of nauplius production in batch cultures in a bioreactor
- Determining first feeding characteristics of opakapaka larvae.

It should also be noted that the work completed took place at PBRC located on the Manoa Campus. The source of spawned eggs was from the opakapaka broodstock facility at the Hawaii Institute of Marine Biology.

Project work group members would collaborate on a related project entitled "Airlift Bioreactor Based Production, Preservation, and Collection of Copepod Eggs" Principal Investigator: Petra H. Lenz and Associate Investigator: Michael J. Cooney that was supported by a grant from the University of Hawaii Sea Grant College Program. The goal of this project is to develop and test a bioreactor for the cultivation of copepods and the production of copepod nauplii and eggs which would have utility for the opakapaka project. The bioreactor was tested on Bestiolina similis, one of the copepod species being used in the rearing of opakapaka larvae and also used for other aquaculture enterprises in Hawaii and elsewhere. The design of the bioreactor takes advantage of the positive phototactic response by the copepodite, water depth and varying temperatures within the growth chamber and collection vessel. It was found that the bioreactor growth chamber supported copepodite growth and reproduction. The bioreactor was also found to be able to efficiently separate the eggs and nauplii from the adult copepods with 10% of the eggs remaining in the bioreactor and hatching into nauplii. This level of retention should keep the bioreactor well stocked over longer periods. The level of separation of the eggs and resulting nauplii found in the collecting chamber (Figure 1) is especially intriguing as it is uniformly nauplii and eggs. Continuous production of nauplii, however, proved to be problematic and would require additional support to finalize the design of the bioreactor.

The initial focus was on larval first feeding and a summary of the temporal changes in total length of opakapaka larvae that took place between fed (copepod nauplii) and not fed treatment groups is summarized in Figure 2. A significant change in total length of the non fed larval group is clearly evident just after 78 hours (3.25 days) post-hatching and is used as an indication of the early signs of starvation setting in. A significant change in gape size (Figure 3) takes place at a later time interval (e.g., 108 hours post hatch) and collectively the data indicates that introduction of live food organisms must take place no later than the third day post-hatch. This information is important particularly with the use of copepod nauplii as their production must be timed in order to get the highest density when the larvae begin to feed. If presented

too early the nauplii develop their capacity to escape (discussed in later section) while developing in the rearing tank. Too late and the larvae already show signs of starvation within 78 hours post-hatching.

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 the various feeding treatments:

- Rotifer only (10 – 20 individuals/ml)
- Copepod nauplii + rotifers (10-20 individuals each/ml)
- Copepod nauplii only (10-20 individuals/ml)
- Phytoplankton only (e.g, Isochrysis galbana)
- no feed control

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. The data summarized in Figure 4 shows the significantly lower survival of the larvae when rotifers are presented as a first live food organism. While the result was not unexpected the treatment was significantly ($P<0.05$) lower than when copepod nauplii were used either in combination with rotifers or when 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 actually be detrimental. Impact of the feeding treatments is mirrored in the resulting total length (Figure 5) of the surviving larvae with the smallest being those fed rotifers only.

Gape size (Figure 6) of larvae presented the different feeding regimens were particularly revealing as there was no change in gape size between larvae fed rotifers only and rotifers combined with copepod nauplii. Larvae presented copepod nauplii only resulted in larvae possessing significantly larger gape sizes indicating a more advance stage of development and when combined with the other results indicates that the nauplii only diet is a superior feeding regime over the others tested.

To address the crossover of opakapaka larvae from copepod larvae to rotifers or an alternative feed has emerged as the next major obstacle to the development of a hatchery protocol for this species. Rearing trials both at PBRC and HIMB have not resulted in any promising results with larval survival plummeting to less than 1% after the first week post-hatching at PBRC and between 10-12 days at HIMB.

Objective 2: Increase hatchery output by improving other tank conditions

No progress was made during the first year of the project due to the timing in hiring of the graduate student and the spawning season of the opakapaka broodstock.

Objective 3: Transfer developed technology to appropriate end users.

As a training exercise first feeding larval rearing trials (same feeding treatments) were conducted with moi (Polydactylus sexfilis) larvae rather than opakapaka larvae during the off season. The data proved to be useful to the hatchery manager of Hukilau Foods and their hatchery operations as some thought about incorporating copepods into the rearing protocol of moi larvae was ongoing for several years. The question about whether there was a definite benefit was provided as a result of that initial training 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 developing a hatchery protocol for an alternative fish species.

Information that was generated directly benefits the larger NOAA supported project as it confirms the information to date and that the utility of the copepod (P. crassirostris) nauplii as the first feeding organism(s) for opakapaka larvae.

Year 2

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

Several challenges were encountered during the many attempts to rear P. filamentosus larvae. These challenges greatly hindered efforts to resolve the "second feeding" issue. At this point all evidence continues to support 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.

Feeding Behavior Experiments:
The limitations of P. filamentosus predatory ability was investigated by presenting the larvae with assemblages of P. crassirostris life stages and with or without rotifers. In addition, predator-prey interactions were filmed at 30 f/s for a period of 1 hour.

Filming Methods:

P. filamentosus and P. crassirostris were recorded at 30 frames per second (fps) with a CCTV video camera (Panasonic Corporation, Kadoma, Osaka, Japan; model WV-BP310) equipped with a Nikkor 50mm lens (Nikon Corporation, Shinjuku, Tokyo, Japan; model 1433). The camera lens was positioned 0.25 m from the observation container and the lens was focused in a plane in the center of the container such that the field of view was 3 cm2. The container was illuminated from above with one 20 watt fluorescent light providing 1,900 lumens of light. Footage was recorded onto 60 minute high definition mini digital video cassettes using a digital high definition videocassette recorder (Sony Corporation, Minato, Tokyo, Japan; model GV-HD700).

Filming took place for 60 minutes. Larval feeding rates were not sufficient to reduce prey density by more than 10% during the 60 minute period. After filming was completed each day, except during trials where larvae were fixed, P. filamentosus larvae were returned to a second rearing container designated for larvae that have undergone observation so as to avoid re-sampling.

The footage recorded was converted to digital audio video interleave format using Adobe Première CS3 (version 3.0) and transferred to a FreeagentTM 1000 gigabyte external hard drive (Seagate Technology LLC, Scotts Valley, CA, USA; 9NK2AM-510). The footage was reviewed and all attacks were identified and examined frame-by-frame. Attacks were categorized into successful, unsuccessful and unknown i.e. the larva captured the prey item, failed to capture the prey item or the outcome was unknown. Attacks with unknown outcomes were not included in the data analysis. A summary of the various feeding treatments and filming events is summarized in Table 1.

P. filamentosus larvae exhibit two chief attack behaviors. The first behavior (Figure 7) is a typical c-start attack where once the larvae target a prey item they curl their body into a “c” or even “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 a c-start. This second type of attack seems to manifest haphazardly and was usually unsuccessful.

From days 3 to 5 post-hatch P. filamentosus larvae were able to capture early stage P. crassirostris nauplii when presented as the only prey item with increasing success (Figure 8). Capture success rates remain equivalent at days 5 and 6 post-hatch before decreasing at day 7 post-hatch. However, perhaps owing to the low number of observations (n of 15), the capture success rate is only significantly different between day 3 post-hatch and days 4-7 post-hatch ($p<0.05$, Pearson’s chi-square test).

The results of filming trials in which P. filamentosus larvae were presented with copepodites as well as nauplii are shown in Figure 9. The capture success rates for naupliar prey in this mixed prey-type experiment were not significantly different from the capture success rates for the single prey-type experiment (Figure 8) where only naupliar prey were presented. However, in these mixed prey experiments there was consistently no capture success for copepodites across all larval ages indicating that their escape mechanism(s) is successful to prevent capture. Although data was not gathered on a stage by stage basis, qualitatively it appeared that late stage P. crassirostris nauplii (NIV-NVI) possessed escape response/sensory abilities that were too advanced for the larval P. filamentosus to successfully capture during the first 2 days of feeding. Mean P. filamentosus mouth gape at first feeding (78 hours post-hatch) is 0.25 mm which should easily accommodate a P. crassirostris NVI with a mean length of 0.176 mm. At 120 hours post-hatch P. filamentosus had a mean mouth gape of 0.36 mm, which should accommodate all stages up to CV and CVI. Such comparisons between prey size and mouth gape provides further evidence that prey sensory ability and/or escape response is the limiting factor here, not prey size.

P. filamentosus larvae were observed to successfully capture and consume B. rotundiformis rotifers from first feeding. Attack "rates" appeared to be lower when larvae were presented with rotifers versus nauplii. However, capture success rate when P. filamentosus larvae were fed rotifers in single-prey type assemblages were significantly higher than nauplii (Figure 10). A mixed rotifer nauplii treatment was not attempted. There are some obvious differences between these two prey types. One of the most important considerations is that the sensory and escape response abilities of copepod prey result in significantly greater difficultly in capture. The rotifer prey is non-evasive and easy to capture in comparison. However, this result further substantiates the hypothesis that the rotifer may not be an appropriate live food organism for opakapaka larvae.

Capture success rates for clownfish (Amphiprion ocellaris) larvae presented with copepod nauplii are also significantly higher than capture success rates for opakapaka larvae (Figure 11). P. filamentosus total length and mouth gape measurements were compared with those A. ocellaris at the same age post hatching. A. ocellaris eggs and larvae are much larger and more developed than opakapaka. A. ocellaris larvae begin feeding at hatching and their average total body length at 6 hours post-hatch was 3.9 mm. Whereas P. filamentosus larvae do not begin feeding until day 3 post-hatch and mean total length at 6 hours post-hatch was 2.5 mm and is still 3.1 mm at 78 hours (day 3) post-hatch. Mean A. ocellaris mouth gape was 0.34 mm at the age of first feeding (e.g., day 1 post-hatch), which is 0.1 millimeter larger than the mean P. filamentosus

mouth gape of 0.24 mm at the age of first feeding (day 3 post-hatch). Based on these results it is not surprising why technologies for the hatchery production have already matured to allow for commercial production of the species.

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

Small temporal and spatial feeding trials provided insight as to the mode in which opakapaka feed. Experiments utilized 5 day post hatch larvae stocked in 100 ml beakers at a density of 10 individuals or 1 individual/ml. In the same beaker 100 copepod nauplii were stocked which equates to 1 individual/ml and the contents were allowed to sit for 3 hours. Two treatments (with light and without light) with three replicates for each treatment were used for the experiment and after the allotted time transpired the number of nauplii consumed by each larvae was quantified under a compound microscope. The results are summarized in Figure 11 and the beakers that were exposed to light had significantly higher number (average 2.5 nauplii per larva) in the gut contents as did those that were kept in the dark. The data demonstrate that the paka larvae rely heavily on sight with regard to preying on food items. The end of the spawning season precluded other experiments (e.g., color, light intensity) to be conducted.

Objective 3: Transfer developed technology to appropriate end users

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.

Spawned eggs are being made available to stakeholders interested in trying their hand on rearing. Syd Kraul of Pacific Planktonics has been receiving eggs and conducting rearing trials over the course of the last year and including this year. He has had only limited success as is being experienced at HIMB and the transitioning to rotifers remains a challenge.

Impacts

Year 1: 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 for Hukilau Foods to pursue an alternative fish species.

Year 2: Unfortunately there were no major impacts with regard to an overall improvement of the hatchery technologies being developed for the opakapaka. However, an impact of another nature is being realized as James Jackson, the graduate student from the department of Zoology successfully defended his thesis on May 18, 2011 and will officially graduate this summer. The project provides the necessary training and experiences needed to produce the next generation of researchers.

Recommended Follow-Up Activities

Additional funding will be solicited in order to refocus efforts on defining a suitable rearing protocol for the rearing of opakapaka larvae. Combine results with the NOAA supported project and summarize information into a manuscript that is to be submitted to a peer reviewed journal for publication.

Publications and Manuscripts Written and Papers Presented

Publication

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.

Presentations:

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 .

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.

Jackson J. 2011. Larval Clownfish Amphiprion ocellaris Predatory Success and Selectivity when Preying on the Calanoid Copepod Parvocalanus crassirostris. Master of Science Thesis Defense. Department of Zoology, May 18, 2011.

3. Pacific Regional Aquaculture Information Service for Education (PRAISE) and Publications, Year 9

General Information

Reporting Period	October 1, 2010 to September 30, 2011 (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	
	Patricia Brandes, Assistant, University of Hawaii at Manoa	

RESULTS AT A GLANCE…

- The dollar value of the PRAISE service is $56,166. The program replied to 473 requests for direct assistance; 4,969 of those queries were emailed to PRAISE patrons. The 706 articles represent 10,953 pages delivered exclusively by email.

- PRAISE staff are completing bibliographies on alternative feeds, open ocean cages and aquaponic systems.

- A new, more user-friendly CTSA website was created and went "live" in June 2011.

- A monthly e-newsletter, Regional e-Notes, 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 new Publications video project profiles various aquaculture activities in the CTSA region, for both stakeholder and public audiences.

Objectives

PRAISE

1. Create a new web portal: Pacific Aquaculture News

2. Develop current bibliographies on aquaculture "hot topics" for inclusion on the News Portal.
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

Objective 1: Create new web portal: Pacific Aquaculture News

The World Wide Web has multiple access points to current aquaculture news. PRAISE staff are with AquacultureHub to provide one access point to this information. It will then be linked via the PRAISE website.

Objective 2: Develop current bibliographies on aquaculture "hot topics" for inclusion on the News Portal.

PRAISE staff are completing bibliographies on alternative feeds, open ocean cages and aquaponic systems. PRAISE staff continue to evaluate and update bibliographies.

Objective 3: Promote Pacific Region information infrastructure.

The PI and Assistant continuously promote and work to expand the online digital resources related to aquaculture. We are attempting to get copyright permission to add more aquaculture-related documents to the University of Hawaii at Manoa's open access digital institutional repository, Scholar Space, which will be linked from the PRAISE website. We also continue to work on getting copyright permission to add more full-text documents to some of our bibliographies, such as 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

In June 2011, CTSA released a brand new website developed under this project. The updated user-friendly site has many new features, most notably an interactive map of the CTSA region and a comprehensive section for commercial producers.

The Center has continued to produce and distribute an online newsletter each month to nearly 1,000 subscribers. The newsletter includes dissemination of CTSA project results, as well as regional aquaculture news and event announcements. In March 2011, the Center began a video series that highlights aquaculture farmers, research groups, CTSA projects, etc., throughout the region. To date, five videos have been viewed by 1,400 people on YouTube, Aquaculturehub, and the CTSA website.

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 FY11 Development Process and the preparation of the Plan of Work, and has worked together with CTSA's Administrative Assistant to compile this Annual Accomplishment Report from project progress and termination reports.

A water quality publication for the "Pacu" and "Biosecurity" projects was created during the reporting period and, in collaboration with NOAA and NMC CREES, the Publications P.I. also conducted a public outreach and education project to promote aquaculture in Hawaii and the CNMI.

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:

7,864 queries averaging 3 minutes each or:

393.2 hours online @ $80/hr =	$	31,456
706 articles @ $35.00 ea. =	$	24,710
Total	$	56,166

In replying to 473 requests for direct assistance, 4,969 of those queries were emailed to PRAISE patrons. The 706 articles represent 10,953 pages delivered exclusively by email. In addition, the staff responded to 9 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 Publications project have helped extend CTSA and other RAC research to industry stakeholders and interested parties throughout the region. The new YouTube video series has provided an opportunity for worldwide promotion of regional aquaculture activities.

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.

4. Development of Captive Culture Technology for the Yellow Tang, Years 2 & 3

General Information

Reporting Period: January 1, 2009 to December 31, 2009; two NCE thru December 31, 2010 (**Year 2 Final Report)** February 1, 2011 to September 30, 2011 (Year 3 Progress Report)

Funding Level

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

Participants: **Charles Laidley, Ph.D.,** Oceanic Institute

Chad Callan, Ph.D., Research Scientist, Finfish Department, Oceanic Institute

Eric Martinson, M.S., Research Associate, Finfish Department, Oceanic Institute

Melissa Carr, B.S., Graduate Student, Marine Sciences Program, Hawaii Pacific University

RESULTS AT A GLANCE…

- Established a reliable year-round supply of high quality yellow tang eggs.

- Developed a larval rearing system and protocols yielding excellent survival of yellow tang larvae through the highly challenging pre-feeding larval period.

- Identified eggs and early nauplii stages of Parvocalanus copepods as a suitable first-feed for yellow tang larvae.

- Developed highly efficient algae and copepod production systems with a mean output approaching 100 million copepod nauplii per day.

- Documented development of yellow tang larvae out to two weeks of age.

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

4. Identify suitable first feed for yellow tang larvae.

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

6. Develop larviculture feeding regimen suitable for rearing yellow tang larvae through metamorphosis.

Year 3

7. Develop suitable methods to transition yellow tang into juvenile settlement phase.

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

9. 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, we 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

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

Yellow tang broodstock collected and quarantined under year one continue to be maintained in both 25m^3 outdoor fiberglass tanks maintained as described previously. Recruits maintained in the smaller tanks were used to conduct replicated trials examining the effects of diet and water source on reproductive performance and egg quality.

The water treatment/source trial was completed in the second year comparing the effects of control fish maintained in outdoor tanks supplied with untreated OI well water to stocks receiving either filtered and degassed OI water, or recirculated ocean water passing through extensive water treatment. Reproductive output and egg quality were similar under all three treatment protocols, while fish maintained in the recirculating aquaculture system using ocean water yielded a remarkable improvement in fish condition over the study period.

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

Under year two of the project we also conducted a replicated diet trial comparing our control mixed diet with that of an in-house formulated feed. The control diet was composed of various commercial marine ornamental pellet and flake foods supplemented with raw including squid, shrimp, peas, spinach, Nori seaweed, and fish eggs. The formulated diet was a high-protein (60%), low-lipid (16%) diet that was 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. The new diet was generally well accepted after acclimation, showing a strong feeding response to the formulation. The formulated diet performed similar to our control (mixed raw diet), providing a simpler, more biosecure, and possibly more consistent diet for yellow tang maintenance.

With the completion of the feed and water trials in year two, we sorted the remaining stocks to establish 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 cyclical egg output with a clear lunar pattern in egg production centered on each full moon (Fig. 1). The combined improvements in egg output and egg quality have greatly increased overall egg supplies over the last year, allowing for significant progress on studies of early-feeding larvae. Total egg supplies peaked at over one million eggs/month, with a mean fertility rate of 80% and a mean egg viability rate of 44% since May 2010.

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

With the development of a steady supply of viable yellow tang eggs toward the end of the first year of the project we began examining embryonic and the early larval stages. Eggs demonstrate a typical development sequence for pelagic spawning marine species with the production of a very small egg and larvae. Hatching typically occurs in about 21 to 22 hours after fertilization. The resulting larvae are significantly smaller (~1.4mm) in length than even the pygmy angelfish larvae (~1.7mm). Newly hatched larvae survive on yolk reserves (endogenous feeding) for the first two to three days of development, after which time they develop functional eyes and mouths and must start feeding on available prey.

As egg supplies grew during the second year of the project we found that newly hatched yellow tang larvae behave quite differently from flame angelfish and other hatchery reared fishes studied to date, thus requiring changes to the larval rearing systems and husbandry protocols. Following hatch, larvae spend the first day at the tank surface, prior to translocation into the water column, on day two, while they complete mouth and eye development in preparation for feeding. Even light aeration (which facilitated hatching) was shown to be highly destructive to these fragile pre-feeding larvae. Although the use of static conditions improves initial survival; deterioration in water quality precluded its practical application. In response we developed an upwelling water delivery system to maintain water quality while providing a less turbulent larval environment. These system improvements now support excellent early (pre-feeding) larval survival enabling us to generate large numbers of larvae through to the start-feeding period.

Objective 4: Identify suitable first feed for yellow tang larvae.

As we began the second year of the project, with the initial availability of viable eggs and hatched larvae we began testing culture methods that had previously been successful for flame angelfish. Although yellow tang larvae filled their guts with microalgae, but they did not appear to consume harvested *Parvocalanus* nauplii, resulting in no survival through the early feeding period. However, by late in the second year as egg supplies improved substantially, along with

continued improvements in overall egg quality, improvements in larval rearing system design, and significant improvements in live feeds supplies, we were able to get yellow tang larvae to begin feeding. Subsequent trials revealed that yellow tang larvae could not eat most nauplii, but are capable of ingesting microalgae and the egg and earlier (i.e., smaller) stages of copepod nauplii. We also tested eggs/nauplii from a second species of local calanoid copepods, *Bestiolina similis*, turned out to be of similar size to *Parvocalanus* copepods but much more difficult to culture at suitable densities.

Objective 5: Scale-up culture of identified first feed to level required for conducting replicated larviculture trials.

With the successful identification of early stage copepod nauplii as appropriate first-feeds for yellow tang larvae, we began scaling up our microalgae and copepod production capacity as needed to facilitate continued development of yellow tang culture development.

During the second, and into the third year of the project we worked hard to renovate and scale up our algae production laboratory to facilitate generating sufficient quantities of high-quality *Chaetoceros* and *Isochrysis* microalgae to meet copepod and larval rearing requirements. The developed system consists of replicated 2L flask and 20L carboy systems for scaling up *Isochrysis* and *Chaetoceros*, and a bioreactor room with 16 independent bioreactor cylinders for scaling up algae production (Fig. 2). The room is supplied with filtered air and water to help maintain algal purity. The air supply system utilizes a dedicated blower system and air delivery system designed to eliminate moisture and filter air down to 0.1μm prior to delivery to algae cultures. The water delivery system automatically dilutes saltwater to 25ppm salinity and treats incoming water through a series of mechanical filtration steps including sinter glass pressure filter, 5μm and 1μm bag filters, a 0.35μm and 0.1μm cartridge filters, followed by UV sterilization. Cylinders are stocked with algae from carboys and run under continuous harvest, at a rate of approximately 40% per day. Cultures are maintained until they become contaminated or otherwise loose productivity.

Under current conditions, the *Chaetoceros* cultures show relatively consistent culture performance, maintaining steady state culture densities of approximately 4 million cells/ml and a mean daily output of 0.56 trillion cells/cylinder/day. The *Isochrysis* cultures appear to perform similarly to that of the *Chaetoceros* culture, but are beset by more frequent culture crashes, yielding about one-half the overall production output at a mean culture density of 2.8 million cells/ml and mean daily output of 0.41 trillion cells/cylinder/day. Aside from the consistency issues, *Isochrysis* culture dynamics appear remarkably similar to that of *Chaetoceros* in terms of culture density and cylinder output. Current algae production from the bioreactor system operating five reactors for

each of the two algae species is 2.8 trillion *Chaetoceros* cells and 2.1 trillion *Isochrysis* cells per day. Air conditioning capacity is the main bottleneck, as operating more than ten cylinders at once leads to rapid increases in room temperature, with culture temperatures increasing above the apparent 25-26ºC upper threshold for culture stability.

Algae from the bioreactors is continuously supplied to 1,000L harvest tanks and further distributed via a pump powered delivery system to the copepod broodstock maturation and nauplii production laboratories. The algae delivery system greatly facilitates algae distribution to copepod production tanks and allows for continuous feeding through the day.

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 (Fig. 2). Tanks are stocked with newly hatched nauplii on a daily basis and harvested after a week to ten days 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. 2). Adults are retained in the production system, while egg and nauplii are harvested daily. Female densities are maintained through regular adult supplementation as needed. Total egg and nauplii production is currently averaging 4.6 million eggs and 17.2 million nauplii/day for a daily total output of 21.9 million copepods per 1500L production tank, yielding an average of 88 million eggs and nauplii per day for larval feeding and system restocking.

