



U.S. Fish & Wildlife Service

Aquatic Animal Drug Approval Partnership

DRUG RESEARCH INFORMATION BULLETIN

Efficacy of Chloramine-T to Control Mortality in Late Fall Chinook Salmon *Oncorhynchus tshawytscha* Naturally Infected with External Columnaris Disease *Flavobacterium columnare*

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Columnaris disease (causative agent, *Flavobacterium columnare*) is an acute-to-chronic bacterial infection with a worldwide distribution capable of infecting most freshwater fishes (Noga 2000). The disease most commonly occurs as an external infection; however, it can also occur as a systemic infection with no visible external signs (Plumb 1999). *Flavobacterium columnare* is more pathogenic at temperatures >15°C, and both mortality and acuteness of disease increase with temperature (Noga 2000). Typically, an external columnaris outbreak requires intervention (e.g., improving water quality or fish culture conditions and/or using chemotherapeutants) to reduce the bacterial load on fish. Several chemotherapeutants have historically been used to control mortality caused by external columnaris, and chloramine-T (CLT) is generally regarded as one of the most effective. Based in part by research conducted by AADAP (Bowker et al. 2008; Bowker et al. 2011; Bowker et al. 2013), Halamid® Aqua (100% CLT; sponsor, Axcentive SARL, Bouc Bel Air, France) was approved in May 2014 by the U.S. Food and Drug Administration (FDA) to control mortality in freshwater salmonids due to bacterial gill disease and in walleye *Sander vitreus* and warmwater finfish due to external columnaris. To expand the existing label to allow use to control mortality in all freshwater finfish due to columnaris, an additional field effectiveness study was required on a coldwater fish species.

In this bulletin, we summarize the results of a study conducted to demonstrate the effectiveness of CLT to control mortality in late fall Chinook Salmon (CS) *Oncorhynchus tshawytscha* fingerlings naturally infected with external columnaris disease. (i.e., 50-100 mg/L H₂O₂ diluted 2:3; 101-150 mg/L H₂O₂ diluted 1:4, and 151-400 mg/L H₂O₂ diluted 1:9). See AADAP SOP MISC 261 for a more detailed description of the analytical procedures.

Methods

The study was conducted from June 8-25, 2021, at the U.S. Fish and Wildlife Service's Coleman National Fish Hatchery in Anderson, CA. Test fish were CS fingerlings (mean length, 7.03 cm; mean weight, 4.2g). A single production raceway plumbed with water from Battle Creek, containing 81,538 CS fingerlings was used as the reference population.

Reference population fish were presumptively diagnosed with external columnaris by the U.S. Fish and Wildlife Service's CA-NV Fish Health Center (FHC; Anderson, CA) via spleen tissue samples from 10 moribund fish cultured on Sheih media with tobramycin. Isolates from the cultures of three of the 10 fish were used for pathogen confirmation via polymerase

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chain reaction (PCR). Completely randomized design procedures were used to assign fish and treatment conditions (treated vs. nontreated control) to test tanks. Circular, fiberglass test tanks (rearing volume, 370 L), plumbed with water from Battle Creek, were stocked with fish from the reference population. Each treatment condition was replicated five times ($n = 10$ test tanks at approximately 200 fish/tank). The study comprised a 1-d acclimation period, 3-d treatment period, and 14-d posttreatment observation period. During the treatment period, CLT was administered to the five treated tanks at a target concentration of 20 mg/L in a static bath for 60 min per day on three consecutive days, and the five control tanks received a hatchery water sham treatment under static-bath conditions. Mortality, general fish behavior, feeding behavior (i.e., non-aggressive, semi-aggressive, aggressive), water temperature, and dissolved oxygen concentration data were collected daily throughout the study. Water samples from one randomly selected treated tank and one randomly selected control tank were collected for CLT dose verification approximately 45 min into each 60 min treatment. Analytical dose verification was conducted with a HACH DR 900 Colorimeter (HACH Co., Loveland, Colorado). If the dose verification results are outside of the required of $\pm 25\%$ of the target CLT dose, dose verification is conducted on water samples from all test tanks. During the treatment and posttreatment periods, moribund fish were sampled from test tanks and cultures from skin, spleen, or gill tissue streaked on Sheih media were confirmed by PCR as *F. Columnare* by staff at the CA-NV FHC.

