CENTER FOR TROPICAL AND SUBTROPICAL AQUACULTURE

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Letter from the Director

Aloha,

You may have read the recent good news about the 12% increase in Hawaii's aquaculture sales. According to the USDA, 2022 production value reached a record high of $89.6 million, about $10M more than 2021. Algal products continue to bring in the majority of revenue. While it is important for us to support and promote environmentally friendly algal culture and other forms of non-fed aquaculture, we cannot ignore the need and opportunities for expansion of fed aquaculture species as well. However, feed source and price remain among the most significant challenges that must be overcome to secure the future growth of the regional aquaculture industry. To this end, CTSA will co-lead a workshop at the University of Hawaii on October 26 to discuss production costs and identify strategies to lower feed costs. Click here for additional information.

In addition to addressing feed challenges, the expansion of aquaculture relies on increased consumer buy-in. I have seen firsthand how collaboration between each sector of the supply chain --from production and processing to distribution and consumer sales-- can improve promotion efforts and consumer sales. On my recent vacation in Taiwan, I was very impressed by the consumer-focused presentation of seafood. As seen in the photo, the milkfish was processed and offered to consumers in a variety of cuts and portions that overcome the typical bony issue. They were able to fully use each piece of the fish without letting any go to waste and, at the same time, create cuts that are attractive for easy meal preparation.

As we get ready to welcome in Seafood Month, it is important to remember that our industry must work together to promote seafood as healthy protein for people and the planet.

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

Register Now for the Hawaii Feed Mill Workshop in October

CENTER FOR TROPICAL AND SUBTROPICAL AQUACULTURE

Sea Grant University of Hawai'i

College of Tropical Agriculture and Human Resources
University of Hawai'i at Mānoa

Feasibility of Feed Mill Operation in Hawaii
End Users Input Workshop
Thursday, October 26, 2023

Hawai'i is the most remote archipelago on Earth; close to 90% of its food, including seafood, is imported. Hawai'i has the water resources and favorable climate conditions for aquaculture development. However, aquaculture development in the islands has faced several roadblocks. A recent survey conducted by the Center for Tropical and Subtropical Aquaculture (CTSA) -funded by a grant from NOAA- indicated that there are two key bottlenecks for aquaculture development throughout the Pacific Island region: strict regulations and a lack of inexpensive/affordable local feed sources.

The limited availability of affordable feed has hindered the expansion of aquaculture in the region. The most recent thorough study on the problem and prospects of livestock feed in Hawai'i was conducted and reported in 1975 by Nishimura (1975). It concluded that feed was the largest cost item in livestock production, representing between 50% to 75% of the total cost of production. Feed costs in aquaculture typically account for between 40% and 60% of production costs (De Silva and Hasan, 2007). Considering transportation, the cost is even higher in Hawai'i. Can feed be feasibly produced in Hawai'i?

Andini Desita Ekaputri, a graduate student under the guidance of Dr. Stuart T. Nakamoto and Dr. PingSun Leung at the University of Hawai'i College of Tropical Agriculture and Human Resources (CTAHR), has developed a spreadsheet to estimate the cost of a feed mill in Hawai'i. CTSA and the University of Hawai'i Sea Grant College Program will hold a workshop on October 26 to present the feed mill spreadsheet and solicit industry input on the feasibility of an aquatic feed mill in Hawai'i. The meeting will be held in Room 219 of the Agricultural Science building on the UH Manoa campus, and online. A detailed agenda will be released soon.

Please register by October 15, 2023: https://forms.gle/hCDHxajJfveuZDXo6
Questions? Contact myma@hawaii.edu or (808) 956-3974

CTSA Project Summary: Advancing Aquatic Health Diagnostic Testing in Hawaii

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Figure 1. Filtering water for eDNA extraction

The following article reports on the progress for disease detection in the Hawaiian fish aquaculture industry. Under the auspices of the CTSA project "Advancing Aquatic Animal Health Diagnostic Testing in Hawaii, "the research group tested new diagnostic tools, some of which were successful and some of which many need further development or may be discarded. Surprisingly, the rapidly developing technology of environmental DNA (eDNA) did not yield robust detections, and new DNA amplification technologies were successful only at detecting major outbreaks.