Objective 6: Develop larviculture feeding regimen suitable for rearing yellow tang larvae through metamorphosis.

The increased supply of eggs/viable larvae and development of a suitable early larval rearing system led to exciting progress in getting newly hatched yellow tang larvae to begin feeding and initiate development. Despite excellent feeding rates (often above 80%) we continue to experience very high mortality rates past day five. Interestingly, the control (non-fed) larvae constantly showed slightly higher survival rates than fed larvae (until yolk exhaustion) suggesting that survival was impacted by factors associated with the startup of feeding. This led us to examine key rearing parameters including tank size, stocking density, background algae and color, microbiology, and water quality.

Unlike for the flame angelfish, early feeding yellow tang larvae showed little impact of tank size over a range of 1L to 1000L on early survival, allowing us to use our optimized

20L system to conduct replicated feeding trials. Also, feeding rates and survival were also not affected by stocking density over a range of 20 to 80 larvae per liter. In trials examining the effect of tank size, we noted a possible effect of tank color. Further exploration revealed that yellow tang larvae are quite sensitive to tank coloration, with improved feeding rates and better early survival in black larval tanks. We also examined preferences in terms of background algae and found much improved initial survival with either *Isochrysis* or *Chaetoceros* microalgae, relative to *Tetraselmis* or no background algae.

Continued high mortality rates after the initiation of feeding led us to focus on microbial effects during the period of first-feeding and gut colonization. Efforts to lower microbial loads using UV sterilization of copepods prior to feeding and the introduction of probiotics failed to improve survival. The switch from well saltwater to sterilized and conditioned ocean water seemed to somewhat improve early survival, while testing with a number of antibiotics revealed substantial improvements in early survival when treated with Neomycin. Follow up studies testing the application of Neomycin in the larval rearing tank water, in the algae cultures, and the copepod cultures all yielded improved early survival (Fig. 3). However, efforts to apply Neomycin to larger scale (i.e., 200L) rearing efforts again yielded little survival after the startup of feeding. Examination of these larvae under the dissecting microscope revealed a reddening of the intestinal region (Fig 4). While histological examination of these specimens by both the Allen Riggs (State of Hawaii, Aquaculture Development Program Veterinarian) and Dr. Jerry Heidel (Oregon State University) failed to provide convincing evidence of inflammation, vascular dilation or hyperemia. The histological preparations do reveal a few necrotic cells in the mucosal lining and lumen of intestine and bacteria attached to some of the gut debris (Fig. 4). The possibility of bacteria-associated toxemia/septicemia appears reasonable although evidence is not very strong.

Although survival rates past the startup of first feeding remain extremely low, we have been able to rear small numbers of yellow tang larvae out to two weeks of age enabling us to begin describing development. Over the first two weeks of development, the yellow tang larvae show dramatic changes in appearance with the rapid growth of both pectoral and dorsal spines and a deepening of the body as they develop into actively feeding pelagic larvae (Fig. 5). Although survival rates are quite low as the larvae enter their second week, the successful transition onto live feeds and rapid rates of development are highly encouraging.

Objective 7: Develop suitable methods to transition yellow tang into juvenile settlement phase.

Studies on post-larval transition into the juvenile settlement phase have not been initiated at this stage of the project. There is insufficient larval survival at this stage of the project to work on the settlement phase of the yellow tang life cycle. Planned studies on methods to settle yellow tang post-larvae requires the successful rearing of yellow tang larvae and generation of sufficient numbers of post-larvae to conduct planned trials.

Objective 8: Establish suitable feeds and holding system to ensure juvenile quality suitable for marine ornamental wholesale market.

Juvenile feed and holding studies have not been initiated at this stage of the project. Planned studies on juvenile feed and holding systems for yellow tang also requires the successful rearing of yellow tang larvae and generation of sufficient numbers of juveniles to conduct planned trials.

Objective 9: 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 a review of work to date in a CTSA Regional E-notes article released in last month and a number of conference and workshop presentations as listed under the publications and papers presented portion of this report.

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:

Rietfors, M.C. 2011. Aquaculture of yellow tang (Zebrasoma flavescens): An investigation of the early rearing environment, first feeding, and larval development. Masters Thesis, Hawaii Pacific University, Masters in Marine Sciences Program. 89pp.

Laidley, C.W., C.K. Callan, M.D.C. Rietfors, M.D. Kline, and E.W. Martinson. 2011. Development of captive culture technology for the yellow tang (*Zebrasoma flavescens*). CTSA eNotes Vol. 3, Issue 3.

Laidley, C.W. Saving the Reefs: Trials and tribulations in developing captive culture technology for coral reef fishes. Rising Tide Workshop, Orlando, Florida, November 18, 2010

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.

Laidley, C.W., C.K. Callan, M. Carr, E. Martinson, K. Pinkerton, M. Kline. Development of copepod culture and its application to the culture of marine ornamental species. Aquaculture 2010, San Diego, CA, March 4, 2010.

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.

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

General Information

Project Period: September 1, 2009 to August 30, 2010; two NCE thru May 31, **2011 (Year 1 Final Report)**
June 1, 2011 – October 31, 2011 (Year 2 Progress Report)

Funding Level

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

Participants: **Robert Howerton**, University of Hawaii Sea Grant College Program.

Clyde S. Tamaru, College of Tropical Agriculture and Human Resources, MBBE

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RESULTS AT A GLANCE…

- Trials testing the feasibility of polyculture of pacu and Chinese catfish experiment were conducted using 14 pacu (per trial) and various amounts of catfish. Based on the body weight and total length, the best treatment for Chinese catfish at this point in time is the 14 pacu + 100 Chinese catfish treatment. The average body weight of pacu that were raised alone (456.4 ± 116.5g) was not quite significantly larger than the pacu grown with 100 Chinese catfish (392.2 ± 69.2g).

- While it is clear that growout of pacu in an aquaculture setting is feasible, its consumption of water alone is unacceptable unless there is a resource available that will be cost effective. The growout in an aquaponic setting was only briefly addressed but the preliminary results looks very encouraging.

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

Objectives

Year 1

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.

Year 2

1. Conduct pilot-scale monoculture growout enterprises in collaboration with private sector farms.
2. Characterize growth and survival of juvenile red pacu in closed recirculating systems.
3. Test market pacu as a food fish species on both Oahu and Maui and conduct survey of ornamental market demand. Major Deliverable: Publication of market survey fact sheet. Demonstration of taste testing with UH culinary schools.
4. Technology transfer to appropriate end users. Major Deliverables: Two workshops will be conducted and at least two fact sheets will be published summarizing the results of all above objectives.

Principal 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. 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 remained.

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 no parasites were observed with biweekly inspections. A total of 150 individuals remain and were used to initiate the polyculture experiment with Chinese catfish. Additional funds for the Year 2 project funds will be needed to complete this particular objective.

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

On November 11, 2010 the polyculture of pacu and Chinese catfish experiment was initiated with the stocking of ten 600 gallon tanks with the following treatments:

- 14 pacu + 0 Chinese catfish
- 14 pacu + 50 Chinese catfish
- 14 pacu + 100 Chinese catfish
- 14 pacu + 200 Chinese catfish
- 14 pacu + 300 Chinese catfish

Fish were fed a diet of Nelson's Silver Cup trout feed to satiation and their growth and survival were monitored at monthly intervals over the course of the remaining project period. The last sample date was completed on March 10, 2011 and the data on their average percent survival, body weight, total length and condition factor index is summarized in Table 1.

Survival over the course of the 113 grow-out period was high (>82%) for both Chinese catfish and pacu amongst all of the treatments. Significantly ($P<0.05$) lower average body weights were detected as was total length amongst the Chinese catfish stocked at the lowest density (e.g., 50 individuals) along with 14 pacu. This trend was also detected with the condition factor index (CFI) which is a measure or robustness. Based on the body weight and total length the best treatment for Chinese catfish at this point in time is the 14 pacu + 100 Chinese catfish treatment. The change in growth for Chinese catfish appears to have begun approximately two months after the initiation of the growout trial (Figure 2).

By the end of the 113 day grow-out period the pacu stocked with 300 Chinese catfish showed the lowest growth rate and while not statistically significant, the lowest average survival (Table 1). This trend is repeated for both average total length and CFI. As observed for the Chinese catfish, the change in growth of the pacu appears to have occurred two months into the grow-out trial (Figure 3). Based on the results obtained, pacu alone or combined with 100 Chinese catfish appears to be the best stocking combination.

When the data are viewed on the basis of pacu biomass (kg body weight/m3 , Figure 4) in relation to the stocking density of Chinese catfish there are no statistical differences in Pacu biomass when the Chinese catfish are stocked at 0, 50, 100 individuals. Biomass drops significantly when the Chinese catfish are stocked at 200 and 300 individuals, accompanied by an increase in mortalities particularly in the 300 Chinese catfish treatment. Total biomass (e.g., pacu + Chinese catfish) of the 14 pacu + 100 Chinese catfish treatment averaged just under 20 kg/m3 and were over 30 and 40 kg/m3 when stocked with 200 and 300 Chinese catfish, respectively. Interestingly the biomass for Chinese catfish continues to increase in a linear fashion with increasing number of fish

indicating that their limits have yet to be reached.

The food conversion ratio (FCR) for the various treatments has been summarized in Table 2. The highest average FCR was obtained when the pacu were being cultured alone and much has to do with their feeding behavior. They are not aggressive feeders and particularly during periods when the water temperatures were low (e.g., < 25 oC) they were very lethargic. However, the FCR of the treatments that include Chinese catfish exhibit good FCR's and were all well below 2 with one treatment group (e.g., Pacu + 200 Chinese catfish) achieving an FCR of 0.99. Using the current feed costs of $0.65/lb for the Nelson's Silver Cup trout feed the range in feed costs for producing pacu alone is estimated at $1.59/lb but when combined with Chinese catfish can be as low as $0.64/lb.

The growout trial was being conducted using a flow through system that resulted in a daily turnover of each tank at a rate of approximately 4.4 times per day per tank. For all ten tanks this equates to a water consumption of approximately 8,360 gallons per day or 31,768 liters per day. Because the trends in growth were clearly detectable the experiment was terminated on the 113th day and the estimated water consumed till that date was approximately 944,680 gallons. Using today's Board of Water Supply rate of $2.79/1,000 gallons the cost in just water is estimated at $2,636.

All were equipped with two 26 gallon ebb and flow biofilters filled with cinder and operated using a bell siphon . Water from the tanks is being airlifted through ½ inch PVC pipe. Aeration is also being provided to the tanks via ½ inch PVC placed on the tank bottom and drilled with holes at approximately 1 foot intervals. Fish were restocked on March 28, 2011 into the various tanks consisting of 1) 20 pacu only, 2) 20 pacu + 100 Chinese catfish, 3) 50, 4) 100 and 5) 200 Chinese catfish only. All treatments are being conducted in duplicate and were conducted using a closed recirculating system (Figure 5). Because the biofilter is a reciprocating ebb and flow system it can also support plant growth and they have been planted with a variety of plants (e.g., bak choi, manoa lettuce, green onions, basil, and chiso). Growth and water quality were monitored as before and the target harvest is 450 grams (1 lb) for pacu and Chinese catfish.

Due to inclement weather resulting in a power surge and also an accident with a standpipe aeration was lost during the night on April 26 resulting in heavy mortalities with the Pacu. No mortalities were observed for the Chinese catfish and the experiment had to be terminated albeit with approximately one month of data as an aquaponic trial. Data on growth was summarized for both Chinese catfish and pacu

and is presented in Table 4. The average body weight of pacu that were raised alone (456.4 ± 116.5g) was not quite significantly larger than the pacu grown with 100 Chinese catfish (392.2 ± 69.2g) two tailed P = 0.080, t = 1.807 which is consistent with the previous results that the pacu fair better when cultured alone and particularly at the higher densities. Although the trial had to be terminated prematurely the pacu raised alone had attained the target harvest size of one pound (e.g., 450 g). As the original stocking of the fish was in November of 2010, the pacu had attained the target body weight of one pound within six months, albeit already being at the average body weight of 88 grams. Clearly the fish does exhibit a relatively fast rate of growth as predicted from the literature. However, it is not able to tolerate the low (< 1.0 ppm) dissolved oxygen levels that tilapia and Chinese catfish can.

As the trial was being conducted under a closed recirculating aquaponic setting the water quality parameters that were obtained are of great interest and is summarized in Table 5. With the exception of the treatment that contained 50 Chinese catfish the average dissolved oxygen levels were already dipping below 5 ppm with the lowest values occurring in the tanks that were stocked with the 200 Chinese catfish. Biomass in this treatment is already estimated at over 20 kg m3 and will require additional aeration to improve the values as the system is being operated as a closed recirculating one. Typical of aquaponic systems is the less than neutral pH values indicating nitrification is taking place and also supported by the high total nitrate and low nitrite values. The high TAN that were observed in the majority of tanks, however, indicates that the bacterial populations that are responsible for nitrification may not have become fully established as the cinder beds are only a month old. However, the low DO levels and observed fouling of the cinder bed due may actually indicate that de-nitrification maybe taking place and that the cinder beds are being overwhelmed with the amount of fish already present. In any event, the trial was terminated and will be put back into place with the start of the second year of the project.

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

Five sub-adult pacu (1 kg body weight) were received at the WCC facility on November 5, 2010 and placed under quarantine. An additional six sub-adult black pacu that are being housed at Plant Quarantine on Sand Island are available for use for this objective. These individuals were confiscated because they did not meet the four inch minimum size allowed for importation and have been held in an aquarium for several months. They have since grown to the size where they can be positively identified as pacu rather than piranha. During the next reporting period these individuals will be moved to the WCC campus and be incorporated into this project. The resulting pacu that survive

the remainder of the growout trials will become potential broodstock to be held at WCC and/or distributed to stakeholders.

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

A comprehensive literature search taken place prior to funding of this project revealed that a significant amount of peer reviewed literature was written in foreign languages (Spanish and Portuguese). This fact resulted in the inclusion of this objective in the work plan for year one. Attempts made to identify someone who may translate these documents have been unsuccessful to date. Work to complete this objective will continue in year two.

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.

Objective 6: Conduct technology transfer to appropriate end users.

During the reporting period a number of workshops were held by project personnel covering aquaponics and these were:

1. Workshop: Aquaponics workshop at Maui Community College with UH Sea Grant. February 6, 2010.
2. Workshop: AQUAPONICS SYSTEMS FOR USE IN YOUR OWN BACKYARD. Waimanalo Homestead Association Feb. 15, 2010
3. Workshop: AQUAPONICS SYSTEMS FOR USE IN YOUR OWN BACKYARD. Honolulu Aquarium Society April 2, 2010
4. Workshop: Aquaponics at the Hawaii State Hospital. Hawaii State Hospital, April 8, 2010.
5. Workshop: Aquaponics at the University of Hawaii. ʻĀina Hoʻōla O Māʻilikūkahi June 7-9, 2010
6. Workshop: CTAHR's traveling road show, Windward Community College, June 15, 2010.
7. Workshop: How to build your own aquaponics system. Windward Community College, July 24, 2010
8. Workshop: July 27, 2010. Chaminade's NHEA Pua Lililehua Project, Palolo Elementary School Teachers, Windward Community College.
9. Workshop: Black Soldier Fly, Windward Community College, July 31, 2010.
10. Workshop: Backyard Aquaponics for the Homestead, Department of Hawaiian

Homelands, Kapolei. August 28, 2010.

11. Workshop: Aquaponics at the College of Tropical Agriculture and Human Resources (CTAHR). October 8, 2010. College of Engineering Sustainability workshop. Windward Community College.
12. Workshop: October 11, 2010. Chaminade's NHEA Pua Lililehua Project, Anuenue Elementary School Teachers, Windward Community College
13. Workshop: Aquaponics at the College of Tropical Agriculture and Human Resources (CTAHR). October 12, Master Gardner's, Maui Cooperative Extension Service.
14. Workshop: Basic Water Quality Testing for Aquaponic Systems March 12, 2011, Windward Community College, Hale Imiloa 123.
15. Workshop: Basic Water Quality Testing for Aquaponic Systems May 21, 2011, Maui Community College.

Year 2

Objective 1: Conduct pilot-scale monoculture growout enterprises in collaboration with private sector farms.

Due to the heavy losses that occurred during the first year work there were no additional fish that could be distributed to our private sector collaborators. All of the financial resources that were allocated for the project had to be adjusted in order to obtain the additional fish needed to complete the co-culture trial during the Year 1 project. The current objective will have to be delayed in order to complete Objective 2 of the current project and to insure that there will be enough fish. It is anticipated that there will need to be another proposal made that would address this objective.

Objective 2: Characterize growth and survival of juvenile red pacu in closed recirculating systems.

During the previous reporting period the grow out trial was conducted using a flow through system and the inputs regarding water use were summarized and they are:

Resulted in a daily turnover of approximately 4.4 times per day per tank.
For ten tanks ≈ 8,360 gallons per day or 31,768 liters per day was used.
Estimated water consumed ≈ 944,680 gallons for the growout trial.
Using today's Board of Water Supply rate of $2.79/1,000 gallons the cost in just water is estimated at $2,636.

Clearly, an open system approach results in significant use of a precious resource. A short trial using a closed recirculating system equipped with an ebb and flow cinder bed (e.g., Aquaponic system) was initiated during the previous reporting period.

Unfortunately, the trial had to be terminated only after a month in duration due to inclement weather resulting in a power surge and loss of much of the pacu. However, the remaining fish (e.g., Chinese catfish) were grown out until harvestable crops were achieved (Figure 1 in appendix) during the initial stages of the current reporting period. As depicted in the photographs the ebb and flow biofilter can easily double as an excellent growbed resulting in a second crop of various vegetables in addition to the fish. All of the tanks have been refitted with shade cloth covers to prevent sunlight penetrating into the fish tank to avoid phytoplankton growth (Figure 2). They are also equipped with the same dual 26 gallon ebb and flow cinder beds with airlifts to provide water to the grow bed. In addition, all tanks have also been refitted with a new aeration system that provides DO levels of 5 ppm or greater. All tanks are currently stocked with tilapia to maintain the biofilters while the project awaits the arrival of the red pacu that have already been permitted and ordered.

Objective 3: Test market pacu as a food fish species on both Oahu and Maui and conduct survey of ornamental market demand.

Samples were provided to three chefs (Ed Kenney Town restaurant, Hiroshi, Alan Wong) and they were asked to evaluate the pacu grown out at WCC as a potential food fish in Hawaii. Hiroshi had by far the most in depth assessment and a copy of his report is attached in the appendix for review. Specific comments were:

Weight of fish	60 oz
Usage skin off	20 ½ oz
Yield	34%

Skin is on the tuff side, also on the thicker side

Pros-very easy to filet, skinning is very easy like mahimahi pulling the skin from the head to tail to skin on the whole. Cons- very hard to skin with knife sashimi style, hard to breakdown the fish on the belly side

Sashimi-excellent crunchy texture, very sweet, nice fatty-ness
Sauteed-very moist, good
Steamed-flaky, very muddy
Fried-belly side slightly muddy, top side very good & moist
Best way to prepare is either sautéed or deep fried.

Overall opinion-you have to like fishes like catfish etc. where it is more muddy in taste but it's an okay fish.

Ed Kenney of Town Restaurant has a weekly event (Wine Down Wednesday at Town Restaurant) where new wines and dishes can be tested with a small group of customers and their views could be captured in addition to the chefs. At this event, different entrees were prepared using pacu and they were:

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Bartolomiol Brut Prosecco (Valdobbiandene)
Fried Pacu, pickles, remoulade

Fontana Candida Frascati '09 (Lalio)
Malvasia/Trebbiano
Steamed Pacu, green tea salt, Fred Lau's Manoa Lettuce and Fred Lum's watercress

Placido Pino Grigio '10 (Toscana)
Pan seared Pacu, hand-cut pasta

Principessa Gavia Gavi '09 (Plemonte) Cortese
Grilled Pacu, Roasted tomato and limu

A copy of both chefs comments as well as the customers is provided in the appendix. In summary similar results were obtained for the steamed preparation which had a muddy taste that was not well received by the patrons. The pacu were not purged prior to delivery and may have contributed to the poor showing in that category. However, the seared preparation were very well received and overall the pacu received high marks as a potential food fish.

Alan Wong did not provide as critical an evaluation as the other two chefs, however, he did mention that the skin, while thick, when deep fried made an excellent side dish and should be considered in future dishes.

The remaining fish was provided to Wah Wah Seafood in China Town where the local fresh fish market could also be tested, albeit, with a small number (n=8) of fish. However, it was well received with all fish being sold the same day, marketed for $15.00 a pound as live whole fish.

A common comment that was made by both chefs and patrons was that the fish was "bony" and this particularly was troublesome in the preparation of filets. An x-ray of a market size pacu was obtained by the project work group (Figure 3).

Objective 4: Technology transfer to appropriate end users.

One newsletter article was published during the reporting period.

Howerton, R., C.S. Tamaru, R. Klinger-Bowen, B. Kai Fox and K. McGovern-Hopkins. 2011. Diversifying Freshwater Aquaculture Products for Hawaii with the Red Pacu (Piaractus brachypomus). Center for Tropical and Subtropical Aquaculture. Vol 3 Issue 6. http://www.ctsa.org/files/publications/June_11_Regional_e-Notes.pdf

A narrated PowerPoint presentation summarizing the Year 1 activities:

Howerton, R., C. Tamaru, R. Klinger-Bowen, B. Fox, and K. McGovern-Hopkins. 2011. Diversifying Freshwater Aquaculture Products for Hawaii: Two Crossover Species, the Red and Black Pacu (*Piaractus brachypomus* and *Colosomma macropomum)* – Year 1. submitted to the Center For Tropical and SubTropical Aquaculture..

WORKSHOPS:

An induced spawning of Chinese catfish was held in collaboration with the College of Agriculture and Human Resources (CTAHR) Local Immigrant Farmer Education (LIFE) program on September 14, 2011 at Maris Garden.

A presentation, Performance of red pacu, *Piaractus brachypomus*, cultured alone or co-cultured with Chinese Catfish, *Clarius fuscus*, is being prepared for presentation at the upcoming CTAHR/HAAA workshop to be held on November 12, 2011 at Windward Community College.

Work Planned

Upon arrival of a new shipment of red pacu (anticipated in the next few weeks), growout trials with available commercial feeds will commence. Growth and survival of pacu will be monitored in monoculture in aquaponic settings. This will fulfill objective one of year one (reason for delay described above).

Sources of black pacu continue to elude us but we remain hopeful to obtain this species for similar growout and survival trials.

Once pacu reach market size (i.e. 1 - 2 lbs), they will be sent to various culinary establishments on Oahu and Maui to conduct additional surveys for marketability. Final summaries of surveys as well as growout trial results will be disseminated via technical handouts and workshops/presentations to end users.

Impacts

The red pacu grew rapidly reaching one pound in six months, signifying a great potential for the Hawaii aquaculture industry. Additionally, the pacu adjusted well under an aquaponic setting, which served as a water conservation measure and expanded into other potential markets (i.e. vegetable crops).

Although a small sample size (N=8) were sold at WahWah Seafoods, the fish were readily sold on the same day commanding a $15/lb price. Continued exposure and surveys of marketability for pacu should only enhance this promising introduction into Hawaii's aquaculture industry.

