Unravelling the Mysteries of Francisellosis and Developing Strategies for Prevention and Mitigation in Cultured Tilapia

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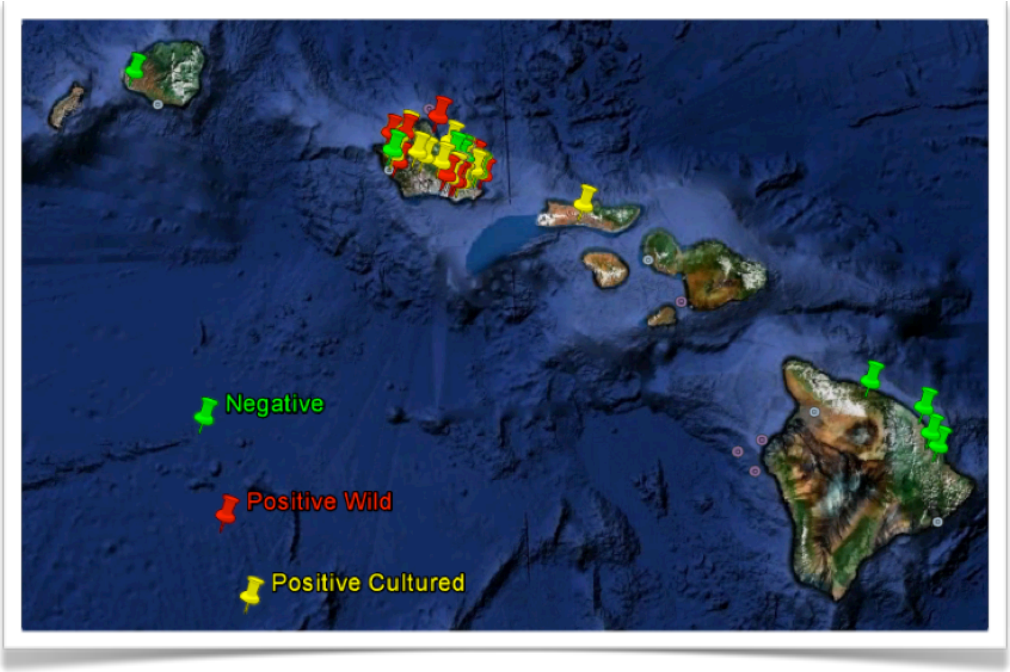
Under the auspices of the Center for Tropical and Subtropical Aquaculture (CTSA) supported projects since 2011, researchers at CTAHR have characterized a pathogen that infect cultured tilapia in Hawaii. This facultative intracellular bacteria is suspected to be responsible for outbreaks of diseases in cultured and feral tilapia species since 1994, and continues to plague farmers and backyard aquaponic practitioners, particularly in the cooler winter months. This organism has been cited in research with many different names – Rickettsia-like organism (RLO), Tilapia rickettsia-like organism (TRLO), and Francisella-like bacteria (FLB). Recent molecular analyses have led to the identification of *Francisella noatunensis* subsp. *orientalis* (syn. *F. asiatica*) or *Fno* (Colquhoun and Duodu, 2011). *Francisella* spp. has been implicated as the causative agent for mortalities in several tilapia species (e.g., *Orechromis* spp., and *Saratherodon* spp) in Hawaii, the continental United States, Taiwan, Latin America and Japan (Hsieh, et al., 2006; Fujita, et al., 2008; Soto, et al., 2009; Soto et al., 2011; Soto, et al., 2013) . Based on intensive PCR testing for *Fno* in Hawaii (Tamaru, et al., 2011), feral and cultured fish have yielded substantial findings impacting Hawaii's tilapia production. Information about the pathogen was generated, such as the existence of asymptomatic carriers – individuals that carry the *Fno* DNA in the spleens for over a year, without showing clinical disease signs. (Tamaru, et al., 2011b).

The major impact of the initial project was a heightened awareness of the *Fno* pathogen among stakeholders both at the commercial scale and with urban gardeners. To follow through, an epidemiology study of *Fno* would provide details of incidence and distribution of the pathogen in Hawaii. Additionally, this research formed the basis for establishing *Fno*-free centers for various cultured tilapia species in the state. Specifically, the CTSA-funded project work group examined these objectives:

- What is the prevalence of *Fno* in cultured and feral tilapia stocks around Oahu and the other Hawaiian Islands?
- What is the distribution of *Fno* in non-tilapia species, in culture and in the wild?
- Is there a way to stop the spread of the disease by removing the diseased individuals from the system?
- Establish *Fno*-free centers for three species of tilapia on Oahu – *Oreochromis mossambicus, O. aureas*, and *O. honorum*.