The Food and Agriculture Organization of the United Nations (FAO) reports that total fish consumption in 2020 was 20.2 kg per capita, more than double of 9.9 kg per capita in the 1960s. Global consumption of aquatic foods (excluding algae) is projected to increase to an average of 21.4 kg per capita, a fifteen percent increase by 2030. Due largely to the advantage of sustainable aquaculture, production of aquatic animals is expected to reach 100 million tons in 2027, rising to 106 million tons by 2030 (FAO, 2022). With aquaculture playing such a vital role in food production, significant constraints caused by disease must be addressed. Globally, the industry loses billions of dollars per year to disease. Moreover, the movement of

live aquatic animals and feed products, while necessary for industry growth, plays a pivotal role in spreading pathogens to new populations and geographic regions. For example, koi herpes virus (KHV) is infectious to all varieties and subspecies of common carp, *Cyprinus carpio*and hybrids such as *Cyprinus carpio*x *Carassius auratus*(OIE, 2022). All life stages are susceptible, and most mortalities occur when temperatures are 23 to 25° C. The KHV was first reported in the United Kingdom in 1996 but spread to Israel within two years (Haenen et al., 2004). Since then, KHV has been reported from almost all countries that culture koi and/or common carp (OIE, 2022). Fortunately, KHV has never been detected in fish imports to Hawaii.

Francisella orientalis(Fo), previously *Francisella noatunensis*subsp. *orientalis*or Fno, a facultative bacterium, has been well studied both in Hawaii and worldwide (Soto et al., 2014; Nguyen et al., 2015; Klinger et al., 2016; Yamasaki et al., 2020). On Oahu, there was a concerted effort to encourage tilapia producers to test for Fo, adapting a protocol from the shrimp broodstock specific pathogen free (SPF) program. However, diagnostic testing is cost prohibitive for most of Hawaii producers. Here we add Fo as part of the PCR testing to determine if it can be detected in system water or in tilapia live feeds, which could potentially reduce the cost of testing and eliminate lethal diagnostic methods.

Vibriosis, caused by *Vibrio*spp., causes severe mortalities in multiple marine fish and shellfish species worldwide. *Vibrio*spp. are rather ubiquitous, and potentially devastating in aquaculture systems (Frans et al., 2011). Zooplankton culture (e. g. copepods) used for fish fry and larval feeds have been shown to harbor bacterial pathogens such as *Vibrio*spp. (Hansen and Bech, 1996). The real-time PCR is a fast methodology that can detect *V. parahaemolyticus*and *V. vulnificus*in live feeds. The assay is highly specific and sensitive even if *Vibrio*is present in very small numbers. It reduces the amplification of DNA from nonviable bacteria and therefore results in fewer false-positive results (Pillot, 2010).

