

Regional  
e-Notes

## Letter from the Director

Aloha!

It is the time of year when the national Regional Aquaculture Center (RAC) program submits stakeholder testimonials to legislators. This is an important part of RAC development process, and I would like to say thank you to the many industry stakeholders who offered to write testimony in support of CTSA.

I would also like to take the opportunity to thank those who submitted comments in response to our request for priority identification. Based on your suggestions, the ad-hoc committee has been working on developing the priority areas for CTSA support in FY2011 research. However, we would like to continually hear from all stakeholders to make sure we are doing the right thing and helping the right area

As always, if you have any suggestions, concerns, or comments, please do not hesitate to let us know. We look forward to seeing you again in the new year!

Mahalo,

Cheng-Sheng Lee  
Executive Director, CTSA

## In This Issue

Letter from the Director

CTSA Biosecurity Update

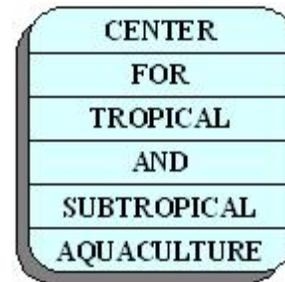
Farmer & Pacific Island Spotlight: Meet Pete!

February AquaClip: US Govt Says Eat More Seafood

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## CTSA Biosecurity Project Update

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

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The continued expansion and diversification of Hawaii and Pacific aquaculture industries is a primary goal of the State Department of Agriculture and the aquaculture extension program housed in College of Tropical Agriculture and Human Resources (CTAHR). A major constraint in achieving this overall goal is the threat posed by various pathogens that can cause catastrophic losses in both vertebrate and invertebrate production systems. As discussed at the United States Department of Agriculture (USDA) Aquaculture Stakeholder Workshop held in Kansas City, MO in April 2008, to prevent the introduction and spread of harmful diseases the aquaculture industry needs *"rapid, automated, and accurate tests to demonstrate that aquatic animals, seed stocks, and products are free from harmful pathogens"*. Likewise, *"improved biosecurity through the use of cost-effective health management plans should lessen the frequency and dispersion of disease problems and improve animal well being and increase system*

*productivity*". In response to these challenges, project working groups were formed in the 2008 to address each of the priority areas and align them with what was obtained through stakeholder input resulting in a proposal submitted to the Center for Tropical and Subtropical Aquaculture (CTSA). An outcome of the initial proposal was a joint award made to the Oceanic Institute and the University of Hawaii at Manoa. Highlights regarding the progress being made under the diagnostic and surveillance priority area forms the basis for this report.

A major objective of the project was to establish a local diagnostic laboratory that would result in rapid turn around time for providing test results by working with existing facilities. This would then allow for achievement of the second and third objectives of the project which are to conduct pro-active screening for specific pathogens under Office International des Epizooties (OIE) guidelines and conducting of initial surveys of disease pathogens (e.g., Tilapia *Rickettsia*-like organism or TRLO and Koi Herpes Virus or KHV) present on farms and in the environment. At the outset the project had to face some of the most daunting fiscal challenges[1] ever experienced in the State's history that would ultimately result in the loss of two key project personnel (Dr.'s Leonard Young and Amanda Lowrey) who were the original architects for this phase of the proposed project. Despite these challenges the project succeeded in forging a collaborative private-public partnership with Moana Technologies LLC, that possesses the molecular capacity to conduct Polymerase Chain Reaction (PCR) assays as it is part of their routine work. In collaboration with Dr. James Brock the project has recently completed the preliminary steps necessary to validate testing samples for KHV and TRLO.

