



## Letter from the Director

Aloha!

In this month's issue of e-notes, we announce the eight projects included in the CTSA FY2010 Plan of Work. The funds for these projects were recently released by the USDA and the proposed research will begin shortly.

Also in this issue is an article from the CTSA Biosecurity project, in which researchers from the project work group present their confirmation of TRLO in asymptomatic carriers. Last but not least, this month's Farmer Spotlight article introduces you to Felix Calvo, a tilapia farmer from Rota CNMI.

As always, if you have any suggestions, concerns, or comments, please do not hesitate to let us know.

Mahalo,

Cheng-Sheng Lee  
Executive Director, CTSA

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## USDA Approves CTSA FY10 Plan of Work, Eight New Projects Begin

The USDA has recently approved the CTSA FY2010 Plan of Work. Under this plan, eight new projects will be funded, and Year Two funding for four continuing projects will be released. The new projects are as follows:

### Assessing Hawaii's Aquaculture Farm and Industry Performance

Led by Dr. PingSun Leung of the University of Hawaii, 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.

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

This two-year project, led by Dr. Maria Haws of the University of Hawaii at Hilo, 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.

Marine Finfish Aquaculture Development in the Northern Marianas Islands  
Dr. Charles Laidley, Oceanic Institute, will lead this project with the primary objective of 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.

#### Seed Production Mangrove Crab *Scylla serrata* in Palau

The overall goal of this project, led by Miguel Delos Santos of Palau Community College, 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.

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

Miguel Delos Santos is the Principle Investigator of this two year project that has a main goal 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 *iganus 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.

#### 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, extension agent Masahiro Ito 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.

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

The overall goal of this project, under PI Kent Kobayashi, 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.

#### Pacific Regional Aquaculture Information Service for Education (PRAISE) and Publications, Year Ten

This project is a continuation of services provided by both CTSA and the University of Hawaii for two decades. The overall goal of this project is to promote the transfer of information within the Pacific aquaculture community, including the acquisition and dissemination of marine science information and data about the Pacific region, especially as it regards aquaculture, and to help develop instructional materials to be used in university level aquaculture education programs.

Each project will begin by the end of the calendar year. If you have any questions about these or any other CTSA funded projects, please do not hesitate to let us know!

#### Detection of Asymptomatic *Francisella* spp. Carriers in Tilapia Cultured in Hawaii

Clyde S. Tamaru<sup>1</sup>, Kathleen McGovern-Hopkins<sup>1</sup>, RuthEllen Klinger-Bowen<sup>1</sup>,  
1 2 2 3

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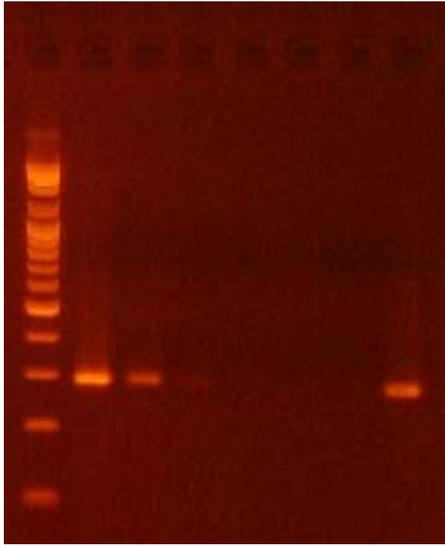


Figure 1. Serial dilution CTSA case #T17. Lane 1: Ladder, Lane 2: vial T17, Lane 3: 10-1, Lane 4: 10-2, Lane 5: 10-3, Lane 6: 10-4, Lane 7: no template, Lane 8: +RLO control.

In 1994, wild and farm-raised populations of tilapia (*Oreochromis mossambicus* and *Sarotherodon melanotheron*) on Oahu began to die of an unknown disease that was similar but not identical to piscirickettsiosis in salmonids. Only tilapia were affected and mortalities occurred only during the cooler months (October to April) of the year. In the years that have followed, mortalities from the disease have been recorded only in Oahu farm-raised tilapia populations and again usually during the months that experience cooler temperatures. The development and widespread availability of molecular detection techniques (e.g., polymerase chain reaction or PCR) identified the disease is caused by a Francisella-like bacteria (FLB) which continues to impact farmed fish and mollusks worldwide.