To protect Hawaii's growing aquaculture industry, we need improved laboratory facilities, diagnostic expertise, control protocols, testing capabilities and permanent data bases. The PCR-based methods of pathogen detection are sensitive and highly specific but they require expensive equipment and reagents, need to be conducted in a highly-controlled laboratory setting, and are time-consuming. Isothermal diagnostic assays such as recombinase polymerase amplification (RPA) or loop mediated amplification (LAMP) are novel approaches of amplifying DNA without the cost and complexities required for PCR (Howson et al. 2017). The loop-mediated isothermal amplification (LAMP) assay is a novel approach of amplifying DNA with high specificity and rapidity under isothermal condition. Additionally, quantification can be achieved by measuring the turbidity of the sample using a photometer (Mekata et al.; 2012). The LAMP method is very rapid, which makes the assay attractive for routine detection, and it is already used to screen shrimp for infectious hypodermal and hematopoietic necrosis virus (IHHNV) (Xia et al. 2015). These properties for the LAMP assay indicate potential for on-site monitoring of infection and preventing spread of disease in aquaculture production. The application of the LAMP assay has the added benefits of reduced analysis time and easy interpretation of results based on a simple colorimetric change, thus offering a faster diagnostic tool compared to PCR. More importantly, LAMP assays are high sensitive and reliable, which has been particularly useful during tilapia lake virus (TiLV) infections (Phusantisampan et al., 2019). The RPA alternative uses recombinase-driven primer targeting strand-displacement synthesis and can be used in point-of-care assays. RPA assays are used with rapid surveillance in livestock outbreaks (Abd El Wahed, 2013) and they are beginning to be used in aquaculture (Liu, 2017). Lobato and O'Sullivan (2017) mentioned that the RPA is an isothermal amplification technique that has already garnered a huge amount of attention and has been exploited for both laboratory-based and portable analysis. It has been used in diverse applications including aquaculture diagnostics. The same authors mentioned applications for hypodermal and hematopoietic necrosis virus (HHNV), *Vibrio owensii*and White Spot syndrome virus (WSSV). Xia et al. (2015) developed a highly sensitive real-time RPA assay for rapid pondside IHHNV detection. Shahin et al. (2018) showed that RPA was more robust than qPCR in the detection of Fo in clinical and field samples. Apparently, RPA is more tolerant to common contaminants that inhibit PCR reaction. The RPA technique for the detection of pathogens in aquaculture can be a good option for faster results with good diagnostic sensitivity.

To date, Hawaii does not have a consistent and continually updated database of emerging aquatic diseases that occur regionally, nationally, or internationally. Acquiring the latest information of aquatic animal diseases can be tedious and with the advent of social media, there are many opportunities for misinformation. There are websites offered by national laboratories, such as the Wild Fish Health Survey, by US Fish and Wildlife Service, which keeps track of freshwater fish diseases in the U.S. International databases include sites such as EBSCO and World Fish Center, and the Office International des Epizooties or OIE (recently renamed World Organisation for Animal Health (WOAH)) (2023), the predominant world organization for emerging pathogens for all animals. OIE is regarded as the international standard for allowing animals to move across borders based on disease events and history. Utilizing such a global database is essential for tracking the latest emerging pathogen (Tedesco et al 2017; Chen et al., 2018). With the assistance of the National Marine Fisheries Service Honolulu office, which tracks regional fish disease outbreaks, along with HDOA Animal Diagnostic Laboratory, HDOA PQ, USDA, and OIE, it is possible to compile all the current information relevant to Hawaii producers in a timely confident manner. This information can be posted to a listserv for anyone interested in receiving pertinent disease information and guidance. Similarly, historical and contemporary data available in such a database, which would also include case information such as environmental and management variables, would be valuable for analysis of trends that could inform future management practices. Preliminary results for detection of most of these pathogens via non-lethal methods has so far proved unsuccessful with real-time or conventional PCR; however, using environmental DNA (eDNA) has potential to detect pathogens with just a water sample

(van der Heijden et al. 2008; Zhang et al. 2020).

This project expands upon previous works by using eDNA to directly assess imported koi shipment water, system water, and live feeds used in fish culture as potential disease vectors. The ultimate goal is to develop a state/region wide database/listserv to communicate up to date information on aquaculture diseases around the world. A quantitative PCR for *V. parahaemolyticus*and *V. vulnificus*was developed and added to the panel of assays available at the UHADL. The UHADL/HDOA became a diagnostic program for aquaculture producers to include histopathologic examination and bacteriology, as needed. Also the expanse of other technology such as RPA or LAMP, which provides quick turnaround results, was compared to traditional diagnostic methods.

This project utilized eDNA assays to detect pathogens in shipping water in the hope of allowing broader non-lethal sampling of the consignment, as opposed to a random sampling of individual fishes to represent the whole population. This eDNA/PCR methodology was also used to detect specific pathogens in live feeds prior to entering the fish systems. The impact of the project was apparent by the expansion of diagnostic testing for diseases affecting aquacultured animals, the determination of potential risks associated with importing animals and feed, and dissemination of knowledge related to aquaculture pathogens to guide biosecurity practices or other methods of mitigating risk.

