# Update on Francisellosis in Tilapia in Hawaii

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- Mitigating The Diseases Of Freshwater Cultured Fish Species In Hawaii And The Pacific Region
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### Aknowledgements

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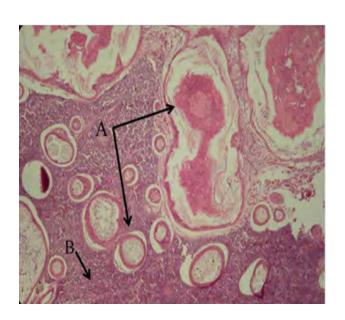


### Rationale for Project

- In 1994, wild and farm-raised tilapia die of an unknown disease.
- Department of Agriculture issues PQ policy 98-09, Section 150A-8, HRS effective November 5, 1998

Enlarged spleen with granulomas typical of an active infection





Histological section of spleen with granulomas (A) and normal spleen tissue (B). Photo courtesy of Dr. Juan Morales, Universidad Nacional of Costa Rica



What is the first thing that comes to your mind when Tilapia is mentioned





# Rationale for Ongoing Research

Continued expansion and diversification of tilapia in Hawaii's aquaculture industry









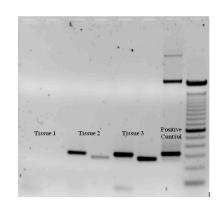
# Conventional Polymerase Chain Reaction (PCR)

And

Real Time PCR

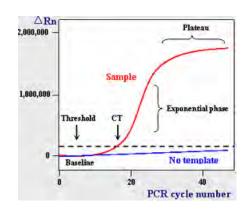








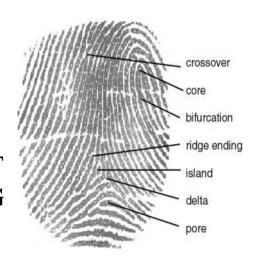




### DNA Sequence

#### <u>10-87 (+TRLO)</u>

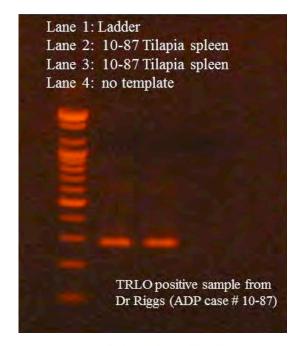
GGATCTACTGCGTTGGATAGCTAGTTGGTGGGGTAA GGGCCTACCAAGGCTACGATCCATAGCTGATTTGAG AGGATGATCAGCCACATTGGGACTGAGACACGGCC CAAACTCCTACGGGAGGCAGCAGTGGGGAATATTG GACAATGGGGGAAACCCTGATCCAGCAATGCCATGT GTGTGAAGAAGGCTCTAGGGTTGTAAAGCACTTTAG TTGGGGAGGAAAGCCTGTGAGTTATAGCTTGCAGG

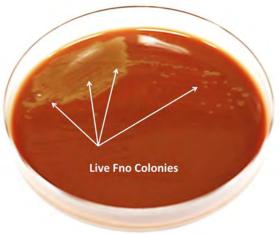


**B**asic Local Alignment Search Tool, BLAST Program Result = Francisella noatunensis subspecies orientalis 99%

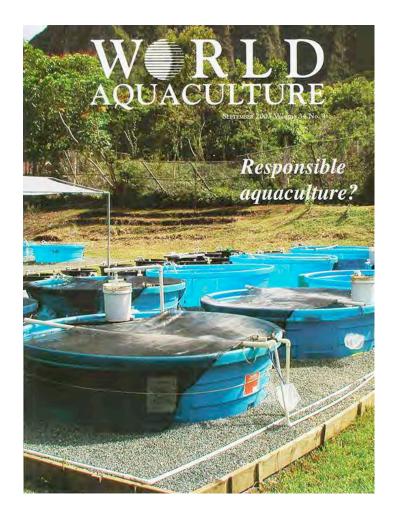
### Update

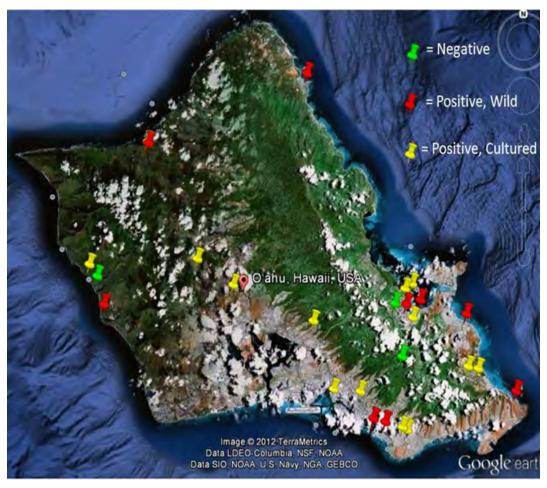
- The pathogen, Francisella noatunensis subsp. orientalis (syn. F. asiatica) or Fno
- Causative agent for mortalities in several tilapia species in Hawaii, the continental United States, Taiwan, Latin America and Japan.
- To avoid confusion RLO, TRLO, FLB and Fno are referring to the same pathogen.