Under the auspices of a Center for Tropical and SubTropical Aquaculture project entitled, "Regional Biosecurity: Operational Biosecurity and Diagnostic Surveillance" and through a collaborative private-public partnership between Moana Technologies LLC, Hawaii Department of Agriculture and the College of Tropical Agriculture and Human Resources, the capacity to detect

FLB utilizing PCR methodology with Francisella sp. primers has been accomplished and validated for use in the islands. Active outbreaks with mortalities being experienced on Oahu have already been monitored. An example of the relative amounts of FLB-DNA in spleen collected from tilapia suffering from FLB disease is summarized in Figure 1. Apparently the amount of FLB-DNA present in this spleen sample can be diluted at least 100 fold.

Although the improved molecular tools for detection and diagnosis of francisellosis have become available, major gaps in understanding the life history of FLB remain. Project work group members have taken advantage of outbreaks, as they occur, and have obtained preliminary information that can be used to direct future research objectives. For example, an urban aquaponic producer in Kaneohe was experiencing persistent mortalities in his tilapia production tank and gross examination revealed granulomas in the gill and spleen. The mixed population consisted of two strains (i.e., golden and "koilapia" Figure 2) and on March 2, 2011 eight individuals (four from each strain) were sacrificed and gill and spleen samples were excised for PCR analyses. An alternative collecting method was also tested swabbing gill tissue on to fast technology analyses (FTA) cards. FTA paper (Whatman Inc., Clifton, NJ) 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 used for polymerase chain reaction (PCR). The results (Figure 3) 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. 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 (i.e., the 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 strain related resistance to FLB and clearly a topic for future investigation.

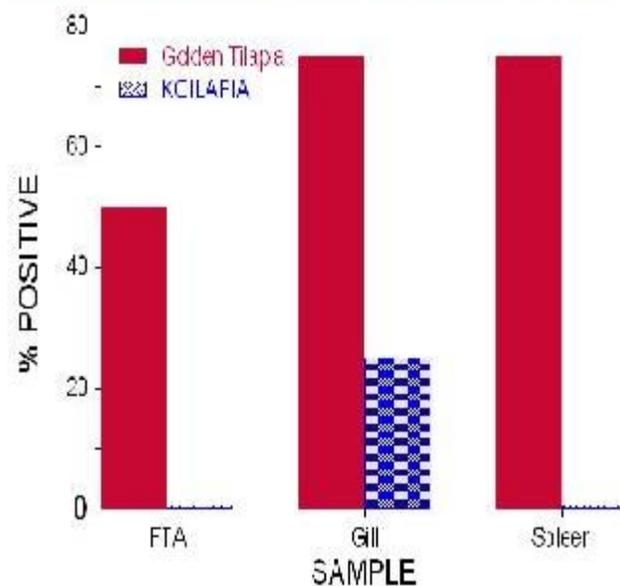


Figure 2 (top). Photographs of two popular strains of tilapia on Oahu; Figure 3 (bottom). The percentage of samples containing FLB DNA from tilapia undergoing active disease outbreak.

An earlier outbreak in an urban aquaponic system located in Waimanalo 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. Mortalities had ceased 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 high numbers of granulomas in the spleen. 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 high 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. A significant decrease in the percentage of FLB-DNA positive gill samples occurs with tilapia. 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.

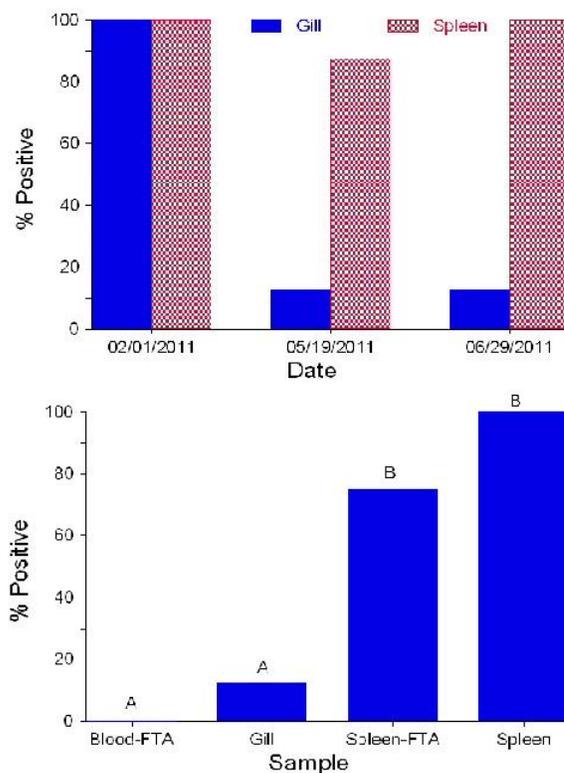


Figure 4 (top). Percentage of positive tilapia individuals for FLB-DNA in gill and spleen detected with PCR. Figure 5 (bottom). Summary of percentage of positive individuals using various tissues and collection techniques. Bars that do not share an alphabet are statistically different from each other.

