An Evaluation of the Effectiveness of Various Florfenicol Treatment Regimens to Control Mortality Caused by *Streptococcus iniae* in Cultured Hybrid Striped Bass

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Authors	James D. Bowker		
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Participants	James D. Bowker*	US Fish & Wildlife Service	Montana
	Vaughn Ostland*	Kent Sea Tech Corporation	California
	Steve Harbell (*Ext. Rep.*)	Washington State University	Washington
Technical Advisor	Jerri Bartholomew	Oregon State University	Oregon

* funded participants

PROJECT OBJECTIVES

The purpose of this research project is to determine whether an alternate (i.e., higher concentration and/or longer duration) treatment regimen (other than 10 mg florfenicol/kg fish body weight administered on 10 consecutive days) is more efficacious in controlling mortality in hybrid striped bass (HSB) caused by *Streptococcus iniae*. The specific objectives for this study are listed below. Objectives that are relevant to Year 2 funding are italicized.

Objective 1.

Trial 1. Using isolates of *S. iniae,* determine which route of infection (immersion or intraperitoneally (IP) injection) of HSB will consistently yield a mean cumulative mortality of 50% in the exposed group, with the least statistical variation among replicates. Also, identify important dose-dependent variables, such as time to onset of first morbidity, time to first mortality, and total cumulative mortality.

Trial 2. Refine methodologies identified in Trial 1 to consistently yield a mean cumulative mortality of 50% in HSB of a different age exposed to isolates of *S. iniae.*

Objective 2.

Using the optimal dose and exposure route described in Objective 1 Trial 2, determine the most effective treatment dose of florfenicol to control mortality in HSB experimentally infected with S. iniae *fed a medicated feed top-coated with either 0, 10, 15, or 20 mg florfenicol/kg fish/day for 10 days. This data will identify the lowest treatment dose that results in the highest survival during the 10-day trial.*

Objective 3.

Using the lowest treatment dose that resulted in the highest survival (identified in Objective 2), determine the most effective treatment duration of florfenicol to control mortality in HSB experimentally infected with *S. iniae* fed a medicated feed for either 0, 10, 15 or 20 days. This data will identify the shortest treatment duration that results in the highest survival.

Objective 4.

Demonstrate and substantiate that the most efficacious treatment regimen identified in Objective 3 is also effective when florfenicol is administered to HSB naturally infected with *S. iniae* (i.e., controlled field trial).

ANTICIPATED BENEFITS

This project will assist and benefit the aquaculture industry by providing information so that prudent decisions can be made about therapeutic treatment regimens to control mortality in HSB caused by *S. iniae*. Currently, the treatment regimen option available to the aquaculture industry is the standard florfenicol dosage (10 mg active drug/kg fish body weight administered daily for 10 consecutive days). Results from this study will determine whether the industry standard or a higher concentration/longer treatment duration is more efficacious in controlling mortality in HSB caused by *S. iniae.* (Note that there is some evidence in the literature, and some anecdotal information, that a higher therapeutic concentration is required to control mortality caused by *S. iniae* in other warmwater finfish [e.g., tilapia].)

A second benefit to the aquaculture industry is the development of a disease challenge model to initiate an outbreak of *S. iniae* in HSB that should be suitable for testing other therapeutants and biologics.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

During the reporting period, three trials were initiated in an attempt to address Objective 2.

1. On November 27, 2007, the PIs met at the Kent SeaTech (KST) Corp. HSB farm in Mecca, California to start a trial to address Objective 2 and determine the most efficacious treatment dose of florfenicol to control mortality in HSB experimentally infected with *S. iniae*. Fish were fed medicated feed top-coated with 10, 15, or 20 mg florfenicol/kg fish/d for 10 d. Fish were randomly allocated to test tanks and treatment conditions were randomly assigned to test tanks. Day one of the 10-d treatment period began one day post-challenge. Due to low mortality, the trial was terminated on post treatment day 3.
2. On January 8, 2008, the PIs again met at the KST HSB farm to start another trial with nearly identical goals and methodologies to the previous trial. Due to low mortality, the trial was also terminated early (on post treatment day 4).
3. To help us direct future research efforts, a third trial was initiated to investigate the influence of fish age and the inclusion of sheep serum in the bacterial growth medium on the ability of *S. iniae* to induce mortality in HSB exposed to this pathogen by immersion. We also found that by using younger fish (8.7 g mean weight), we were able to improve cumulative mortality, although we were still unable to reach our target mortality of 50%. Furthermore, the addition of sheep serum to the growth medium did not increase overall mortality. Further research is planned to refine the HSB immersion challenge model to achieve our target mortality pattern of 50%.