The SAS PROC GLIMMIX (logit link) procedure was used to compare mean cumulative mortality in control tanks to that in treated tanks. Treatment levels were judged statistically significant if $P < 0.05$.

Results

At the end of the study (Figure 1), mean percent cumulative mortality (\pm SD) in treated tanks, 3.9 ± 6.6 , was not significantly less ($P = 0.2509$) than mean percent cumulative mortality (\pm SD) in control tanks, 9.5 ± 5.8 . Upon further examination of mortality data, it was clear there was unusually high mortality in one of the treated tanks (Tank 8; T8) that was suspected to have been caused by a systemic *F. columnare* infection. Dose verification data revealed that Tank 8 was underdosed with CLT on Exposure Day 1, with a dose of 11.91 mg/L, which was outside of the requirement of $\pm 25\%$ of the target CLT dose. Upon further examination of the fish health data for T8 and the dose verification performed on all tanks on study day 1, it was concluded that the infection in T8 was systemic and therefore, susceptibility of fish to CLT was reduced. For this reason, the statistical analyses were re-run without mortality data from T8 (Figure 2) and it was determined that mean percent cumulative mortality at the end of the study (study day 17) in treated tanks (0.9 ± 0.5) was significantly less ($P = .0231$) than that in control tanks (9.5 ± 5.8), when T8 data was removed from the statistical analyses.

The overall mean analytically verified CLT concentration administered to treated tanks was 18.4 mg/L (within 10% of target dose). Chloramine-T was not detected in control tanks.

Mean water temperature (17.9°C ; range, $17.6 - 18.5^{\circ}\text{C}$) and mean dissolved oxygen concentration (9.2 mg/L; range, 8.5 – 9.5 mg/L) during the study were suitable for rearing healthy CS.

General fish behavior was considered mostly normal in all ten test tanks with a couple exceptions. Abnormal behavior consisted of one fish suspended and spinning in the water column, and some lethargic behavior. During the treatment and post-treatment periods, fish were characterized as feeding semi-aggressively.

Discussion

In this study, CLT administered at 20 mg/L for 60 min daily in a static bath on three consecutive days was effective in controlling mortality caused by external columnaris in fingerling CS for a period up to 14-d posttreatment. A Final Study Report has been prepared and submitted to the FDA Center for Veterinary Medicine for review. If accepted, we will request that CVM consider the effectiveness technical section complete for use on all freshwater finfish, and we will encourage the sponsor to expand the drug label to allow use to control mortality of all freshwater finfish caused by columnaris.

