CENTER FOR TROPICAL AND SUBTROPICAL AQUACULTURE

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Letter from the Director

Aloha,

Yesterday, CTSA held our annual Board of Directors meeting, one of the final steps in the FY25 development process. As we finalize last year's Plan of Work for submission to the USDA, we are also beginning the FY26 development cycle. Please take a few moments to complete the priority area survey described below and share your thoughts on regional development. As I often express in these letters, advancing our regional industry requires all of us working together.

As a federally funded USDA program, it is CTSA's responsibility to invest public resources in projects that address critical gaps and advance aquaculture development. Our structure ensures accountability and transparency while remaining industry-driven. Projects are selected based on regional stakeholder priorities, and funding is administered through established and standardized grant mechanisms. Maintaining this framework is essential to fulfilling our mission and upholding the trust placed in us. We recently received a request to deviate from these procedures in order to pursue a partnership. While collaboration is central to our success, it must operate within the parameters that govern our program. We remain committed to identifying constructive paths forward that align with our federal mandate and our shared goal of strengthening aquaculture.

On that note, I would like to thank everyone who visited the CTSA booth at Hawaii Agriculture Awareness Day at the Capitol (including Senator H.M. Richards III, pictured). I truly appreciated the many conversations I had, which reinforced a clear message: we must produce more of our own food. With water among our state's most abundant resources, aquaculture provides a direct pathway to strengthening food security. Discussions with prospective collaborators centered on training the next generation to responsibly steward our waters and expand local seafood production.

At the federal level, Hawaii Congressman Ed Case has co-sponsored legislation supporting aquaculture development. The national industry has the potential to support 1.6 million jobs and generate billions in revenue; however, the United States imports approximately 85 percent of its seafood despite strong domestic demand. Regulatory complexity and policy uncertainty --including delays in passing a new Farm Bill-- continue to constrain growth. As currently drafted, the pending Farm Bill includes provisions such as the establishment of a USDA aquaculture advisory committee, which could strengthen coordination and provide clearer strategic direction for the sector.

Encouragingly, consumer interest in locally produced seafood continues to grow, and our partners at UH are finalizing the hiring of new aquaculture faculty. Moving forward, we must align resources, deepen collaboration, and stay focused on stakeholder-identified priorities. By doing so, we can reduce barriers to responsible development and build a more resilient domestic seafood industry.

Mahalo,
Dr. Cheng-Sheng Lee
Executive Director, CTSA

CTSA FY26 'CTSA Priority Areas and Species' Survey

CTSA is gathering input on regional aquaculture development and would like to hear from you, our valued industry stakeholders!

Each Spring, CTSA releases a Request for PreProposals based on industry input from our 'Funding Priorities' survey. We request that you please click here to take our FY2026 survey.

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FY26 Funding Priorities Survey

Last year, we redesigned our priority areas survey to identify the highest-priority species for development in Hawai‘i and the U.S. Affiliated Pacific Islands (USAPI). Based on stakeholder input, the top seven species for Hawai‘i and the top three species for USAPI were selected as funding priorities for our FY25 'Request for Pre-Proposals.' Building on that process, this year's survey asks respondents to rank the species that were identified as top priorities in last year's survey.

Our goal is to focus on species that have already been initiated in the region and require additional support to reach full commercial potential or to address key challenges within established industries. Accordingly, the first section of the survey asks you to rank the previously selected top-priority species. This will help us assess the **top** priorities for our next round of funding.

This survey also lists the other priority areas included in our FY25 RFP-P, and requests that participants indicate whether or not each area remains a priority for regional aquaculture development and funding support. There is an option to write in new priority areas that were not identified in FY25. Please complete the survey by April 15; it should only take a couple of minutes and will greatly assist our process. We will release the FY2026 Request for Pre-Proposals in the April issue of e-Notes, and are developing the priority areas with essential input from our Industry Advisory Council, Technical Committee, Board of Directors, and stakeholders like you!

If you have any questions or need assistance completing the form, please email ctsa@hawaii.edu.

CTSA Project Results: Using eDNA for Detection of *Francisella orientalis* in Tilapia Aquaculture Settings

By RuthEllen Klinger-Bowen, Jenee Odani, and Yong Li
University of Hawaii CTAHR

For three decades, the bacteria, *Francisella orientalis* (Fo), has been causing morbidities and mortalities in Hawaii's tilapia. Since 2010, tilapia farmers have utilized best management practices to mitigate losses. These include buying known Fo-free stocks, culling moribund individuals, quarantining and conducting diagnostic testing on new stocks, keeping water temperatures above 23°C (73°F), and implementing various biosecurity measures. These practices have reduced losses due to Fo.