Additionally, by addressing the requests for technical assistance from the aquaponic stakeholders there has been one notable impact that has resulted as a result of this project and it is:

Palolo Elementary School: A Model of Improvement.
http://www.staradvertiser.com/news/hawaiinews/20110328_Palolo_Elementary_a_model_for_improvement.html

Project work group members were instrumental in establishing both the system for use and training the trainers to use aquaponics to teach STEM skills in the classroom. An unsolicited letter of appreciation from the Dean of Chaminade University is presented in the appendix as evidence of the impact that was achieved as a result of the two workshops held for the teachers at Anuenue and Palolo elementary schools.

Recommended Follow-up Activities

Due to the loss of the red pacu during the previous reporting period the comparison of the different commercial feed types have yet to be initiated and a request has been made to focus on the completion of that task before moving to the next species (e.g., black pacu). In addition, a major consideration is to conduct feed trials using closed recirculating systems and include an aquaponics component to this task. The rationale being the flow through system that was used is no longer consistent with the overall theme of reuse, renew and recycle that will lead to a more sustainable production technology. Likewise, preliminary trials with tilapia using two commercial feeds clearly show differences in resulting amounts of nutrients (Table 5) and while no differences could be detected in two varieties of lettuce, kai choi did show differences in growth performance depending on the commercial feed used for the fish. Follow up trials utilizing freshwater species should seriously consider the incorporation of the aquaponic technologies when conducting research.

Publications and Manuscripts Written and Papers Presented

Stakeholder input from the aquaponics community has been extremely high regarding alternative species for use in this type of systems. For that reason the aquaponics activities have now become part of this particular project.

Technical Handouts:

Fox, B. K., R. Howerton, and C. S. Tamaru. 2010. Construction of Automatic Bell Siphons for Backyard Aquaponic Systems. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa. Biotechnology, June 2010, BIO-10. http://www.ctahr.hawaii.edu/oc/freepubs/pdf/BIO-10.pdf

Klinger-Bowen, R., C.S. Tamaru, B. K. Fox, K. McGovern-Hopkins, and R. Howerton. 2011. Testing your aquaponic system water: A comparison of commercial aquaculture methods. Center for Tropical and Subtropical Aquaculture. Technical Report. 16 pages.

Newsletter Articles:

Howerton, R., C.S. Tamaru, R. Klinger-Bowen, B. Kai Fox and K. McGovern-Hopkins. 2011. Diversifying Freshwater Aquaculture Products for Hawaii with the Red Pacu (Piaractus brachypomus). Center for Tropical and Subtropical Aquaculture. Vol 3 Issue 6. **http://www.ctsa.org/files/publications/ June_11_Regional_e-Notes.pdf**

Tamaru, C.S., B. Fox, M. Lee, K. McGovern-Hopkins, R. Klinger-Bowen, H. Ako, C. N. Lee, S. Khanal, J. Sugano and T. Radovich. 2010. Challenges and Opportunities for Aquaponics in the College of Tropical Agriculture and Human Resources. Hānaiʻai / The Food Provider Dec 2010. http://www.ctahr.hawaii.edu/sustainag/news/articles/V6-Tamaru-aquaponic.pdf

Tamaru, C.S. 2010. Expanding and Diversifying CTAHR's Aquaculture Extension and Outreach Capacity. CTAHR Research News. March 2010, Volume 6, Issue 3(47). http://www.ctahr.hawaii.edu/site/downloads/crn/CTAHR_Research_News_Mar_10.pdf

Presentations:

Tamaru, C.S., B. Fox, T. Radovich, Y. S. Kim, S. Khanal, H. Ako, J. Sugano, K. McGovern-Hopkins and R. Klinger-Bowen. 2010. Aquaponics at the College

of Tropical Agriculture and Human Resources (CTAHR). Zero Emissions Conference. The World Congress on Zero Emissions Initiatives . September 13-17, 2010. Honolulu, Hawaii,

Tamaru, C.S., B. Fox, H. Ako, T. Radovich, J. Sugano, K. McGovern-Hopkins and R. Klinger-Bowen. 2010. Aquaponics at the College of Tropical Agriculture and Human Resources (CTAHR). Hawaii Agriculture Conference. September 23, 2010. Ihilani Resort and Spa, Ko`Olina, Honolulu, Hawaii.

Tamaru, C.S., B. Fox, H. Ako, T. Radovich, J. Sugano, C.N. Lee, K. McGovern-Hopkins and R. Klinger-Bowen. 2010. Aquaponics at the College of Tropical Agriculture and Human Resources (CTAHR). Growing Together in Hawaii, 1st Annual Statewide University of Hawaii Master Gardener Conference, October 15-17, 2010. Ala Moana Hotel, Honolulu, Hawaii.

Howerton, R., C. Tamaru, R. Klinger-Bowen, B. Fox, and K. McGovern-Hopkins. 2011. Diversifying Freshwater Aquaculture Products for Hawaii: Two Crossover Species, the Red and Black Pacu (*Piaractus brachypomus* and *Colosomma macropomum)* – Year 1. Narrated Presentation submitted to the Center For Tropical and SubTropical Aquaculture.

6. Developing a value-added product "half-pearls" from the blacklip pearl oyster Pinctada margaritifera in Pohnpei (the Federated States of Micronesia), Year 1

General Information

Reporting Period: July 1 2010 to June 30, 2011 (**Year 1 Final Report)**

Funding Level:

Year	Amount
1	**$47,979**
2	$43,528
Total	$91,507

Participants: **Masahiro Ito**. Ph. D. College of Micronesia Land Grant Program

Ms. Yuko Kibe, GIA (Gemological Institute of America) Certified Pearl Grading Expert

Mr. Belenko Halverson, Mr. Clayton Maluwelgiye Half-pearl and round-pearl grafting technician and extension aide of COM.

Pakin Community Association (PCA), Non-Governmental Organization of Pakin Atoll

RESULTS AT A GLANCE…

- Year 1 of this project has demonstrated the successful transition of a micro-economic development plan into larger scale business opportunities through technology transfer.

- Approximately 700 oysters, 1,400 oysters, 200 oysters and 500 oysters were implanted with half-pearl nuclei at Nett Point, Pakin, Pingelap and Pweniou, respectively. All of the grafting operations were done by the project's Micronesian technicians and 43 trainees who participated in training sessions at each farming location.

- Half-pearl pendant and pearl shell accessory making on-site training sessions were also conducted by the project's core technicians. The total number of participants in the jewelry making training sessions were 24 at Nett Point (including 3 trainees from Pingelap), 110 (including 30 school children) at Pakin, and 20 at Pweniou.

Pingelap Municipal Government, Pingelap Atoll in Pohnpei State

Pweniou Pearl Farm

Objectives

Year 1 and 2

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.

Work Progress and Principal Accomplishments

Although the commencement date of this project was July 1, 2010, all the pearl oysters had been maintained at the COM's demonstration pearl farm at Nett Point. Also community extension work including announcement, liaison, preparatory field work, day-to-day animal care and equipment and materials had to be done well before the commencement of this project. All these preparation work have been done by the COM with no cost of the CTSA's project funding. Soon after the project proposal approved, the first activity was carried out in August at the COM's pilot farm and marine laboratory at Nett Point, Pohnpei. Half-pearls were harvested from the oysters that had been grafted in the previous year for the purpose of using those half-pearls for demonstration and training of pearl grading and accessory making immediately after the grading expert's arrival. Another half-pearl harvest was conducted in June 2011 to perform grading practices by the project staff and trainees and to prepare for actual display and sale of half-pearl pendants and earrings in August 2011

Objective 1: Conduct half-pearl seeding and produce half-pearls.

During the first quarter of the Year 1, approximately 700 half-pearls were harvested in August 2010 from 170 oysters that had been grafted in the previous year. These harvested 170 pairs of pearl shells were used for the half-pearl grading training and accessory making sessions. These half-pearls from older 6 – 7 years old pearl oysters which had been rejected for the round-pearl operation were also used to compare those half-pearls from the 2-year-old virgin oysters for teaching the trainees about the differences in quality (Appendices 3 – 1 and 3 – 2). In June 2011, approximately 1,200

half-pearls from 400 virgin oysters were harvested after 10 months cultivation. As described above, half-pearl harvesting and nucleus implantation work commenced from the first quarter by the project's technicians as well as trainees at farming sites at Nett Point and Pakin Atoll. The seeding work with skill training sessions continued intermittently during the subsequent quarters in November, December, January, March and June at either Nett or Pakin. Additional two farming communities, Pingelap Atoll 150 mile east of Pohnpei and Pweniou Island at the southeast corner of Pohnpei, joined the half-pearl seeding operations in October, February and April, which were also performed by the project technicians.

During the Year 1, total numbers of approximately 700 oysters, 1,400 oysters, 200 oysters and 500 oysters were implanted with half-pearl nuclei at Nett Point, Pakin, Pingelap and Pweniou. Average of 2 – 4 nuclei with 11 mm, 12 mm or 13 mm sizes were implanted per oyster and they were either half-round or half-drop shape. All the grafting operations were done by the project's Micronesian technicians and trainees who participated training sessions at each farming location.

Objective 2: Demonstrate and train half-pearl seeding techniques by COM staff to selected youths from pearl farming communities.

During the first quarter of the Year 1 at Nett Point, the first demonstration and skill training were conducted over two weeks at Nett Point (Appendix 4 – 1). Half-pearl grafting demonstration and training continued during the Year 1, two sessions at Nett Point, five at Pakin Atoll (Appendix 4 – 2), one at Pingelap Atoll and two at Pweniou Island. Since the commencement of this project, two islands communities of Pingelap Atoll 150 mile east of Pohnpei and Pweniou Island at the southeast corner of Pohnpei outer reef have joined pearl farming and half-pearl skill training activities. Three trainees from Pingelap participated to the COM's skill training program, who were selected by their municipal government to learn farming operation as well as half-pearl grafting skill and accessory making. Pweniou Island is owned by a single family with five – ten family members, which is supported by their relatives of the village community of Kitti municipality. As of this reporting date, one teenage girl of this family participated to the project's training session and learned the half-pearl grafting skill by the project technicians.

At Nett Point, half-pearl harvesting and nucleus implantation operations were conducted in the first, the second and the fourth quarters. Total of approximately 700 oysters were used for the half-pearl grafting and skill training by the two of the project's Micronesian technicians who also supervised half-pearl grafting practices of 16 local

trainees. At Pakin Atoll, total of 1,400 oysters were seeded half-pearl nuclei for both demonstration and skill training from the first to the fourth quarters of this project. Among those 23 participants of the training sessions, three teenage girls were selected by the Pakin Community Association to continue receiving specialized training by the Micronesian technicians including the round-pearl grafting skills. Total numbers of participants were; 16 at Nett Point, 23 at Pakin, 3 at Pingelap and 1 at Pweniou. Duration of these demonstration and training work varied from 3 days to 15 days at each session and total of 10 sessions were carried out in the Year 1. Total number of approximately 700 oysters, 1,400 oysters, 200 oysters and 500 oysters were used for these demonstration and skill training work at Nett Point, Pakin, Pingelap and Pweniou. The number of host oysters varied from 2 to 50 oysters a day per trainee, which was based on the judgments by the project's core technicians because each trainee had different talent and capability. The technicians had been trained by a master technician in the past three years, who also acquired half-pearl pendant making skills.

Objective 3: Demonstrate half-pearl pendants and accessory making by COM staff.

For the half-pearl pendant and pearl shell accessory making, on-site training sessions were conducted by the project's core technicians along with grafting work in the Year 1; two sessions at Nett Point (Appendix 5 – 1), six at Pakin Atoll (Appendix 5 – 2), one at Pingelap Atoll and two at Pweniou Island. Total numbers of participants were; 24 at Nett Point including 3 trainees from Pingelap, 110 people including 30 school children at Pakin, and 20 at Pweniou. Duration of these demonstration and training work varied from 2 days to 15 days at each session and total of 11 sessions were carried out in the Year 1. As described above, several trainees from Pohnpei and outer islands participated hands-on training, which included preparations of tools and equipment for half-pearl pendants and pearl shell handicraft making, practices of half-pearl pendant and shell earring making onsite the farms at Nett Point. The project's demonstrations and training also extended to the outer islands such as Pakin, Pingelap and Pweniou. The pearl grading expert and the project seeding technicians worked side-by-side to grading raw materials to finished products.

Because of differences in economic situations and infrastructure between Nett Point (Pohnpei) and Pakin Atoll, techniques of making half-pearl pendant were modified. At Nett Point, ordinary electric machines and tools were used to make sample pendants and earrings with metal fittings and chains (Appendix 5 – 1). On the other hand, there is no public electricity supply at Pakin Atoll for using electric machines and tools such as hand-held grinder, sander, drill and sewing machines, non-energy hungry tools were the main tools such as manual grinder rotating by a hand, hack-sew, files, sandstone and sandpapers. For drilling hole to the shells and pendant-top, a small hand-held drill

was powered by a small generator. Pendant fitting were made of a strong string, which is usually used for the seaweed cultivation line, woven into a strap. This is a similar techniques to the Japanese traditional "Kumihimo" strap or string-weaving technique (Appendix 5 – 2), which does not require any metal fittings, rings or chains to wear the half-pearl pendant. If more generators are available to Pakin community, people can also use more electric apparatus. The old-fashioned Kumihimo is an alternative to the modern method of jewelry making but its final product gives us more artistic and unique impression (last photo of Appendix 6 – 2).

Sample products of half-pearl pendants and earrings from this project are different from finely made half-pearl (Mabe) jewelry which requires a complex processing: removing the non-nacreous shell and nucleus, cleaning and polishing organic blemish inside the half-pearl, stuffing a hollow with resin, capping with polished nacreous plate, cutting accurately along the pearl shape and attaching golden frame and ring. This processing technique is impossible for the outer island people to make a jewelry-cut product. Therefore, the project adopted simple technique by cutting through the half-pearl with the polished shell intact. As sample products received favorable reactions by the foreign tourists, local people and some of the jewelry businesses overseas, the project's processing technique could be viable. 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. The Year 1 of this project has shown the Micronesians can build expertise in this area, laying a foundation for pearl industry development by reviving the half-pearl productions.

Objective 4: Conduct quality assessment of half-pearls and pearl shell-related accessories.

In August 2010, the half-pearls were harvested from approximately 200 oysters at Nett Point. These oysters had been had been implanted with half-round, half-drop and half-heart nuclei in the previous year. This made possible for the pearl grading expert to conduct training the Micronesians for grading the half-pearls and accessories immediately after her arrival to Pohnpei. During the final month of this project, approximately 1,000 pieces of half-pearls were also harvested from 250 oysters, with which the grading work was done by the trainees who had received training by the pearl grading expert.

Coloration of half-pearls reflects the nacre color of the shell in each species, e.g. white-silver and yellow-golden of P. fucata and P. maxima, brownish-blue of P. penguin, bluish of P. sterna. The blacklip pearl oyster P. margaritifera have the unique

luster and color from silver or grey to dark green or purple. The darker color, particularly bluish color, is considered to be fetching the higher prices in the round-pearl market so that the Year 1 targeted to produce darker and/or bluish color half-pearls. For this purpose, a pearl grading expert taught Micronesian core technicians and trainees for how-to and where-to look at the inner sided of pearl shell and selecting host oysters for grafting.

The pearl grading expert also demonstrated basic round-pearl grading methods of the GIA pearl grading, which included four of the project's extension aides and three trainees, and conducted onsite training of half-pearl grading for the participants (Appendix 6 – 1). A preliminary quality assessment of the half-pearls from previous year was completed during the second quarter, in which the GIA's standard of round-pearl grading (grades A to D) on luster, flaw, color, nacre thickness and shape was modified to grades A to C so as to develop grading of half-pearls. Unlike the round-pearls, a better shape of the half-pearls including half-round and half-drop is defined by sharpness or clearness of outline. In order to simplify grading the half-pearls, the project adopted three grades: A = superior, B = good, C = inferior. Grade A has darker and/or lighter color such as blue, green or mixed color with high luster and none or minute blemish on the surface by a naked- eye examination. Grade B has darker and/or lighter color with high luster but minor blemish visible by a naked eye; or has whitish color with high luster without blemish visible by naked eye. Grade C has medium luster with distinct blemish visible by a naked eye. The grading expert also conducted pricing of the half-pearls and pearl shell-related products at farm gate prices, for example, which ranged from $5, $10, 20, $30 and $40 per half-pearl pendant for domestic display-sale, and also depended on the design or jewelry-cut (Appendix 6 – 2). A trial display and promotional sales of the half-pearls and other value-added products has conducted in August, 2010 which forms a part of Year 2 activities..

Impacts

The Year 1 of this project shows a model of micro-economic development into a larger scale business development by technology transfer project which can readily be acquired and generate income by the skill training program itself. Although the first display-sale in Pohnpei was small from $500 plus from 24 pieces by as of this reporting date, there are more than one thousand pieces being in a process to become jewelry-cut value-added products by the project's training sessions. More public exposures in Pohnpei and active sales promotions of the Micronesian brand half-pearl jewelry and handicrafts for overseas market are planned in the Year 2. The international enquires for purchase, which could be worth approximately $12,000, have already received for

ordering jewelry-cut half-pearls produced by the islanders who participated to the project's training.

As of this reporting date, half-pearl grafting operations were conducted at four locations although the proposal described only two (Nett Point and Pakin Atoll). This was because the PI has been engaging not only on this half-pearl project but also on the pearl industry development task in Pohnpei. The half-pearls are value-added products to the pearl farming business, which has less operation cost by shorter cultivation period and has relatively easy nucleus implantation technique compared to the round-pearl production, thus additional neighboring island communities have also requested to participate to the project's activities. 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.

In elsewhere like Tanzania in Africa, Kiribati and Tonga in the South Pacific, similar projects were implemented in mid 2000 by the governments or local communities funded by international organization. However, no penetration has accomplished in the international jewelry market with a significant quality and quantity. According to the recent economic modeling of half-pearls for rural small-scale aquaculture (SPC/QDPI workshop 2008, unpublished data), gate prices of the half-pearls from the blacklip pearl oyster were expected to fetch from $10, $7 down to $2 each for the half-round and from $12, $7 to $2 each for half-drop shapes for A, B and C grades, respectively. In Pohnpei, domestic customers tended to purchase the products in the range of $5 to $40 at trial sales, which is encouraging forecast. As of this reporting date, the project conducted sample display and sale of the jewelry-cut half-pearl pendants and earrings in August in Pohnpei, which fetched total of $525 for 24 samples, the average sale of about $20 per item, all of which were produced by 10 trainees. This project suggests that the technology transfer and skill training practical to generate income immediately to the island people.

Recommended follow-up activities

Although curiosities and enthusiasms by the island people, who have been requesting half-pearl accessory making demonstration and skill training, it was difficult from the beginning for this project to make frequent visits to the outer islands such as Pakin, Pingelap and Pweniou. This was simply because of our strict budget (zero budget of this project for travel to/from outer islands) and of depending all on the COM extension

service's for all the travel costs. Therefore, the PI invited as many trainees as possible from these islands by their own costs during the major training activities at Nett Point (Pohnpei). However, participations by the trainees were limited up to 10 – 15 people per session as they were also financially difficult to come to Pohnpei. To maximize the opportunity, we built strong mutual trust among the outer islanders so they provided our project team with generous free-accommodation and supplemental meals which helped the project work onsite the outer islands. The sample half-pearl accessories actually sold locally after the end of Year 1 and participating individuals and communities became more aware of income generation by this project. The Year 2 needs to take several opportunities to participate to local public events such as the World Food Day (October), Christmas (December) and Pohnpei Culture Day Fest (March) for displays and sales of more resultant half-pearl jewelry products . As the project's branding promotion has begun for the Micronesian brand, the Year 2 should shift its activities to actual half-pearl jewelry making business by the project participants.

Regarding the half-pearl grafting skill training, the project's Micronesian technicians demonstrated their skills were at high level being recognized by the local people and by overseas buyers from the display and sales of products. The project should continue grafting training for the local apprenticeships to create core technicians on each island and community. This is the only way to develop sustainable pearl business development in Pohnpei and the Year 2 project will be a precursor to a model of income generation project from value-added products.

The outer island communities still need financial support from the outside, such as the State and Federal governments for pearl farming infrastructure improvements. However, this project has already shown that such small families and communities were able to generate income from zero to small amount of money which enabled them to continue participating to the training for making the value-added products. Therefore, the Year 2 should provide material support, such as pendant and earring fittings, machines and tools, packing, displaying and delivering materials, and should continue to lead a way of half-pearl jewelry business into both domestic and overseas.

Publications and Manuscripts Written and Paper Presented

Manuscripts

Ito, M. 2011. Aquaculture research, extension and training by the Land Grant Program at College of Micronesia: From 2001 to 2010. Abstract, the 5th National

Aquaculture Extension Conference, Memphis, USA.

Literature Cited

Antoine Teitelbaum, 2007. Pearl oyster products jewelry marketing workshop. June 26-July 2, 2007, South Tarawa, Kirobati, Secretariat of the Pacific Community, 16 Pages.

Southgate, P., J. Rubens, M. Kipanga and G. Msum. 2006. Pearls from Africa. Pearl Oyster Information Bulletin 17, Secretariat of the Pacific Community, Pages 16-17.

7. Developing a value-added product "half-pearls" from the blacklip pearl oyster Pinctada margaritifera in Pohnpei (the Federated States of Micronesia), Year 2

General Information

Reporting Period	July 1, 2011 – September 30, 2011 (Year 2 Progress Report)
Funding Level	Year Amount
	1 $47,979
	2 $43,528
	Total $91,507
Participants	**Masahiro Ito,** Director and Chief Scientist, Aquaculture Development, College of Micronesia Land Grant Program
	Belenko Halverson, Clayton Maluwelgiye Half-pearl seeding technician and extension aide of COM.
	Pakin Community Association (PCA)
	Pweniau Pearl Farm

RESULTS AT A GLANCE…

- In the first quarter of Year 2, two of the project's Micronesian technicians were capable of training half-pearl seeding skills to 12 trainees without the supervision of a foreign master technician while they grafted 3,000 oysters.

- High luster and unique colors with simple designs of Micronesian branded half-pearl accessories are gaining good reputations and positive responses from both domestic customers and overseas pearl traders and the jewelry industry.

- 24 items (totaling $525) were sold in a day during the first promotional sale in Pohnpei in August 2011.

Objectives

Years 1 and 2

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

Anticipated Benefits

During the Year 2, the resultant products will be sold after grading and pricing to the market both domestic and overseas. Approximately 5,000 pieces of half-pearl earrings and pendants are expected from the participating farms before the end of this project, which could be valued between $50,000 and $100,000 in total, at average of $10 – $20 per piece particularly through overseas marketing efforts. As the technology transfer is the most important output, the project's Micronesian technicians will continue training other local youths. Those local youths who are trained will be trainers to create more half-pearl technicians at each outer island. On- farm training sessions of the half-pearl accessory making will spread among the participating island communities. Unified grading and pricing standard demonstrated by the project may take more years to spread and to be established among the farms if it develops into a sustainable commercial activity.

Work Progress and Principle Accomplishments

As for the objectives 1 and 2, half-pearl seeding and/or harvesting operations were conducted. Two of the project technicians demonstrated and trained local youths for half-pearl seeding techniques. During the first quarter of this project, the half-pearl seeding work was conducted at four farms: Pweniau Island (x580) and Pingelap Atoll (x1,080) in July, Nett Point (x500) in August and Pakin Atoll (x800) in September. The sizes of nuclei implanted were 11 – 12 mm in diameter for half-round, 14 mm by 22 mm for half-drop and 12 mm by 13 mm for half-heart. During the first quarter, two Micronesian technicians conducted half-pearl grafting for 1,100 oysters in total. In addition, they also trained total of 12 young apprentices from four communities, who practiced half-pearl grafting by using 1,530 oysters. Pweniau Island is located on the barrier reef in the southeastern corner of Pohnpei and is owned by a single family whose small farm has also been maintained by volunteers of their relatives from nearby

community. As of this reporting date, one teenage girl of this family participated to the project's training session and learned the half-pearl grafting skill by the project technicians. With supervisions of the project technicians, three teenage girls at Pakin community and two young men at Pingelap continued to practice the half-pearl grafting skill.

