

An evaluation of the effectiveness of various florfenicol treatment regimens to control mortality caused by *Streptococcus iniae* in hybrid striped bass

Year 2 Progress/Termination Report

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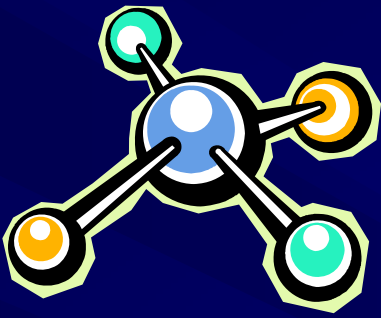
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WRAC Florfenicol Project

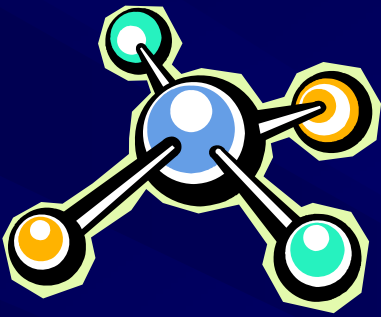
- Evaluate alternative treatment regimens to determine whether the standard dose is most effective for controlling mortality in hybrid striped bass (HSB) caused by *S. iniae*
- Conduct preliminary studies using naïve HSB experimentally infected with *S. iniae*
- Confirm findings in studies using production HSB naturally infected with *S. iniae*





Study Objectives

1. **Develop a *S. iniae* disease challenge model suitable for testing the effectiveness of florfenicol on HSB**
2. **Evaluate the effectiveness of 10, 15, and 20 mg drug/kg fish/d for 10 d on HSB challenged with *S. iniae***
3. **Evaluate the effectiveness of most effective dose for 10, 15, and 20 d on HSB challenged with *S. iniae***
4. **Demonstrate the effectiveness of the most efficacious treatment regimen to control mortality in HSB naturally infected with *S. iniae***



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4. **Demonstrate the effectiveness of the most efficacious treatment regimen to control mortality in HSB naturally infected with *S. iniae***

Trials completed through Yr 2

➤ **Objective #1**

- **Trial 1 – Injection challenge model development – Feb 2007**
- **Trial 2 – Immersion challenge model development – Feb 2007**
- **Trial 3 – Immersion challenge model refinement – Sept 2007**

➤ **Objective #2**

- **Trial 4 – Florfenicol trial – various doses – Nov 2007**
- **Trial 5 – Florfenicol trial – various doses – Jan 2008**
- **Trial 6 – Immersion challenge model – refinement – Aug 2008**
- **Trial 7 – Florfenicol trial – various doses – Sept 2008**

Year 1 Progress Summary

- **Evaluated *S. iniae* immersion and injection disease challenge models (tested various doses)**
- **Further evaluated *S. iniae* immersion model (tested one dose for various exposure durations)**
- **Repeated immersion challenge to evaluate reproducibility**
- **Agreed that the immersion challenge model was suitable for addressing Study Objectives 2 and 3**

Year 2 Progress

Objective #2

- Conducted 2 trials to evaluate the effectiveness of 10, 15, and 20 mg drug/kg fish/d for 10 d on HSB challenged with *S. iniae*

- $H_0 \mu_{0 \text{ mg/kg}} = \mu_{10 \text{ mg/kg}} = \mu_{15 \text{ mg/kg}} = \mu_{20 \text{ mg/kg}}$



- Trial 1 – Started trial to address Objective #2 – **Nov 2007**
- Trial 2 – Started another trial to address Objective #2 – **Jan 2008**

Objective #2

Specific study objectives

- Experimentally challenge naïve HSB with *S. iniae* at the mid-dose level ($3 \text{ E}+07$ CFUs/mL) for 30 min.
- Administer medicated feed to replicate ($n = 3$) groups of test fish at 0, 10, 15, or 20 mg florfenicol/kg fish body weight
- Determine florfenicol concentration that resulted in lowest (statistically and biologically) cumulative mortality 14 d posttreatment



Trial #1 & 2

General study procedures



- Prepared challenge inoculum from working seed using standard procedures
- Sedate reference population to measure length and weight
- Randomly allocate fish to tanks (30 fish/tank)
- Randomly allocate treatment conditions to tanks (include a positive and negative control)
 - Data collection
 - ❖ Fish size, daily mortality, fish health, water temperature, and DO concentration
 - ❖ Feeding behavior – graded on a 5 pt ordinal scale (1 = worst, 5 = best)
 - ❖ Mortality monitored during the 10-d treatment period and the 14-d posttreatment period

