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Regional e-Notes ~ October 2012

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Letter from the Director

Aloha,

During my first meeting with Dr. Maria Gallo (CTAHR dean and CTSA's newest board member), she asked me the question "since we are surrounded by so much water, why don't we do more aquaculture in the region?" Indeed, I agree that we should use our abundant natural resources to produce more seafood and address the issue of food security.

We are incredibly fortunate to live in an area that has easy access to clean warm water, as well as nutrient-rich deep ocean water. These resources can be sustainably used to increase our seafood supply. Unfortunately, we have not fully explored the potential to apply them to our food production systems, and are essentially squandering away the resources we do not use.

CTSA has a limited budget, and we must ensure that it goes to support research and demonstration efforts that will have the greatest return on our investment. The Center is consistently pursuing opportunities to build capacity and help our regional stakeholders realize the limitless potential of aquaculture. However,

In This Issue

Letter from the Director

Island Farmer Spotlight Video: Linda Gusman

New CTSA Publication:
Francisellosis in Tilapia

CTSA Project Report: Biosecurity

Farmer Announcements

October AquaClip

Quick Links

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we can only do so much. I implore our friends in Hawaii, Palau and other U.S. Affiliated Pacific Islands to collaborate with CTSA and make a serious commitment to work towards achieving our common goal of regional self-reliance.

As always, our goals cannot be achieved without effective partnerships. If you have any questions, comments, or suggestions, please feel free to contact me.

Mahalo,

Cheng-Sheng Lee
Executive Director, CTSA

Island Farmer Spotlight Video: Linda Gusman, Kualoa Ranch



In the latest installment of CTSA's Island Farmer Spotlight Video series, we visit Linda Gusman's tranquil farm in the Kahalu'u area of Oahu, where she grows shrimp, tilapia, and grass carp in earthen ponds and tanks.

[Click here to view the video!](#)

New CTSA Publication "Francisellosis in Tilapia"

Researchers RuthEllen Klinger-Bowen, Clyde S. Tamaru, Kathleen McGovern-Hopkins, and Bradley K. Fox of the University of Hawaii College of Tropical Agriculture and Human Resources collaborated on a new publication that takes an in-depth look at Francisellosis, the disease affecting many tilapia farmers in the state of Hawaii.

[Click here to download the publication on the CTSA website.](#)

CTSA Project Report: Update on Francisella Infections in Cultured and Wild Tilapia Species in Hawaii

and Wild Tilapia Species in Hawaii

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 Esteban Soto

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Moana Technologies, LLC



Under the auspices of the Center for Tropical and SubTropical Aquaculture (CTSA) supported projects the capacity for conducting conventional and real time PCR testing for a pathogen, Francisella-like bacteria (FLB) that impacts tilapia populations in Hawaii and abroad have recently become available to the aquaculture industry in Hawaii. This is being accomplished through a collaborative public private partnership between three institutions and they are the College of Tropical Agriculture and Human Resources (CTAHR) of the University of Hawaii at Manoa, Moana Technologies, LLC, based on the island of Oahu and Ross University School of Veterinary Medicine located in the West Indies. Progress made has led to the

delineation of an intracellular pathogen, *Francisella noatunensis* subsp. *orientalis* (syn. *F. asiatica*) or *Fno*, which has been implicated as the causative agent for mortalities in several tilapia species (e.g., *Oreochromis* spp., and *Saratherodon* spp) in Hawaii, the continental United States, Taiwan, Latin America and Japan. To avoid confusion FLB and *Fno* are referring to the same pathogen.

A major leap in knowledge reported previously was the identification of asymptomatic carriers of the pathogen's DNA in both cultured and feral tilapia stocks on Oahu. The discovery also underscores the challenges of dealing with this particular pathogen as such carriers do not exhibit any external signs of harboring the disease and purchase and/or sharing of these individuals appears to be one of the most common means of dispersing the disease among aquaculture and aquaponic stakeholders on Oahu. The project work group has been carrying out additional field investigations to understand the epidemiology of *Fno* the results of which are a necessary first step in the development of a disease management strategy for this particular pathogen in Hawaii and region. Information being obtained is to be disseminated to the scientific community through the peer reviewed publication process but is also to be shared with aquaculture stakeholders via the classical means of extension and outreach and forms the basis for this report.