From June (final month of the fourth quarter of Year 1) to September (first quarter of Year 2), half-pearl harvests were conducted both in Pohnpei and other islands resulting approximately 1,800 oysters in total; 600 oysters at Nett, 700 at Pakin, 245 at Pingelap and 280 at Pweniau. On August 21, a trial display and promotional sales of jewelry-cut half-pearl products, such as earrings and pendants, were conducted in Kolonia, a main town of Pohnpei. Those harvested half-pearls were graded by the PI and project technicians; designing, cutting, polishing and coupling were done by the trainees and the technicians and project staff; and the pricing of final products was done by the PI. Because the display and sale was focused mainly on the round-pearls, only 40 pieces of pendant and 20 pairs of earrings made by the trainees from Pweniau and Nett were presented. Total of 14 pieces pendant top and 10 earrings fetched $525. Half-pearl accessory making is ongoing at Nett, Pakin and Pweniau for local sales for Christmas season in December and Culture Day Festival in March next years as well as responding to the purchase requests for the jewelry-cut half-pearls from overseas jewelry traders.

Work Planned

(Milestone 4 – 12 months, Year 2): Additional operations of half-pearl grafting may be conducted at Nett in October, 2011 and June 2012, at Pakin in March and August 2012, at Pingelap in June 2012 and at Pweniau in November, 2012 and June, 2012. These operations are planned at same timing of half-pearl harvest on each farming site. Skill training also coincide the harvest – grafting work period. Most of the half-pearl grafting work will be done by the trainees under supervisions of the project technicians. Although the accessory making is shifting from Nett Point demonstration farm to each farm, Nett farm continue to serves as a training center for half-pearl grading, accessory making and pricing final products. Difficulties in traveling bimonthly to/from outer islands by the project staff, post-grafting monitoring of the host oysters will be carried out by care-takers or owners at each farm. The project will organize charity sales in Pohnpei of half-pearls and related accessories on behalf of participating farms and individual trainees (e.g. Christmas season, Culture Day Fest in March and other events). As purchase inquiries for jewelry-cut half-pearls have been places from overseas pearl traders and jewelry businesses, the project plans to hold half-pearl business development seminar/meeting.

Impacts

The project conducted pearl display and sale on August 21 in Pohnpei, which included small quantity of the jewelry-cut half-pearl pendants and earrings made by the trainees, which fetched total of $525 for 24 samples in a 5-hour sale at average of roughly $20 per item. Although the round-pearls in loose, which were price-tagged from $5 to $500 per piece, were the main in the display and sale, small number of hundred half-pearl products received attentions from local customers. In the outer islands of Pohnpei, average farm gate price at $5 per half-pearl could offer sufficient profit to cover annual operation cost of 5,000 round-pearl farming when the farm harvests annually 2,000 pearl oysters which bear 8,000 pieces of half-pearls. This indicates that the project's half-pearl technology transfer and skill training are appropriate to generate income immediately to the island people. A team of three or four trainees can make total of 20 pieces of fine quality pendants in a day, if they divide the whole process into grinding shells, designing, cut and curving, drilling, polishing and coupling. The trainees from Pweniau sold 14 items for $250 of half-pearl pendants and earrings. Although this was very small amount of money, it was their first income from the project's training sessions. The Pweniau farm purchased additional tools and materials for their half-pearl accessory making. Displays and sales of half-pearls in Pohnpei will be conducted separately from those of the round-pearls. Only value-added pearl shell products such as half-pearl pendants and earrings, pearl-shell tags, bracelets and broaches will be displayed and sold because of a different nature of marketing from the round-pearls. The half-pearls are value-added products to the pearl farming business, which has less operation cost by shorter cultivation period and has relatively easy nucleus implantation technique compared to the round-pearl production. Other outer islands have also requested to participate to the project's activities. However, this project does not have capacities in financially and logistically to involve more than those who have already participated to.

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

Ito, M. 2011. Aquaculture research, extension and training by the Land Grant Program at College of Micronesia: From 2001 to 2010. Abstract, the Fifth National Aquaculture Extension Conference, Memphis, USA.

Ito, M. 2011. Circle and spot formation mechanisms and changes in luster, color and roundness by grafting methods on the blacklip pearl oyster, Pinctada margaritifera.

Proceedings of the Fifth International Gemological Symposium, Gems & Gemology 47 (2): 148, Carlsbad, USA.

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

General Information

Reporting Period: February 1, 2010 to April 30, 2011 **(Year 1 Final Report)**
May 1, 2011 to October 31, 2011 (Year 2 Progress Report)

Funding Level

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

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RESULTS AT A GLANCE…

- PCR assay successfully developed and validated through a collaborative private-public partnership. The assay has resulted in a much improved understanding about certain aspects of the life history of FLB that is laying the ground work for future development of a disease management program for this emerging pathogen.

- Substantial amount of new information obtained, including tissue distribution of the pathogen during a clinical outbreak, discovery of asymptomatic carriers of the disease, tissue distribution of pathogen in asymptomatic carriers, indication of the existence of resistant tilapia strains, indication that the pathogen is not vertically transmitted, direct evidence of the existence of the pathogen.in feral stocks of tilapia on Oahu.

Objectives

Year 1

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 KHV 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.

Year 2

1. Increase capacity of local diagnostic laboratory to include detection of *Piscirickettsia*-like organism (PLO) that infects tilapia.
2. Conduct survey for *Piscirickettsia*-like organism in farmed and feral tilapia populations statewide
3. Extend survey to the whole CTSA region.
4. Produce technical handouts of resulting information. Information obtained is to also be included in Operational Biosecurity handouts.
5. Conduct follow up workshop to disseminate information and begin discussions on the challenges and opportunities of establishing a health certificate program for farmed tilapia.

Anticipated Benefits

The anticipated benefits of the proposed project will be a much improved understanding of the life history of the pathogen. Such information will be essential to the development of a disease management strategy that should result in an overall improvement in tilapia production state wide. Potential impacts maybe farther

reaching than just preventing statewide impacts as the pathogen affects other fish species both in the wild and cultured.

Principle Accomplishments

Year 1

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

A collaborative partnership with Moana Technologies, Inc. was made during the previous reporting periods and PCR technologies for detecting both KHV and TRLO were established and validated. Currently, the average turn abound time for results after submission is approximately 3-7 working days. An example of a report that is received after samples have been submitted is provided as a separate attachment. In this particular case multiple samples were submitted and the reports shows the testing results for each sample vial that is labeled only with a vial number and no other information. Using the vial number the sample can then be traced back to obtain information (e.g., sample date, tissue type, fish type, sample location, size, water temperature, number of individuals) specific for the sample. In the report #'s 86,87,88 refer to gill, fin and kidney samples, respectively, obtained from a single feral koi individual that was caught under the Vineyard Street Overpass and euthanized on 12/24/2010. Samples 89-98 are composite (n=5 individuals) gill samples obtained using a non lethal sampling technique from a single holding tank from Kodama Koi Farm quarantine facility (Figure 1 Appendix) obtained on 1/6/2011. All individuals survived the sampling protocol and were returned to the quarantine tank from which it had come from. In this manner a total of 50 individuals were sampled in approximately two hours and with the pooling of samples only 10 samples need to be analyzed by PCR testing instead of 50 which greatly reduces the cost of analyses. Sample #'s 99-102 are gill swabs taken using Fast Technology for Analysis (FTA) cards for nucleic acids and represent an alternative method of obtaining samples that was also field tested on the last four individuals sampled using the non-lethal sampling technique. Initial results indicate that there may be merit in the use of this sampling technique as it offers advantages of being easy to apply and for long term storage of samples.

As described in a previous progress report, a PCR test for detecting tilapia rickettsia like organism (TRLO) was also established and validated during the current reporting period. The test and results will be reported on separately as it is being used to achieve the objectives of the Year 2 project.

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 (KHV)

As reported in a previous progress report, a workshop to inform both koi and tilapia stakeholders entitled, "What is happening with the culture of koi and tilapia in Hawaii?" was completed on July 17, 2010 at Windward Community College. A photograph of workshop participants and workshop evaluation is provided in the Appendix (Figure 2).

While the workshop was well received and the activity is an indication that the objective was completed, it should be noted that the participants were dominated by tilapia stakeholders and another workshop specifically for koi producers was held and will be reported on under Objective 6.

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 reference laboratories. We received a positive KHV control sample using gill tissue and preserved in absolute ethanol on October 8, 2010 from:

Norihisa Oseko
Director of Diagnostic & Training Center for Fish Diseases
Fisheries Research Agency, Japan
422-1 Nakatuhamaura, Nanse, Watarai, Mie 516-0193, Japan
Tel +81-599-66-1830 Fax: +81-599-66-1962
E-mail: ohseko@fra.affrc.go.jp

DNA was extracted from the KHV positive carp gill tissue and resulted in a positive PCR test result with the OIE KHV PCR primer. (See Appendix)

In addition to establishing the methods for detection of KHV 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. Dr. Riggs is the aquatic veterinarian with the State Department of Agriculture that oversees the Shrimp Broodstock Specific Pathogen Free (SPF) monitoring program and similar collection, processing and reporting protocols were established in conjunction with the PCR testing that is being done by Moana Technologies. The same kind of transparency already being practiced with the Shrimp SPF program is to be adopted with the KHV program.

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

Survey of streams on Oahu revealed feral koi in several of them and some of these were collected by cast net. It should be noted that the water temperatures for all of the samples (feral and cultured) ranged between (23 - 25 C) and well within the range that is reported to be optimal for the expression of KHV. Fish were transported live from the stream and back to Windward Community College where they would undergo sampling. Initially a lethal sampling method was used (n=4) that allowed kidney to be assayed. The use of the lethal sampling protocol would be discontinued as it became obvious the fish in the wild were not KHV positive. Only gill samples are obtained and tested with the non-lethal method of sampling. The advantage is that the individuals are not sacrificed and can be therefore used for other things. A total of 14 feral koi were sampled with no detection of any positive for KHV. (Table 1)

Four commercial retail and wholesale vendors that are located on Oahu were sampled over the course of the project (Table 2). A total of 125 individuals representing a variety of age groups and strains were sampled over the course of the project. Interviews with the vendor indicate a wide range of sources (e.g., breeders) of the koi being sold in Hawaii. As with the feral koi all samples were negative for KHV and positive for CO-I. 1

Owners of koi from four locations (Table 3) collaborated in allowing their koi to be sampled over the course of the project. Only gill samples were taken using the non-lethal sampling protocol. The sampled fish were returned to their holding facilities and no reported mortalities have been reported as a result of the sampling protocol. These koi are being considered cultured by hobbyists and their koi strictly for viewing. A total of 55 individuals were sampled and as with all other koi tested they were all negative for KHV and positive for the CO-I.

It has been reported that KHV DNA has been found in other cyprinids such as goldfish and the availability of both comets and goldfish offered the opportunity to have these sampled. A total of 30 individuals (n=15 for each) goldfish and comets were sampled and subjected to KHV testing via PCR. As with all other samples all individuals were negative for KHV and positive for the CO-I fish control. (Table 4)

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

One newsletter article was produced during the reporting period that focused on raising awareness of the capacity to conduct PCR testing for KHV in Hawaii was now available. The title of the newsletter article was:

Tamaru, C.S., K. McGovern-Hopkins, R. Klinger-Bowen, B. Fox, A. Riggs, T. Low and N. L. Antonio and J. Brock. 2011. Improved Capacity for the Diagnostic Surveillance of Two Aquatic Pathogens at the University of Hawaii College of Tropical Agriculture and Human Resources and the State Department of Agriculture. Center for Tropical and Subtropical Aquaculture.

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.

On January 9, 2011 project work group members Allen Riggs DVM and the principal Investigator held a workshop with the Hawaii Goldfish and Carp Association at Salt Lake Elementary School.

The title of the presentations made were:

Tamaru, C.S., A. Riggs, T. Low, K. McGovern-Hopkins, R. Klinger-Bowen, B. Fox. 2011. Regional Biosecurity: Diagnostic Surveillance for Koi Herpes Virus Disease (KHVD). Hawaii Goldfish and Carp Association , Salt Lake Elementary School January 10, 2011.

Riggs, A. and T. Low. 2011. Overviews on Koi Herpes Virus (KHV) and the Specific Pathogen Free (SPF) Shrimp Broodstock Program. Hawaii Goldfish and Carp Association , Salt Lake Elementary School, January 10, 2011.

Indirectly related to the work being done under the current project is in the area of increasing Hawaii's self sustainability. During the reporting period project work group members are also involved in the production of presentations and workshops in this area and they are:

Conference Presentations:

Tamaru, C.S., B. Fox, H. Ako, T. Radovich, J. Sugano, K. McGovern-Hopkins and R. Klinger-Bowen. 2010. Aquaponics at the College of Tropical Agriculture and Human Resources (CTAHR). Hawaii Agriculture Conference. September 23, 2010. Ihilani Resort and Spa, Ko`Olina, Honolulu, Hawaii.

Tamaru, C.S., B. Fox, H. Ako, T. Radovich, J. Sugano, C.N. Lee, K. McGovern-Hopkins and R. Klinger-Bowen. 2010. Aquaponics at the College of Tropical Agriculture and Human Resources (CTAHR). Growing Together in Hawaii, 1st Annual

Statewide University of Hawaii Master Gardener Conference, October 15-17, 2010. Ala Moana Hotel, Honolulu, Hawaii.

Workshops:

1. AQUAPONICS SYSTEMS FOR USE IN YOUR OWN BACKYARD. Waimanalo Homestead Association Feb. 15, 2010
2. AQUAPONICS SYSTEMS FOR USE IN YOUR OWN BACKYARD. Honolulu Aquarium Society April 2, 2010
3. Aquaponics at the Hawaii State Hospital. Hawaii State Hospital, April 8, 2010.
4. Aquaponics at the University of Hawaii. ʻĀina Hoʻōla O Māʻilikūkahi June 7-9, 2010
5. CTAHR's traveling road show, Windward Community College, June 15, 2010.
6. How to build your own aquaponics system. Windward Community College, July 24, 2010
7. Black Soldier Fly, Windward Community College, July 31, 2010.
8. Backyard Aquaponics for the Homestead, Department of Hawaiian Homelands, Kapolei. August 28, 2010.
9. Aquaponics at the College of Tropical Agriculture and Human Resources (CTAHR). October 8, 2010. College of Engineering Sustainability workshop. Windward Community College.
10. Aquaponics at the College of Tropical Agriculture and Human Resources (CTAHR). October 12, Master Gardner's, Maui Cooperative Extension Service.

Year 2

Objective 1: Increase capacity of local diagnostic laboratory to include detection of *Piscirickettsia*-like organism (PLO) that infects tilapia.

Through the established collaborative partnership with Moana Technologies, Inc. the PCR methodology for detecting PLO was established and validated. A copy of the procedure is available upon request. Currently, the average turn abound time for results after submission is approximately 3-7 working days. The protocol is dependent on the primers which were described by Hsieh et.al., (2007)[1] and they are:

FLB16S180f: 5′-GCG-GATTAA- AGG-TGG-CCT-TTG-C-3′ (forward primer)
FLB16S465r: 5′-CCT-GCA-AGC-TAT-TAA-CTC-ACAGG- 3′ (reverse primer).

[1] Hsieh et al., 2007. PCR and in situ hybridization for the detection and localization of a new pathogen *Francisella*-like bacterium (FLB) in ornamental cichlids. *Diseases of Aquatic Organisms* **75**, 29-36.

As with the development of the KHV PCR assay a negative control that detects the presence of fish DNA and in this case Cytochrome Oxidase (CO-I) is also employed in order to provide confidence that a negative result does indeed have a sample when being assayed. The second required component of the assay is a positive control specimen and that was provided by Dr. Allen Riggs and consists of a spleen preserved in ethanol from a clinically documented case of TRLO (ADP case # 10-87). Results of PCR assays for the pathogen and CO-I using the positive control is presented in the appendix (Figure 1).

The assay was further validated by extracting the DNA from the band visualized on the gel for PLO and submitted to the Greenwood Molecular Biology Facility, University of Hawaii at Manoa for sequencing. The resulting nucleotide sequence was determined for that specific band:

10-87 (+TRLO)

GGATCTACTGCGTTGGATAGCTAGTTGGTGGGGTAAGGGCCTACCAAGGCTACG
ATCCATAGCTGATTTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCC
AAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGAAACCCTG
ATCCAGCAATGCCATGTGTGTGAAGAAGGCTCTAGGGTTGTAAAGCACTTTAGTT
GGGGAGGAAAGCCTGTGAGTTATAGCTTGCAGGAA

The sequence was then run through a Basic Local Alignment Search Tool (BLAST) that compares the sequence that was obtained with other known sequences from a data base. The results of that analysis are presented in the Appendix (Figure 2) and a high degree of overlap (Maximum 99%) was obtained with sequences coding for a *Francisella* like bacteria (FLB). Apparently what was previously known as PLO is really FLB based upon DNA sequence analyses and that terminology will be used during the remainder of the report. A similar analyses was also conducted using the sequence obtained for CO-I and was also reported on during Year 1. The results show a high degree of overlap with Cytochrome Oxidase obtained from *Oreochromis mossambicus*. Both results provide definitive evidence that the developed PCR assay is specific for the target pathogen and control DNA and forms the basis for future analyses.

Objective 2: Conduct survey for *Piscirickettsia*-like organism (PLO now called FLB) in farmed and feral tilapia populations statewide

From this point on HTPLO, TRLO and FLB are considered synonymous. An example of a report (CTSA Progress Report_31) that is received after samples have been submitted is provided as a separate attachment. In this particular case multiple samples were submitted and the reports shows the testing results for each sample vial that is

labeled only with a vial number and no other information. Using the vial number the sample can then be traced back to obtain information (e.g., sample date, tissue type, fish type, sample location, size, water temperature, number of individuals) specific for the sample. In the report #'s 259, 260, 261,262,263, and 264 are samples that were submitted to Moana Technologies and consist of filter paper (#259) that was exposed to water from Enchanted Lake and composite samples of spleen from five tilapia individuals also obtained by cast net from Enchanted lake (260-264), respectively. With the exception of the filter paper sample (259) all other samples are scored as positive for the presence of FLB DNA indicating that at least one individual per vial possessed DNA from the pathogen. Although long suspected, this is the first direct evidence that the pathogen exists in wild populations of tilapia on Oahu.

Project work group members have been taking advantage of outbreaks, when they occur, and have obtained preliminary information that can be used to direct future research objectives. An active outbreak on a farm in Waimanalo, where fish mortalities were occurring, allowed for addressing a basic question as to what tissue can be used to be confident as to the detection of TRLO in an individual. Tissue samples (gill, fin, spleen) from ten individuals that were morbid but still alive were sampled on 12/14/2010 and submitted for PCR analyses. The results are summarized in the Appendix (Figure 3). As seen in the figure the use of a fin clip resulted in only 40% of the individuals being detected as positive while the use of spleen resulted in 100% detection. A gill biopsy resulted in 90% detection of TRLO which is not statistically different when using spleen. All ten individuals sampled where morbid and found to possess ulcerated spleens and the desired outcome would have been a sampling protocol that resulted in 100% detection of the pathogen. While this was achieved when using spleens it also requires a lethal sampling method to be used. Using the gill biopsy or non-lethal sampling protocol, one individual resulted in an apparent false negative result and while statistically not significantly different from the results using spleen does raise some additional questions on the strategy that will be employed when using PCR testing as a surveillance tool for these two specific pathogens.

Another outbreak this time in an urban aquaponic system located in Waimanalo on Oahu was sampled on February 1. 2011. Mortalities were occurring with individuals possessing granulomas in gill and spleen characteristic of the disease. In this case, however, the tank was emptied and the surviving fish were moved to a separate aquaponic rearing system located in another location in Waimanalo. Mortalities had ceased altogether prior to the population being sampled again on May 19, 2011. At this point in time specimens (n=8) were sacrificed but were asymptomatic for FLB with the exception of possessing moderate to low numbers of granulomas in the spleen that

were detected using wet mounts and viewed with a compound microscope. Spleen tissue was preserved in 10% formalin and processed for histopathology which revealed the presence of FLB in the infected spleens. The same tank was re-sampled (n=8) on June 29, 2011 with fish remaining asymptomatic with the exception of moderate to low numbers of granulomas in the spleen. Gill and spleen samples from both May and June were subjected to PCR analyses and the results are summarized in Figure 4. Apparently a significant decrease in the percentage of FLB-DNA positive gill samples occurs with tilapia becoming asymptomatic for the disease. This is consistent with the observation of fish beginning to breathe and feed normally as impairment of the gills is drastically reduced during recovery. From their feeding and overall behavior, one could easily make the assumption that the infected individuals had fully recovered from the disease episode. However, as seen in the results from both May and June, a very high incidence of individuals retain FLB-DNA in their spleen indicating that the pathogen was present but at a subclinical level. It remains to be determined whether these individuals are infective and what stressors might trigger a renewed outbreak. All of these questions are areas that require future examination.

Because of the availability of an asymptomatic population of tilapia we also examined what tissues and techniques might be useful for detecting infected individuals using the PCR assay that was available. The same individuals sampled in June were also used to examine if Fast Technology for Analyses (FTA® Whatman Inc., Clifton, NJ)[2] cards and blood might be suitable for identifying positive individuals. FTA paper is specially treated to bind and protect nucleic acids from blood, plant and animal tissue extracts from degradation. For analysis, a small disc is punched from the FTA paper containing the DNA sample of interest, washed, dried and assayed using the PCR test. The PCR results are summarized in Figure 5. Clearly blood collected using the FTA® cards are not suitable for use as all samples resulted in a negative result. In contrast, when spleen is used directly in the PCR assay all of the individuals were found to be positive for FLB-DNA. The low percentage (e.g., 1/8 or 12.5%) of individuals that were detected using gill tissue directly is consistent with the results obtained in the previous month. When spleen samples are collected using the FTA® cards a lower (e.g., 6/8 = 75%) percentage of individuals are detected with FLB-DNA than when spleen is used directly (e.g., 100%). The difference, however, is not statistically significant indicating that the use of the FTA® card may still have some utility.

A third outbreak taking place in an urban aquaponic system in Kaneohe was experiencing persistent mortalities in the production tank and gross examination

[2] http://www.whatman.com/References/GenSolveDataSellSheetLR.pdf

revealed granulomas in the gill and spleen. The mixed population consisted of two strains (e.g., golden and "koilapia") and on March 2, 2011 eight individuals (e.g., four each for each strain) were sacrificed and gill and spleen samples were excised and preserved for PCR analyses. In addition to gill tissue being preserved in ethanol they were also swabbed and samples preserved using FTA® cards. The results (Figure 6) indicate that use of FTA® cards were not as sensitive in detecting positive individuals as only 2 of 4 individuals were detected as being positive versus 3 of 4 when using gill or spleen tissue directly. A more interesting observation from this case was that one would expect that during an active outbreak, with mortalities occurring, the majority of individuals within the confines of the same tank would be infected. That was not the case as the majority of individuals positive for FLB were of only one strain (e.g., golden). The results can only be considered preliminary because of the low sample size (N=4) for each strain analyzed. The implication, however, is that there may be some resistance to FLB exhibited by at least one strain of tilapia and clearly a topic for future investigation.

