

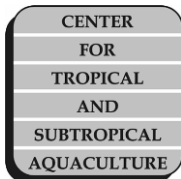


Update on Francisellosis in Tilapia in Hawaii

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Windward Community College





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Aknowledgements

- Shermiah Iaea
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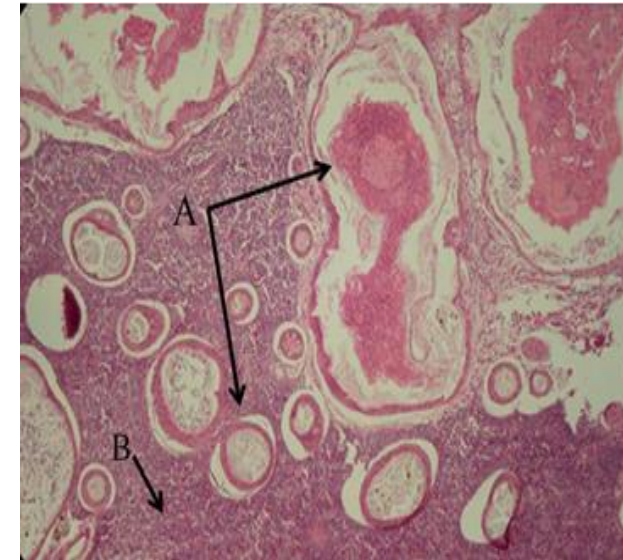




Rationale for Project

- 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

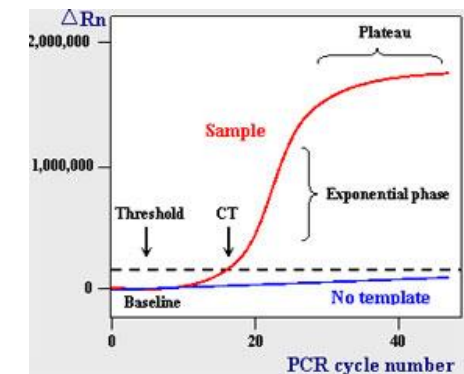
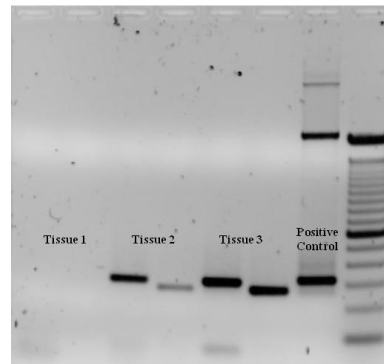
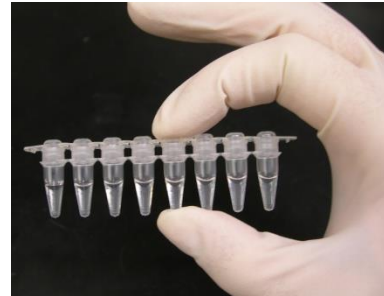




Conventional Polymerase Chain Reaction (PCR)

And

Real Time PCR





DNA Sequence

10-87 (+TRLO)

GGATCTACTGCGTTGGATAGCTAGTTGGTGGGGTAA
GGGCCTACCAAGGCTACGATCCATAGCTGATTTGAG
AGGATGATCAGCCACATTGGGACTGAGACACGGCC
CAAACCTCCTACGGGAGGCAGCAGTGGGGGAATATTG
GACAATGGGGGAAACCCTGATCCAGCAATGCCATG
TGTGTGAAGAAGGCTCTAGGGTTGTAAAGCACTTTA
GTTGGGGAGGAAAGCCTGTGAGTTATAGCTTGCAG
GAA

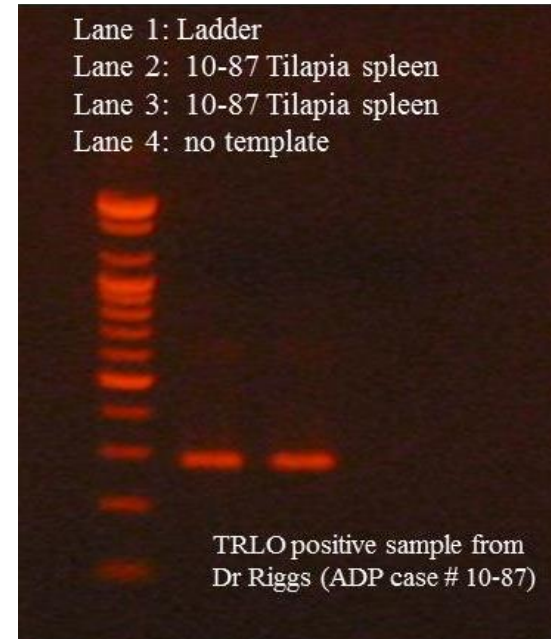


Basic Local Alignment Search Tool, BLAST
Program Result = *Francisella noatunensis*
subspecies *orientalis* 99%