For KHV it was necessary to obtain a positive control for this pathogen as it is an OIE listed and reportable pathogen. KHV DNA was obtained from Dr. Kei Yuasa, Diagnostic & Training Center for Fish Diseases, National Research Institute of Aquaculture, Japan which is one of two OIE reference laboratories for this pathogen. The KHV positive samples consisted of one vial of extracted DNA from wild common carp gills naturally infected by KHV and a second vial that contained ethanol-fixed gill tissue of carp that had been experimentally infected with KHV (isolate No.: NRA0301). Moana Technology developed a PCR test protocol principally following the protocol listed in the OIE Aquatic Animal Manual[2]. Availability of a positive control for TRLO was a bit more involved. Several pieces of tilapia spleen preserved in ethanol that was clinically and later confirmed by PCR to be TRLO positive was provided by Dr. Allen Riggs. DNA was extracted from the spleen samples and is currently serving as our positive control for all future PCR tests for TRLO on fish samples.

The selectivity of a PCR test is based upon the use of short sequences of nucleic acids called primers that are complementary to the DNA region of the genome targeted for amplification. The primers for KHV are well documented and described in the OIE manual. The primer sequence for the current PCR assay for TRLO was obtained from a pre-publication manuscript entitled, "Identification and isolation of *Francisella*-like bacteria (FLB) from tilapia (*Oreochromis mossambicus*) for the first time in Hawaii" authored by researchers at the Hawaii Institute of Marine Biology Ichiro Misumi, Jennifer M. Kishimori, Teresa D. Lewis, Ashley M. Smith, and Jo-Ann C. Leong. The manuscript reports PCR primers suitable for the PCR detection of extracted TRLO DNA and was synthesized in the Greenwood Molecular Biology Facility, University of Hawaii at Manoa.

In addition to being able to identify the nucleic acid sequence for a specific pathogen the validity of a PCR test is also dependent on having some assurance that a negative result truly means that the nucleic acid (DNA or RNA) in the sample is not present and had not been degraded prior to or during the laboratory processing phase of the test being done. One way to address this issue is to include a control PCR test for each tissue sample analyzed that measures the presence of DNA or RNA sequences from the host animal whose origin will also be present in the sample. If the PCR test is positive for the control DNA or RNA sequences, then confidence is high that degradation of the nucleic acid has not taken place between the time the tissues were collected from the host and the extracted DNA sample was subjected to nucleic acid amplification. The choice for this DNA marker was part of the gene encoding for an enzyme known as cytochrome oxidase subunit I (CO-I) that is currently being used routinely as a barcode for identifying fish species[3].

Equipped with positive controls for the target pathogens and the appropriate primers, the validation of the PCR assays have been completed and one of the results for KHV is summarized in Figure1. Likewise, there is a separate band of DNA that corresponds to the DNA sequence encoding for CO-I approximately 700 bp that is also present in the same sample. The combination of results

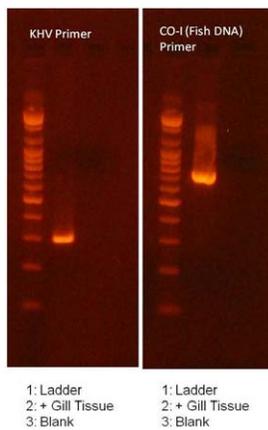


Figure 1. Photographs of gels to visualize selected DNA sequences and amplified by PCR.

indicate that the gill tissue was positive for the KHV and that there was no indication of substantial degradation of the DNA present in the sample. The same kind of testing has been completed for the TRLO with similar results.

To further validate the PCR assay is specific for KHV the DNA bands that were detected using the KHV primers and the CO-I primers the bands were extracted from the gels first purified and submitted to the Greenwood Molecular Biology Facility, University of Hawaii at Manoa, where they would undergo nucleotide sequencing. The resulting sequences were then entered into the BLAST[4] database to determine whether any similar sequence alignments occurred in the database and the results were a 99% maximum identity to the Cyprinid herpes virus for the KHV band and a 97% maximum identity to cytochrome oxidase from Perciformes sp. and a 96% maximum identity to *Oreochromis mossambicus* for the CO-I band. A similar process was also used for the band detected using the TRLO primer and when processed through the same BLAST program resulted in

99% max identity to a *Francisella*-like bacteria (TRLO). Based on information retrieved from the BLAST database, there is significant similarity found from the positive bands for KHV, TRLO and CO-I and further validates the specificity for the PCR tests that are now available for the project work group.