Trial #1 & 2

Challenge procedures

- 30 min post-transfer, fish in all test tanks received a 3 hr static bath salt treatment (15 – 20 ppt)
- Next day, fish received immersion challenge
 - Test tank water level reduced to 3”
 - 1 L of broth culture added to each tank; negative control received 1 L sterile THB
- Exposure duration – 30 min,
 - water in-flow resumed
 - (monitored fish for behavioral distress)



Trial #1 & 2

Study parameters

➤ Trial #1

- Mean length and weight:
 - ❖ 22.3 cm ± 1.46
 - ❖ 124.7 g ± 26.04 (n = 120)
- Water temp = 25.9°C
(range, 23 – 26.7°C)
- Water in-flow – 2 L/min

➤ Trial #2

- Mean length and weight:
 - ❖ 18.6 cm ± 1.60
 - ❖ 59.9 g ± 15.97 (n = 120)
- Water temp = 26.6°C
(range, 25.6 – 27.1°C)
- Water in-flow – 4 L/min

Trial #1 - Results

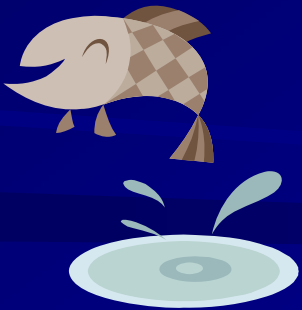
	0 mg/kg	10 mg/kg	15 mg/kg	20 mg/kg	Neg control
Challenge dose = 2.65 E+07 CFUs/mL					
Mean Mortality	0.2	0.2	0.3	0.3	0.0
Std Dev	0.53	0.55	0.59	0.77	0.00
% Mean Mortality	0.8%	0.6%	0.9%	1.0%	0.0%
Feeding behavior	3.2	3.2	3.2	3.4	4.5

Due to low mortality and morbidity, trial terminated on
posttreatment day 3

Trial #2 - Results

	0 mg/kg	10 mg/kg	15 mg/kg	20 mg/kg	Neg control
Challenge dose = 2.03 E+07 CFUs/mL					
Mean Mortality	0.2	0.1	0.0	0.0	0.0
Std Dev	0.57	0.33	0.00	0.00	0.00
% Mean Mortality	0.8%	0.2%	0.0%	0.0%	0.0%
Feeding behavior	3.4	4.3	4.9	5.0	5.0

Due to low mortality and morbidity, trial terminated on posttreatment day 4



Trial #1 & #2

Fish Health

- Reference population and controls - No bacteria detected in cultures
- Challenged fish – *S. iniae* recovered from all dead fish, levels described as “large to massive #’s of colonies
- Pathogen sensitive to florfenicol (agar disc diffusion)
 - (zone of inhibition = 26 - 27 mm)
- Attempts to determine MIC via broth dilution–**unsuccessful** (due to technical constraints)
- Histological evaluation – not done due to early trial termination



Trial #3

Objective

- **To evaluate the effect of:**
 - Presence/absence of 5% sheep serum in challenge broth - effect on mortality of younger (smaller)
- **Findings:**
 - Using younger fish resulted in greater mortality;
 - However, we did not see a difference in fish mortality between *S. iniae* challenge broth w/ and w/o 5% sheep serum

Objective #2

Summary and conclusions

- Immersion challenge model – insufficient mortality to test study hypothesis (i.e., it didn't work!)
- Scheduled another trip to KST for Sept 22, 2008
 - Technical Team meeting planned
 - Prepare higher *S. iniae* broth concentration
 - Use smaller fish
- Trip cancelled due to situation at KST



Future of WRAC Project

- **Due to situation at KST, alternatives were explored to continue project:**
 - **Options to continue at KST not practical**
 - **Difficult (impossible?) to proceeding w/o V. Ostland (or an equivalent expert)**
 - **Considered other collaborators/study locations w/in and outside Western Region**
 - **“Best” alternative - terminate project and return unspent \$\$**

Additional project to Benefit Western Regional Aquaculture

- **Negotiated with CVM to:**
 - **Complete safety data requirements to allow use of Aquaflor[®] at 15 mg flor/kg fish b.w. for all FW finfish**
 - **Conduct study on HSB under “near-GLP” conditions**
 - **Planned to conduct study at KST in conjunction with WRAC project activities (w/ V. Ostland as co-Investigator**
 - **Seeking alternative study site (e.g., USDA-ARS, SNARC; Miss. State Univ)**



Questions??

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