A common feature of the previous three reported outbreaks was the high incidence of the golden strain being infected and interviews conducted with affected producers indicated a common source of this particular variety. Composite samples (n= 5 individuals) of spleen were obtained on 08/26/2011 from three separate tanks located on a farm located in Waianae that was suspected of being the source of infected tilapia. All fish were of the golden variety and at the time of sampling did not show any clinical signs of the disease. The samples were assayed for FLB using the PCR assay and all samples were found to be positive. A follow up sampling was conducted on 09/08/2011 in which spleens were obtained from ten individuals from each tank and subjected to PCR analyses. In this manner an estimate of prevalence of asymptomatic individuals in each of the tanks could be estimated and surprisingly, two of the tanks had only 1 of 10 individuals being positive (i.e., 10% prevalence) and the remaining tank had all 10 individuals (100% prevalence) being positive. It is challenging to make any conclusions about the low prevalence in two of the three tanks and one can hypothesize that the fish are actually healing from a previous infection? Obviously this question will have to be addressed in future work.

Each of the spleen samples that undergo PCR testing also undergo a wet mount preparation where a small piece of spleen is placed on a microscope slide with a drop of water. It is then squashed using a coverslip and viewed under a compound microscope under a 4X objective. The presence of granulomas in the spleen are recorded and a correlation between the relative amount of granulomas present in the spleen and the percentage of positive FLB samples is summarized in Figure 7. Out of

16 samples that had little or no granulomas only one PCR positive result was detected. This indicates that the lack of granulomas, while not definitive, has a possible use as a good and quick field indicator of the presence or absence of the pathogen. At the higher levels of granulomas present only a 50:50 chance of correctly indicating the presence of FLB was achieved. This poor correlation of the higher levels of granulomas present is a bit surprising as one would have expected for a more positive correlation .As many of the samples obtained were from asymptomatic carriers the poor correlation may possibly indicate some degree of recovery from a clinical outbreak and clearly will require additional work to uncover the significance of the observed results.

To date a total of 11 separate sites have been sampled during the reporting period and 7 of the 11 sites have been found to possess positive individuals for FLB. The different locations where FLB positive samples are located on the island of Oahu is summarized in Figure 8 in the appendix. Of the seven sites that positive cases were detected one of these (Enchanted Lake) is from feral stocks and one from an aquaculture operation and all of the others are from aquaponic systems. Clearly, the pathogen is spread throughout the various reaches on Oahu and provides justification that the current Department of Agriculture PQ Policy 98-09, Section 150A-8, HRS effective November 5, 1998: that states: Oahu shipments presented to PQB should not be certified for movement to other islands should remain in effect and be actively enforced. This also raises challenges as to how producers will need to work collaboratively to rid the disease from cultured stocks. There are locations (e.g., Windward Community College) where tilapia have been surveyed that have been shown to be clear of FLB. Additional sites also include the golden variety indicating that the pathogen can and should be managed to result in disease free stocks in the cultured varieties. This obviously will need to be addressed in future work.

On 10/11/2011 the farm known to possess FLB positive individuals was revisited and from a single tank five females and five males were sampled. All individuals were of the golden variety. In this case, females that were removed from the tank were first placed into a five gallon bucket (Figure 9) in the hope that any fry being carried in their mouth would be expelled and could then be sampled. Only one female of the five to be tested possessed fry. In addition to the fry being preserved samples of her ovary and also spleen were also preserved and underwent PCR testing. Four other female individuals also had their ovary and spleen submitted for testing. In addition, five male individuals had their testes preserved along with their spleens and submitted for PCR testing. Only two of the five females were found to be positive for FLB using spleen. Their ovarian samples did not result in a positive result indicating that during the asymptomatic phase the pathogen is restricted to the spleen. All five males had spleens that were positive for FLB while all of the testicular samples resulted in a

negative result also indicating that during this phase of an infection the pathogen is restricted to the spleen. The combination of these initial results indicate that the pathogen is not vertically transmitted although a similar sampling protocol would have to be done during an active infection.

In addition to the sampling of fish in the tank, 500 ml of water from the tank was filtered using coffee filter paper (Figure 9). A section of the filter paper was cut out and preserved in ethanol and submitted for testing using the PCR assay. A positive result was obtained using this sample and while the data is preliminary at best because of it only being conducted once, the result is consistent with the report of it being facultative and maybe free swimming or excreted in the feces. Clearly this result will need to investigated further.

Objective 3: Extend survey to the whole CTSA region.

No progress made.

Objective 4: Produce technical handouts of resulting information. Information obtained is to also be included in Operational Biosecurity handouts.

No progress made with the exception of a newsletter article that was published in CTSA's Regional Notes during the reporting period.

Objective 5: Conduct follow up workshop to disseminate information and begin discussions on the challenges and opportunities of establishing a health certificate program for farmed tilapia.

A planned workshop has been combined with the Hawaii Aquaculture Aquaponic Association annual meeting and is to be held on November 12, 2011 at Windward Community College. A copy of the workshop announcement is being attached to this report.

Project working group members were called upon to support the ongoing efforts to provide technical assistance to the growing number of aquaponic stakeholders and a workshop entitled, CHALLENGES AND OPPORTUNITIES OF SOIL-LESS FARMING IN HAWAII was held on July 23, 2011. The workshop was organized and facilitated by project work group members and the speakers and topics covered were:

Jim Hollyer: Update on Farm Food Safety.
Harry Ako: Economics of Aquaponics
Hydroponic Producer - Richard Ha: Hamakua Springs Country Farms, Hawaii

Hydroponic Producer - Paul Singelton: Waipoli Hydroponic Greens, Maui
Aquaponic Producer - Tim Mann: Friendly Aquaponics, Hawaii
Aquaponic Producer - Fred Lau: Maris Garden, Oahu
Tisha Uyehara: Director of Marketing and Special Projects for Armstrong Produce
Over 170 people were in attendance.

Work Planned

The major focus of the next reporting period will be the completion of the survey of the pathogen throughout the state and through the region. The information obtained will also be summarized and submitted to a peer review journal for publication. In addition, the data is to be summarized into a technical handout that would complete Objective 4. Last but not least is to secure additional funding as the current project has yielded a considerable amount of new information regarding this emerging pathogen that will need to be followed up on if a successful management plan is to be developed.

Impacts

The work on developing PCR technology for detecting KHV is in its beginning stages and clearly from the results to date it appears that the pathogen is not presently in any of the populations that have been investigated. The major point is whether this status can remain this way and whether a program or activities can be undertaken that insures the disease does not impact the koi industry in Hawaii. The information and capacity being developed might be turned into an opportunity that would result in added value to koi producers in the state in the form of achieving SPF status and forms the basis for future efforts to be described in the "Recommended Follow-Up Activities" section..

It is apparent to Hawaii's populace that growing more of our own food would increase the security of the food supply, conserve energy used in transportation and distribution, reduce the risk of introducing invasive pests, and even improve nutritional content by providing fresher foods. It would also stimulate Hawaii's economy and create jobs, concurrently decreasing the $3 billion food deficit incurred by the State annually to buy imported food. Furthermore, increasing locally produced food needs to be done in a manner that does not compromise our State's fragile ecosystem, and yet strengthens our communities. This "triple bottom line" concept – where our economic, community and environmental goals are in balance is the foundation of the Hawai'i 2050 Sustainability Plan and is at the core of CTAHR's vision and mission. Over the course of the project work group members would be called upon to extend

their efforts beyond just the focus of the core project but to address stakeholders expectations that came to light during the reporting period.

In response to stakeholders, in 2010 our team conducted 9 workshops outside of the core project each of which were filled to capacity and represents a contribution in kind of the current project. Our growing clientele list encompasses both urban gardeners and commercial producers with an ever increasing number of stakeholders in education. Educators view aquaponics as a viable tool to teach Science, Technology, Engineering and Math (STEM) skills because it connects so many facets of science and engineering. When a classroom is equipped with a simple system, opportunities for hands on experiences occur and complex topics such as the nitrogen cycle in an aquatic environment or pH mediation are transformed from text in a book to something a student can see, touch, smell and even taste. To top things off, the technology is consistent with the Three R's for protecting the environment, (e.g., reduce, reuse and recycle) people can easily become engaged in this national movement by producing their own food aquaponically. The strategy of broadening audiences and tapping on research projects are typical examples of how extension specialists have become expert "scroungers" to counteract dwindling funding with increasing demands (Patrico, 2011). Impacts of these efforts have been felt in partnerships with the State Hospital , Palolo Elementary School and Maris Garden .

Recommended Follow-up Activities

With the challenge of detecting KHV using PCR technology being surmounted an opportunity has been created in that the project work group might be able to establish the validity and utility of a Specific Pathogen Free certificate of health status for a specific OIE listed pathogen (e.g., Koi Herpes Virus). The project work group is currently participating in proficiency testing for KHV using PCR with the Veterinary Laboratory Agency (Contract No., QAL/1033). Utilizing the collaborative partnerships already formed between CTAHR and the State Department of Agriculture discussions have already commenced with Taro Kodama of Kodama Koi Farm as being a logical site for establishing an SPF surveillance protocol where the challenges and opportunities for conducting such a program can be assessed by all stakeholders. Such an effort, however, would require additional support as the effort requires multiple sampling over a two year period and at a level that assures statistical validity of the sampling protocol. A preproposal in response to the 2012 Requests for Preproposals by CTSA was submitted to solicit additional support necessary to complete that goal.

Publications and Manuscripts Written and Papers Presented

Bright, L., L. Ohai, C. S. Tamaru, B. Fox, K. McGovern-Hopkins and R. Klinger-Bowen. A Hawaiian Herbal Medicine Cabinet Through Aquaponics. 23rd Annual College of Tropical Agriculture and Human Resources & College of Engineering Student Research Symposium. Agricultural Science Building, University of Hawai'i at Mānoa. April 8-9, 2011.

Klinger-Bowen, R., C.S. Tamaru, B. K. Fox, K. McGovern-Hopkins, and Robert Howerton. 2011. Testing your aquaponic system water" A comparison of commercial aquaculture methods. Center for Tropical and Subtropical Aquaculture. Technical Report. 16 pages.

Tamaru, C.S., K. McGovern-Hopkins, R. Klinger-Bowen, B. Fox, A. Riggs, T. Low and N. L. Antonio and J. Brock. 2011. Improved Capacity for the Diagnostic Surveillance of Two Aquatic Pathogens at the University of Hawaii College of Tropical Agriculture and Human Resources and the State Department of Agriculture. Center for Tropical and Subtropical Aquaculture. http://www.ctsa.org/upload/note/Feb_11_Regional_e-Notes634345679631163967.pdf

Tamaru, C.S., K. .McGovern-Hopkins, R. Klinger-Bowen, B. Fox , A. Riggs , T. Low and N. L. Antonio and J. Brock. 2011. Detection of Asymptomatic Francisella spp. Carriers in Tilapia Cultured in Hawaii. Center for Tropical and SubTropical Aquaculture Regional Notes, Volume 3, Issue 7. July 2011. http://www.ctsa.org/files/publications/July_11_Regional_e-Notes.pdf

Tamaru, C.S., B. Fox, T. Radovich, Y. S. Kim, S. Khanal, H. Ako, J. Sugano, K. McGovern-Hopkins and R. Klinger-Bowen. 2010. Aquaponics at the College of Tropical Agriculture and Human Resources (CTAHR). Zero Emissions Conference. The World Congress on Zero Emissions Initiatives . September 13-17, 2010. Honolulu, Hawaii

Tamaru, C.S., J, Brock, N. L. Antonio, ,A. Riggs, K. McGovern-Hopkins, R. Klinger-Bowen, B. Fox, 2011. Update on the Tilapia Rickettsia Like Organism (TRLO) infecting tilapia on Oahu. CTAHR/HAAA Workshop. November 12, 2011, Windward Community College.

9. Regional Biosecurity: Operational Biosecurity and Diagnostic Surveillance (OI component), Year 1

General Information

Reporting Period: June 1, 2010 to September 30, 2011; no cost extension through December 31, 2011 (Year 1 Progress Report)

Funding Level

Year	Amount
1	**$50,000**
2	$49,997
Total	$99,997

RESULTS AT A GLANCE…

- Literature review is under development.

- List of aquatic organism stakeholders is under development.

- Draft survey plan has been developed.

Participants

Charles Laidley, Ph.D., Oceanic Institute

Kim Pinkerton, M.S., Research Associate, Finfish Department, Oceanic Institute

Eric Martinson, M.S., Research Associate, Finfish Department, Oceanic Institute

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

3. Complete specific biosecurity assessments of specific farm operations for each of the major culture species.
4. Write a project report reviewing species-specific operational biosecurity for specific farm operations in Hawaii and the Pacific Region.
5. 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 Principal Accomplishments

Year 1

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

Aquaculture is the fastest growing sector of the global food production industry, and like other sectors is moving toward greater intensification and commercialization of aquatic production. Also like other food production sectors, the frequency and intensity of disease issues increases rapidly as aquaculture efforts concentrate and expand in footprint. Further, the diversity of culture species makes disease diagnosis and early detection of a growing list of pathogens and substantial challenges. Disease is already a major constraint in the culture of many culture species with large economic and environmental impacts.

Given the large diversity of cultured species and potential pathogens, there is an increasing demand to develop and maintain effective biosecurity programs. Most definitions of biosecurity center on a suite of procedures that protect living organisms from contracting, carrying, and spreading pathogens or other non-desirable organisms.

A series of earlier workshops convened at the Oceanic Institute by Dr. Cheng Sheng Lee under the NOAA sponsored aquaculture development program may have helped set the stage for growing interest in aquaculture biosecurity, a field with continues to grow in both importance and refinement.

Under the first objective of this project, we have been re-examining the literature on biosecurity and specific disease issues for species of relevance to aquaculture and the aquatic animal industry in Hawaii. Despite the small size of the industry (approaching $30 million/yr) the industry appears quite diverse. The major culture areas identified for our focus include:

- Macroalgae culture
- Shellfish culture
- Shrimp culture, particularly the broodstock industry
- Marine Fish culture – amberjack, moi, halibut, opakapaka, tuna, etc.
- Freshwater fish culture – Tilapia
- Aquaponics – a relatively new growth area in Hawaii
- Marine ornamental culture, collection, and import/export
- Freshwater ornamentals – mainly cultured
- Hawaiian Fishponds
- Public aquaria and displays
- Stock enhancement
- Research and Development – multispecies, multioperational
- Wild fishery auction
- Seafood restaurant and retail trades

Under each we are currently compiling a list of cultured/traded species and stakeholders in Hawaii. For each we are also compiling a review of basic culture/husbandry methods, determining the source of aquatic organisms, conducting a literature review of known pathogens and diseases, and major biosecurity protocols and approaches for each. Due to staffing reassignments while waiting for project startup we have been somewhat delayed in completing this initial review process. Ms. Kim Pinkerton, the research associate originally assigned to the project has returned to the mainland, with Mr. Eric Martinson assuming her role in the project. Mr. Martinson is now completing the literature review and otherwise coming up to speed on the project.

We have also drafted a series of questionnaires, survey plans, and flow diagrams have been drafted for use in operational biosecurity assessment. A common stumbling block on many farms is the inability to break down the concept of biosecurity into

understandable and simple steps that be consistently practiced. Efforts have been made to help communicate the importance of a biosecurity plan and aid in biosecurity plan development. The goal of these forms is to provide a template that can be tailored to an operation's specific goals, facilities, and practices. These forms will help an operation develop their biosecurity plan by following a series of steps. These steps are:

- Step 1: Document general information – information about your facility
- Step 2: Identify your goals
- Step 3: Identify potential risks
- Step 4: Identify measures to limit risk identified

Effectiveness of a biosecurity plan will be maximized when strategies are tailored for specific sites, with consideration given to the diverse range of environmental impacts, aquaculture systems, species cultured, geographical location, farm size, financial situation, and production goals. Development and implementation of a biosecurity plan should be a continuous process so that new approaches can be considered as technology changes and new diseases emerge. The current Hawaii aquaculture regulations do not require a producer to have a biosecurity plan on file with the Hawaii Department of Land and Natural Resources; however, a formal plan is required to claim OIE compliance per Article 4 of the 2009 Aquatic Animal Health Code. There are also many benefits to establishing and implementing a formalized plan. One primary benefit is that the plan will force an organization to evaluate all of the potential sources of risk that face their current operation.

No two aquaculture facilities are the same, which means that the specific risks each facility faces and the best steps to minimize those risks will also be different. The goals of these surveys and assessment tools will identify each organization's specific goals, facilities, and practices.

In addition to survey forms and questionnaires a series of decision trees/diagrams are being developed to help operations decide what critical control points are for their specific operation and for deciding which pathogens and diseases are of concern to their operation. To further aid in helping individual operations assess their risks, a risk evaluation table has been created. This risk evaluation table should help enumerate different levels of risk and tolerance facing an operation.

Currently these survey plans are in draft form and we are conducting on-site evaluations assessing the practicality and importance of the results generated from completing these exercises. We are now contacting various operations and setting appointments to meet with these operations. Operations have been selected in a best

effort to survey the different aquaculture sectors on each of the Hawaiian Islands giving a cross sectional assessment of aquaculture operational biosecurity goals and risks. In an effort to reach out to different operations a comprehensive list of aquaculture operations in Hawaii has been created. A map of the different Hawaiian Islands with locations of aquaculture operations has provided a useful tool.

During the project we also received kind correspondence from A. David Scarfe of the American Veterinary Medical Association relaying some of their ongoing efforts to refine aquatic animal biosecurity plans and programs that are practical and effective. Included in his correspondence was a draft process of integrated steps for developing, implementing, auditing and certifying a biosecurity program intended to prevent, control, and possibly eradicate disease in any epidemiological unit. The approach has a series of nine formal biosecurity process/steps with associated questions for the farmer and appropriate documentation and records ranging from hazard identification and prioritization (step 1) to Veterinary authority verification and endorsement (step 9) (see appendix 1).

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.

Site evaluations of aquaculture, research, and other animal holding or processing facilities on each of the main Hawaiian Islands is scheduled to begin this November. The survey forms and tools developed under objective one will be utilized to both complete a general biosecurity assessment of operations in Hawaii and also help develop best management strategies and practices for the different aquaculture sectors in Hawaii. To date we have compiled a comprehensive list of best management practices that cover general aquaculture operations that may be used by all operations and we will further organize and highlight the best management practices that are applicable to each individual's operation.

In conducting these site evaluations the questionnaires will be used to assess how bio-secure an organization and those of their suppliers are. They survey tools will

- Allow estimates of the risks associated with various scenarios to enable an operation to manage biosecurity issues now and take action, if wished, needed or

required, to raise standards by establishing future targets and taking practical steps to achieve them

- Establish a guide to good practice in some areas such as sourcing stock and enable an organization to identify how current practices might be improved
- Provide background information on topics such as disease identification and vaccination

By working alongside aquaculture operators and conducting survey plans outlined in objective one will identify where an operation currently stands on biosecurity issues, and if appropriate set future targets that will help in understanding key issues. These tools and exercises will enable each operation to develop plans that might help prevent the introduction of unwanted organisms on to a site or to detect them and prevent them from spreading once they are present.

Work Planned

None to report.

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, Manuscripts, or Papers Issued, Approved, or Presented

None to date.

10. Adapting Aquaponics Systems for Use in the Pacific Islands, Year 1

General Information

Reporting Period: August 1, 2010 to September 30, 2011; no cost extension thru December 31, 2011 (Year 1 Progress Report)

Funding Level

Year	Amount
1	**$34,012**
2	$34,012
Total	$68,024

Participants

Kent Kobayashi,, Dept. of Tropical Plant and Soil Sciences, CTAHR, University of Hawaii at Manoa.

Adam Baker, Dept. of Molecular Biosciences and Bioengineering, CTAHR, University of Hawaii at Manoa.

James Hollyer, Agricultural Development in the American Pacific, CTAHR, University of Hawaii at Manoa.

Harry Ako, Dept. of Molecular Biosciences and Bioengineering, CTAHR, University of Hawaii at Manoa.

Clyde Tamaru, Dept. of Molecular Biosciences and Bioengineering, CTAHR, University of Hawaii at Manoa.

Kiara Sakamoto, student, Dept. of Molecular Biosciences and Bioengineering, CTAHR, University of Hawaii at Manoa.

RESULTS AT A GLANCE…

- Three systems were constructed in American Samoa and run alongside farmers and a local project work group member, who is working with the farmers to ensure that their systems remain productive.

- In addition to extension, beginnings were made in the understanding of the biochemistry of aquaponics systems. This led to affordable designs and management schemes that were transferrable to farmers.

- Chinese catfish were successfully tested as alternative fish for aquaponics and were used to improve upon denitrification problems suffered by some farmers; these problems were causing decreased yield and lengthened growout times. Planting and harvesting schemes were developed to cut growout time in half.

Objectives

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.

Anticipated Benefits

Anticipated benefits will be organized in terms of accomplishments in the lifetime of the current project and consequent benefits to the industry.

Beginnings were made in understanding the biochemistry of aquaponics systems. Understanding the biochemistry allowed adaptation of some aspects of hydroponics technology developed for Hawaii and allowed for building of cost effective systems. This allowed lowering the cost of a 1.2 m X 30 m module/raceway for $3,500 instead of 4 times the amount for other systems (Ako and Leung, 2011).

This also allowed sizing guidelines. For 48 vegetable plants, 2.5 kg of fish are needed to be held in 200 L of water and would have to metabolize about 40 g of feed per day. The working group's experience in American Samoa may be used as a counter example. When we arrived we found a nice looking (and expensive) Rakocy system (see appendix). It was growing five lettuce plants, four of which were severely stunted. There were a few fish in a large tank and they are not fed during the weekends. In contrast we were able to construct three starter units and each produced 96 lettuce plants per 6 weeks. In Hawaii, one large farm, two moderate sized farms, and a multitude of backyard farms were started .

The biochemistry of aquaponics systems also implied a management scheme. In part this scheme is an adaptation of recirculating aquaculture technology. The example cited above of the Rakocy system may again be used as a counter example. It is based on a biochemistry that is not understood and no management scheme is associated with it. A management scheme is associated with the CTSA aquaponics scheme (Ako and Baker, 2009a; 2009b). The system has a biofilter, unlike the traditional scheme which only has a solids remover. Ammonia, nitrite, pH, and dissolved oxygen (DO) are monitored routinely to maintain fish well being and nitrate levels are monitored as well to ensure the fertility of the water. The CTSA scheme is associated with an extension educator who needs to guide the farmer through learning this biochemistry. A farmer in training is shown below doing water quality tests (see appendix).

A publication was produced (Ako and Sakamoto, 2011) and this publication also shows a picture of this farmer's first crop (see appendix). It stands in contrast to the Rakocy system which was not associated with extension teaching. The extension educator was stationed in Samoa for an entire crop cycle.

On a humorous note, we are called the Wizard of Oz because we are somewhat remote (being in Hawaii) and we benevolently suggest things that inevitably, almost by magic, lead to success.

Chinese catfish were successfully tested. These proved to be excellent aquaponics fish and the work is still ongoing. The Chinese catfish allowed work on denitrification, or loss of nitrate fertilizer to the air due to anaerobic, denitrifying bacteria. Benefits to a farmer and the industry will be described later and are prevention of fertilizer loss to the atmosphere.