Update

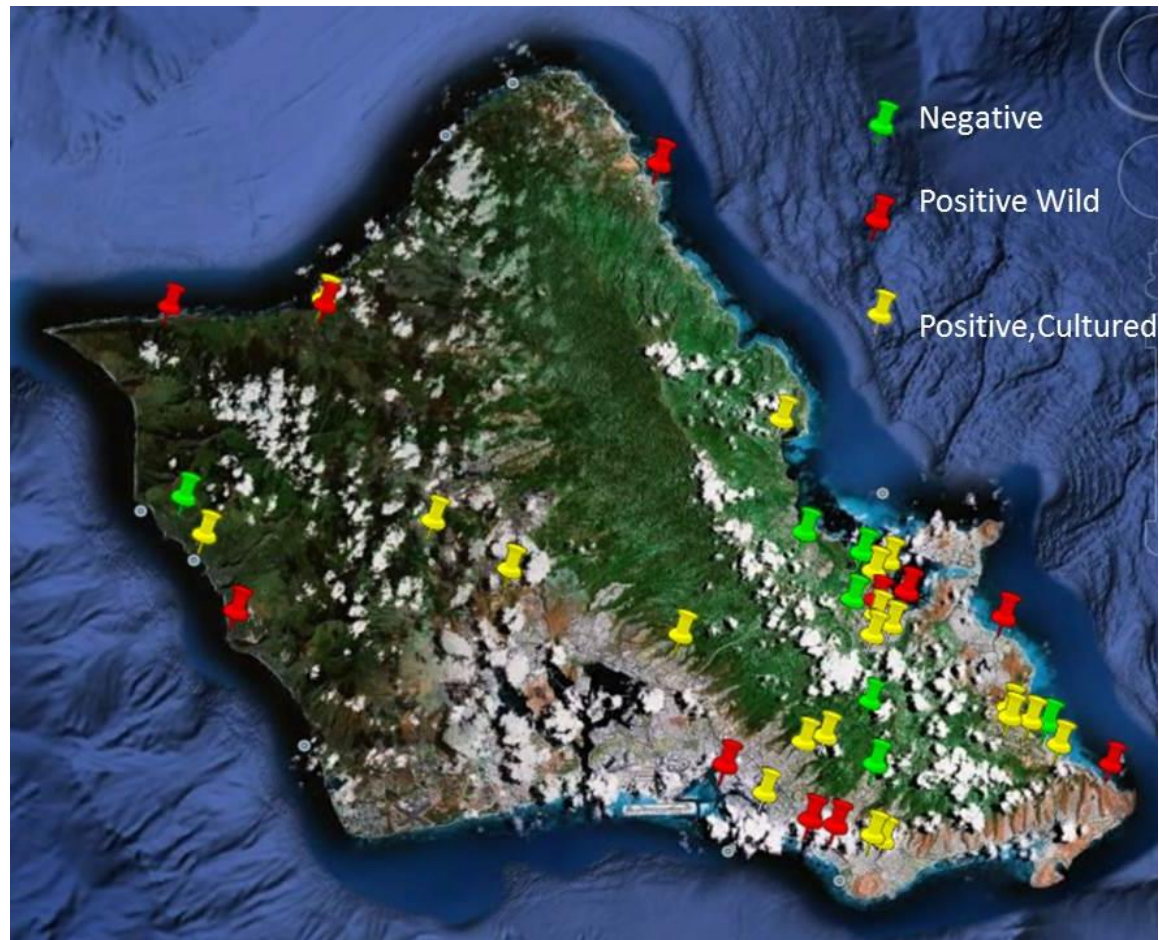
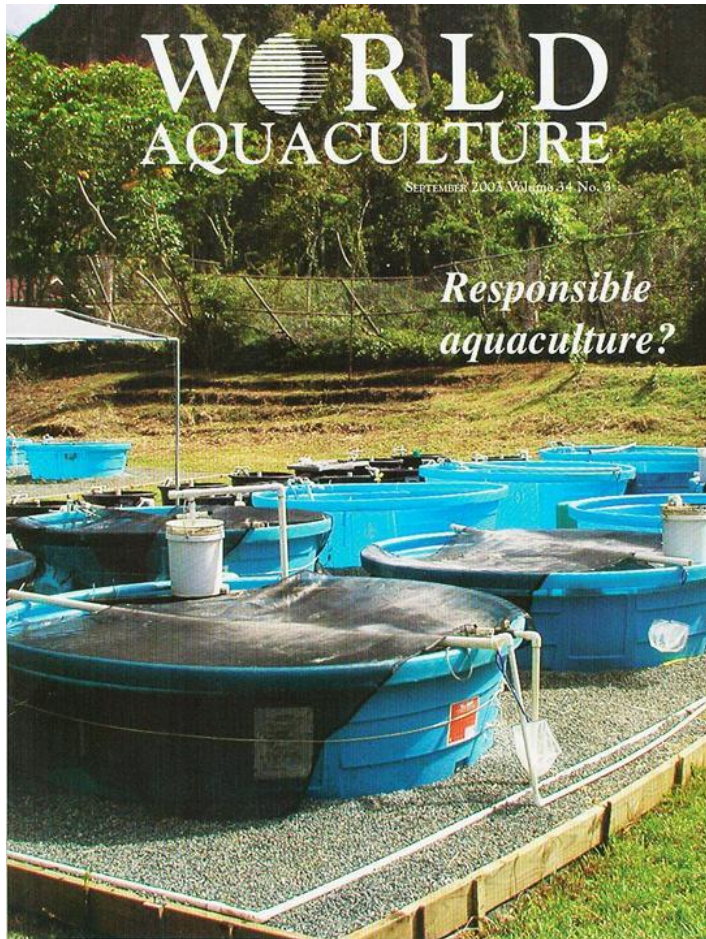
- The pathogen, *Francisella noatunensis* subsp. *orientalis* (syn. *F. asiatica*) or Fno
- Causative agent for mortalities in several tilapia species in Hawaii, the continental United States, Taiwan, Latin America and Japan.
- To avoid confusion RLO, TRLO, FLB and Fno are referring to the same pathogen.