Experimental Design and Methods

Objective 2 Trial 1

To ensure disease challenge consistency throughout this project, bacterial "working seeds" were produced and frozen for all subsequent challenges. From a single working seed, an immersion challenge inoculum was prepared and used to seed a sufficient volume of *S. iniae* broth to dose groups of fish in their respective test tanks at a pre-determined (approximate) dose. The objective of this trial was (1) to initiate a disease outbreak sufficient to evaluate the effectiveness of an antibiotic, and (2) treat fish infected with *S. iniae* with various concentrations of florfenicol (i.e., 0, 10, 15, or 20 mg florfenicol/kg fish body weight) to determine which of the tested doses resulted in the lowest mortality at the end of the study.

The *S. iniae* bacterial working seed was prepared using standard procedures. Immersion challenge inoculate was prepared and administered to naïve HSB (mean length, 22.3 cm; mean weight, 124.7 g) that had not been previously exposed to *S. iniae*.

The trial was designed to test the following null hypothesis: Ho: $\mu_{o\ mg/kg} = \mu_{10\ mg/kg} = \mu_{15\ mg/kg} = \mu_{20\ m/kg}$ (no difference in mean percent total mortality between the four treatment conditions). In addition to the four treatment conditions, an untreated negative control (fish not challenged with *S. iniae*) was included in the trial to assess incidental mortality (hence, there were five treatment conditions). Three replicates of each treatment condition were allocated among 15 tanks in a completely randomized design. Completely randomized procedures were also used to allocate 30 fish into each test tank. Treatments were administered 1 d after all fish had been challenged with *S. iniae*. In addition, all fish that died were necropsied and brain tissue streaked on blood agar plates to presumptively confirm *S. iniae* infections. Mortality, fish behavior, appetite behavior (graded on a scale of 1 = no interest in feed to 5 = all fish break surface during feeding), water temperature, and dissolved oxygen were collected/observed/measured daily during the 10-d treatment period and the proposed 14-d posttreatment period (note: due to lack of mortality and signs of morbidity, the trial was terminated on posttreatment day 3). At the end of the posttreatment period, mean mortality associated with each treatment condition was evaluated by statistically comparing mean percent total mortality between treatment groups.

Results: During the challenge phase of the study, fish were exposed to a dose of approximately 2.65 E+07 *S. iniae* colony forming units (CFUs)/mL. At the end of the trial, mean relative mortality in each treatment condition was extremely low (approximately 1%). Large numbers of *S. iniae*–like colonies were cultured from brain tissue of all fish sampled during the trial. Mean water temperature and dissolved oxygen concentration during the study was 25.9°C (±1 SD = 0.59) and 14.1 mg/L (±1 SD = 1.79), respectively. Fish behavior was characterized as normal, and feeding behavior ranged from 3.2–3.4 for all disease challenged fish and 4.5 for negative control fish.

Objective 2 Trial 2

The *S. iniae* bacterial working seed was again prepared using standard procedures. Immersion challenge inoculum was produced and administered to naïve HSB (mean length, 18.6 cm; mean weight, 59.9 g) that had not been previously exposed to *S. iniae.* The experimental design and methodologies used in Trial 2 were virtually identical to those used in Trial 1.

Results: During the challenge phase of the study, fish were exposed to a dose of approximately 2.03 E+07 *S. iniae* CFUs/mL. At the end of the trial (the trial was terminated on posttreatment day 4), mean relative mortality in each treatment condition was extremely low (i.e., 0–1%). Large numbers of *S. iniae*–like colonies were cultured from brain and head kidney tissue of all fish sampled during the trial. Mean water temperature and dissolved oxygen concentration during the study was 26.6°C (±1 SD = 0.26) and 13.6 mg/L ± (±1 SD = 1.78), respectively. Fish behavior was characterized as normal, and feeding behavior ranged from 4.3–5.0 for all disease challenged fish fed florfenicol medicated feed, 3.4 for the positive control fish, and 5.0 for negative control fish.