Planting and harvesting schemes were developed. These were associated with a 2 or 3 week nursery period where valuable grow bed space was not taken up by tiny seedings. This was followed by a 3-4 week growout period and kept the growbeds fully occupied. Increases in profit are obvious. With a 3 week nursery and a 3 week growout period, twice the occupancy of grow beds may be associated with twice the profits.

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.

Chinese catfish were tested as aquaponics organisms. They were well suited to aquaponics because they are air breathers. One would not risk losing one's stock in the event of an electrical outage and the aeration stops. They thrive and grow better when crowded. They are, however, slightly negatively affected by DO levels lower than three. At this levels, their feeding rate declines. A review of the literature suggests that air breathing fish use their gills when DO levels are high and only breathe air when DO levels are low. Thus, while they survive low DO, their tanks should probably be aerated so that they would grow well. In our test, they grew well and had a desirable feed conversion ratio about 1.2-1.4 at the sizes we tested them.

Chinese catfish allowed us to do denitrification studies because they could live, eat, and metabolize (albeit at lower rates) at low DO. When DO was held at 0.5 mg/L all nitrate disappeared from the fish tanks reducing the fertilizer value of the fish water. When minimal aeration was applied, DO could be adjusted to 1.0 mg/L. At these levels no nitrate was present again suggesting quantitative denitrification.

The tipping point was a DO of 2.0. Sometimes nitrate-nitrogen was 40 mg/L and sometimes it was 20 mg/L. Aerated control had nitrate nitrogen levels of 60 mg/L. At a DO of 3 mg/L, grow tray nitrate-nitrogen was about 20 mg/L and about 50 in aerated control grow trays. Vegetable growth was not significantly different at these two nitrate levels. This means that aquaponics grow trays require a management scheme that ensures that DO is 3.0 or higher.

The studies were triggered by data obtained by Clyde Tamaru from a farmer (below). One can see that DO values are very low in System 1-4 fish tanks but rise in Growbeds 3 and 4, presumably due to ebb and flow gravel biofilters which can aerate somewhat. One can see that nitrate is very low in Systems 1 and 3, and not very good in other systems as well. The farmer said his vegetable growth was "slow". On our advice, he raised his DO values to above 3 mg/L and on average 5 mg/L using water jets, the pH remediated itself, nitrate-nitrogen levels rose to 36. Growout times dropped to 3 weeks.(see appendix)

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 begun to be addressed as we have just received feed from Samoa for testing.

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.

We have developed a scheme for lettuce. Day 0-2 is for spouting. Days 3-Week 2 is nursery. Week 2-3 is extended nursery which we need to repeat before we make a recommendation. It is done with plantlets in Oasis cubes in pans over fluorescent light gratings so that roots can reach downwards. Weeks 4-6 are growout. We note that spouting must begin 3 weeks before harvest.

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 was the major objective. It began with acquiring materials prior to going to Samoa. There is little surface freight to Samoa and surface freight rates are similar to the very high air freight rates. As much as possible we purchased materials in American Samoa, presumably shipped by surface during the infrequent shipments. Ian Gurr and Francis Leiato were very helpful and found that some of the things we needed were available in Samoa. Prices were twice those in Honolulu. We met Ian when he was inspecting out demonstration site in Manoa as he was getting an M.S. in sustainable agriculture here at UHM. The items unavailable in American Samoa had to be taken over and we soon exceeded our luggage capacity. We had to pay air freight rates which exceeded $6.6 per kg. The list included small items such as staples and screws and was very long.

The first order of business after arriving in American Samoa was clearing customs. Receipts are needed for large items. Fortunately, we had the receipts which we FAXed over. We made a set of three PowerPoint presentations at several sites and handed out hard copies of the PowerPoint presentations as well as the Hawaii instruction manual for aquaponics in lieu of the workshop manual promised. The substitution was adequate and serves as the "Bible" in Samoa.

This was followed by building alongside with Samoan clients. Two of many photos are shown below (see appendix). We found that Samoans have carpentry skills as most are farmers and building went smoothly.

Next fish were purchased from fish farmers. Aquaculture in Samoa in underdeveloped because there is no fish feed available. The cost of shipping fish feed to Samoa would be about $6.6/kg. The fish were stocked at the appropriate densities as directed by the nutrient flux hypothesis. Water catchment containers which we used as fish tanks.

We then taught farmers how to feed their fish and how to do water chemistry tests to ensure the health of their fish and the fertilizer value of the fish water. The farmers all seemed very happy with this especially because their vegetables were the best they had ever seen or eaten. They were prepared for us and had already prepared marketing plans. Plans had also been made to form coops ahead of time as well.

As a postscript, one of the farmers must come to Hawaii to get surgery. We have been taking him around to the various operations here as well as the various vendors. We consider this as part of our continuing duties.

Work Planned

Complete the test of the Samoa produced fish feed. Questions are rate of fish growth compared with a leading American feed and vegetable growth parameters. Chemical methods often offer shortcuts to assessments of feed effectiveness in vegetable growth.

Continue to examine alternate vegetables such as kai choy, basil, and so on. A fine line must be walked between specializing and becoming efficient in commercial production of a crop and providing customers with a variety of vegetables, the one stop shop concept.

Make a return visit to American Samoa to cement progress made in the first trip and to assist any new farmers who may want to do aquaponics.

Work with interested Hawaii aquaculturists who may wish to expand into aquaponics or agriculture farmers who may wish to do the same.

Impacts

The primary impacts are designing and building aquaponics systems that are affordable and which work. No disrespect for the designer of the original systems but this work takes the concept further and adds to it a management scheme.

Due to work presented in the 2009 workshop on the economical aquaponics system and the guidelines on operating parameters of aquaponics systems, Mari's Garden was started. This farm has been continually helped. As of the 2011 workshop, the farm was operating 28 X 30 m X 1.2 m raceways for lettuce. Production was conservatively reported to be 2,500 kg/week. Prices were about $6.6/kg. Cucumbers were also produced and a section of Whole Foods was devoted to Mari's Garden produce. They report difficulty in supplying demand. Tilapia are sold through channels developed by CTSA farmers and the price receive is $13.20/kg. A standing stock of about 30,000 animals is maintained. At last visit, seven jobs were created by this farm. This farmer shared some of his midterm successes and his PowerPoint is attached.

Two moderate sized farms in Hawaii are too new to generate reliable impacts from as are the two new farms in American Samoa.

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

Ako, H. Adapting aquaponics systems for use in the American Pacific Islands. Presentation made at the public presentation of research projects for CTSA, June 18, 2010.

Ako, H. and A. Baker, Nutrient fluxes in aquaponics systems. Presentation made at the Aquaponics Workshop, Honolulu, Hawaii, November 21, 2009a.

Ako, H. and A. Baker. 2009b. Small-scale lettuce production with hydroponics or aquaponics. College of Tropical Agriculture and Human Resources, Publication No. SA-2. University of Hawaii, Manoa, Hawaii, USA.

Ako, H. and A. Baker. 2010. Aquaponics in the Pacific: Studies Using a Nutrient Flux Approach. Aquatips in Regional e-Notes for the Center of Tropical and Subtropical Aquaculture 2(3): 2-3.

Ako, H. and P.-S. Leung, Preliminary Economics of Aquaponics. Presentation made at a workshop in Honolulu, Hawaii, July 23, 2011, Windward Community College.

Ako, H. and K. Sakamoto. 2011. Pacific Island Spotlight: Transferring Aquaponics Knowledge to American Samoa. Aquatips in Regional e-Notes for the Center of Tropical and Subtropical Aquaculture 3(8):2-6.

11. Alternative Methods for Marine Copepod Production in Hawaii, Year 2

General Information

Reporting Period	January 1, 2011 to October 31, 2011 (Progress Report)
Funding Level	$25,106
Participants	**Clyde S. Tamaru,** Ph.D., Department of Molecular Biosciences and Bioengineering, University of Hawaii-Manoa Karen Brittain, Hawaii Institute of Marine Biology Benjamin Alexander, Hawaii Institute of Marine Biology

RESULTS AT A GLANCE…

- The major algal species (e.g., Isochrysis galbana, Chaetoceros sp. and Tetraselmis sp) that can be used to culture copepods grew similarly irrespective of whether the source of seawater was Instant Ocean® or natural seawater from Kaneohe Bay.

- The calanoid copepod, P. crassirostris, and the harpatacoid copepod, E. acutifrons perform similarly irrespective of whether the culture was done in artificial or natural seawater.

- To date only live phytoplankton can be used as a suitable food item for culturing copepods albeit in artificial seawater.

Objectives

1. Conduct short-term controlled experiments to assess the adaptation of the copepod cultures to artificial seawater and artificial feed particles..
2. Conduct short-term controlled experiments aimed at intensification of copepod cultures (greater animal density, target: 20 animals/mL, expansion to 300–500 liter volumes, more intensive support and maintenance) in artificial seawater with artificial feeds.
3. Assess and document results of long-term culture comparisons.

4. Technology Transfer: Document experimental results in a summary report for use in workshops or technical publications, and prepare an extension bulletin in less formal language with photos describing practical protocols as appropriate.
5. Technology Transfer: Present public workshop sessions at which results will be presented, hands on demonstrations provided and stakeholders will be invited to suggest further directions for project development.

Anticipated Benefits

Anticipated benefits of the proposed project is the demonstration that artificial seawater can be used for the culture of at least two species of copepods. This is particularly important with regard to circumventing challenges with biosecurity as well as being able to conduct hatchery operations in areas that are not associated with the shoreline or access to seawater. This is particularly true for marine ornamental producers that are operating in locations where there is limited access to natural seawater.

Work Progress and Principle Accomplishments

Objective 1: Conduct short-term controlled experiments to assess the adaptation of the copepod cultures to artificial seawater and artificial feed particles.

Copepod culture currently relies heavily on the production of live phytoplankton and for that reason some initial small (2-Liter) trials comparing phytoplankton growth using artificial seawater (e.g., Instant Ocean®) versus filtered natural seawater obtained from Kaneohe Bay were conducted. The use of Instant Ocean is not an endorsement of the product but reflects that it is an easily accessible commercially available product. There are a myriad of artificial seawater formulations and rather than assess the value of all of them it was decided to focus on one that was easily obtainable and ready to use. These comparisons, however, are important to conduct because the goal of the inventor of Instant Ocean (William Kelley) was not to replicate natural seawater exactly, but to use its fundamental chemistry as a guideline to create a synthetic marine environment that would sustain life reliably and consistently from one aquarium to the next.

The most important of the phytoplankton species used for culturing our initial target calanoid copepod, Parvocalanus crassirostris is Isochrysis galbana. Standard protocols being used the Hawaii Institute of Marine Biology (HIMB) bottomfish hatchery facility were followed with regard to micronutrients , aeration, and

sterilization procedures. The only difference in the culture process is the source of seawater used. Phytoplankton were quantified using traditional methods (e.g., a hemocytometer and a compound light microscope). Three species of phytoplankton (e.g., Isochrysis galbana, Chaetoceros sp. and Tetraselmis sp) have been tested to date. All of the algal species examined grew similarly irrespective of whether the source of seawater was Instant Ocean® or natural seawater from Kaneohe Bay. The results obtained with I. galbana is summarized in Figure 1 in the appendix. Typical of the results with all species tested no significant differences could be detected between the use of natural or artificial seawater.

Objective 2: Conduct short-term controlled experiments aimed at intensification of copepod cultures (greater animal density, target: 20 animals/mL, expansion to 300–500 liter volumes, more intensive support and maintenance) in artificial seawater with artificial feeds.

Two species of copepods are the target of the ongoing investigations and they are the calanoid copepod, Parvocalanus crassirostris and the harpatacoid copepod, Euterpina acutifrons. Investigations testing various treatments were conducted in plastic buckets (20-L) in a static fashion supplied with a continuous source of aeration. When a treatment called for the use of artificial seawater the phytoplankton that was used as a food source had also been cultured in artificial seawater. The temporal changes in copepod density was quantified by randomly taking 1 ml aliquots (n=4) with a glass pipette and using an 8x lube the various stages of copepods present within the pipette could be counted and recorded. The number of adult copepods and copepodites were grouped together as they are difficult to distinguish from each other using the methods described but can easily be differentiated from the nauplii stage (Figure 2).

The initial experiment examined the use of artificial seawater versus natural seawater for culturing the calanoid copepod. Duration of the growth trial was 14 days and was completed in January of 2011. Average water temperature was 22.6 ± 0.6 C, salinity was 35.9 ± 0.6 ppt, pH was 8.5 ± 0.3 and Total Ammonia Nitrogen was 0.6 ± 1.0 ppm.

Temporal changes in nauplii and adult copepod densities that took place during the trial is summarized in Figure 3 found in the appendix. The data was also subjected to statistical analyses (unpaired t-test) and while the observed mean nauplii densities were found to be significantly different (two tailed P=0.022, t =2.32) the mean number of adults observed between the two treatments was not (two tailed P=0.77, t=0.29). It should also be pointed out that the highest average nauplii densities obtained when using artificial seawater was over 20 individuals per ml which is a suitable quantity to obtain when conducting larval rearing trials with larval fish species that rely on this

type of live food organism as an initial feed item. The average total (nauplii + copepods) number of individuals was not significantly different between the use of artificial or natural seawater for their culture medium (two tailed P=0.07, t =1.78) although they approach being significant. While there may be subtle differences between the results obtained using artificial seawater and natural seawater for culturing P. crassirostris overall the data indicate that there are no major differences in the use of one or the other in culturing this species of copepod..

It is anticipated that because of the inherent costs of using artificial seawater in both material and labor to produce it, one strategy that might be incorporated by the end user is to dilute the solution in order to maximize its use. Thus knowing the effects of varying salinity on calanoid copepod production would be useful. Culturing P. crassirostris at various salinities was conducted by diluting the artificial seawater with tap water resulting in five different salinity treatments (e.g., 30, 20, 15, 10, 7 ppt). All treatments were conducted in triplicate and conducted as static systems and provided with a continuous source of aeration. I. galbana cultured in artificial seawater was used as the food source and provided at a density of 2x105 cells/ml. The results obtained over the course of a two week growout period are summarized in Figure 4 found in the appendix. Both nauplii and adults calanoid copepods did not grow well below salinities of 15 ppt. Similar growth profiles were observed for both nauplii and adult stages when cultured at salinities of 15 and 20 ppt. However, clearly both nauplii and adult production was highest using the 30 ppt treatment and indicates that the range of salinities that will be optimal for copepod production will be restricted to salinities of 30 ppt or higher.

The harpatacoid copepod, E. acutifrons was also investigated over the course of the reporting period and also found to perform similarly irrespective of whether the culture was done in artificial or natural seawater. E. acutifrons was also provided with a variety of phytoplankton species (e.g., 1.0 x 105 cells/ml) to assess their growth performance using all artificial seawater conditions. The trials were done similarly to those reported for the calanoid copepod and three phytoplankton species (Tetraselmis sp, Chaetoceros sp., and I. galbana) were examined as to their abilities to support the growth of this harpatacoid copepod. Results of the trials are summarized in Figure 5 found in the appendix. Nauplii densities of 20 individuals per ml were recorded using both Tetraselmis and Chaetoceros as a food source and no differences in performance could be statistically detected. A similar situation was also observed in the adult populations. Nauplii production was significantly less with the use of I. galbana and also translated into a lower output of adults. Results are consistent with what has been reported previously and also based on personal experiences of project personnel.

The use of only artificial seawater can apparently support growth of this harpatacoid copepod.

A similar kind of trial was also conducted with the calanoid copepod but in this case only two species of phytoplankton (I. galbana and Chaetoceros @ 2.0 x 105 cells/ml) were used. Temporal changes in nauplii and adult densities are summarized in Figure 6 found in the appendix. Results obtained were consistent with our previous results using natural seawater in that while the Chaetoceros sp. used does result in growth of the calanoid copepod it does not perform as well as when using I. galbana.

Alternative feeds were investigated in their abilities to support growth and production of both calanoid and harpatacoid copepods during the reporting period. Attempts at using some of the more innovative artificial feeds like V8 vegetable juice that has been reported to be successful for another marine harpatacoid copepod as well as crushed nori were also attempted. The V8 vegetable juice and crushed nori did not result in any production of the two copepod species being investigated contrary to some of the results that have been reported.

Commercial algal paste such as Nannochloropsis oculata produced by Reed Mariculture has been shown to be highly effective in supporting rotifer production to the point where live phytoplankton can be totally replaced using this product. Likewise, the company produces a variety of other algal pastes that have been reported to be suitable for culturing other species of organisms (e.g., feather duster worms). The results of those trials using the calanoid and harpatacoid copepods being investigated are summarized in Figure 7 and 8. Unfortunately all of the commercial algal paste tested failed to result in any significant ability to support copepod growth and or production for both copepod species.

Objective 3: Assess and document results of long-term culture comparisons.

A 40-L culture using only artificial seawater for the culture of both phytoplankton and the calanoid copepod was initiated on December 2, 2010. It is being run as a static system at a salinity that ranges between 30 - 33 ppt and supplied with a continuous source of aeration. I. galbana is used as a source of food and provided at a density of 2.0 x 105 cells/ml on a daily basis. Density of the nauplii and adults are monitored as previously described. At approximately two weeks duration the culture is completely drained, tank cleaned and culture restocked using the same individuals harvested. A summary of the temporal changes in nauplii and adult density is provided in Figure 9. The typical bloom of nauplii that occurs with each restocking of the tank is routinely observed followed by a rise in the number of adults. This nauplii

bloom is one of the mechanisms that can be used to provide nauplii stages of the copepod to first feeding fish larvae that require this particular live food organism. Clearly, the culture of this calanoid copepod can be done using only artificial seawater and for extended periods of time. Future work should include focus on the recycling of the artificial seawater as it represents a substantial investment and warrants its reuse.

Objective 4: Technology Transfer: Document experimental results in a summary report for use in workshops or technical publications, and prepare an extension bulletin in less formal language with photos describing practical protocols as appropriate.

A draft technical handout was prepared but the final document remains uncompleted. Target is to produce a news letter article for the upcoming CTSA newsletter and complete the technical handout as part of a CTAHR extension activity.

Objective 5: Technology Transfer: Present public workshop sessions at which results will be presented, hands on demonstrations provided and stakeholders will be invited to suggest further directions for project development.

No progress made during the reporting period. Planned workshops will be incorporated with other extension activities to be undertaken at HIMB and CTAHR.

Work Planned

A significant delay in obtaining the commercial algal paste due to permit acquisition while resolved has delayed completion of the last technical task needed to be completed. The delay resulted in not being able to complete the technology transfer activities but will be made up as part of our extension and outreach activities in the College of Tropical Agriculture and Human resources. Future work will be to complete a newsletter article that is to be submitted for printing in CTSA Regional Notes. Second task is to complete the technical handout that would provide information on production of phytoplankton and copepods using artificial seawater. Lastly a hands on workshop is to be held that would introduce methods of culturing copepods and phytoplankton utilizing artificial seawater. These activities will have to be covered by the PI utilizing his extension and outreach resources provided by CTAHR.

Impacts

The results obtained from the current project has utility for culturists that require the use of copepods for use as a live feed organism in rearing of marine organisms. The most significant results being the demonstration that the phytoplankton and copepods can be grown using artificial seawater equally well as when using natural seawater. This would be of particular interest to culturist who do not have access to natural seawater. A major shortfall of the project, however, was not being able to identify an alternative feed that can be used to culture copepods resulting on the continued reliance on live phytoplankton only. This is unlike what has been achieved with the use of preserved phytoplankton for rotifer production that has downsized the production footprint and costs for hatchery operations that originally called for phytoplankton culture.

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

Jackson, J. 2011. Larval Clownfish Amphiprion ocellaris Predatory Success and Selectivity when Preying on the Calanoid Copepod Parvocalanus crassirostris. MS Defense, University of Hawai'i at Manoa, Department of Zoology, 5/18/11.

12. 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 31, 2011; no cost extension through January 31, 2012 (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:	Dr. Darren Okimoto, University of Hawaii – Manoa Campus Sea Grant College Program Extension Leader and Ephraim Ellsworth Temple, the local extension Agent in American Samoa.
Industrial Partners:	Aquaculture finfish farmers in American Samoa

RESULTS AT A GLANCE…

- A simple, at-home feed manufacturing system was created in American Samoa, and increased the feed processing speed at the local community college. Farmers are using the feed processing technology and equipment and making feed for their fish.

- The nutritional composition data of locally-available products and byproducts generated by this project has provided a valuable database in formulating sustainable cost-effective feeds for the aquaculture industry in American Samoa. A simple diet for tilapia was created.

- A children's feed manual (English & Samoan) containing simple ingredient proportions for batching and feed making was created and published, along with an adult feed manual. A training workshop was conducted where both children and adults learned about fish farming and making tilapia feed on the farm site.

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. This project will create jobs, provide a food source, and start a feed industry that will utilize a fishmeal and tropical starch resource in American Samoa which is currently being exported to foreign countries

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 carbohydrate ingredients and a tuna meal by-product were identified by American Samoa co-PI and were collected and prepared for analysis by American

Samoa Community College students. Samples were sent to OI for analysis as potential candidates for inclusion in tilapia diets the tuna meal and dried and ground carbohydrate ingredients sent to OI for analysis included; Giant Cavendish banana w/and w/o skin, Bluggoe banana w/skin, banana leaf, Fa'i banana stalk, breadfruit w/ and w/o skin, cassava, and taro. When the prepared ingredients were received from American Samoa they were submitted for analysis, attained fresh carbohydrate substitutes, banana (raw) & breadfruit (cooked & raw) ingredients were freeze dried submitted for analysis. The fresh substitute carbohydrates were used for the testing of feed manufacturing processes. In addition all-purpose flour was attained and submitted for analysis.

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.

Analysis has been completed for gross energy, amino acids and fatty acids of the ingredients sent from American Samoa. This completes the laboratory analysis portion of the project.

The plant ingredients all have similar cooked nutrient profiles, with low amounts of crude protein, fat and fiber. Energy content was about the same, 4100 calories/g. The plant ingredients locally available are mainly carbohydrate in content (c.a. 90%) to provide the starch portion of the diet. The Fa'i stalk is the exception, being high in fiber and ash with about half the carbohydrate levels of the other plant ingredients and is not as suitable as a tilapia feed ingredient. The slightly higher protein and fat level of the banana leaf may have been influenced by the cooking process with other proteins. The levels of essential amino acids and fatty acids in the tuna meal provide sufficient amounts for nutritional requirements for tilapia diets. (Appendix Tables 1-4)

Objective 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.

The standard feed processing manual for adult tilapia farmers with a simplified list of local ingredients available in American Samoa is more than 60% complete.

The list of locally available ingredients and byproducts and their nutritional composition has been completed. (Appendix Tables 1 & 2).

A tilapia diet has been formulated (Table 6) and was test manufactured by the students of American Samoa Community College. The nutrient analyses of the resulting diets are compared to the NRC (2011) nutrient requirements for tilapia (Appendix Table 5). The manufactured diets provide sufficient essential amino acids, fatty acids, minerals and vitamins to meet the needs for tilapia growth. Using the prior mentioned fresh substitute carbohydrates and all-purpose flour (objective 1) and the analysis provided, a feed formulation was developed and tested using the appropriate fresh substitutes.

The feed processing instructions and process flow diagram for making aquaculture feeds using the local ingredients have been completed. The feed making quality control test protocols for mixer testing, particle size determinations and pellet water stability testing have been written and the flow process diagrams of these are in progress.