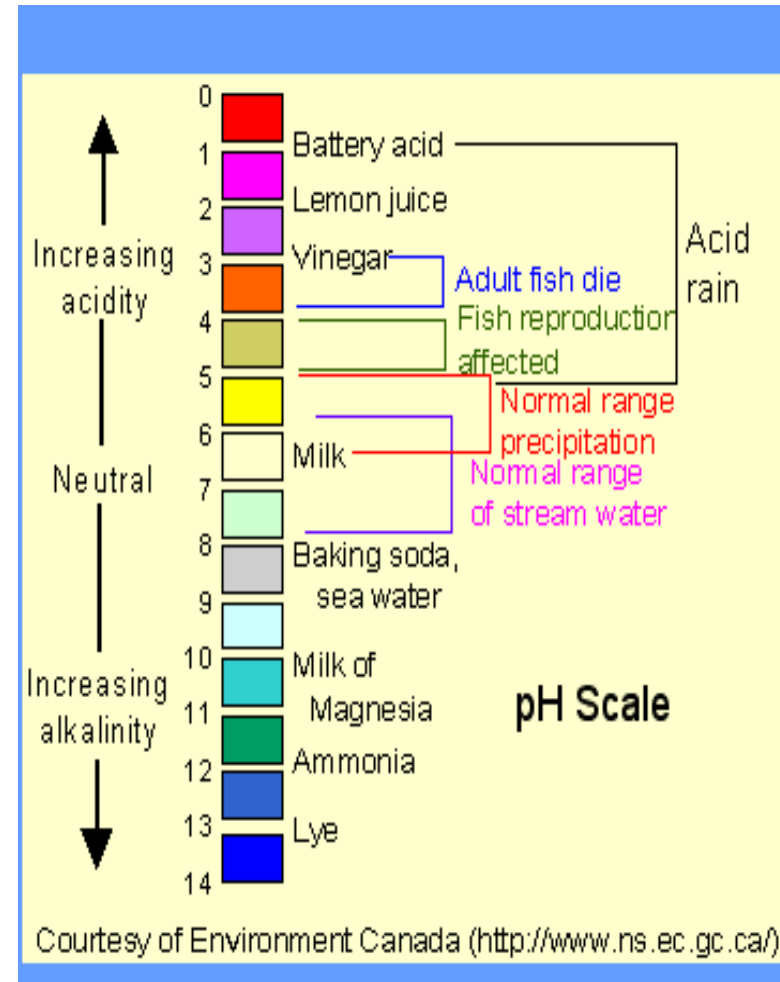
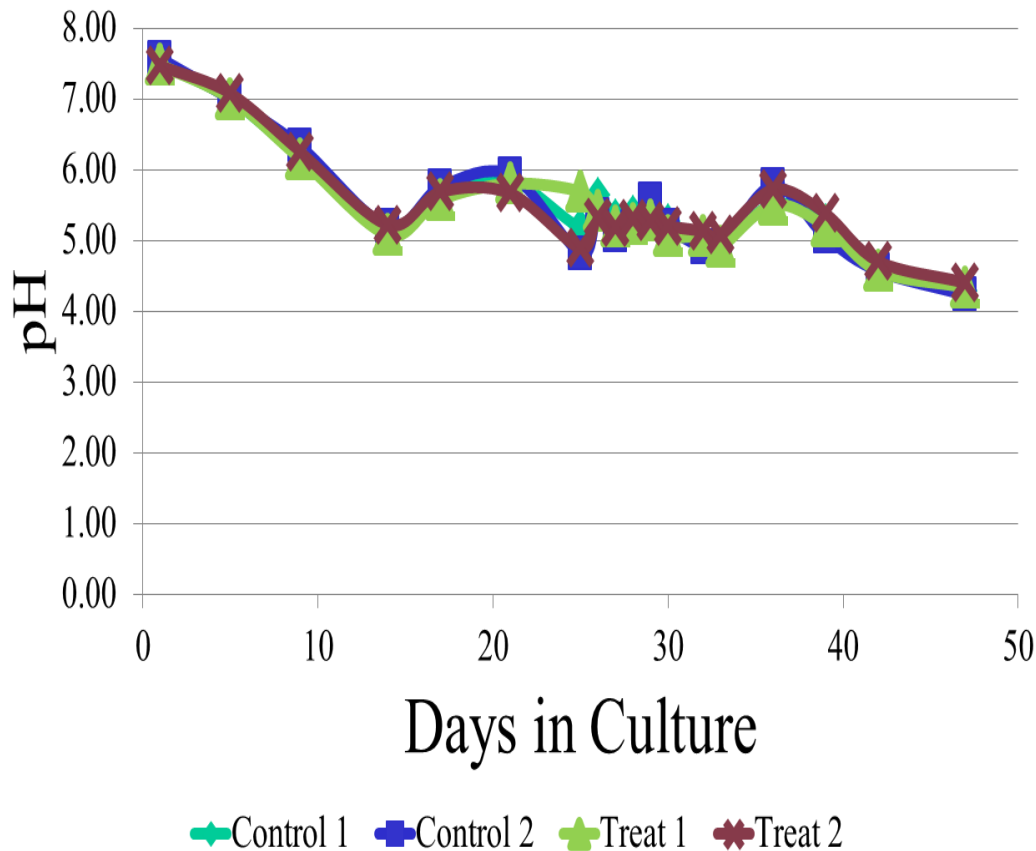
Distribution of Positive Cases on Oahu