To date, a total of 23 sites on the island of Oahu have been found with Tilapia that were positive for *Fno* DNA using a combination of conventional and real time PCR testing. Nine of these locations consisted of wild caught (Figure 1, red push pins) tilapia that were exclusively made up of the black chin variety (*Saratherodon melanotheron*). An additional 14 sites (Figure 1, yellow pushpins) consisted of both commercial farms and backyard aquaponic producers that possessed positive *Fno* individuals. An additional three locations possess tilapia that have tested negative for *Fno* and one of the sites was Nuuanu Reservoir where the species sampled consisted entirely of *T. rendalli*. The other location is an upcoming commercial farm located on the Waianae coast (Figure 1, green pushpin) and the last is our project workgroup laboratory located on the Windward Community College campus in Kaneohe. Clearly, the pathogen is widely distributed on and around Oahu but there apparently are pockets where it also does not exist which is welcome news as it forms the basis for developing centers from which *Fno* free stocks can become established.

Using the tilapia samples collected on

Using the tilapia samples collected on Oahu and from which spleen tissue was subjected to both conventional and real time PCR testing for Fno we are able to obtain our first glimpse of the distribution of the pathogen amongst the various tilapia species present on Oahu (Figure 2). Amazingly 66.7% of the black chin tilapia (*S. melanotheron*) collected from the wild were found to be positive for Fno. Clearly this large pool of infected individuals presents a major challenge in the establishment of Fno free centers. The percentage of positive black chin tilapia was significantly higher than the 45.2% of the golden variety (*O. mossambicus*) that are being cultured by most of our aquaculture and aquaponic stakeholders. What is of interest, however, is the significantly lower percentage (20.0%) of positive koilapia (*O. honorum*) variety that is also being cultured and in many cases together with the golden variety. Determining the reasons for the observed differences in prevalence between the various tilapia species obviously will require further investigations. Both the blue (*O. aurea*) and nile (*O. niloticus*) samples tested were all found to be negative but both of these strains are housed at our Windward Community College laboratory that is currently Fno free after almost two years of monitoring. These stocks have yet to be introduced to infected individuals and it remains to be determined how they will respond to being exposed to the pathogen.



The project work group has been extremely fortunate to be able to work with the various stakeholders who not only willingly provide samples but work together to obtain additional information about this pathogen. A backyard aquaponic system that experienced a clinical outbreak of Fno in February of 2011 provided the first evidence of the existence of asymptomatic carriers of Fno. Rather than depopulate the owner decided on salvaging the survivors but would allow our working group to periodically obtain samples from the system and that would ultimately lead to the confirmation of the existence of asymptomatic carriers of Fno. The original and additional samples have been tested using both conventional and the more sensitive real-time PCR test and there is good agreement between the two methods. Sampling was allowed to continue over the course of a whole year and the data obtained has been summarized in Figure 3. While there appears to be a decrease in the percentage of individuals possessing Fno DNA in their spleens at the last sampling point all values are not statistically different from each other. Clearly once the clinical infection has subsided the presence of individuals possessing Fno DNA in their spleens (e.g., asymptomatic carriers) persists for an extended period of time. It remains to be determined if these individuals remain infective and/or have become immune to the disease. The work being conducted is clearly generating more questions that will ultimately need to be answered.



An opportunity arose during clinical outbreaks in two separate raceway systems at a commercial aquaponic farm.

Depopulation of the infected stocks is not a



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feasible option when at commercial scale as the number of fish that might recover still represents a substantial economic savings, albeit a reduction in overall profit. In a commercial aquaponic setting there is also the added dilemma of compromising the plant crop production with the sudden removal of the nutrient stream that drives the plant production portion of the system. Not surprising the decision made is to ride out the outbreaks the first of which was confirmed to be an Fno infection using conventional and real time PCR on February 15, 2012. Approximately a month later the outbreak would subside (e.g. cessation of mortalities) and as anticipated resulted in a large pool of asymptomatic carriers. A second clinical outbreak would occur in a separate raceway and confirmed on May 3, 2012 and presented an opportunity to culture the pathogen from two different stages of an Fno infection although from different culture systems. On May 14, 2012 fish from the two raceways (n=12 RW4 and n=8 RW8) were euthanized and spleens aseptically removed and placed in sterile microcentrifuge tubes that were then packed in shipping containers along with ice packs and shipped to Dr. Soto whose laboratory is at Ross University-

School of Veterinary Medicine in the West Indies. Upon receipt of the tissues he attempted to culture all of the samples and was not successful for all 12 samples obtained from RW4 but was successful with 7 out of 8 (87.5%) of the samples from RW8. A photograph of one of the successful cultures is provided in Figure 4. The possibility does exist that transshipping of the specimens may have impacted their ability to be cultured. None the less, the results raise some obvious questions as to how the pathogen can cause an infection and clearly an area that will require further study.