From the formulation, the available ingredients were used to determine the best hand processing procedure. Using substitute fresh carbohydrates, all-purpose flour and American Samoa tuna meal, varying mix orders and varying moisture additions a final formulation and processing procedure was determined. Test diet was processed and allowed to dry before being submitted for analysis.

An illustrated children's feed manual has been completed by the AFN staff w/drawings on a simple feed processing techniques for making tilapia feed. The final feed formulation and processing instructions, determined from objective 3, were compiled for the use in the manual. Copies of the manual were taken to American Samoa and given to the children of the farmers during the workshop mentioned in objective 4.

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.

The transfer of a basic simple feed processing technology has been made to the children and a more advanced but simple feed processing technology has been accomplished w/ the adults. However, there is a lot more work and training of the Sea Grant staff and the tilapia farmers in advanced feed processing techniques to be able to get this technology from the lab scale to the pilot scale. Then what is needed is to guide interested American Samoan entrepreneurs on what is needed to take this process to the

commercial scale of feed production (there was one such individual who attended the workshop).

Work Planned

1) Complete the adult manual, write final report, and make recommendations for future.

2) Write a proposal for additional training of the Sea Grant staff and start sourcing additional scale up lab processing equipment for increased capacity and add QC testing equipment and training in order to increase the quality of their feed processing techniques, and their finished products.

Impacts

1) Create a small scale local base community feed manufacturing industry which will build up and expand the local production of tilapia culture. With the increased feed processing capacity that the project has provided the farmers are now coming in to make their feeds. This capacity needs improvement and a second phase needs to be addressed and funded. A larger mixer (100lb capacity) is needed, larger scales, larger meat grinder to take whole bread fruit, moisture meters and temp probes or guns.

2) 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. Workshop attendee who was not a tilapia farmer expressed interest in getting into the feed manufacturing business, in contact with him and will work together in the future.

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

Children's Feeds Manual published 10/19/2011

13. Aquaculture of Opihi, Year 1

General Information

Reporting Period: August 1, 2010 to September 30, 2011; no cost extension through December 31, 2011 (Year 1 Progress Report)

Funding Level

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

Participants: **Warren Dominy**, Aquatic Feeds and Nutrition, Oceanic Institute

Addison Lawrence, co-P.I., Wildlife and Fisheries Science, Texas A and M University

Chris Bird, co-P.I., School of Ocean and Earth Science and Technology, University of Hawaii

Vernon Sato, co-P.I., retired

Harry Ako, co-P.I., Molecular Biosciences and Bioengineering, University of Hawaii

RESULTS AT A GLANCE…

- Researchers have learned to capture opihi with the lowest mortality possible, and hold them without killing them when moving them from tank to tank.

- Researchers have learned to grow and feed opihi their natural food.

- An artificial diet which can keep opihi indefinitely has been generated.

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

1 Continue testing feeds with adjusted nutritional profiles, with different attractant ingredients, and different methods of presentation. Begin identification of optimal nutrient and attractant levels by systematically varying each individually.

2 Rate the flavor of cultured opihi with a panel of experienced opihi eaters. Beside taste, aquacultured opihi will be tested for texture and/or mouth feel.

3 Work on sustaining the life cycle in culture. Focus on improving management practices. On land growout systems will have to be recirculating.

4 Transfer technology to a few motivated stakeholders via extensive, hands on extension and an industry manual summarizing the developed techniques for opihi aquaculture.

Anticipated Benefits

This project is a long term aquaculture project. Benefits will occur far in the future. Ultimately successful aquaculture of the opihi could lead to an industry producing extremely high value products (current prices are $150/4 L shells on). Current data suggest that opihi do not require large growout volumes or sophisticated facilities.

Work Progress and Principal Accomplishments

Year 1

Objective 1: Collect wild giant opihi and establish a broodstock holding facility.

This project started off with challenges in the first experiment. It started off with a 52% mortality 4 days after the first batch of animals was collected (Table 1). Such a high mortality would make the rest of the project difficult. The cause of the mortality was hypothesized to be physical damage to the animals while scraping them off the rocks during collection. More careful collecting and use of experienced collectors led to more reasonable survivals.

Next, moving opihi from one tank to another tank because an issue. Opihi climb and stick to the sides of tanks and aquaria under culture. They must be scraped off the sides with a modified putty knife or butter knife to move them to a different enclosure. In the first trial, only 53% of the animals survived the move (Table 2). This would be unacceptable in the long run. Hence plastic and nylon sheets were auditioned for both animal handling purposes and as substrates for growing biofilm. A rough polyethylene won the competition. It was manufactured as a drop cloth for painting. When animals need to be moved, they are pushed from the back side of the plastic and they fall off. They may be then picked up and moved. No mortalities due to moving occurred after this.

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.

A first step in feeds development is noting what animals eat in the wild. We began this by doing a stomach content analysis. Much of the stomach contents were unidentifiable sludge or bacterial clumps but the identified materials were benthic diatoms. They are listed in terms of abundance in Table 3.

Tanks with plastic coverings (Fig. 1) received sand filtered water and were on the beach and could have received organisms from sea spray. These were left in the sun and a brown layer of benthic diatoms grew. This plastic sheet could be transported to campus at Manoa and when left in the sun, the diatoms continued to grow. We called this "biofilm" and found that it contained similar organisms as found in stomach contents.

A feeding trial was conducted using "biofilm" as feed. The trial consisted of 8 animals fed over a period of 5 days. Animals ate avidly, clearing areas of biofilm on the tank and ended up consuming a mean of 0.47 ± 0.13 g dry matter/bodyweight/day and this could sustain them for as long as we observed until the biofilm deteriorated.

We then began a series of feed preference tests. We started with sea urchin feed. Feeds were fed and amounts consumed per evening were measured. In the first series of tests, a preference was shown for feeds containing marine protein meals (fishmeal or squid meal) over marine organism homogenates (mussel or squid homogenate). In the second set of tests, no preference could be determined between the squid and fish meals. In the third set of tests, a gelatin diet containing fish and soy meal was preferred over a gelatin diet containing soy and corn meal. Betaine, a feed attractant in other applications was found not to be attractive. In a fourth set of experiments,

biofilm incorporated into an agar diet proved to be highly attractive and GABA (gamma amino butyric acid) and DMPT (dimethyl propiothetin) were not attractants nor was Spirulina. One would not want to have a randomly recruited biofilm which one would have to grow be part of one's feed. Biofilm was found to be replaced with a commercial preparation of *Porphyra* eaten in Hawaii as nori. This yields a completely artificial diet that sustained opihi for as many as 45 days. Specific results of all of the feed trials are presented in the presentation attached.

The nutrition of the diet must next be worked on.

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.

Start of this objective was delayed. Year 2 work has not begun.

Work Planned

As indicated we have found feeds that are palatable enough to sustain life for opihi. We need to work on the nutrition of the feeds so that the opihi will mature in captivity. Captive maturation is more sustainable than catching ripe animals from the wild. After this we intend to finish Year 1 objective 3 and continue on to Year 2 objectives.

Impacts

Being able to capture animals without killing them is important. Being able to handle them without killing them is important for the research as well. Being able to hold them on biofilm, which seems to be close to their natural food is invaluable. The biggest breakthrough is having an artificial feed for opihi. This is a necessary jumping off point for further studies.

None of this work is of use to farmers yet. In the longer term it could all become important.

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

Ako, H. with N. Hua, Aquaculture of the Giant Opihi. Presentation at the joint CTAHR/Hawaii Aquaculture and Aquaponics Society, Windward Community College, Honolulu, Hawaii. November 12, 2011.

Ako, H. and N. Hua, Aquaculture of the Giant Opihi. Presentation at the Hawaii Malacological Society, Honolulu, Hawaii. October 5, 2011.

14. Collection and Health Certification of Coralgrouper Broodstock in the Mariana Islands (UOG Component)

General Information

Reporting Period	January 4, 2010 to October.1, 2011 (Progress Report)
Funding Level	$42,818
Participants	**Hui Gong,** Ph.D. University of Guam
	John W. Brown, Ph.D. University of Guam

RESULTS AT A GLANCE…

- Unfortunately, the inability to obtain the live broodstock from the wild thus far has left the research group to anticipate that there will be a significant delay in completing this project.

Objectives

1. To contract with local fishers for a minimum of 20 live, adult or near adult Plectropomus leopardus and 20 live adult P. laevis caught from the local wild population.
2. To test the resulting captive stocks of these two species for the two most common classes of grouper viruses: viral encephalopathy and retinopathy also known as Viral nervous necrosis (VER/VNN), and a group of four iridoviral diseases.
3. To maintain these stocks in quarantine and monitor them for any other diseases for a six month period.

Anticipated Benefits

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. Coralgrouper might be a good option as a potential candidate for meeting such need. The results from project would provide the preliminary investigation for such potentiality.

Work Progress and Principle Accomplishments

Objective 1: To contract with local fishers for a minimum of 20 live, adult or near adult Plectropomus leopardus and 20 live adult P. laevis caught from the local wild population.

Recruiting a qualified graduate student as the research assistant to work on the project has not been working out as planned. Numerous attempts through multiple channels in obtaining the live brooder have been unsuccessful. Our initiated contacts with the local fisherman for getting the coral brooders, and feedback indicated there would be great difficulties technically in obtaining the live broodstock. Therefore, In addition to continuously contacting the professional fisherman for live coral reef fish, currently, we are starting a cooperative effort with Underwater World, the commercial aquarium on Guam, to join their live collecting expeditions on a weekly basis. Their senior technician feels that it may be possible for them to obtain some coralgroupers in the Spanish Steps area south of Apra Harbor. We are also investigating the possibility of cooperating with the Land Grant aquaculture program and the Division of Marine Resources in Palau both groups have indicated their willingness to assist us in our efforts to obtain the coralgroupers needed to complete this project.

Objective 2: To test the resulting captive stocks of these two species for the two most common classes of grouper viruses: viral encephalopathy and retinopathy also known as Viral nervous necrosis (VER/VNN), and a group of four iridoviral diseases.

We put together the required reagents for the RT-PCR procedures, and ordering is under the way. Our molecular biology lab is set up and capable of PCR disease diagnosis.

Objective 3: To maintain these stocks in quarantine and monitor them for any other diseases for a six month period.

No action has been taken in this part.

Work Planned

We hope that the live coralgrouper brooders can be collected in the next month or two, so that we will move forward with disease diagnostics and quarantine monitoring. Because of inability of obtaining the live broodstock from the wild so far, we anticipate that there will be a significant delay in completing this project and would like to request a 12 month no-cost extension.

Impacts

The results form this research would help us to understand if the coral grouper is suitable for being consider as the good candidate of aquaculture species, and would provide useful information to both academia and industry in aquaculture. Coralgroupers offer the possibility of serving three markets: providing fish of the restoration of natural reef populations, providing fry for local farmers and providing SPF fry or broodstock to the Asian industry.

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

Not Available.

15. Determining aquaculture bottlenecks of Pacific threadfin (*Polydactylus sexfilis*): Increasing fry survival, growth and quality, Year 2

General Information

Reporting Period: October 1, 2010 to September 30, 2011 (Year 2 Progress Report)

Funding Level:

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

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

Chad Callan, Ph.D., Research Scientist, Finfish Department, Oceanic Institute

Eric Martinson, Research Associate, Finfish Department, Oceanic Institute

RESULTS AT A GLANCE…

- Identified low environmental iodide availability as route cause of high moi broodstock mortality rates.

- Demonstrated that goiters, and consequent broodstock losses can be reversed using either water or dietary iodide supplementation.

- Developed and tested a practical sausage diet that maintains highly valuable broodstock populations in excellent condition and has further improved overall broodstock performance.

Objectives

Year 1

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.

Years 1 and 2

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 and NOAA is helping create hatchery technologies supporting the development of commercial moi production operations in Hawaii, and the first commercial open ocean fish farm in the United States. Although current production methods support year-round egg supplies enabling large scale fingerling production, the species appears particularly prone to thyroid deficiency issues, leading to high rates of broodstock turnover and possible impacts on egg quality and larval performance. Therefore this project was initiated to provide practical solutions for improving broodstock health and fingerling production capacity. The overall benefit of this project is an enhanced broodstock holding protocol that greatly improves retention of highly valuable moi broodstock, increases egg availability, and lowers operational costs of hatchery operations.

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.

Under year one of the project we documented that despite steadily improving survival rates, increasing proportions of the moi broodstock population at OI was succumbing to goiter related mortalities. Earlier analytical studies examining saltwater derived from the OI/SLP well system showed that most of the iodide was in an oxidized form (IO_3), which affected the ability of moi to synthesize sufficient quantities of thyroid hormones leading to goiter formation.

Decreasing goiter associated mortalities over the last two years likely reflects inclusion of water and dietary treatment regimens developed under this project. Year one project efforts also showed that goiters are slow to develop, taking several years to

manifest and are not found during growout and early broodstock conditioning. As goiters are only seen in older broodstock, peaking after three years of captive maintenance, they can greatly impact egg supplies as it is the older fish which typically generate the majority of eggs and generally produce eggs of the highest quality. The rates of goiter formation have remained similar between male and females,, suggesting that the disease is likely a function long-term maintenance in low iodide waters associated with bore-hole water supplies which appear to oxidize available iodine supplies.

In earlier work with Dr. Grau's team at the Hawaii Institute of Marine Biology we found 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 using normal ocean water. Further, iodide supplementation of broodstock through either the water or diet, increased egg thyroid hormone content, showing that either approach is appropriate for mitigating thyroid deficiencies. We also saw a significant decline in broodstock mortality associated with goiters in both iodide supplementation groups. The dietary route, in addition to being less expensive and easier to administer, also appeared to be more effective in reducing goiter appearance. Although iodide supplemented formulated feeds (both in-house and commercial Vitalis™ feed formulations) reduced goiter formation and increased egg thyroid hormone content, these diets proved inferior at maintaining required egg quality necessary for commercial moi fingerling and were therefore returned to the high-quality mixed raw feeds.

Therefore in July 2010 we randomly sorted the moi broodstock into two production tanks (~55 fish per tank) and initiated the current trial. Under this trial, half of the moi broodstock population (i.e., one tank) are being maintained on alternate feedings of smelt, squid and shrimp (control diet) while the other half of the broodstock population were switched to a vitamin and iodide supplemented diet in mid-September 2010, using the same proportions of smelt, squid and shrimp using a food grinder and sausage maker as a vehicle for dietary supplementation.

During the pre-trial sorting procedure we removed ten moi with identifiable goiters and stocked them in a separate tank to see if we could reverse the goiter process. Initial efforts to reverse the goiter by addition of potassium iodide at 0.14 g/kg diet was insufficient, with seven of the ten goitered fish being lost to the goiters. Subsequent doubling of the iodide dose to 0.3 g/kg diet was effective with the remaining three fish

surviving to the present with the apparent reversal of the externally observable goiter. Therefore the 0.3 g/kg diet dosing was used in the long-term diet supplementation trial. The diet transition to the sausage diet resulted in a temporary reduction in feed rate relative to the control treatment (raw smelt/squid/shrimp) and the tanks earlier feeding rates (Fig. 1). However, the fish quickly acclimated to the new sausage diet and have slowly increasing feed rates, while stocks maintained on the control diet tend to feed slower and appear to be experiencing a gradual decline in overall feed rates. Over time this has led to a growing differential in overall feed consumption with the sausage diet fed stocks now consuming 62% more food than the control raw diet fed stocks.
During the feed supplementation trial there were ten mortalities in the control (raw diet) group, six with obvious goiters and one mortality in the sausage treatment group that appeared unrelated to goiter formation (Fig. 2).

Spawning output of the supplemented sausage diet fed group started off lower than the control (raw) treatment group and experienced a decline in egg production in October and November, immediately after diet transition (Fig. 3). Following acclimation to the new diet, spawning output was restored in the diet supplemented group with a mean fecundity of 9.8 million eggs/month at 86% fertility rate and 62% viability rate, compared with 4.4 million eggs/month at 76% fertility and 47% viability in the control group. This difference in fecundity and egg quality between groups has grown markedly over the summer 2011 reproductive period with the iodide and vitamin supplemented sausage diet yielding over five-times the average monthly egg output.

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 iodide supplemented groups in earlier trials failed to show any significant change in hatchery performance. However, each of these treatment groups likely contained fish at various stages of goiter formation.

Subsequent trials conducted with broodstock populations carefully screened for the appearance of goiters yielded three test groups. A 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 , again confirming that neither low iodide levels in eggs from goitered broodstock, nor eggs from broodstock provided water iodide supplementation affected hatchery survival or growth of early larvae.

A final trial being conducted currently (October/November 2011) is examining both the effect of broodstock diet iodide supplementation and larval rearing water iodide supplementation on hatchery performance of moi.

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 and survival 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.

As referred to above, an ongoing hatchery trial is currently examining the effect of adding iodide to the hatchery water, both separately and in combination with broodstock dietary iodide supplementation to determine potential effects on larval survival and growth. The trial should be harvested in mid-November.

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, the May 2010 industry stakeholder workshop, and the May 2011 industry stakeholder web presentation. More complete technology transfer will occur at the end of the project with the completion of hatchery trials under objective number three.

Work Planned

Remaining activities under this project include:

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

2. 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 iodide supplementation of larval feeds and water.

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

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 increase the retention of highly valuable broodstock, increased 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 have stocked 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.

16. DNA- Based Identification and Selection of High-growth Tilapia in Hawaii, Year 1

General Information

Reporting Period: August 15, 2010 to October 31, 2011; no-cost extension through December 15, 2011 (Year 1 Progress Report)

Funding Level

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

RESULTS AT A GLANCE…

- Testing and establishment of the MtDNA CR PCR and DNA sequencing protocol has been conducted.

- 10 local tilapia populations have been classified based on their DNA.

- Discussion and collaboration with local tilapia farmers has been initiated.

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

Liang Wu, Ph.D. University of Hawaii at Manoa

Awat Yousif, Yvonne Lee, Gavin Iwai, Graduate Student, University of Hawaii at Manoa

Objectives:

Year 1

1. To identify and classify wild and captive tilapia strains and hybrids in Hawaii by DNA-based methods.

Year 2

2. To develop DNA-based testing tools for selecting high-growth tilapia by using existing strains or hybrids in Hawaii.
3. Distribute educational materials and research results

Anticipated Benefits

Results from Year 1 research work will provide the genetic basis of selecting suitable tilapia strains or hybrids for the Year 2 project. Results from Year 2 project will provide useful directions of tilapia broodstock breeding for the local tilapia aquaculture community. In the long term, this project will have significant impacts on Hawaiian aquaculture and technology development for a sustainable industry. Successful accomplishment of the project is expected to benefit the local tilapia farmers with better broodstock selection techniques, therefore boosting local tilapia production and aquaculture activities.

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 have been collected from ten populations (sites), including four wild populations in Oahu islands and six aquaculture and experimental facilities. Fin clip samples from 30-50 fish were collected from each site, and possible strain identifications or pictures of the tilapia fish were taken (Figure 1). A total of 560 tilapia fin samples were collected, and preserved in 100% ethanol. Genomic DNA was isolated from caudal fin clips. Briefly, tissue samples were digested with 0.5 μg/μl proteinase K at 55ºC overnight. The resulting solution was centrifuged; supernatant was extracted by phenol-chloroform and precipitated in ethanol and dissolved in 1X TE buffer. The quality and concentration of DNA are assessed by spectrophotometer and agarose gel electrophoresis, and DNA samples have been stored at 4ºC until use. We have established the method of genomic DNA isolation.

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. Representative DNA samples from each population of tilapia were amplified with primers encompassing the 5′ end of COI gene. Polymerase chain reactions (PCR) for COI barcoding were conducted with the primers combination:
VF2: TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC
FishR: CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA

The total volume of PCR reaction is 25 μL and included: 12.5 μL of 10% trehalose, 4.00 μL of ultra pure water, 2.5 μL 10× PCR buffer 0.1% Triton X-100, 0.5 μL of each primer cocktail (0.01 mm), 0.5 μL of each dNTP (10 mm), 0.2 μL of Taq DNA Polymerase (5U/

μL) and 1.0 μL of DNA template. PCR program for COI were: 94 C for 5 min, 35 cycles of 94 C for 30s, 50 C for 40s, and 72 C for 1 min, with a final extension at 72 °C for 10 min. The amplified fragments with expected size of 619 bp was checked by agarose gel electrophoresis (Figure 2) and subjected to direct sequencing using ABI prism 377XL DNA sequencer. Sequence trace files were assigned by using the GAP4 program, sequences are aligned with ClustalW online program. Sequence alignments are presented in Figure 3. The maximum-parsimony algorithm (Swofford and Berlocher, 1987) using MEGA4.0.2's default models recommended by FISH-BOL bioinformatic tool. We have tested more than 100 tilapia samples from amplified COI DNA fragment. Representative PCR amplification and DNA sequencing alignments were shown in Figure 2 and 3.

DNA Analysis on Mitochondrial DNA Control Region: The control region (CR) of the mitochondrial (mt) DNA sequence is known to have more rapid mutations over the course of species evolution than other part of the mt DNA sequences. The mtDNA CR sequences have been used for analyzing 42 tilapiine species (Nagl et al, 2001). To confirm and validate the COI data, we also developed PCR and DNA sequencing protocol of mtDNA CR. PCR for mtDNA CR were carried out using the primer.
ORMT-F, 5'-CTAACTCCCAAAGCTAGGAATTCT-3';
ORMT-R, 5'-CTTATGCAAGCGTCGATGAAA-3'.

The total reaction volume was 25μL and included the following components: 2.5μL 10 × PCR buffer, 0.5 μL of each primer (0.01 mM), 0.5μL of dNTPs (10 mM), 0.2μL of Taq DNA polymerase (5U/μL), and 1.0μL of template DNA. The PCR program consisted of a denaturation step for 3 min at 94 °C, followed by 35 cycles of 94 °C for 30s, 54 °C for 40s and72 °C for40 s; and a final extension step of 72 °C for 10 min. Negative controls were used in all PCR reactions to make sure that no contamination took place in reaction system. All PCR products were separated on 1.5% agarose gels. Images were photographed under UV light with imaging system. The agarose gel contained DNA bands was cut off and the DNA bands were recovered with the DNA purification kit. After purification, amplified PCR products were sequenced by the ABI PRISM 377 sequencer (Figure 4). Sequence alignments are presented in Figure 5 for MtDNA CR.

DNA Sequences Data Analysis: The sequences are aligned by clustalW2 program. All the sequences have been analyzed and compared with published tilapia species data including the O. aureus, S. melanothern, T. rendalli, O. mossambicus, O. urolepis reported by Nagl, S et al (Genbank Accession #AF328851, AF328854, AF328851,AF328843, AF296493, AF328843), S. melanothern, O. niloticus reported by Falk (Genbank Accession # AF484717, AF485083), O. mossambicus and Hybrid tilapia

reported by D' Amato, M.E (Genbank Accession #AY833459, AY833481). Representative sequence alignments are presented in Figure 4 and 5 for COI and mtDNA CR. Neighbor-joining (NJ) tree are constructed with Kimura Two-parameter distance model by MEGA version. All transitions and transversions are calculated in the tree. Genetic distances are quantified within and among species using the Kimura two-parameter (K2P) distance model by MEGE version 4. The relationships between the populations are analyzed with MEGE 4. Preliminary identifications of ten populations are presented in Table 1.