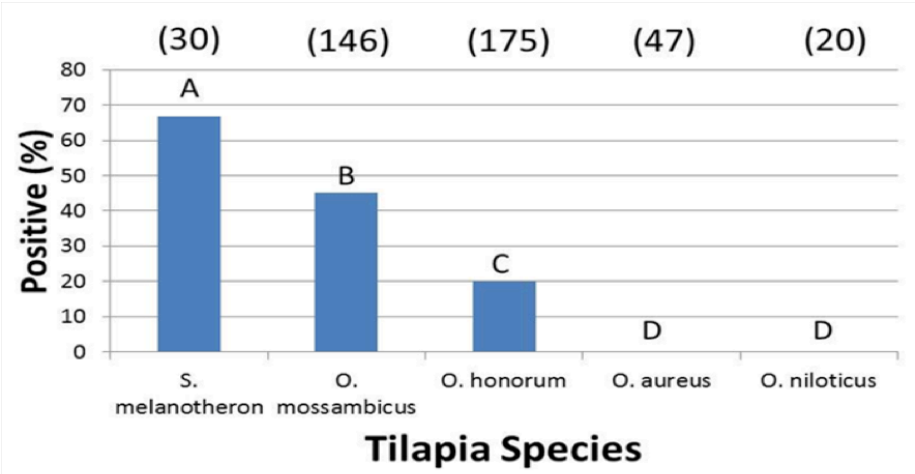
Figure 1. Distribution of positive and negative cases of Fno 2010 to present.



Distribution in Hawaii's Tilapia

Fno has been causing disease outbreaks in warm water species worldwide (Birkbeck et al., 2011; Colquhoun and Duodu, 2011). Sampling of cultured and /or feral tilapia around Oahu and the other main islands revealed the majority of positive *Fno*-DNA fish were from Oahu (Figure 1). The exception was one positive cultured tilapia sample on Molokai. Based on this initial data, *Fno* appears to be confined to Oahu. All public reports of *Fno* outbreaks, only occurring on Oahu since 1994, collaborate with this finding. In addition, at least three tilapia species (cultured *O. mossambicus*, *O. honorum*, and feral *Saratheodon melanotheron*) have been found to possess *Fno* DNA with varying degrees of prevalence (range 20% - 67%) on Oahu (Figure 2).

Figure 2. Distribution of Fno among Tilapia Species.



Distribution in Other Species

Eleven introduced and endemic fish species were sampled around Oahu for detection of *Fno*-DNA (Table 1). All specimens were cohabiting with *Fno* positive individuals with the exception of *Pangasius sutchi*. This fish was obtained from a farm that experienced an *Fno* outbreak but was being cultured in tanks isolated from the infection. The positive *Clarias fuscus* result were obtained from fish originating in Hawaii but analyzed in a separate investigation. This data allowed detection of *Fno*-DNA in a feral barracuda, and a formalin-fixed paraffin block tissue of a cultured Chinese Catfish (Soto, et al, 2012). However, caution is advised upon interpretation of this data as these samples were analyzed with PCR methodology that detects the *Fno*-DNA. It does not imply these animals are infective to other species, which would require isolation of the infective organism by bacterial culture techniques.

Scientific name	Common Name	Source	N	PCR Result
Chelon engeli	White mullet	Introduced	12	Negative
Mugil cephalus	Striped mullet	Native	12	Negative
Polydactylus sexfilis	Moi	Native	12	Negative
Kuhlia sandvicensis	Aholehole	Native	12	Negative
Sphyraena barracuda	Barracuda	Native	4	Positive*
Poecilia sphenops	Liberty mollie	Introduced	10	Negative
Amphilophus citrinellus	Midas cichlid	Introduced	12	Negative
Amphilophus labiatus	Red devil	Introduced	12	Negative
Gambusia affinis	Mosquito fish	Introduced	10	Negative
Clarias fuscus	Chinese catfish	Introduced	5/7	Positive **
Pangasius sutchi	Tuna catfish	Introduced	1	Negative