Dec 2010 – Current



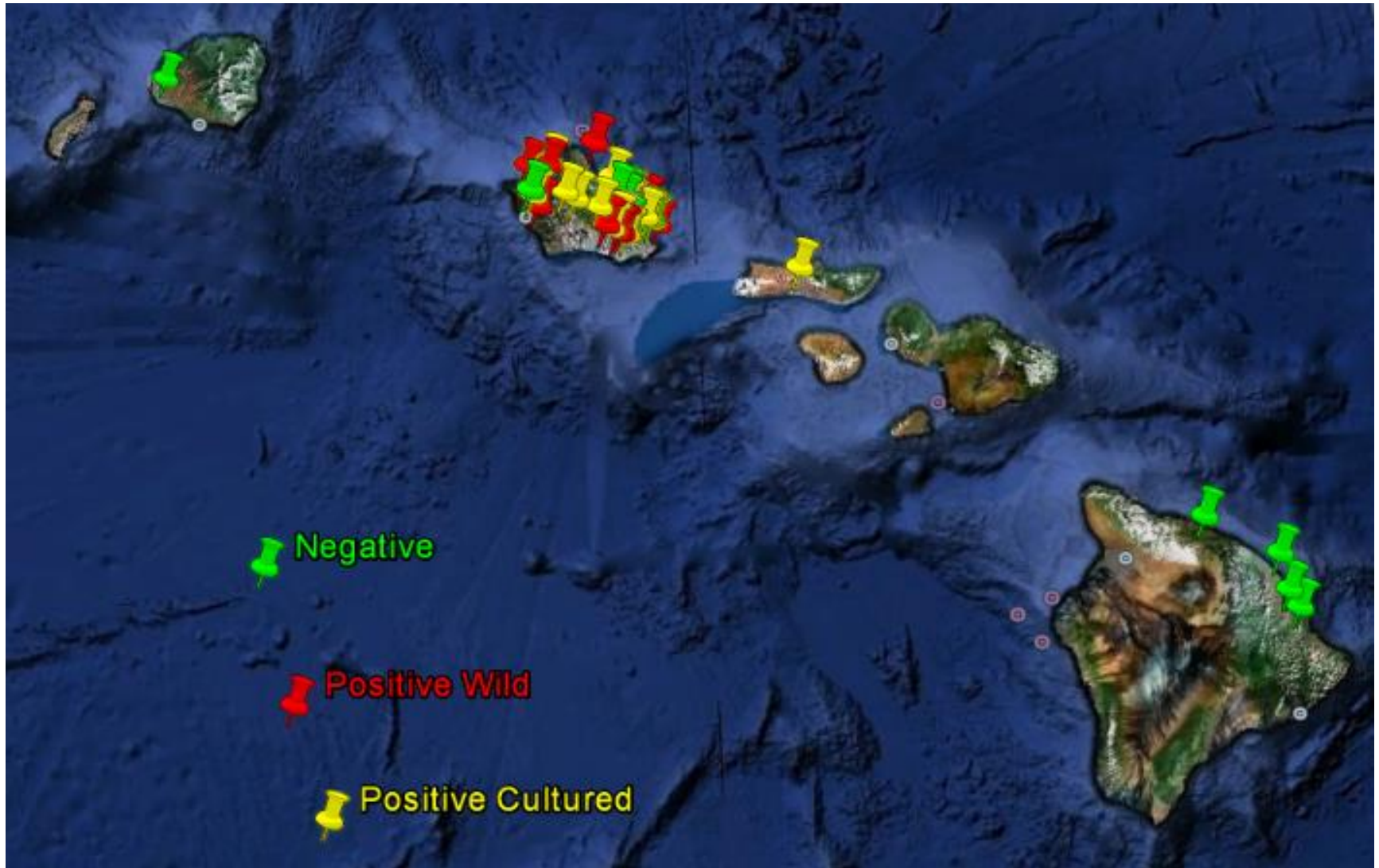


Temporal Change in pH Without Remediation



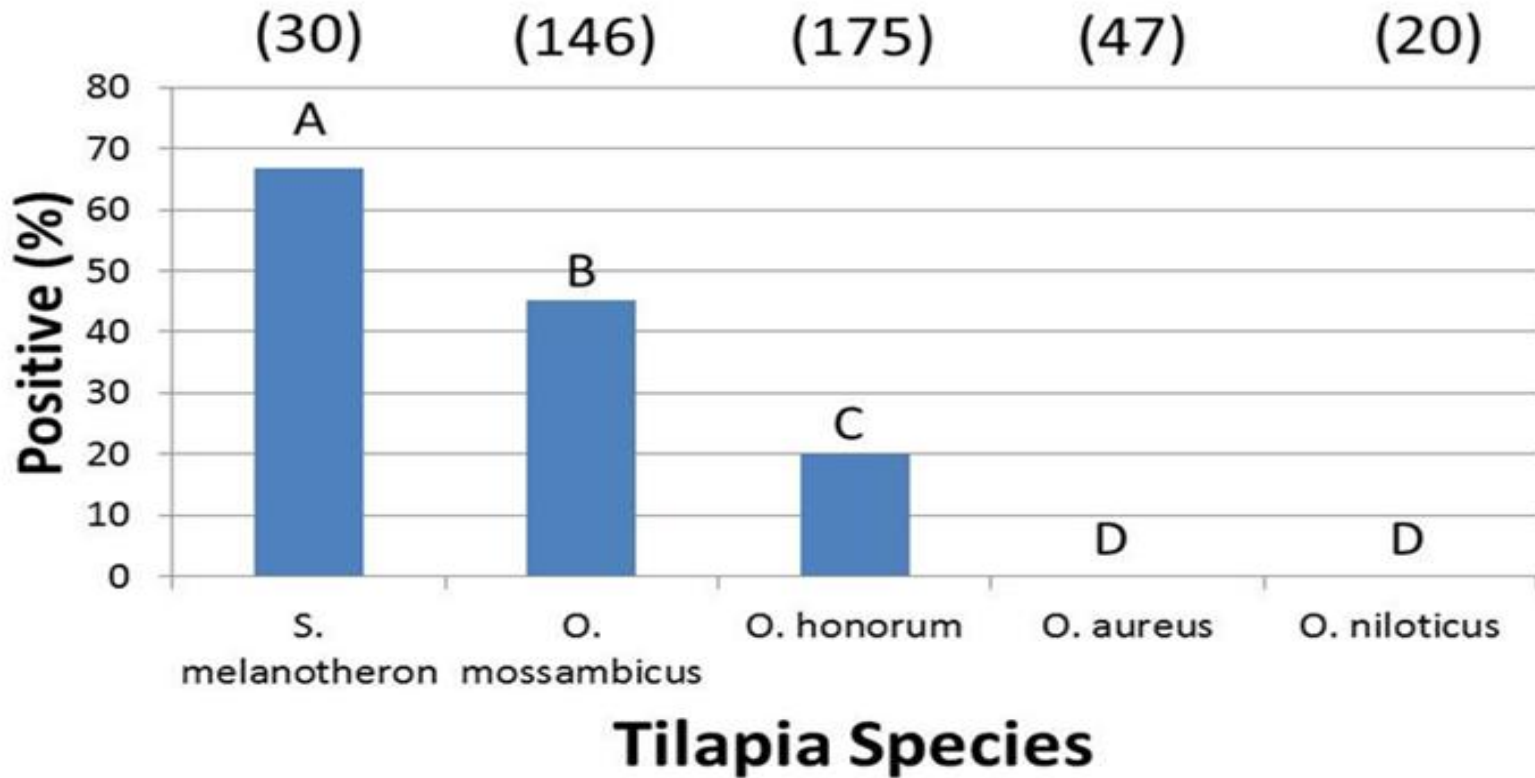


Distribution of Positive Cases in Hawaii Dec 2010 – Current



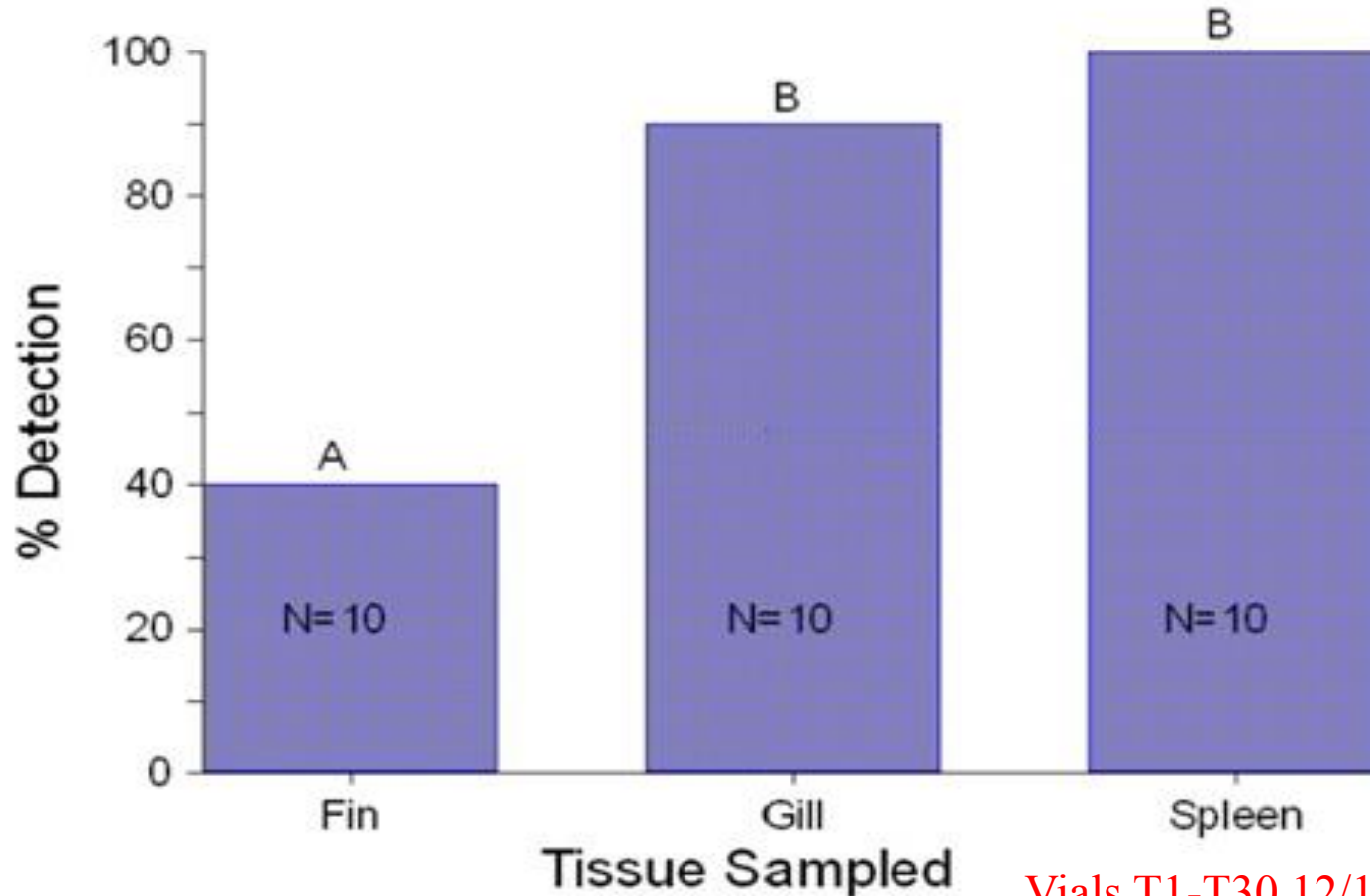