The availability of an asymptomatic population of tilapia allowed the project work group to determine what tissues and techniques are most useful for detecting infected individuals. The same individuals sampled in June were also used to examine if FTA cards and blood were suitable for identifying positive individuals. The PCR results are summarized in Figure 5. Clearly blood collected using the FTA cards are not suitable as all samples were negative. In contrast, when spleen is used directly in the PCR assay, all 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 (i.e., 6/8 = 75%) percentage of individuals detected FLB-DNA than when spleen is used directly (i.e., 100%). The difference, however, is not statistically significant indicating that the use of the FTA card may still have some utility.

Our collaborative partnership has successfully developed the capacity to detect a Francisella-like bacteria using PCR technology. This is the same

pathogen that has been reported to infect tilapia on the continental U.S. as well as many parts of the world. Data obtained from outbreaks on Oahu have begun to reveal the necessary information if we are to devise a disease management program for this particular pathogen. Preliminary data indicates that there may be strains of tilapia that are more susceptible to the disease, identification of which strains would be very important to the industry both in Hawaii and abroad.

Gill tissue apparently is only useful for detecting positive individuals using PCR during an active disease outbreak (i.e., when mortalities are occurring). That similar results might also be obtained by just visual examination of the gills during an active infection indicates that the utility of gill tissue for detection of this pathogen at the molecular level is not cost effective. Furthermore, the utility of gill tissue detecting individual carriers of the pathogen from an asymptomatic population is questionable.

Blood as a suitable tissue was being considered from the standpoint of having a non-lethal method of sampling. However, its poor performance in the current investigation clearly rules out its usefulness in that capacity. At present only the spleen and a lethal sampling protocol appears to be viable as a dependable method for use with asymptomatic tilapia.

The use of the FTA cards represented a quick and fast means of collection and preserving DNA and although it did show some promise for one tissue (e.g., spleen) it was still not as good as using the tissue directly. Additionally, obtaining spleen still requires lethal sampling, which further

diminishes the utility of FTA cards for the detection of FLB.

One of the more significant observations obtained by project work group members is the existence of asymptomatic carrier individuals once the clinical outbreak has subsided. Although long suspected, this is the first known report that such a situation exists with this particular pathogen and adds to the current growing body of knowledge. Apparently when an infection subsides, for reason(s) that remain to be determined, replication of the pathogen in gill tissue begins to wane allowing fish to feed and take in oxygen normally. However, a high percentage of these same individuals have FLB-DNA in their spleens for a prolonged period after infection. The results indicate that the pathogen remains in the spleen albeit at a subclinical level. One implication of this result is the immediate need to locate and maintain stocks that do not harbor the pathogen as the carriers can easily be mistaken for healthy individuals. Infected individuals could unknowingly be transported to other locations (e.g. farms) where in all likelihood they will infect their cohabitants. Designing how to obtain and distribute tilapia stocks that are FLB free would assist Hawaii's industry as this disease continues to result in major losses of stock. The significant losses experienced in previous years resulted in the transportation of Tilapia between Oahu and the rest of the Hawaiian Islands to be restricted (PQ Policy 98-09 Section 150A-8, HRS - November 5, 1998) and currently remains in effect. The rationale for the restricted policy is clearly supported by the current results. However, the current restriction in movement of tilapia stocks has and will continue to hamper the development and growth of tilapia aquaculture in the islands and clearly designing a means in which tilapia stocks can be rid of and remain FLB pathogen free becomes a very high priority for the project work group.

## Pacific Island Farmer Spotlight: Felix Calvo



Feeding Time: Felix Calvo tends to tilapia on his Rota farm.

Felix Calvo's tilapia farm is tucked deep in the jungle on the northwest side of Rota, Commonwealth of the Northern Mariana Islands (CNMI). Every day, Felix or one of his family members travels to the farm to maintain the large concrete tank of several thousand tilapia. His mission in doing so, aside from providing fish to his customers, is to demonstrate "a good way of introducing a sustainable food resource" to his fellow Pacific Islanders.