Table 1. Fish species targeted for PCR analyses to detect Fno

*4 individuals pooled into one vial for PCR

**Soto, et al., 2012

Remediation

The basic information discussed here and in our publications (Tamaru, et al., 2011) provides a means to mediate the disease before, during and after an outbreak. A case in point is the work by Co-PI Dr. Soto, who demonstrated that both temperature and salinity are important factors in the persistence of *Fno* in both sea- and freshwater environments. The results indicated that *Fno* can persist for longer periods of time and at higher numbers in seawater with its persis-

tence is inversely related to water temperature. Moreover, the pathogenic properties of the bacteria in the water environment decreases after only 24 h and becomes non-infective after 2 days in the absence of the fish host (Soto and Revan, 2012). This finding was tested in a backyard aquaponic system that experienced an *Fno* outbreak. Conventional wisdom on how to disinfect the system would be to remove the plants, euthanize the remaining fish, chlorinate the solid media grow beds and fish tank and start anew. This is a major undertaking for a backyard system and a significant financial burden in a commercial system, so alternative means in dealing with an outbreak are desirable. Working collaboratively with the backyard aquaponic producer the remaining fish were euthanized and represented the only thing that was done to the system. Plants were maintained and the water continued to be recirculated for two weeks after which naïve fish were restocked into the system.

According to the published results the time period should be more than sufficient to render *Fno* non-infective. Fish were monitored over the course of eight months using conventional PCR and no individuals tested positive over the course of the sampling period (Figure 3). Fish continued to be monitored from April to November with no signs of *Fno* recurring, indicating a possible means of mitigating the pathogen.

In freshwater aquaponic systems, high temperature (i.e. above 30ºC), chlorination, and chemicals are significant influences in biofilm formation, which would allow *Fno* to persist even after the fish are removed. Therefore, we recommend if an *Fno* outbreak occurs in your system, certify your system's biofilm has been disrupted, even after the two week fallowing period. This

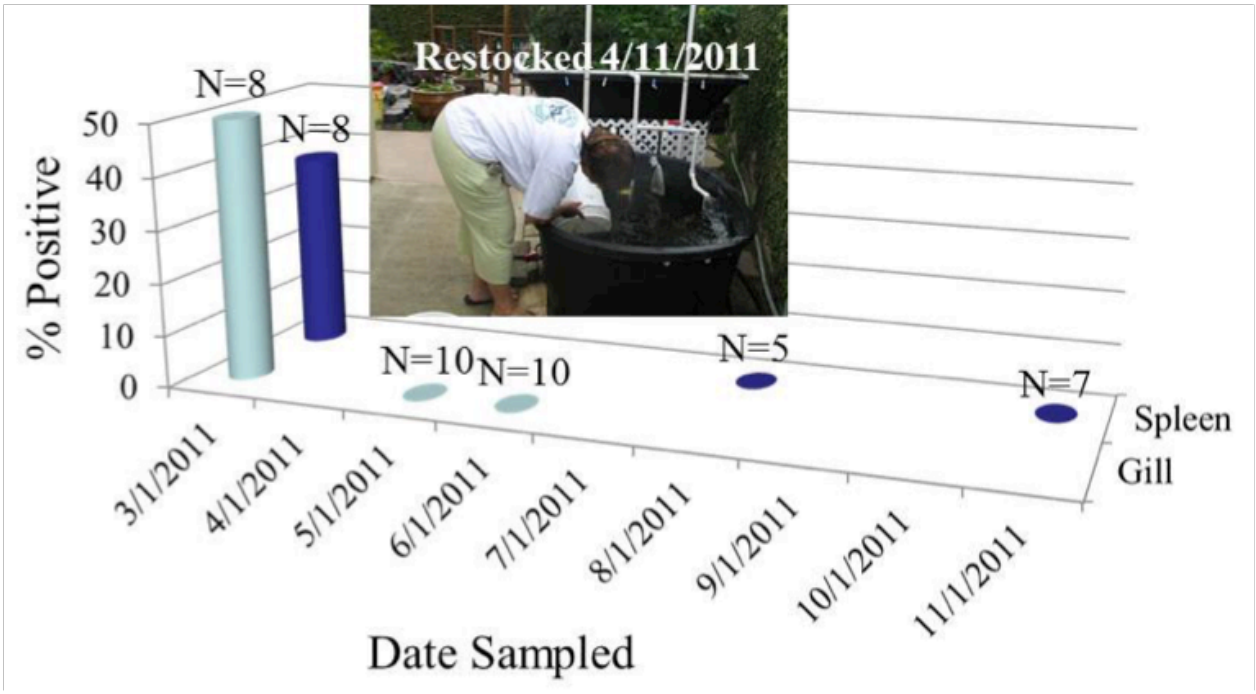


Figure 3. Temporal change in Fno DNA positive and negative individuals in a backyard aquaponic system after removal of infected stock and restocking with naïve individuals.

can be accomplished by raising the temperature above 30°C, chlorination (city water flush), or adding hydrogen peroxide.