DNA-based Species Identifications: Compared with morphology methods, DNA-based approach is practical and accurate. DNA barcoding by using the region of the mitochondrial COI gene is widely used for fish identifications. However, studies found that COI sequences failed to distinguish the close relationship of certain species. The comparison of intraspecific variability for five populations between mtDNA COI and CR regions indicates that mtDNA CR shows higher nucleotide diversity than COI in this study. Phylogenetic tree based on mtDNA CR data successfully separated all the sequences into different groups. The phylogenetic tree based on COI was consistent with the result form mtDNA CR. However, COI failed to differentiate the O.aureus and O.niloticus. Therefore, we employed mtDNA CR as the main DNA method to classify most tilapia species.

MtDNA CR sequence analysis identified all those samples into seven distinct groups. The group O. aurues includes 44 sequences from three populations. Group O. mossambicus includes 26 sequences from two populations. Those populations that were closest to the original imported stocks representing O. aureus and some samples of O. mossambicus were relatively pure, and other populations indicating various degrees of introgression and hybridization. Oreochromis mossambicus was the first species to be widely distributed. As a result, most O. mossambicus stocks were unmanaged and some imported stocks of this species have escaped to the wild. However, O. mossambicus still has the potential contribution for the breeding of tilapia.Of all the populations examined, 7 populations showed some degree of introgression and hybridization. Some hybrid populations included the genes both of O. niloticus and O.aureus (population M) probably related to the hybrids known to have been imported. Based on the genetic diversity and phylogenetic tree analysis, it was found that the population K has a high average number of nucleotide differences and showed characteristics of O. niloticus, probably from hybrid O. mossambicus x O. niloticus, which had been imported from Taiwan in 1980.

Work Planned

We will continue data analysis and revise the manuscript, and prepare the final report for the first year of the project objective by December 2011. Based on the DNA testing results, we have been planning for a growth trial for the second year experiment. DNA testing will be employed for the selected tilapia strain/hybrids for the grow-out experiment.

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 in the state of Hawaii.

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

Pan G and Yang J. 2010. Analysis of Microsatellite DNA Markers Reveals no Genetic Differentiation between Wild and Hatchery Populations of Pacific Threadfin in Hawaii. International Journal of Biological Science 6:827-833.

Yang J, Wu L, DuPonte M, Okamura WM, Tagawa AW, Tamaru C, Ako H, Lee CS. 2011 DNA-based identifications of feral and farmed tilapia in Hawaii. Hawaiian Aquaculture and Aquaponics Association Annual Meeting (Honolulu, November 2011).

17. Pacific aquaculture development and extension support for the U.S. affiliated Pacific islands of the Federated States of Micronesia, FY2010

General Information

Reporting Period	July 1, 2011 to September 30, 2011 (Progress Report)
Funding Level	$47,157.00
Participants	**Masahiro Ito,** Director and Chief Scientist, Aquaculture Development, College of Micronesia Land Grant Program
	Belenko Halverson, Clayton Maluwelgiye; extension aide and pearl seeding technician of COM.
	Pakin Community Association (PCA)
	Pingelap Municipal Government; Pingelap Atoll in Pohnpei State
	Pweniou Pearl Farm
	(Yap component) Steven Young-Uhk; aquaculture extension agent of CRE, COM-Yap

RESULTS AT A GLANCE…

- Half-pearl grafting demonstration and skill training for the local youths by the project's Micronesian technicians both in Pohnpei and at the outer islands has continued. Trainees selected from each farm began grafting work under the trainer's supervision onsite.

- Half-pearls and related accessories were sold on behalf of participating farms and individual trainees for the first time in Pohnpei.

- Negotiations have commenced with the Yap State Government R & D (Resources and Development) to ensure the R & D providing a grant support and MRMD (Marine Resources Management Division) to provide logistic support such as larval & grow-out tanks for the sea cucumber hatchery development.

Objectives

1. Extension support and technical assistance.
   Assist the development of an economically sustainable aquaculture industry in the U.S.-affiliated Pacific islands of FSM:
   1-a; Provide technical advices on the pearl and sea cucumber hatchery operations in Pohnpei.
   1-b; Coordinate and conduct research sea cucumber tagging/marking experiments for restocking programs in Pohnpei and Yap.

2. Technology transfer.
   Transfer hatchery-based aquaculture technologies and specialized pearl culture skills:
   2-a; Coordinate and supervise technology transfer of pearl oyster and sea cucumber grow-out operations.
   2-b; Coordinate and provide training to local people on pearl seeding skill and related farm management (half-pearls), pearl culture (round-and half-pearls) and value-added products making (half-pearls and pearl-shell accessories).

3. Information dissemination
   Coordinate and administrate active CTSA projects in the proposed region:
   3-a; Coordinate and advise communities and stakeholders on the pearls and value-added products in Pohnpei and the sea cucumber resource enhancement programs in Pohnpei and Yap.
   3-b; Coordinate and conduct marketing development by promotional sales and displays of pearls and value-added products made during this project period

Anticipated Benefits

(Pearl component): The project will build local capacities in pearl culture practices at four sites in Pohnpei, three outer islands and one main island; create at least six half-pearl seeding technicians whose skills produce high quality half-pearls; and achieve actual income generation by the participating farms and individual trainees. Technology transfer forms a major output, particularly half-pearl seeding and farming techniques between the Micronesians. Therefore, this extension support ensures that the seeding and harvesting operations will be conducted at least once a year at each farm in Pohnpei. The project aims to spread the seeding operations and trainings as quickly as possible by the two Micronesian core technicians created from the previous CTSA projects such as CTSA-fy06 (pearl quality improvement) and CTSA-fy09 (year 1). In

order to support income generations by those trainees, the PI will coordinate to demonstrate local display-sale events in Pohnpei and will advise export planning of the pearls and pearl-related products during the commercialization process. Assessments of quality of harvested half-pearls and hand-made accessories will be done by the PI and the project staff, who were trained by a pearl grading expert during the year 1 of CTSA-fy09 project. It is expected that several thousand half-pearls will be produced in the year 2, which should be sold in the domestic and international markets.

(Sea cucumber component): Restocking of the sandfish is targeted issue to be conducted as research and extension support in Pohnpei. Tagging experiments will be implemented at the two sites in Pohnpei lagoon and demonstration and trial tagging will be implemented in Yap as a part of regional technology transfer and skill training between the Micronesians. The PI will provide expert advices on developing a hatchery facility and operation plans in Yap. As a rapid dissemination of extension support work, the PI will negotiate with the COM central office to provide the sea cucumber hatchery skill training onsite in Pohnpei for the COM Yap aquaculture extension agent. Considering the Yap's conservative traditions and hierarchy in the society, who tend not to accept the outsider's instructions particularly from the younger trainers, this will be more effective and save great amount of spending from the CTSA funding rather than sending the Micronesian technicians (Pohnpeians) over to Yap.

Work Progress and Principal Accomplishments

(Pearl component): The pearl component conducted demonstrations and skill training for the round- and half-pearl nucleus implantation (grafting or seeding) as well as half-pearl accessory making at four sites; Nett Point, Pakin Atoll, Pingelap Atoll and Pweniau Island (Objective 2b). Detailed information was given in the CTSA-FY09 (year 2) Annual Progress Report. The PI negotiated to COM for employing a retired master grafting technician for a short term teaching from Australia. The round-pearl grafting was for the first time taught by the retired master grafting technician to four Micronesian apprentices including two teenage girls from outer islands. Total of 800 oysters were implanted with the round-pearl nuclei by those trainees. Two Micronesian technicians supervised training of the half-pearl seeding skill for 12 trainees as a technology transfer between the Micronesians, in which they grafted 3,000 oysters in the first quarter of this project. Approximately 1,800 oysters were harvested for half-pearl accessory making training (Objective 2b). 5,000 each of 8 – 10 months old hatchery-produced and grown blacklip pearl oysters (Pinctada margaritifera) at Nett farm were delivered to Pingelap and Pweniau for their grafting operations in 2013 –

2014 (Objective 2-a). In August, half-earl products such as pendants and earrings produced from the skill training were also sold for the first time in Pohnpei as a part of Micronesian Pearls display and sale in Kolonia (Objective-3b). This trial display and promotional sales of jewelry-cut half-pearl products, such as earrings and pendants, were conducted in Kolonia, a main town of Pohnpei. Those harvested half-pearls were graded by the PI and project technicians; designing, cutting, polishing and coupling were done by the trainees and the technicians and project staff; and the pricing of final products was done by the PI. Because the display and sale was focused mainly on the round-pearls, only 40 pieces of pendant and 20 pairs of earrings were presented, which were made by the trainees from Pweniau and Nett. Total of 14 pieces pendant top and 10 earrings fetched $525.

(Sea cucumber component): The Sea cucumber component in Pohnpei, approximately 3,000 juveniles sandfish (H. scabra) produced from hatchery work in earlier this year have been kept alive in two Habitat Simulators, 2,500-liter raceway tanks with semi-closed recirculating seawater system (Objective 1-a). They are used for tagging experiments in early next year (Objective 1-b). Aquaculture project of the black teatfish also began searching the broodstock in September. Contrary to previous stock survey by others in 2004, significantly high abundance of the black teatfish were found from the leeward barrier reef areas near the hatchery in the northern Pohnpei lagoon; total of 140 large broodstock each with 6 – 8 pound wet weight were collected in two separate daytime search (Objective 1-a). During the first visit to Yap, the PI coordinated to meet all stakeholders who expressed their interests, concerns and supports to the sea cucumber aquaculture as an important export business opportunity (Objective 3a). Mr. Steven Young-Uhk (COM-Yap aquaculture extension agent) agreed to coordinate local stakeholders, yap State Government and community leaders for preparation of a new hatchery construction at a former ice-plant building in Kaday village the west coast of Yap. He will also secure necessary funding/grant from Yap R & D and other international organizations for purchasing hatchery equipment and materials. Yap MRMD will provide in-kind supports such as lending raceway tanks (4 – 5 tanks), seawater suction pumps and other equipment.

Work Planned

Pohnpei component continues grafting training and accessory making for the local apprenticeships on each island and community. The PI will organizes local sales of the value-added pearl products and half-pearls will be repeated for local sales activities such as Christmas sale, Pohnpei Culture Day Fest and the Easter Holiday sale as well as exporting to overseas jewelry makers and accessory traders. It is expected to produce

approximately 5,000 or more pieces half-pearls next year resulting from this year's grafting work. Half-pearl accessory making is ongoing at Nett, Pakin and Pweniau for local sales for Christmas season in December and Culture Day Festival in March next years as well as responding to the purchase requests for the jewelry-cut half-pearls from overseas jewelry traders. In the subsequent quarters, more training sessions for half-pearl accessory making and grafting are planned at each location either using the oysters implanted round-pearl nuclei, the ones vomited nuclei or virgin oysters.

For the second visit to Yap, the PI prepares system design and a list for equipment and materials for the sea cucumber hatchery as well as project development planning and hatchery work plan during the stakeholders meetings. The sandfish tagging techniques and work plan will also be explained for trial tagging which is proposed during the third visit in March next year.

(Milestone 4 – 6 months):

- Supervise blacklip pearl oyster hatchery operations and sea cucumber hatchery operation including juvenile grow-out work at Nett Point in Pohnpei. As of this reporting date in October, larval rearing commenced with 15 million D-veliger larvae and work is in progress, expecting that 2 – 5mm sizes spat (juvenile pearl oysters) will be transferred to Nett, Pakin and Pweniau in mid-December.
- Trial spawning of the black teatfish was conducted in October with approximately 10 million fertilized eggs. However, hatchery runs to produce juveniles will commence from March – April, 2012.
- Coordinate and demonstrate pearl harvesting, re-grafting and related farming work at Nett in Pohnpei and supervise pearl farm management training onsite.
- Continue coordinating skill training work on the half-pearl seeding and accessory making at Nett, Pakin, Pingelap and Pweniou in Pohnpei.

(Milestone 4 – 12 months):

- Coordinate and conduct promotional sale & display of pearls and value-added products in Pohnpei.
- Coordinate and provide expert advices to communities and stakeholders meeting on skill training, product development and farming management at Pakin Atoll, and Pingelap Atoll and Pweniou Island in Pohnpei.
- Supervise sea cucumber tagging experiments in Pohnpei and demonstrate tagging techniques in Yap. Tagging experiments of the sandfish for both adults (broodstock) and juveniles will be conducted in November and in January next year at Nett Point and Pweniau Island. Tagging techniques will be demonstrated in Yap during the third quarter.

(Milestone 7 – 12 months):

- Supervise sea cucumber hatchery operation and juvenile grow-out work at Nett Point in Pohnpei. The sandfish spawning runs will be planned in February – March next year and the produced juveniles will be used for a medium scale ocean grow-out and restocking project at Nett Point in Pohnpei. The COM Yap aquaculture agent will participate to the hatchery operation as a part of technology transfer program among the Micronesians

Impacts

(Pearl component): Branding promotion is the most important issue as the Micronesian branded round- and half-pearls. Jewelry-cut half-pearls are the most frequently enquired by the overseas buyers. Therefore, the PI encourage the farmers to obtain some tools and machines for making their own jewelry-cut, value-added products, of which seed money could be earned from the promotional sales of their products from the training work during this project. During the pearl display and sale on August 21 in Pohnpei, small quantity of the trainees' jewelry-cut half-pearl pendants and earrings were also presented. Total of $525 for 24 samples were sold in a 5-hour sale at average of roughly $20 per item. Although the round-pearls in loose, which were price-tagged from $5 to $500 per piece, were the main in the display and sale, small number of hundred half-pearl products received attentions from local customers. The trainees from Pweniau sold 14 items for $250 of half-pearl pendants and earrings. Although this was very small amount of money, it was their first income from the project's training sessions. The Pweniau farm purchased additional tools and materials for their half-pearl accessory making. Displays and sales of half-pearls in Pohnpei will be conducted separately from those of the round-pearls. The half-pearls are value-added products to the pearl farming business and the operation cost has less than those round-pearl operation by shorter cultivation period and has relatively easy nucleus implantation technique compared to the round-pearls. Other outer islands have also requested to participate to the project's activities. However, this project does not have capacities in financially and logistically to involve more than those who have already participated to. The PI contemplates to hold a meeting towards the end of this project for communities and business owners who may concern to join pearl business development in Pohnpei.

(Sea cucumber component): Over the last decade in Yap, a hatchery-based aquaculture project has never been implemented. During the first visit to Yap, the PI coordinated to meet all stakeholders who expressed their interests, concerns and supports to the sea

cucumber aquaculture as an important export business opportunity if it is implemented. Traditional leaders and elders concerned about lack of aquaculture skill training for youths in their communities, so the hatchery training could be one of the opportunities for them.

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

Ito, M. 2011. Aquaculture research, extension and training by the Land Grant Program at College of Micronesia: From 2001 to 2010. Abstract, the Fifth National Aquaculture Extension Conference, Memphis, USA.

Ito, M. 2011. Circle and spot formation mechanisms and changes in luster, color and roundness by grafting methods on the blacklip pearl oyster, Pinctada margaritifera. Proceedings of the Fifth International Gemological Symposium, Gems & Gemology 47 (2): 148, Carlsbad, USA.

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

General Information

Reporting Period	December 1, 2009 to September 30, 2011; no-cost extension through February 29, 2012 (Progress Report)
Funding Level	$50,000
Participants	**Hui Gong,** Ph.D. University of Guam John W. Brown, Ph.D. University of Guam

RESULTS AT A GLANCE…

- Collected the tuna fish roe samples during both dry and wet seasons through a Guam tuna fish loining company.

- Established the baseline nutritional information of tuna fish roe, including proximate analysis (protein, total lipids, carbohydrates and ash) and fatty acids analysis, etc.

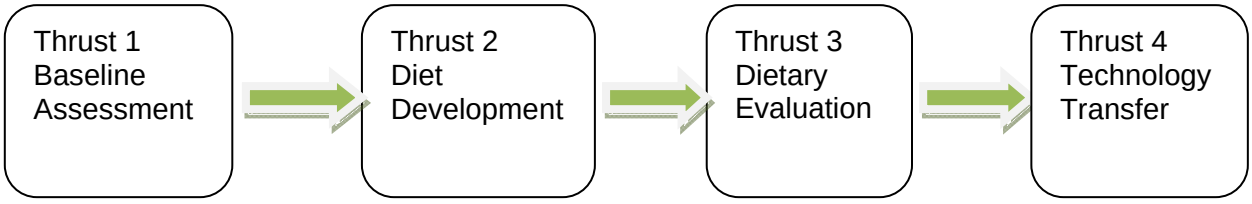
- Verified the tuna roe samples were free of major shrimp viruses using PCR method.

- Formulated and developed the semi-moist diets using tuna fish roe samples for the acceptable texture, storage and nutritional composition through a series of trials.

- Established the protocols for evaluating the reproductive traits through preliminary experiment.

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. Four thrusts are illustrated in the flow chart below,



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.

1.1 Randomly collect samples of tuna fish roe from the different loining operations on Guam (dry and wet seasons).
Completed.

1.2 Analyze the samples, for proximate analysis (protein, total lipids, ash), fatty acids profiles, etc.
A series of experiments on drying procedure were conducted to check for the freezing dry conditions in processing the tuna fish roe and compare the results between conventional oven drying and freezing drying procedures. Six freezing dried tuna fish roe samples were shipped to two labs in University of Hawaii for biochemical analysis.
a) Agricultural Diagnostic Service Center, University of Hawaii, Manoa

1910 East-West Road, G.D. Sherman Lab 134, Honolulu, Hawaii 96822. Tel: 808-956-6706, Fax: 808-956-2592 Contact: Ray Uchida, ADSC@ctahr.hawaii.edu

b) University of Hawaii at Manoa/CTAHR, Department of Molecular Biosciences and Bioengineering, 1955 East-West Road, #218, Honolulu, HI 96822 Tel: 808-956-3541, Fax: 808-956-3542 hako@hawaii.edu; page.iida@gmail.com

Results of freezing dry experiments are summarized in Appendix A and Nutritional values are presented in Appendix B.

1.3 Check the samples 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 in house.

The specific primers and other related reagents were obtained, PCR diagnosis WSSV, IHHNV, TSV, YHV and IMNV were performed on the 6 pooled fish roe samples using the published PCR diagnostic protocol. The results indicated the absence of the listed shrimp viral pathogens in the Tuna fish roe samples. PCR summary is in Appendix C.

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

According to the nutritional baseline information of tuna roe obtained from biochemical analyses, maturation diets were formulated by incorporating tuna fish roe as the major ingredient and supplementing with other ingredients as needed, for example, flour, soy meal, mineral and vitamin mix, binders etc. Feed ingredients were identified for making the semi-moist diets, and several trials have been conducted to achieve the most suitable texture and water stability. The feed and some major feed ingredient were also sampled and submitted to Agricultural Diagnostic Service Center, University of Hawaii, Manoa for proximate analyses. In addition, N% and C% in the diet were analyzed at UOG soil lab in checking the consistence and formulation adjustment. Results are listed in Appendix B.

Semi-moist fish roe maturation diet were made every the other day and kept in freezer at -20°C until ready to feed. Twenty minutes prior to each feeding, the diet was taken out from the freezer, and cut in small pieces (2 gram or so) in order to feed shrimp.

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.

Thirty shrimp of each sex were randomly sampled prior to stocking. Shrimp were stocked into maturation tanks (30 ton, 23.6 m2). Stocking rates were 142 males (27.6g ±

3.12g) and 100 females (33.3g ± 2.67g) in each tank. Shrimp in the treatment tank were fed with commercial diet and fish roe semi-moist maturation diet (200g/time × 4 times). The control tank was fed with squid (200g/time × 2 times) supplemented with twice daily feedings with commercial maturation diet. Fish roe semi-moist diet were made on daily basis and fed to the shrimp in the tanks. One month after feeding the designated diets, the average weight of females reached 47.5g, and the overall reproductive traits were then further evaluated.

The gonadal development of female shrimp was evaluated twice a week and the number of females reaching stage 2 – stage 4 in gravidity in each tank was recorded by the developmental stage.
Stage 1: No development of ovary
Stage 2: Early development of ovary
Stage 3: Incompletely ripe
Stage 4: Completely ripe

Each evening, females were checked for natural mating at 8:00 p.m. from each tank, and after sourcing, mated females were transferred to individual spawning tanks keeping separate the two treatments (control and fish roe group).

Spawning tanks (121L) were cleaned and prepared, and filled with 100L filtered seawater and plastic cover was put on each tanks. The temperature of the spawning tanks ranged from 27.5 to 28.9 °C. Every morning at 6:30 am we started to count eggs and nauplii at the N3-N4 stage. The counts from each spawning tanks were based on multiple volumetric sampling. Seawater in each spawning tank was thoroughly mixed and three samples were very quickly (100 ml each) collected. The number of eggs and nauplii in each sample were counted, and the average for three samples was used to extrapolate to the 100L tank volume to obtain the total number of eggs and nauplii present per female.

After the nauplii were discarded, the spawning tanks would be cleaned, dried and filled water for next run of spawning.

% Fertilization rate:
After the counting of the number of eggs/female was finished, a sample of the eggs in each spawning tank was checked for the % fertilization rate. A random sample of eggs (about 100 -150 eggs) was put onto a slide to evaluate the % fertilization rate under the microscope. We counted total number of eggs on each slide and counted the number fertilized eggs to calculate the % fertilization rate for each spawning female.

% Hatching rate:
The % hatching rate was calculated as the number of total nauplii divided by the number of total eggs x 100 for each spawning tank.

This part of experiment is still ongoing.

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

Work Planned

We plan to complete the maturation experiment and the maturation performance evaluation by the end of January. In the last month of the project, we will organize a workshop open to public and stakeholders in Guam and finish the final report.

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

Not Available.

19. Evaluating an engineered biological treatment process for the application of aquaculture waste and wastewater

General Information

Reporting Period: October 1, 2011 to May 31, 2011 (Midterm Status Report)

Funding Level: $20,000

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

Joshua L. Irvine Graduate Student University of Hawaii at Manoa

RESULTS AT A GLANCE…

- The characterization study of aquaculture waste/wastewater composition has been completed. The following were analyzed and will be included into the final report: total organic carbon, ammonia-nitrogen, nitrate-nitrogen, total suspended solids, pH. A biodegradability based on biological TOC removal is in progress.

- The biological treatment technology process performance for synthetic wastewater has been evaluated. At this time, the evaluation for actual aquaculture wastewater is in-progress.

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

Work Progress

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

The literature review will be included into the final report.

Objective 2: Wastewater Characteristic Study

The characterization study of aquaculture waste/wastewater composition has been completed. The following were analyzed and will be included into the final report: total organic carbon, ammonia-nitrogen, nitrate-nitrogen, total suspended solids, pH. A biodegradability based on biological TOC removal is in progress.

Objective 3: Evaluate the process performance of biological treatment technologies for organic and nitrogen removal using aquaculture wastewater

The biological treatment technology process performance for synthetic wastewater has been evaluated. At this time, the evaluation for actual aquaculture wastewater is in-progress. The results from this study will be incorporated into the final report.

Objective 4: Develop a design and operational criteria to meet treated wastewater discharge and reuse goals (to be determined)

The design and operational criteria will be determined as soon as the entire process performance is evaluated.

Challenges encountered

Equipment failure used to prepare biological media and unexpected renovation resulting in construction work at our Gilmore lab facilities, all contributed to shifting our work timeline schedule by 3 months. During the period of December 2010 to February 2011, we were unable to do valuable experiment work. In March 2011, trials testing with synthetic wastewater began, and the findings prove promising. The proposed process has thus far achieved nearly ~90% organics and >98% nitrate removal. Testing has begun using actual aquacultural tank wastewater. There is sufficient time to produce meaningful results, which will be included into the final report that will be submitted by August, 2011.