Sampling of koi both in the wild and being cultured has already commenced with approximately 200 individuals already assayed with no detection of any positive cases to date. The most notable sampling to date was done at Kodama Koi Farm[5] one of the largest koi producers in the country. Using a non-lethal gill sampling method summarized in Figure 2 50 individuals from a tank of approximately 1,000 individuals were biopsied successfully with zero mortalities. All samples were negative for KHV using the PCR assay. Introduction of KHV into Hawaii and the farm itself is one of the greatest threats to the industry and developing a protocol for the early detection of this pathogen would obviously be of value for both farm and customer alike. The project working group will continue to work with Kodama Koi and others in the freshwater ornamental industry in developing a protocol that might lead to specific pathogen free (SPF) status similar to what has already been achieved with Hawaii's marine shrimp broodstock growers.



Figure 2. Non-lethal gill biopsy used for detecting KHV in a koi population at Kodama Koi Farm, Hawaii

Although the work on TRLO is the focus of the second year of the CTSA project the availability of the PCR assay and access to fish that were undergoing a TRLO outbreak allowed project work group members to address some additional questions regarding the use of the PCR technology in detection of this particular pathogen. A herpes virus characteristically becomes embedded in the host's genome. A *Rickettsia* is a genus of bacteria whose survival depends on entry, growth, and replication within the cytoplasm of the host cells. Our interest is the development and use of non-lethal sampling protocols for conducting surveillance of the disease and identifying an appropriate tissue which would allow this to be achieved becomes an important objective that needs to be completed. An active outbreak 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. 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 were morbid and found to possess multifocal granulomata in the spleen and various other organs and the desired outcome would have been a sampling protocol that resulted in 100% detection of the pathogen. While this was achieved when using the spleen 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. While clearly a good beginning these and other challenges await our project work group in the development of an effective means of developing a cost effective diagnostic surveillance program that will result in achieving the overall goal of a continued expansion and diversification of Hawaii's aquaculture industry.

[1] <http://www.bizjournals.com/pacific/stories/2008/11/24/story2.html?b=1227502800%255E1737354>

[2] <http://www.test.oie.int/en/manual-of-diagnostic-tests-for-aquatic-animals/>

[3] [http://www.smbb.com.mx/congresos%20smbb/acapulco09/TRABAJOS/simposios/simposio\\_marina/MICHAEL\\_MORRISEY.pdf](http://www.smbb.com.mx/congresos%20smbb/acapulco09/TRABAJOS/simposios/simposio_marina/MICHAEL_MORRISEY.pdf)

[4] <http://blast.ncbi.nlm.nih.gov/Blast.cgi>

[5] <http://www.kodamakoifarm.com/lover/start.php>

## Farmer & Pacific Island Spotlight: Meet Pete!

By Meredith Brooks, CTSA



Pete Arriola of Dandan, Saipan, in front of his shrimp tank.

It was a bright, sunny December day when I met aquaculturist Pete Arriola at his home in Dandan, Saipan. With a warm smile and friendly hug, he greeted me and immediately handed me a banana picked from a tree in his front yard. I devoured the succulent fruit in a matter of seconds, and asked him what his secret was to growing the most delicious banana I had ever eaten. His response: "It's the fish water."

Using aquaculture effluents for irrigation is just one of the ways Pete is turning his modest aquaculture system into a demonstration for sustainable living in the Mariana Islands. In the back yard of his home, Pete raises tilapia and white shrimp in a system that he created to serve as a working demonstration site for other islanders interested in farming.