Figure 1. Collection of biofilms from tilapia tank sides.

Figure 2. Filtration of water sample through syringe filters.

Previous CTSA-funded projects have examined ways to detect Fo before an outbreak occurs or without the sacrifice of animals. Detection of Fo was determined with invasive diagnostics that involve euthanizing individual tilapia and removing the spleen and/or anterior kidney for DNA extraction. The DNA is tested for Fo with PCR methods. Non-lethal techniques were attempted with gill and fin tissues and using FTA cards (Tamaru et al. 2011), with little success. However, in a challenge study in which a known quantity of Fo bacteria was introduced into a closed system, PCR detection of the bacteria in the water was positive (Klinger et al. 2016). It has also been suggested that Fo may still be present in the biofilms of tilapia systems even during the warmer months (Soto et al. 2015).

Through the CTSA funded project, "Assessment of Hawaiian Aquaculture for Disease Mitigation and Control", we evaluated whether non-lethal techniques such as analyzing eDNA from system water and/or biofilms could effectively detect pathogens, particularly Fo. Detection of Tilapia Lake Virus (TiLV) may also be possible through eDNA methods. eDNA is an emerging field of study that detects DNA (or RNA) fragments from biological cells that naturally sluff off in the environment. In recirculating fish systems, collection of water and substrate, such as biofilm, is ideal for this methodology.

Three tilapia facilities were sampled in February/March 2024 (winter) and August/September (summer). Three one-liter water replicates were collected from the tilapia systems, and one liter of distilled water was

taken as a control at each sampling event. Also, three replicate samples of biofilm were collected from the tank sides or biofiltration units (Figure 1).

All water samples were then filtered through a series of filters (5 µm, 1 µm, and 0.22 µm) to obtain target pathogens for PCR testing (Figure 2). The winter and summer samples of water and biofilm were extracted for DNA for detection of Fo and extracted for RNA for detection of TiLV, which was processed with real time PCR.

While water analyses yielded abundant DNA for Fo detection, they did not yield sufficient quantities of RNA for reliable PCR analysis of TiLV. One possible explanation for the low RNA yield is that the conventionally accepted eDNA method for water filtration specifies a 0.22 µm filter, and the majority of animal RNA viruses are smaller than 200 nanometers (200 nm = 0.2 µm; TiLV particles = 50-100 nanometers). All biofilm samples yielded adequate quantity and purity of RNA and DNA for PCR analysis. Results from the PCR analysis for Fo and TiLV in water and biofilm samples are listed in Tables 1 – 4.

Farm 2 historically has been Fo-free, while Farms 1 and 3 have had Fo-positive fish, with Farm 3 tilapia experiencing mortalities during this study. While Fo was detected in water during a known Fo bacteria challenge study (Klinger et al 2016), it was not detected in this study in water samples and was detected in only one of the biofilm samples during the mortality event, when 9/10 sampled spleens were positive for the bacteria. Fo was detected in all three water samples in the summer sampling period but in none of the biofilm samples from Farm 3. Therefore, although Fo can be detected with eDNA from water and biofilms, at this time the methodology is not robust, as it was less reliable than sampling the fish's spleens. Environmental sampling of water and biofilms (non-lethal techniques) for TiLV detection was unsuccessful. It is unknown if TiLV resides in biofilms. At present, TiLV has not been detected in tilapia aquaculture in Hawaii.

The application for eDNA/RNA for pathogen detection is under scrutiny by the World Organization of Animal Health (WOAH), which generally requires direct animal sampling for specific pathogens (WOAH 2022) for listed diseases. While TiLV is a listed aquatic disease, Fo is not. We conclude that eDNA sampling may be a complement (i.e. suspicion of a pathogen) to animal sampling; however, it is not recommended for certification of disease-free status.

References:

Klinger-Bowen, R., C. Tamaru, K. McGovern-Hopkins, Y. Li, and E. Soto. 2016. Dynamics of piscine francisellosis differs amongst Tilapia species (Oreochromis spp.) in a controlled challenged with Francisella noatunensis subsp. orientalis. J. Fish Dis. 39(11):1305-1312.

Soto, E., I. Halliday-Simmonds, S. Francis, M. Kearney, J. Hansen. 2015. Biofilm formation of Francisella noatunensis subsp. orientalis. Vet. Micro. 181 (2015): 313-317.

