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Efficacy of Chloramine-T to Control Mortality Due to a Natural Infection of External Columnaris Disease *Flavobacterium columnare* in Juvenile Late Fall Chinook Salmon *Oncorhynchus tshawytscha*

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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 (Post 1987). *Flavobacterium columnare* is more pathogenic at temperatures >15oC, and both mortality and acuteness of disease increase with temperature (Post 1987; 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. The label (i.e., approval) was then expanded to control mortality due to external columnaris in walleye *Sander vitreus* and warmwater finfish. To further 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.

Methods

The study was conducted from July 9-29, 2024, at the U.S. Fish and Wildlife Service's Coleman National Fish Hatchery in Anderson, CA. Test fish were CS fingerlings (mean length, 8.3 cm; mean weight, 6.0 g). A single production raceway plumbed with ozone treated water from Battle Creek, containing 77,123 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 imprints of gill tissue samples and body lesions which were evaluated using Gram stain from 10 moribund fish. Only four of the ten fish assessed were presumptively diagnosed with *F. columnare.*

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Another 10 fish were assessed the following day at which point all ten fish were presumptively diagnosed with *F. columnare*. Isolates from kidney tissue samples cultured on Tryptone Yeast extract with salts (TYES) media from those 10 fish were used for pathogen confirmation via polymerase chain reaction (PCR). Completely randomized design procedures were used to assign fish and treatment conditions (treated vs. nontreated control) to test tanks. The study was single blinded, and evaluators did not know the treatment condition of each tank. Circular, fiberglass test tanks (rearing volume, 332 L), plumbed with treated 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 lasted 21-d and comprised of a 2-d acclimation period, 5-d treatment, 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 alternate 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 30-45 min into each 60 min treatment. Analytical dose verification was conducted with a HACH DR 900 Colorimeter (HACH Co., Loveland, Colorado) to ensure the dose was within ±25% of the target CLT dose. During the treatment and posttreatment periods, moribund fish were sampled from all test tanks. Liver, kidney, and spleen were examined visually and characterized as normal or abnormal. Gill tissue samples were taken from all fish sampled. If a body lesion was present, a sample was collected.

Imprints of all gill tissue and body lesion samples were evaluated via gram stain. During the treatment and post-treatment periods, a kidney swab was collected from all fish sampled and cultured using Sheih-T media with tobramycin. Kidney swabs were also cultured using Tryptic Soy Agar (TSA) media in a subset of fish to evaluate the presence of secondary pathogens. Kidney culture isolates or gill tissue samples from a subset of fish collected were confirmed by quantitative polymerase chain reaction (qPCR) as *F. Columnare* by staff at the CA-NV FHC.

A main effects general linear model with a logit link function was used to analyze the effect of treatment concentration (0 or 20 mg/L CLT) on the percent cumulative mortality of test fish (family = binomial; Wolfinger and O'Connell 1993). Percent cumulative mortality in each test tank was calculated by dividing the number of dead or moribund fish removed from each test tank by the initial total number of fish in each test tank, multiplied by 100. Mean percent cumulative mortality of CS fingerlings was compared between test tanks (n = 5) and control tanks (n = 5) on all treatment days and all post-treatment days. Treatment levels were judged statistically significant if $p < 0.05$. All analyses were performed using R Statistical Software (v4.4.2; R Core Team 2021).

Results

Mean percent cumulative mortality at the end of the study (study day 19) was not statistically different ($P = 0.650$) in treated tanks (90%; range = 78-98% per tank) compared to control tanks (97%; range = 93–100% per tank; Figure 1). There was a slight difference in mean percent cumulative mortality between treated (30%; range = 26-34% per tank) and control (42%; range = 37-49% per tank) tanks during the treatment period ($P = 0.360$) as well as between treated (85%; range = 73-92% per tank) and control (94%; range = 88-96% per tank) tanks during the post-treatment period ($P = 0.106$), however, those differences were not statistically meaningful. Given that there was no statistical difference found between mean percent cumulative mortality in test fish and treatment concentration (0 and 20 mg/L CLT), additional statistical models were not analyzed.

The results of the qPCR confirmed the diagnosis of *F. columnare* in all 20 fish tested (acclimation period = 7; treatment period = 7; and post-treatment period = 6). Of the 20 fish tested via qPCR, 15 samples were collected from kidney isolates (all positive for *F. columnare*) indicating the presence of an internalized (systemic) infection of *F. columnare*. These results from the subset of study fish tested, in addition with the high mortality in all test tanks imply that most of the study fish likely had a systemic infection.

The overall mean analytically verified CLT concentration administered to treated tanks was 20.6 mg/L (within 25% of target dose). Chloramine-T was not detected in control tanks.