Distribution of Fno Among Tilapia Species



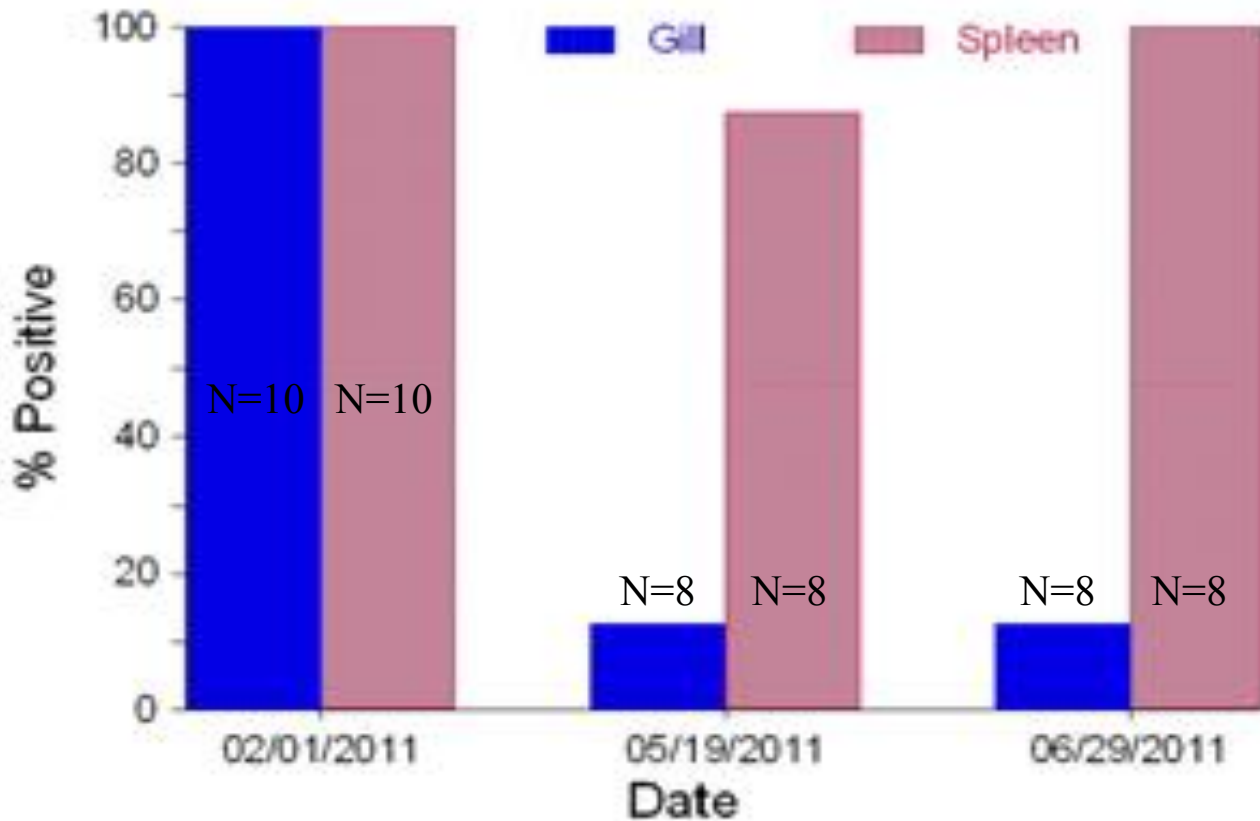


Tissue distribution during an active Fno infection



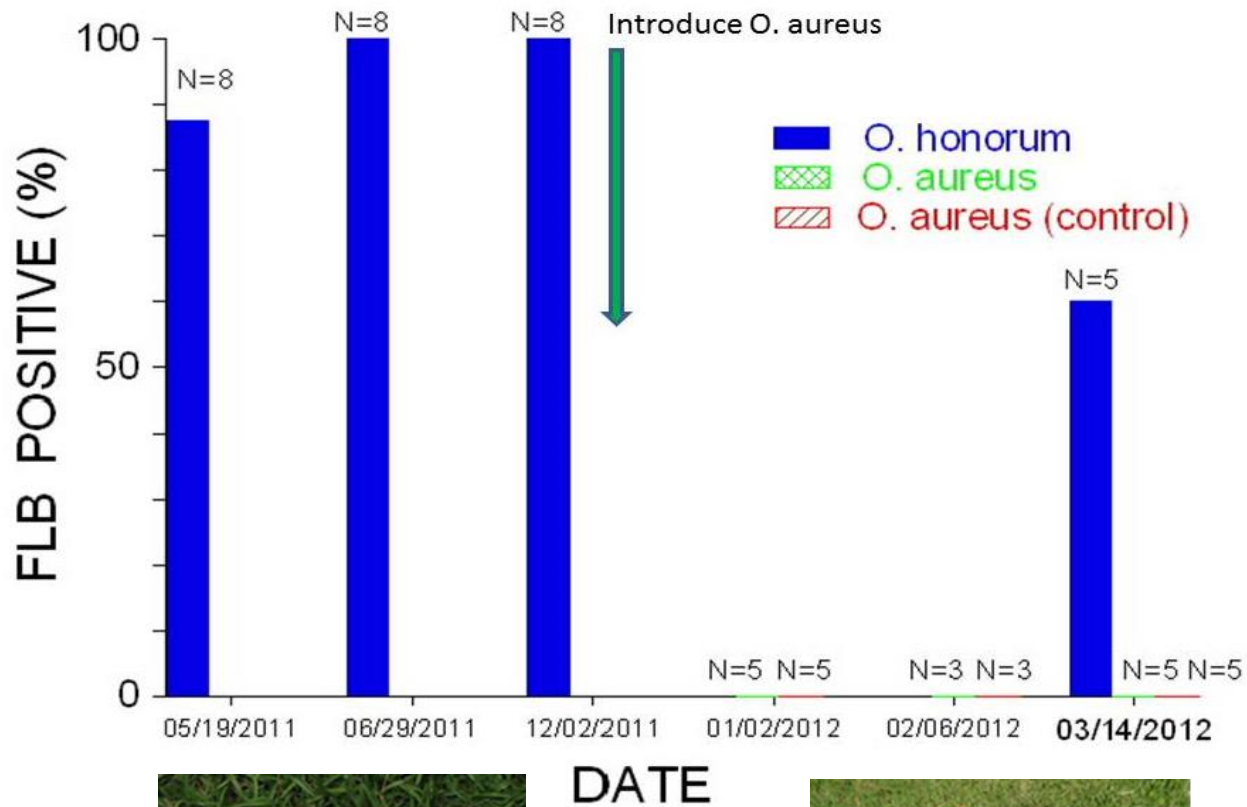


Change in Tissue Distribution of FLB-DNA During and After An Active Infection