Tamaru C., K. McGovern-Hopkins, R. Klinger-Bowen, B. Fox, A. Riggs, T. Low, N. Antonio, & J. Brock. 2011 Detection of asymptomatic Francisella spp. carriers in Tilapia cultured in Hawaii. CTSA Regional e-Notes July 2011. 3(7) 4p.

WOAH. 2022. The use of environmental DNA methods for detection of WOAH listed aquatic animal diseases. A discussion paper developed by the WOAH Aquatic Animal Health Standards Commision (Aquatic Animals Commission). February 2022. 10 p.

Table 1. Winter Sampling for Fo detection via PCR.

Farm #	#Positive/Total # water samples	#Positive/Total # biofilm samples
1	0/4	0/3
2	0/4	0/3
3	0/4	1/3

Table 2. Summer Sampling for Fo detection via PCR.

Farm #	#Positive/total # water samples	# Positive/Total # biofilm samples
1	0/4	0/3
2	0/4	0/3
3	3/4*	0/3

*All water samples collected from system water were positive for Fo. The control (DI water) was negative for Fo.

Table 3. Winter Sampling for TiLV detection via PCR.

Farm #	#Positive/Total Water Samples	#Positive/Total Biofilm Samples
1	N/A	0/3
2	N/A	0/3
3	N/A	0/3

N/A = not applicable due to insufficient RNA to be detected for PCR.

Table 4. Summer Sampling for TiLV detection via PCR.

Farm #	#Positive/Total Water Samples	#Positive/Total Biofilm Samples
1	N/A	0/3
2	N/A	0/3
3	N/A	0/3

N/A = not applicable due to insufficient RNA to be detected for PCR.

AquaClip: Preserving heritage and habitat - MCBH continues fishpond and shoreline restoration

The Environmental Division at Marine Corps Base Hawai'i (MCBH) has worked extensively over the years to remove invasive mangrove from the historically and culturally significant Nu'upia Fishponds, restoring ecological balance and vital coastal ecosystems. These fishponds hold deep cultural importance and provide critical habitat for native species. As environmental stewardship moves forward with caring for the Nu'upia Fishponds, MCBH is grateful for the opportunity to continue working alongside Paepae o He'eia and our broader community partners to further enhance restoration efforts, strengthen cultural connections, and ensure these treasured resources thrive for generations to come.

The American mangrove, one of the most common species found on O'ahu, was originally introduced in 1922 for sediment control but has since expanded across many coastal areas of the island. Mangroves are considered invasive species in Hawai'i and spread quickly overcrowding shallow coastal areas diminishing water oxygen levels.

"From 1994-2000, 30 acres of mangrove were removed from the Nu'upia Fishponds to restore and enhance wetland ecosystems," said Lance Bookless, natural resources program manager with MCBH's Environmental Division. Since the mangrove removal, MCBH's Environmental Division has continued to tend and monitor the Nu'upia Fishponds and surrounding areas twice a year through events such as Weed Warriors, further preventing the resurgence of mangrove in the area.

To continue protecting and preserving the Nu'upia Fishponds, MCBH and Paepae o He'eia, a nonprofit organization that cares for the He'eia Fishpond, an ancient Hawaiian fishpond located in He'eia, Ko'olaupoko, O'ahu, signed a Memorandum of Understanding (MOU) on May 15, 2023, officially establishing an educational, restorative, and environmental centric partnership. The first community workday at Nu'upia Fishponds from the MOU was on Oct. 7, 2023, and was centered around the first steps of restoring an archeological site within the Nu'upia Ponds, a fish wall, by removing an invasive species known as pickleweed.

"Here, at the Nu'upia Fishpond complex, there's not a whole lot of mangroves left within the fishpond, due to the MCBH environmental department doing a good job of tackling mangrove and the other invasive species that we have surrounding the ponds," said Angela Hi'ilei Kawelo, executive director of Paepae o He'eia.

With the Environmental Division's sustained monitoring for mangrove resurgence within the Nu'upia Fishponds area, organizations like Paepae o He'eia can move on to the next steps of fishpond restoration such as preserving archaeological sites. "These fishponds are unique, and they were places of traditional Hawai'ian aquaculture that fed and sustained communities," said Hi'ilei Kawelo. "The restoration of them [fishponds] is important because we don't want to lose that history, and they also provide important habitat for native birds and fish."...

Source: DVIDS // Full Article

This newsletter is written and prepared by the CTSA Information Specialist Meredith Brooks.
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 active grants 2022-38500-38099, 2023-38500-40707, and 2024-38500-42306. The regional aquaculture centers integrate individual and institutional expertise and resources in support of commercial aquaculture development.
CTSA was established in 1986 and is administered by the University of Hawaii.

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