Pete believes that the key to sustainability in the CNMI is expanding small-scale aquaculture farming, and he passionately explained to me his desire to show people how to grow fish to supplement wild catch for their families and neighbors. "Sooner or later, we need to have a replacement for ocean fish on the island because the fish are going away. Tilapia is necessary for replacement of those ocean fish."

When asked what the greatest benefit of being a farmer is, Pete replied "I'm not going to go hungry. My father taught me that if everything goes wrong, you have to go back to the basics of life, which is knowing how to farm using the resources of the land and the ocean."

Pete, who has a full-time job outside of aquaculture, started his operation ten years ago but shut down at one point for a few years because of the high cost of energy and high cost of feed in the CNMI.

In the CNMI, electricity is expensive at 45 cents per kilowatt hour. In fact, energy cost is the first issue any farmer will tell you about when asked about the difficulties of farming in the Western Pacific. However, alternative energy projects can shed some light on the expense. The U.S. Department of Agriculture at Northern Marianas College provides funding for local farmers under the Western Sustainable Agriculture Research and Education (WSARE) program. WSARE's goal is to promote sustainable agriculture through competitive grants that link farmers and researchers across the western region, which includes 13 states and four Pacific Island territories.

Through the program, Pete received a grant of \$10,469 for his project called the "Demonstration of the

Feasibility Study of Solar Energy in Sustainable Agriculture to Address the High Cost in Conventionally Produced Electricity." He is hoping that alternative energy will allow him to avoid closing down again.

Between the alternative energy project and his efficient use of effluents, Pete is proving that sustainable farming can work in the Western Pacific and can, in fact, make the job easier and more enjoyable. "I like to do this because its a good way to relieve stress," Pete said with a smile. "I just love to raise fish."

## AquaClip: "US Government: Eat Fish Twice a Week"

By Steven Hedlund, SeafoodSource editor: [www.seafoodsource.com](http://www.seafoodsource.com). January 31, 2011.

For the first time, the U.S. government is advising all Americans, including pregnant and breastfeeding women, to eat seafood at least twice a week for heart and brain benefits. Previously, the twice-a-week recommendation was limited to heart patients.

On Tuesday, the U.S. Department of Agriculture and the U.S Department of Health and Human Services released the *2010 Dietary Guidelines for Americans*, as required by Congress every five years. The guidelines serve as the basis for federal nutrition policy.

In the guidelines, the agencies said: "Moderate evidence shows that consumption of about 8 ounces per week of a variety of seafood, which provide an average consumption of 250 milligrams per day of EPA and DHA, is associated with reduced cardiac deaths among individuals with and without pre-existing cardiovascular disease."

They continued: "In addition to the health benefits for the general public, the nutritional value of seafood is of particular importance during fetal growth and development, as well as in early infancy and childhood.

In a report last June, the U.S. Dietary Guidelines Advisory Committee declared that Americans eat too little seafood and should be encouraged to eat more for better brain development in babies and heart health in adults. The committee, made up of more than a dozen nutrition experts, was tasked with recommending changes to the dietary guidelines for Americans.

"Consumption of two servings of seafood per week ... is associated with reduced cardiac mortality from [coronary heart disease] or sudden death in persons with and without [cardiovascular disease]," said the report.

Jennifer McGuire, the National Fisheries Institute's registered dietitian, said the mainstream media's coverage of the recommendation may be even more beneficial than the federal nutrition policy itself. "We know the media is the No. 1 source of nutritional information for consumers," she told SeafoodSource on Monday. "Now there's clear, undisputed recommendations for the media to stick to."

McGuire added that the new dietary guidelines may lead the U.S. Food and Drug Administration and Environmental Protection Agency to change their seafood-consumption advisory for methylmercury, which warns pregnant and breastfeeding women to limit seafood intake to 12 ounces per week.

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More more information on the *Guidelines*, please visit [www.dietaryguidelines.gov](http://www.dietaryguidelines.gov)

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 2005-38500-15720, 2006-38500-16901, 2007-38500-18471, and 2008-38500-19435. 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.