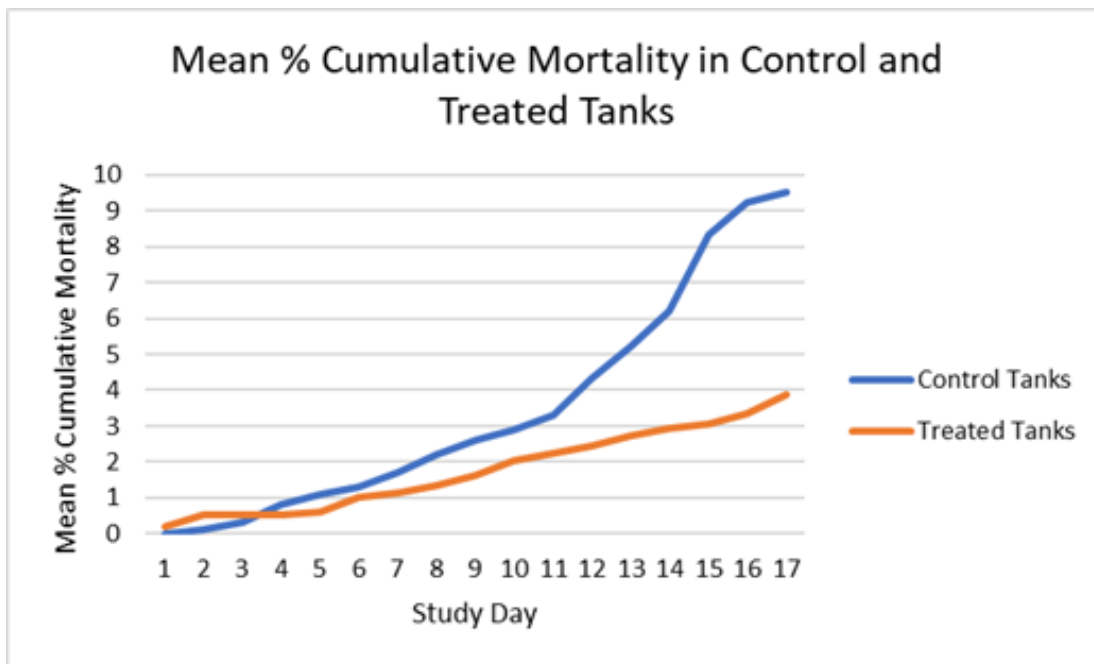


Figure 1. Mean percent cumulative mortality of CS in treated and control tanks during the study. All tanks included in analyses. Test tanks were treated on study days 1- 3.

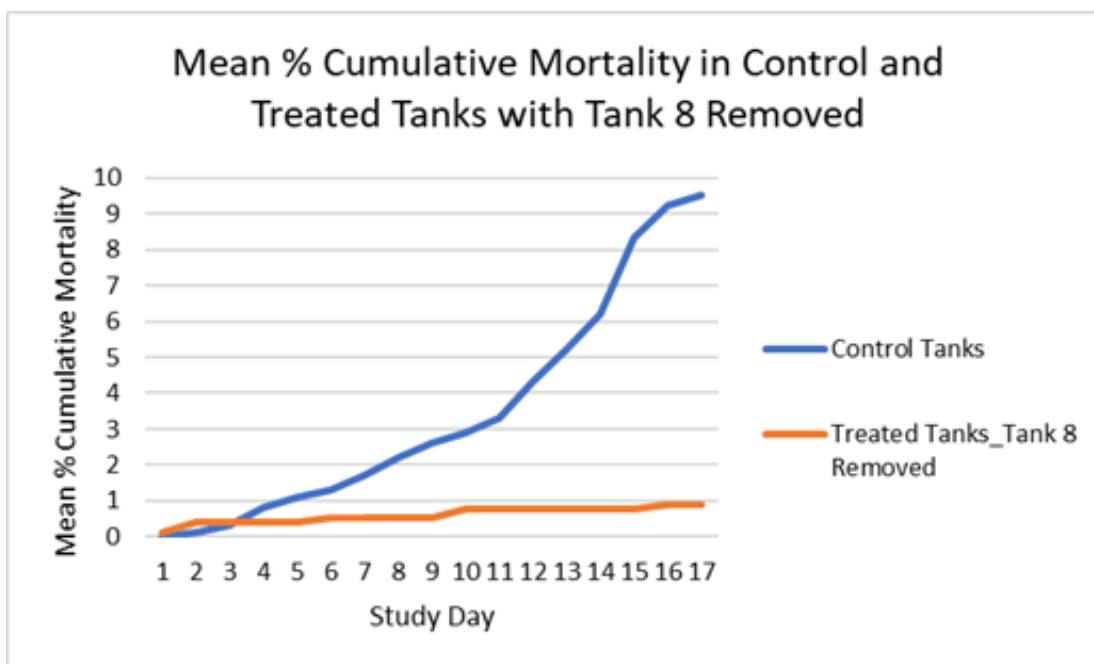


Figure 2. Mean percent cumulative mortality of CS in treated and control tanks during the study. Tank 8 (T8) removed from the analyses. Test tanks were treated on study days 1-3.

Acknowledgments

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