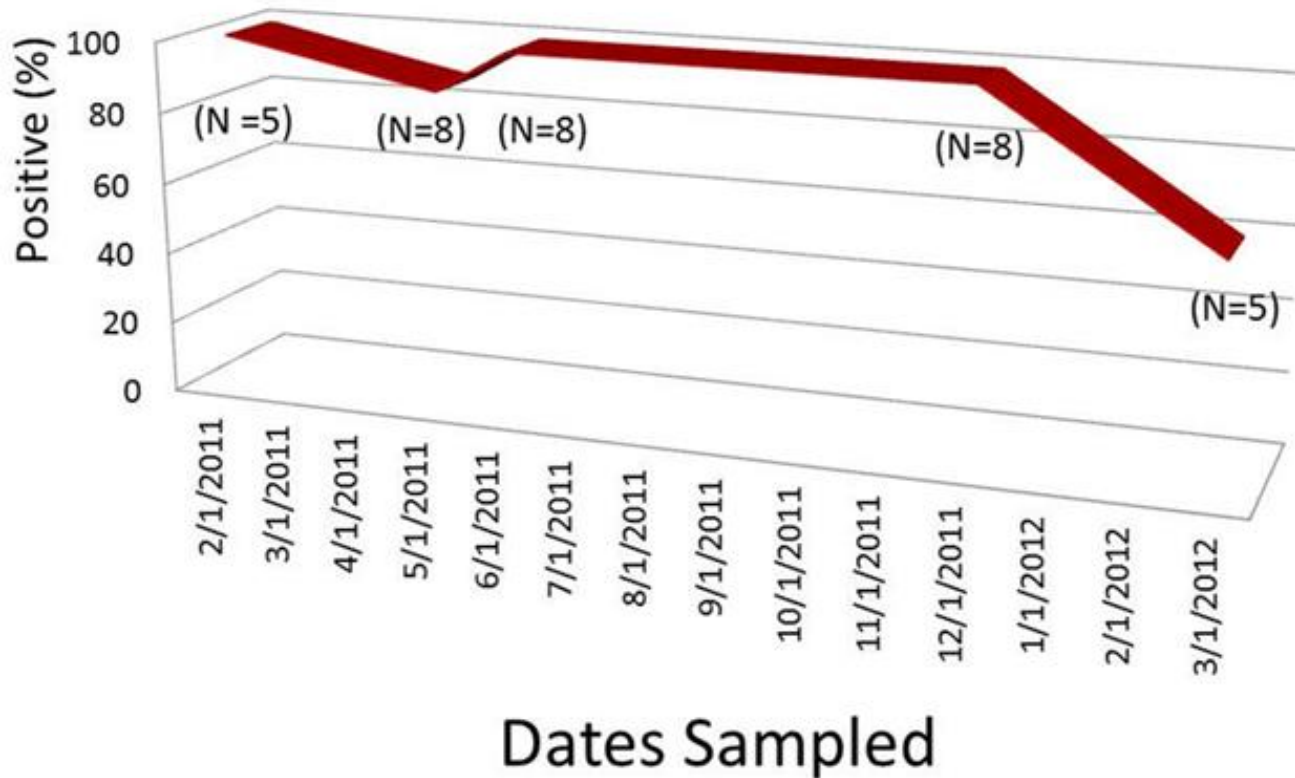


Blue Tilapia Challenge Test





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





Blue Tilapia Challenge Test, Maris Garden

May 3, 2012 RW8 Fno Outbreak

June 9, 2012 215 *O. aureus* Stocked Fno (-)