Fno-free Centers

Transporting tilapia between Oahu and the rest of the Hawaiian Islands is currently restricted (PQ Policy 98-09 Section 150A-8, HRS- November 5, 1998) and the corresponding benefits are supported by our results. However, the current restriction in movement of tilapia stocks has and will continue to hamper tilapia aquaculture in the islands and clearly designing a means in which tilapia stocks can be rid of and remain *Fno* pathogen free became a high priority for our project work group.

Through previous studies on Oahu, the most common means of dispersing the *Fno* bacteria and creating disease outbreaks was the purchase and/or sharing of carriers among aquaculture and aquaponic stakeholders on Oahu. To curtail this activity, we asked volunteers from the aquaculture and aquaponic community to participate in the testing of their farms in hopes of becoming *Fno*-free centers. This entailed sampling their farm's tilapia species on site and would follow the Specific Pathogen Free (SPF) protocol that was established by the Hawaii State Shrimp Surveillance Program. Volunteers would be required to:

- have at least one of the three targeted tilapia species on site
- allow third party sampling (including euthanizing and dissection for spleen tissue)
- allow sampling every six months of each species' populations
- permit exposure via publications/workshops if their site was classified as *Fno*-free after two years

Testing was balanced with the costs of the PCR analysis and perceived market demand and return on investment. In order to insure quality assurance in the sampling process, the project work group members were responsible for obtaining samples and processing them for conventional PCR testing. This activity was part of CTAHR's aquaculture extension and outreach program already implemented and part of the college's land grant mission. All results from samples collected by project personnel were submitted to Dr. Jim Brock of Moana Technologies, LLC, Aiea, Hawaii for conventional PCR testing. Results of the PCR testing was reported simultaneously to the PI and state aquatic veterinarian Dr. Allen Riggs (since 2013, Dr. Lei Yamasaki). The State Department of Agriculture is monitoring all positive results as this is an emerging pathogen of interest. In short, there is an official public record of the testing and test results for each sample processed by project personnel. Each client that has samples tested will receive a written communication of the test result and can be used as documentation of testing and test results as needed.

To date, Hapa Farms in Kaneohe (www.hapafarmshawaii.com) and B&B Nursery (in Waianae) have gone through the protocols with *Fno* testing of their tilapia stocks. After two years of sampling, both farms remain *Fno*-free. The *O. aureas* and *O. honorum* raised at Hapa Farms are listed as *Fno*-free on their website to gain momentum as a value added product. The *O. mos-*

sambicus raised by B&B Nursery has also been *Fno* negative for two years, and remain the only known *Fno*-free source of this species on Oahu. Since then, we've gotten many inquiries of where to get *Fno*-free stock, as well as farms wanting to go through the 2 year requirement testing. The Hawaii State Animal Diagnostic Laboratory in Aiea is presently the third party sampling agency, with Moana Technologies performing the PCR testing.

Summary

With the increased interest in backyard and commercial aquaponics, stakeholders have become increasingly aware of *Fno* and the necessary biosecurity measures to become *Fno*-free. This CTSA funded research on *Fno* became one of the ten most cited articles in the Journal of Aquatic Animal Health in 2013-2014 (Soto, et.al, 2013). The distribution of the *Fno* pathogen that plagues cultured tilapia on Oahu can possibly be brought under control. This will require the collaboration of all stakeholders, as the main means of pathogen distribution is through the purchase and/or trading of infected stocks. This work is preliminary as it details distribution of *Fno* DNA with PCR methodology; however, the status of infectivity (through isolation of the organism) and its prevalence with other species has yet to be determined. Therefore, we encourage implementation of best management practices, such as covering your fish tanks to prevent possible contamination by birds and other wildlife and not receive *Fno* or unknown *Fno* infected fish in your systems.

Article content is the sole responsibility of the authors. For more information about this article, contact Ruthellen Klinger-Bowen, email: rckb@hawaii.edu, or Clyde Tamaru, email: ctamaru@hawaii.edu

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