During the project, two separate importations of koi arrived in Hawaii from multiple farms in Japan. The first consignment of ten fingerlings were collected and gill, spleen, and posterior kidney were sampled for PCR analysis of KHV. Water (3 – 1L bottles) was also collected for eDNA to determine if KHV could be detected in the imported shipment water. The second importation of koi from Japan were too valuable to sample tissues, so only water from the shipping bags were sampled for eDNA extraction (Figure 1). Neither sampling effort detected KHV in any of the tissues or shipment water.

To determine the presence of Fo, three tilapia farms participated in the summer collections, while five farms participated in the late winter/early spring collections. Table 1 describes Fo presence in tilapia from each farm. Note that eDNA from system water was negative for Fo at all farms during both collecting seasons.

While the detection of Fo with qPCR was the primary objective, tilapia tissues were also taken for histology to create a pictural publication of normal tilapia tissues. Granulomas in spleen tissues were categorized from ND (not detected), 1 (light infestation), 2 (moderate infestation), or 3 (heavy infestation). Tilapia spleens were also sectioned for gross examination under light microscopy and microbiology. Although granulomas could be observed under the microscope, no Fo growth was evident with bacteriological (culture) methods. However, it is well known that Fo is difficult to grow in culture when no outbreaks are occurring.

The observation of spleen granulomas with histology and/or by light microscopy in cases that were not positive for Fo with PCR merely shows granulomas can be caused by a wide variety of pathogens or environmental insults. Therefore, scoring granulomas by histological or light microscopy should not be used solely to assess the Fo status of tilapia.

A new loop-isothermal amplification (LAMP) assay, designed by graduate student Taylor Peterson, provides results in as little as 10 minutes. The LAMP assay targets the iglC gene of *Francisella orientalis*, which is the same target as the qPCR created by Soto et al (2010). A limit of detection test was tested in triplicate using a 1:10 serial dilution for a total of ten dilutions of genomic DNA of the Fo LADL-0735A. The LAMP was able to detect 100 pg (picograms) of DNA, therefore during a large disease outbreak, it can detect Fo if disease is rampant and at a high level. We also verified the specificity of the assay by running the LAMP against a panel of closely related species to Fo, as well as bacterial species that are common in fish. The LAMP was highly specific, only detecting strains of Fo and had zero cross-reactivity to the other pathogens.
We also used the samples acquired in the farm survey to test the LAMP as a surveillance tool in the future. The LAMP was not able to detect low level infection that qPCR was able to detect, which is 1 pg. Therefore, for future surveillance work, UHADL should use the qPCR by Soto et al. (2010) instead of Peterson's LAMP. However, we emphasize that the LAMP would be good for fast and reliable detection during a disease outbreak.

In the course of this research, we developed qPCR assays for*Vibrio parahaemolyticus* and *Vibrio vulnificus*. Live feeds from two facilities in summer 2022 and three facilities in March 2023 were sampled. System water was also collected in triplicate for eDNA analysis. The DNA of live feeds including Artemia, rotifers, copepods, diatoms, and marine algae were extracted (Figure 2) and tested for *V. parahaemolyticus* and *V. vulnificus*using real time PCR methodology. eDNA from system water was also tested for both Vibrios. All samples collected at both time intervals were negative for *V. parahaemolyticus*and *V. vulnificus.* Moina and Gammurus that are used to feed tilapia were collected and tested for Fo. These live feeds and system water (3-1L bottles plus one L DI water used as a control) were negative for Fo with qPCR methods.

The maintenance of a University-housed database/listserv of morbidities/ mortalities occurring regionally, nationally and internationally will alert our producers to what pathogens are occurring and where (location will only be given at the national and international) in a timely and efficient manner. Similar to the listservs UH provides to swine and poultry producers, maintaining a database and listserv to communicate with