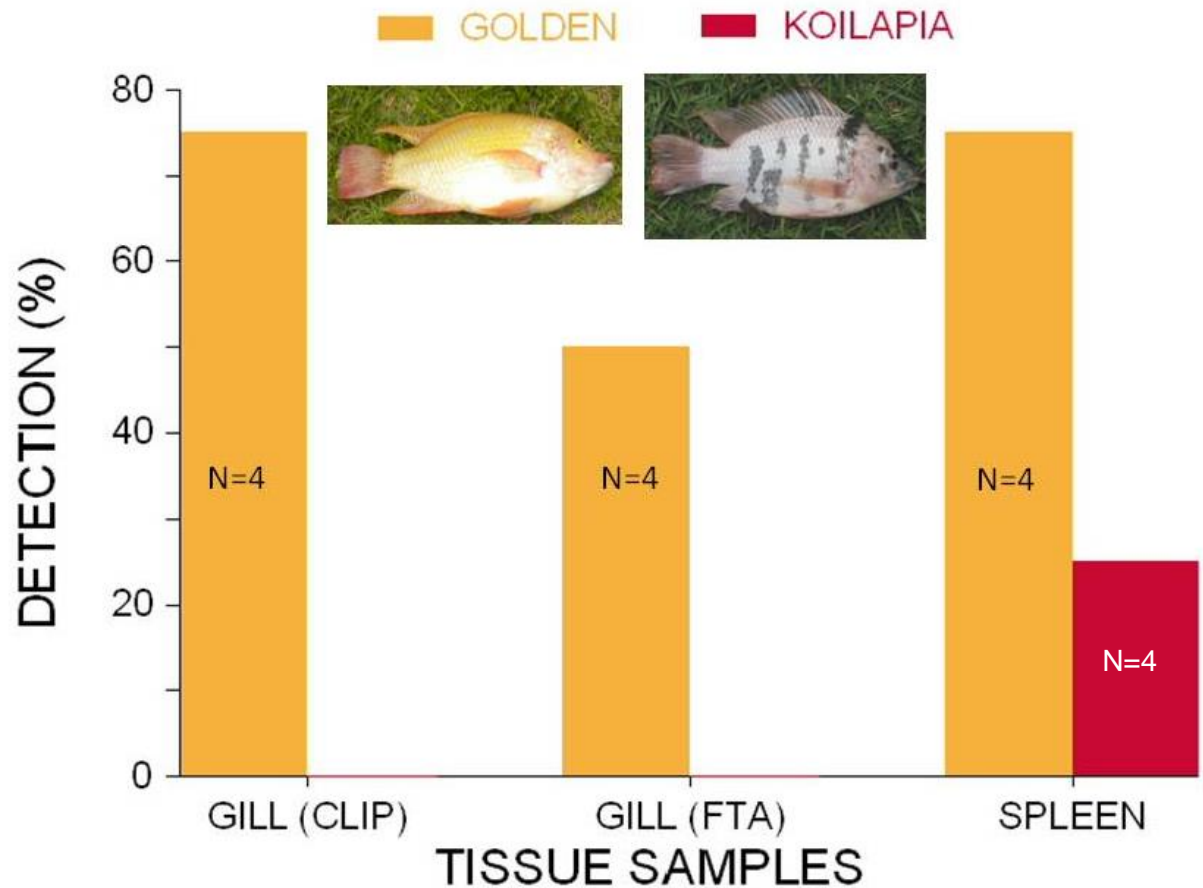
Sept. 19, 2012 *O. aureus* Fno (+) No mortalities