Felix sells his fish locally, mainly to island residents who emigrated from Asia. According to him, tilapia must still overcome a negative reputation long held in the CNMI. However, the fish are slowly gaining in popularity and there has recently been an increase in local demand. One solution Felix offers to help change the reputation is taste testing, which has been championed in Hawaii by Chef Alan Wong. "Let them taste the kind of dishes that can be produced with tilapia and see how good it is," explained Felix. He went on to mention that the numerous health benefits of eating fish can also greatly help the island with some of its more prominent health concerns, such as heart disease.

For all of the benefits aquaculture can provide, there are also challenges facing CNMI farmers that make production expansion difficult. According to Felix, the greatest challenges of farming on Rota are the unavailability and high price of supplies, as well as the high cost of transportation. These are issues he hopes the local and national government can help to alleviate so the industry can attract other potential entrepreneurs and expand. Another possible opportunity to attract farmers, according to Felix, might be the establishment of a local diagnostic laboratory to assist farmers with health problems.

"We can get a quicker response [with a local laboratory] so that we can act quicker to [solve the] problems that we find. Some of our fish are dying and we don't have a laboratory that can respond to our needs immediately," Felix explained. "Those are the things that I would like to see, more responsive assistance. Having these types of services will put people who are considering starting an aquaculture venture at ease. They can say that way their investment is worth their time and money because they have a linkage where it will not go to waste."

In addition to providing assistance to farmers, Felix would like to see local industry leaders emphasize aquaculture education in primary and secondary schools. To his delight, CTSA is currently working together with the Northern Marianas College CREES team to implement a NOAA-funded aquaculture curriculum at Rota Middle School and Rota High School.

When asked what the best part of his job is, Felix responded: "I love being a farmer, so I'm doing what I like to do. Working with nature, working with the environment...you are your own boss. You can [also] be satisfied that you are contributing to the community with the food you are producing."

## AquaClip: Keawa Nui Shrimp Farm on Molokai

By Megan Stephenson, July 2, 2011. <http://theMolokaiDispatch.com>.

After a professional rugby stint, winning multiple Ka'iwi Channel canoe races, pilot training, and starting restaurants and construction companies, John Austin has found a new calling: shrimp.

Relatively new to the business, Austin moved to Molokai three and a half years ago to run Keawa Nui Farm, managing the operation nearly single-handedly.

Keawa Nui looks more like a hobby farm than a shrimp plantation, with horses, cattle, sheep and chickens scattered around the salt ponds. Growing up a fisherman's son in New Zealand, Austin said he wanted to return to what his childhood was like.

"I bought this farm so my daughter could grow up the way I did," Austin said, his voice becoming emotional when talking about five-year-old Madeline.

He bought the 80-acre shrimp farm - one of two on the island -- taking over a lease from Kamehameha Schools. Austin's shrimp are a hit with chefs around the state and even the mainland, and he ships out thousands of pounds of crustaceans every month.

In each circular pond, about the size of half a baseball diamond, Austin raises between 20,000 and 65,000 shrimp. That's 12 to 200 shrimp per square meter...

### ...Shrimp Sales

If you eat at a Roy's restaurant on Oahu or Maui, order a shrimp dish - it came from Austin's farm. Will Hawkins, operations manager of Oyster & Seafood Specialty's Inc. on Oahu, which distributes Austin's shrimp, said Molokai shrimp is a big draw for the chefs and customers on other islands.

"John's right there in the center of our niche," he said. "Not only do people - customers, chefs - want this produce, it's a fantastic product and helps...put people to work [on Molokai]. It's a sense of culture."

Keawa Nui ships about 400 pounds a week to Maui, spread around 15 restaurants, and sells on the mainland as far as Las Vegas. Austin said he also has a strong following locally, occasionally setting up shop on a truck in Kaunakakai on Fridays, offering his shrimp at wholesale prices.

"I don't believe in shipping the good stuff away and keeping junk [locally]," he said. "People go die hard for the shrimp we sell."

[Click here](#) to read the full article.

The Center for Tropical and Subtropical Aquaculture (CTSA) is one of five regional aquaculture centers in the United States established and funded by the U.S. Department of Agriculture's National Institute of Food and Agriculture (NIFA) under grants 2006-38500-16901, 2007-38500-18471, 2008-38500-19435, and 2010-38500-20948. The regional aquaculture centers integrate individual and institutional expertise and resources in support of commercial aquaculture development. CTSA was established in 1986 and is jointly administered by the Oceanic Institute