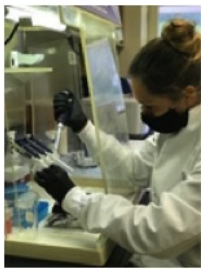
aquaculture producers will help develop an efficient and integrated industry state-wide and region-wide. Trends for morbidities/mortalities outbreaks were determined through coordination with the Hawaii aquaculture producers and the USGS National Wildlife Health Center Honolulu Office. Data was coded by assigning a unique accession number for each event and results were secured in keeping with HDOA and UH-ADL policies on client confidentiality. De-identified information will continue to include the pathogens detected, environmental factors (water temperature, D.O., pH, and biomass), farm records (operation type, species, origin of animals, etc.), history of diseases (year, diagnostic methods utilized, and results), and treatment applied (drugs, probiotics, etc.) to determine factors associated with outbreaks. Confidential data collected included GPS coordinates of farms to facilitate tracking of pathogen spread. Information from national/international disease databases was also incorporated to serve as an early warning system for our aquaculture producers via a listserv for rapid communication.

Sampling water for eDNA analysis during a non-disease event yielded negative results for Fo and KHV. Therefore, using eDNA to detect these pathogens during non-outbreaks is not a useful tool, and sampling fish tissues is still the best method for PCR detection. Also, this project assured the participating producers thatthe biosecurity measures put in place are working to prevent specific pathogens entering their facility. They also have the wherewithal to mitigate pathogen spread if fish morbidities/mortalities occur. When these individual farms received their complete test results, they were satisfied that their farm samples were coded by a unique accession number and results are secured per HDOA and UHADL policies on client confidentiality. In the future, veterinarians, other health professionals, or producers will benefit from a manual on fish sampling and histology to ensure the best diagnostic technologies are applied.

Click here to read the full article including all figures, table, and citations

Survey for Aquaculture Producers in Hawaii

The University of Hawaii is conducting an initial query to Hawaii aquaculture stakeholders to better understand aquatic animal disease and production concerns, and how you respond to morbidities/mortalities at your facility. This is a preliminary questionnaire intended to guide future stakeholder engagement aimed at improving ability to identify and address the needs of the aquaculture community. This questionnaire is provided by the University of Hawaii Manoa and the Hawaii Department of Agriculture, with funding provided by CTSA. Click here to take the survey.



AquaClip: USDA's New Shellfish Pilot Crop Insurance Program Provides Oyster Producers with More Coverage Options

The U.S. Department of Agriculture (USDA) announced that oyster producers are now eligible for protection against losses due to named storms, excessive heat during a low tide event, freeze during a low tide event, or low salinity due to excessive rainfall. The new Shellfish crop insurance program is an actual production history-price component (APH-PC) coverage policy for container-grown oysters commercially cultivated for the fresh half shell market. This new program will be available beginning with the 2024 crop year, and producers have until Nov. 30 to purchase this policy.

“We are always in contact with the entire agriculture community to hear their needs and provide opportunities to strengthen and expand crop insurance resources.” said Marcia Bunger, Administer of the USDA’s Risk Management Agency (RMA). “This policy offers our oyster producers needed protection from environmental challenges they face, and it also allows those who purchase additional levels of coverage to insure for a higher price based on personal sales records.”

For the APH-PC coverage policy, the producer selects the percentage of the average yield to insure—from 50% to 75%. Additionally, the producer also selects the percentage of the crop price to insure, between 55% and 100%, as established annually by RMA. If producers purchase additional levels of coverage, they may also increase their price guarantee using their historical sales records. The program uses county loss triggers combined with producers’ sales records to confirm a loss has occurred. The insurance guarantee is based on the number of oysters expected to be harvested between Jan. 1 and Dec. 31.

Insurability requires a minimum of four years of records and a minimum seed size of four millimeters.

This new Shellfish crop insurance program is available in select counties in Alabama, California, Florida, Maine, Maryland, Massachusetts, Mississippi, New York, North Carolina, Rhode Island, South Carolina, and Virginia.

RMA also offers crop insurance for certain aquaculture categories through crop insurance programs for Group Risk Plan oysters and cultivated clams, and the Whole Farm Revenue Protection (WFRP) plan of insurance. WFRP provides a risk management safety net for all commodities in an operation under one insurance policy.

Source: USDA // More Information

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 2020-38500-32559 and 2022-38500-38099. 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 University of Hawaii and the Oceanic Institute of Hawaii Pacific University.

Center for Tropical and Subtropical Aquaculture
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