Objective 2 Trial 3. Effect of Fish Size and Sheep Serum on Cumulative Mortality

The experimental design and methodologies used in Trial 3 were similar to those used in previous trials except that feeding behavior was not monitored. The main difference in this trial was that younger fish (mean weight of 8.7 g, mean length of 8.7 cm) were used and duplicate rather than triplicate tanks were used to reduce the number of animals necessary for this exploratory research. We also sought to determine whether the addition of sheep serum to the growth medium would improve bacterial growth and result in an increase in cumulative mortality.

Results: Our findings indicate that we can improve cumulative mortality of immersion exposed HSB if we use younger fish (mean cumulative mortality, 41.7%), although under the conditions employed for this trial we were still unable to produce 50% cumulative mortality. Furthermore, the addition of 5% sheep serum to the growth medium did not contribute to increased mortality (mean cumulative mortality, 38.3%), but it did improve observed growth values (expressed as OD600) of *S. iniae,* although a concomitant reduction of viable bacteria per ml was observed in the Todd-Hewitt Broth (THB) culture in the presence of sheep serum. This reduction in viable bacterial numbers *in vitro* was also evident when the challenge dose (CFU/ml tank water) was confirmed in the water 2 min after the addition of *S. iniae*.

USEFULNESS OF FINDINGS

1. Due to insufficient mortality among disease-challenged fish, findings from the second set of trials failed to address Objective 2. Usefulness of these findings are minimal. The trials will need to be repeated employing younger fish and exposing fish to a more concentrated suspension of *S. iniae*.
2. We anticipate that findings from the a trial in which sufficient mortality (~ 50% total mortality) is attained in the 0 mg/L control fish will address the question of which florfenicol dose (10, 15, or 20) is the most efficacious.

WORK PLANNED FOR NEXT YEAR

All remaining trials are planned for next year (Year 3) to evaluate the effectiveness of different dose and treatment duration regimens of florfenicol to control mortality in HSB caused by *S. iniae*.

1. The first trial will evaluate the effectiveness of 0, 10, 15, and 20 mg florfenicol/kg fish body weight to control mortality in HSB caused by *S. iniae* (experimentally induced in test fish).
2. The second trial will evaluate the effectiveness of the treatment dose identified in the previous trial as the most efficacious for 10, 15, or 20 d to control mortality in HSB caused by *S. iniae* (experimentally induced in test fish).
3. The third trial will evaluate the most effective treatment regimen (most efficacious dose and most efficacious treatment duration) to control mortality in HSB naturally infected with *S. iniae*.

Results from the remaining trials should provide us with the "most" efficacious treatment regimen to control mortality in HSB experimentally infected with *S. iniae,* and a demonstration of the treatment regimen effectiveness using production HSB naturally infected with *S. iniae*.

In addition, a study to demonstrate the safety of florfenicol in HSB is planned for Year 3. Note that this study is outside of the WRAC-funded project, but will contribute to the data required by the Center for Veterinary Medicine (CVM) to expand the use of AQUAFLOR® to allow use at a higher florfenicol dose per kg fish body weight. In this study, fish will be fed florfenicol-medicated feed at a dose of 0, 15, 45, or 75 mg florfenicol/kg fish body weight for 20 d. Resources for this study will be provided by the USFWS, Kent SeaTech Corporation, and Intervet/Schering-Plough Animal Health Corporation.

IMPACTS

Successful completion of the trial to address Objective 2 will provide HSB culturists with the option to control mortality in their fish caused by *S. iniae* with the most efficacious concentration of florfenicol.

PUBLICATIONS IN PRINT AND PAPERS PRESENTED

Publications

Bowker, J. Evaluating the effectiveness of various dosages of Aquaflor®. *Waterlines* Newsletter of the Western Regional Aquaculture Center. Autumn 2006.

Presentations

Bowker J and Ostland V. Effectiveness of Aquaflor® to control mortality in hybrid striped bass caused by *Streptococcus iniae*. Presented at Aquaculture America 2008, February 2008, Lake Buena Vista, FL.

Ostland V and Bowker J. *Streptococcus iniae* challenge model development in hybrid striped bass, Research Progress Update. Presented at the 14th Annual Aquaculture Drug Approval Coordination Workshop, July 2008, Bozeman, MT.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
1/07–8/07	29,864		12,000	24,000		36,000	$65,864
9/07–8/08	30,910		12,000	25,000		37,000	$67,910
Total	60,774		24,000	49,000		73,000	$133,774