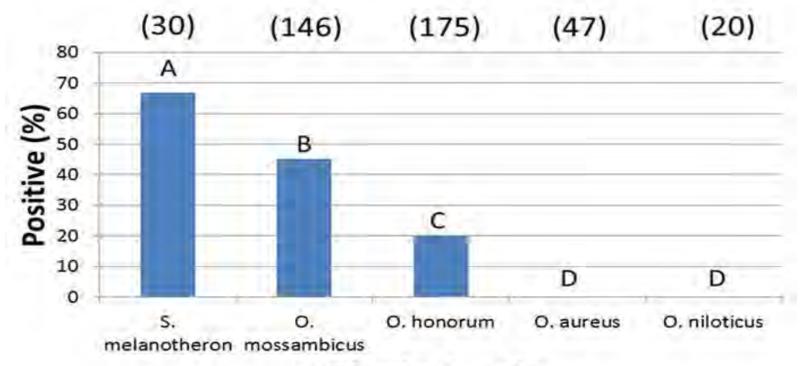


### Distribution of Positive Cases on Oahu Dec 2010 – Current





### Distribution of FLB Among Tilapia Species



#### **Tilapia Species**





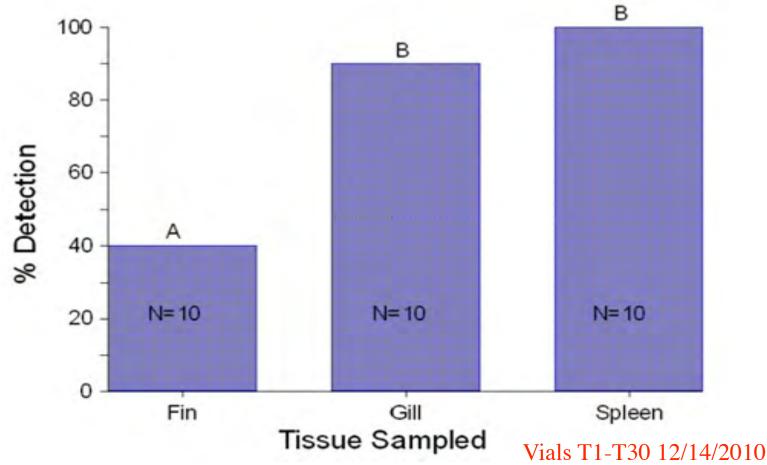




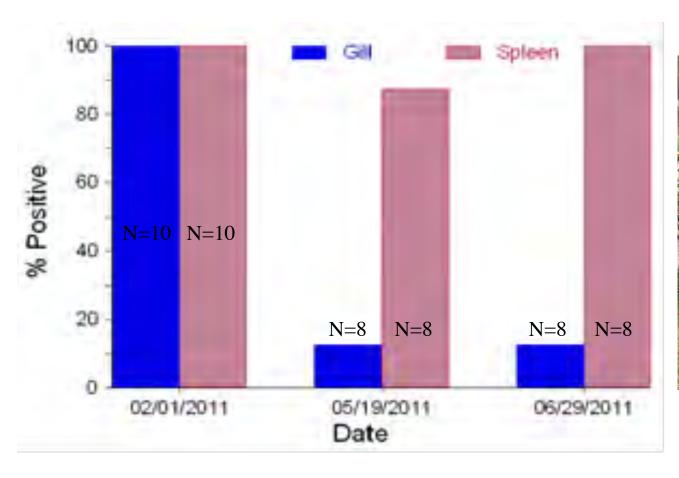


# Tissue distribution during an active Fno infection





## Change in Tissue Distribution of FLB-DNA During and After An Active Infection

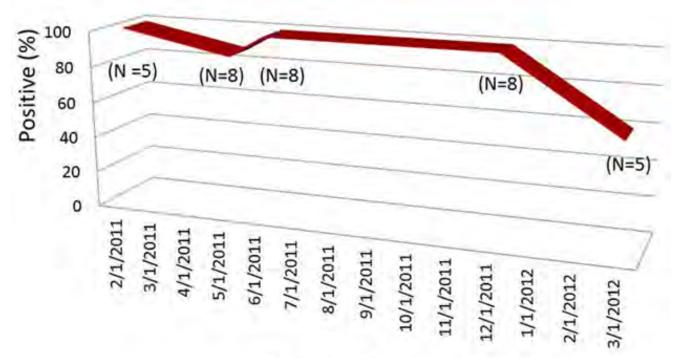




### Blue Tilapia Challenge Test



### Temporal Change in FLB-DNA Positive Individuals in a Tilapia Population





**Dates Sampled** 

### Blue Tilapia Challenge Test, Maris Garden (ongoing)





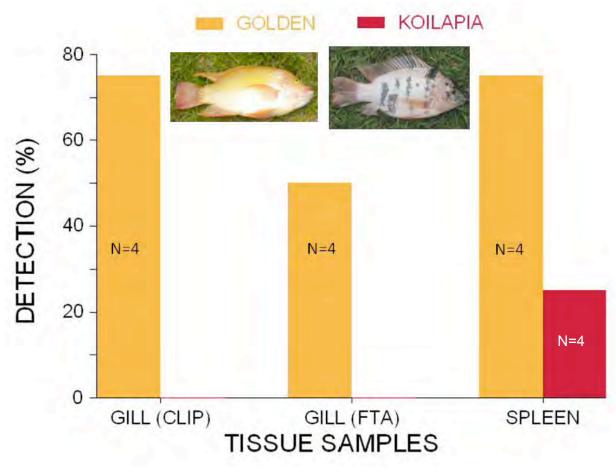




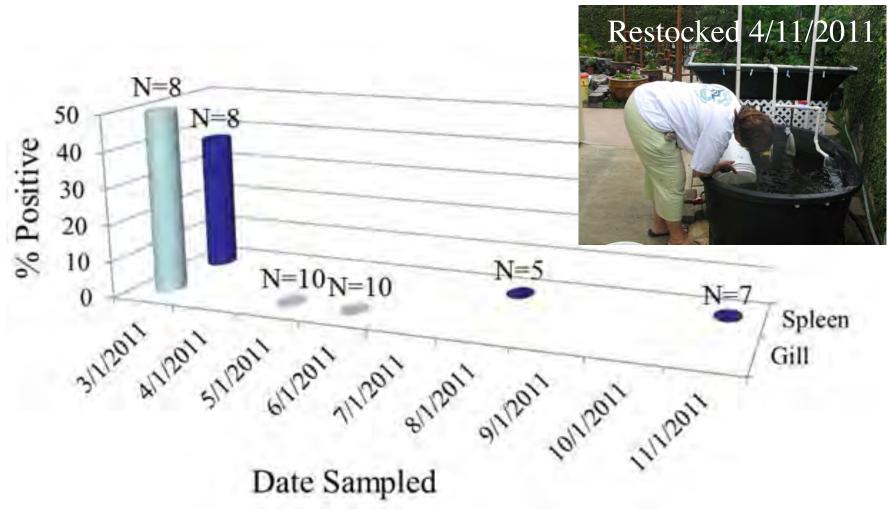
### Species and tissue distribution of FLB during an active infection







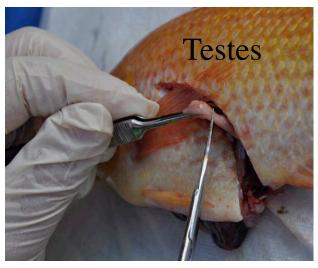
### Temporal Change in Positive Individuals for FLB-DNA After De-population and Restocking



#### Distribution of FLB-DNA among gonadal tissues







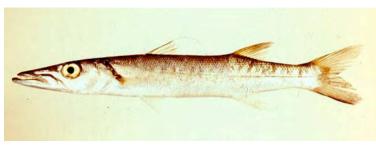
Individual	Spleen	Ovary
Female – 1	Positive	Negative
Female – 2	Positive	Negative
Female – 3	Negative	Negative
Female – 4	Negative	Negative
Female – 5	Negative	Negative

Individual	Spleen	Testes
Male – 1	Positive	Negative
Male – 2	Positive	Negative
Male – 3	Positive	Negative
Male – 4	Positive	Negative
Male – 5	Positive	Negative

Fry being mouth brooded by Female-1 negative for FLB



### Can other fishes have Fno?





Sphyraena barracuda

Clarias fuscus







### Summary

- Delineation of the pathogen as *Francisella noatunensis* subsp. *orientalis*.
- Demonstration of the existence of asymptomatic carriers of Fno-DNA that can persist for at least a year after a clinical outbreak.
- Other cultured and wild fish species can carry Fno DNA.
- Wild tilapia (S. melanotheron) populations around Oahu are positive ( $\approx 60\%$  prevalence) for Fno-DNA

### Summary - Continued

- Significant differences in prevalence of Fno among various tilapia species.
- Preliminary data suggest that Fno is not vertically transmitted.
- Depopulation and restocking of clean stocks maybe an effective means mitigating the disease
- Opportunities do exist to establish Fno-free centers



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