One of Dr. Soto's discoveries was that the pathogen's ability to live outside of the fish host was limited to only a few days (Soto and Floyd, 2012). This information was put to use with another backyard aquaponic owner who would allow his fish to be depopulated without immediately replacing the fish for a period of two weeks. During that time the nutrients available to the plant crops remain elevated enough to support their growth. The fish tank was then restocked with naïve tilapia that were produced at our Windward Community College facility. Fish were periodically removed, tissues sampled and subjected to conventional and real time PCR. Results of this particular trial is summarized in Figure 5. During the initial samplings the project work group used gill tissue in the hopes of conserving the number of fish. This was taking place before the confirmation of asymptomatic carriers was known and that the only tissue to confidently detect Fno DNA was spleen. In any event, during the latter stages of the monitoring process only the lethal sampling protocol and spleen tissue was used in the analyses. Although the PCR testing was terminated seven months after restocking the system has yet to show any indication of a return of Fno to the system. The result indicates that a cost effective means of ridding a closed recirculating system of the pathogen might be as simple as removing the fish for a period of time that would not allow the pathogen to establish itself. While the strategy appears to be effective it does have its limitations in that it is useful for smaller systems (e.g., backyard units) and that its success relies on a source of clean/naïve stock to replace the infected ones.

While much has been accomplished there is a lot more work that remains to be done. Future work is focused on determining whether other fish species that cohabitate with infected stock both in the wild

and cultured can be infected. Likewise, determining the distribution of infected tilapia throughout the state and the region are priority areas that are targeted to be addressed.

Literature cited:

Soto, E. and F. Revan. 2012. *Culturability and persistence of Francisella noatunensis subsp. orientalis (syn. Francisella asiatica) in sea- and freshwater microcosms. Microbial ecology. 2012 Feb;63(2): 398-404*

Industry Announcements & Reminders

Shout-out to NAA Members: Make the Connection

Is it worth 15 minutes of your time to reach hundreds of potential buyers? If the answer is yes and you are an individual member of the National Aquaculture Association, take the time to complete the attached NAA U.S. Aquaculture Directory listing form. The Directory will be a go-to resource when buyers call the office or visit the website to identify suppliers of eggs, fingerlings, stockers, ornamentals, food fish, shellfish, aquatic plants, feed, equipment, and supplies. The listing is especially important for food fish producers.

The Directory listing is only available to NAA Individual or Sponsor members. If you are not currently a member of the NAA, now is the time to join and take advantage of being listed in the Directory. Membership information is available on the NAA website, www.thenaa.net or by contacting the NAA office at naa@thenaa.net or 870-850-7900.

FDA-Approved Fish Drugs

The FDA Center for Veterinary Medicine has issued a letter to aquaculture professionals reminding them that not all drugs currently marketed for fish are approved. And even if a marketed product has the same established name (active ingredient) as an FDA-approved drug, that doesn't mean it's also FDA-approved. If a product currently marketed for fish isn't listed, it's not FDA-approved, and therefore, hasn't been shown to be safe and effective in fish.

[Click here to read the October 16, 2012 FDA CVM Letter to Aquaculture Professionals.](#)
[Click here for additional information about approved drugs for aquaculture.](#)

AquaClip: Initiative Promotes Aquaculture to Fight Hunger

By staff at www.seafoodsource.com. October 2, 2012.

A United Nations-backed initiative that will form partnerships among governments, development agencies and universities has been launched to better understand how aquaculture can help low-income countries fight hunger.

The initiative - Aquaculture for Food Security, Poverty Alleviation and Nutrition (AFSPAN) - will examine low-income food-deficit countries in Africa, Asia and Latin America to develop sustainable fishing policies and support the livelihoods of millions of small-scale fish farmers. It will also design strategies for improving the impact of aquaculture on food and nutrition security and poverty alleviation.

AFSPAN will also develop new ways to quantify the contribution of aquaculture with systematic and quantitative assessments, and establish strategies for improving the impact of aquaculture on food and nutrition security and poverty alleviation.

The three-year project will be funded by the European Union for approximately EUR 1.3 million, which will be managed by the Food and Agriculture Organization (FAO) in partnership with a global alliance

of 20 development agencies, governments and universities.

"The project will work closely with fish farming communities and will focus on field research in many major aquaculture countries in the developing world," said Rohana Subasinghe, a senior expert of the FAO on aquaculture and project coordinator. "It will develop tools and methodologies to help key partners to develop policies geared to improving aquaculture's contribution to food and nutrition security."

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 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 and the University of Hawaii.

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