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Mean water temperature (21.0°C; range, 22.1 – 18.4°C) and mean dissolved oxygen concentration (8.4 mg/L; range, 9.0 – 7.1 mg/L) during the study were suitable for rearing healthy CS, but on the warmer end of their preferred temperature range (Mark Provencher, *personal communication*; EPA 2025).

General fish behavior was characterized as normal in all ten test tanks. Overall fish appetite behavior was described as slightly less than "semi-aggressive" across all tanks.

Discussion

Despite there being a slight difference in mean percent cumulative mortality between the treated and control tanks at the end of the study (treated = 90% vs. control = 97%; P = 0.650), CLT was not effective in controlling mortality in juvenile CS caused by external columnaris in this study. The fish health data collected during the acclimation period (study day 0.1) revealed the presence of *F. columnare*-like isolate growth on kidney cultures indicative of external columnaris, in nine out of the ten fish assessed suggesting that at least some portion of the reference population had an internalized (i.e., systemic) *F. columnare* infection prior to the start of the study despite minimal clinical signs of external columnaris observed. Isolate growth can take days to develop and was not discovered until study day 5 (7/15/2024). By the time the systemic infection was identified in the fish tested from the reference population, the treatment period had been completed. Quantitative polymerase chain reaction confirmed the internal infection of *F. columnare* in all seven isolates (collected from kidney cultures) of fish (n=7) tested from the reference population as well as confirmed the presence of *F. columnare* in 13 fish tested from the study population (8 fish with internal and 5 fish with external infections; given the unsuccessful study results we limited the number of qPCR samples run as a cost saving measure). Since CLT is a topical bath treatment, it is not expected or designed to effectively treat internal bacterial infections. This makes the timing of the treatment and catching the disease before it progresses to a systemic infection imperative for an effective treatment with CLT. A Final Study Report has been prepared and submitted to the FDA Center for Veterinary Medicine for review. The study was not accepted based on a combination of factors related to treatment timing, disease progression, and culture conditions which confounded the assessment of the effectiveness of the CLT treatment. In future studies on CLT the pre-treatment presumptive diagnosis thresholds will be reevaluated to better ensure timely treatment that will more likely lead to successful studies. A successful study in a coldwater fish species is still required to expand the columnaris indication to cover all freshwater-reared coldwater finfish and to achieve an effectiveness technical section complete for all freshwater-reared finfish.

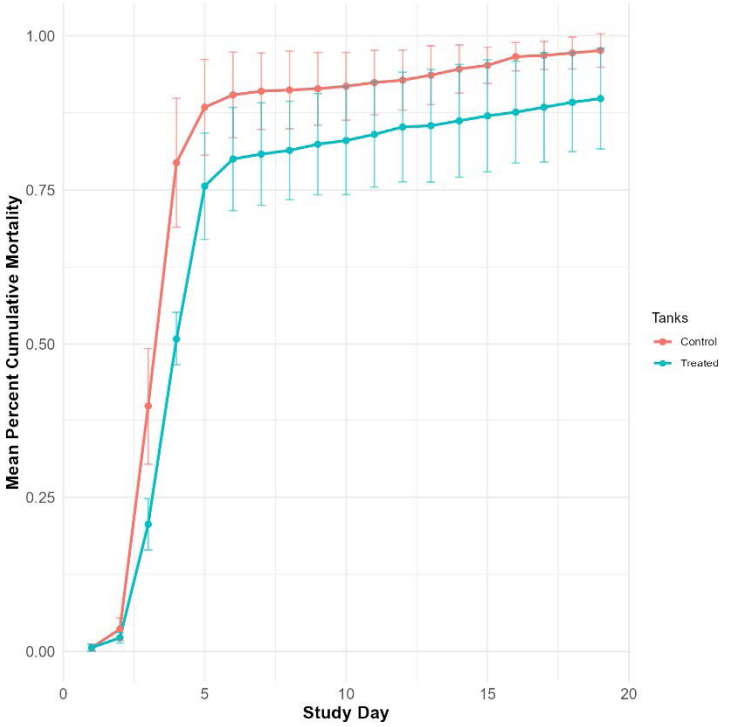

Figure 1. Daily mean percent cumulative mortality of juvenile Chinook Salmon in treated (n=5) and control (n=5) test tanks for the duration of the study (19 days) conducted at Coleman National Fish Hatchery in July 2024. Treated tanks are denoted in blue and control tanks are denoted in pink, with daily mean percent cumulative mortality indicated by a colored point (n = 38). Error bars represent + 1 Standard Deviation (SD).

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