Feb. 15, 2013 *O. aureus* No mortalities



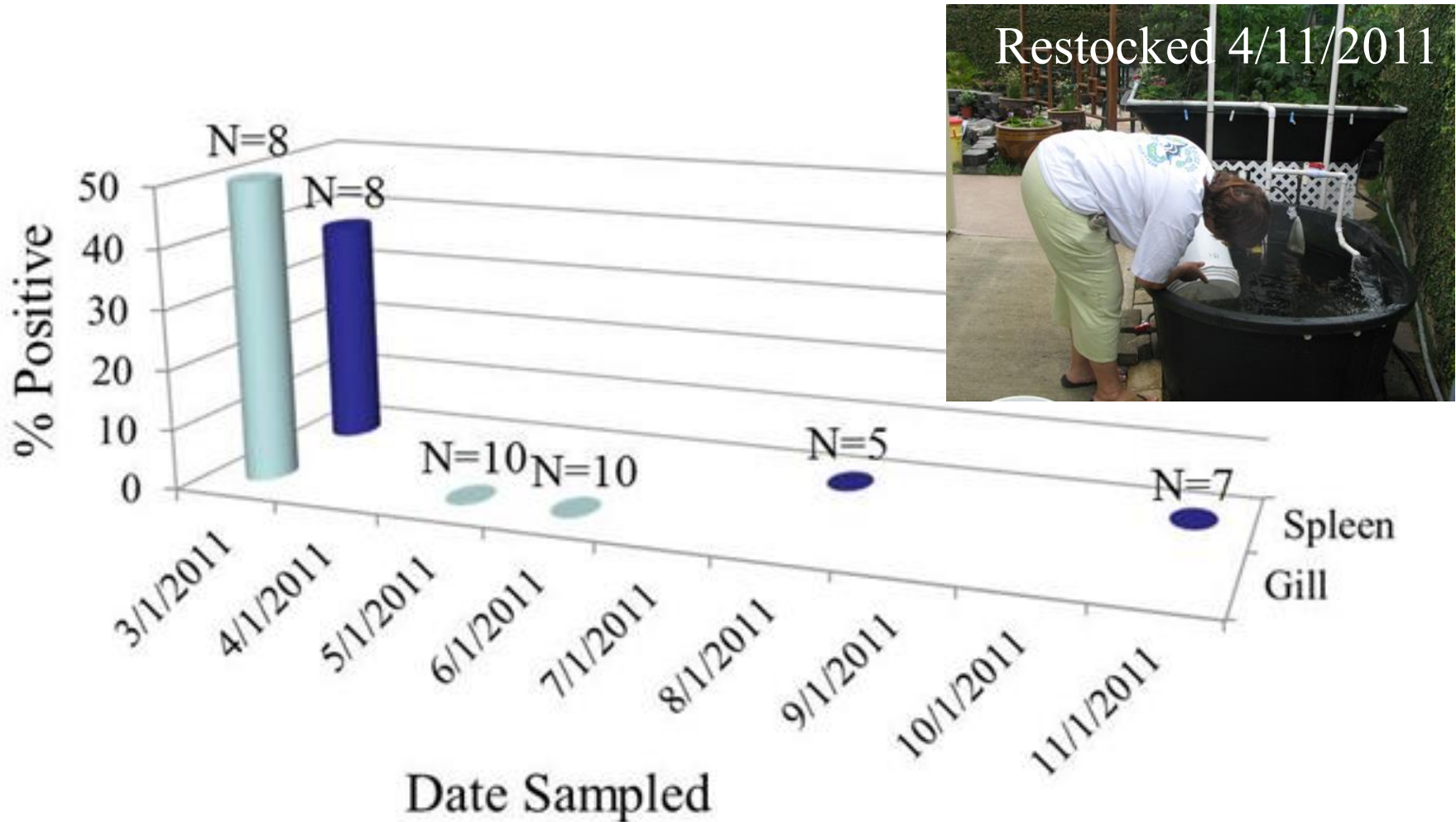


Species and tissue distribution of FLB during an active infection





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



Distribution of Fno-DNA among gonadal tissues



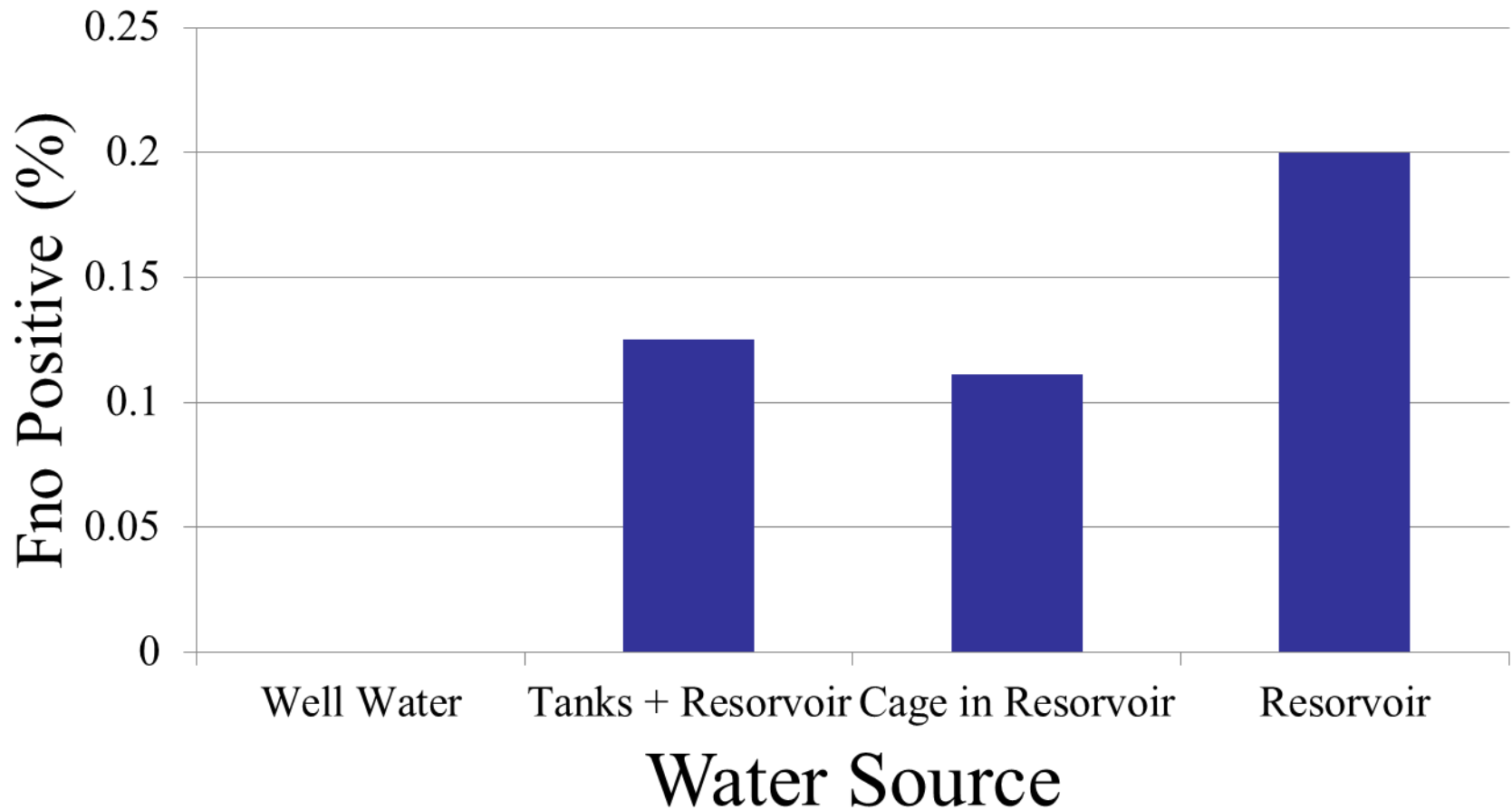
Individual	Spleen	Ovary
Female – 1	Positive	Negative
Female – 2	Positive	Negative
Female – 3	Negative	Negative
Female – 4	Negative	Negative
Female – 5	Negative	Negative

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



Comparing Sources of Culture Water





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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