

**Efficacy of Chloramine-T to Control Mortality in Tiger Musky *Esox lucius* × *E. masquinongy* Naturally Infected with External Columnaris Disease *Flavobacterium columnare***James D. Bowker*¹, Molly P. Bowman¹, Geoffrey Eckerlin², and Andrew Noyes²¹*U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program
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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 coolwater fish due to columnaris, an additional field effectiveness study was required on a fish species other than walleye.

In this bulletin, we summarize the results of a study conducted to demonstrate the effectiveness of CLT to control mortality in Tiger Musky (TIM) *northern pike* *Esox lucius* × *muskellunge* *E. masquinongy* fingerlings naturally infected with external columnaris disease.

Methods

The study was conducted in July 22 – August 8, 2014 at the New York Department of Environmental Conservation South Otselic Hatchery (SO Hatchery), South Otselic, New York. Test fish were TIM fingerlings (mean length, 13.1 cm). A single production tank plumbed with water from the Otselic River

containing 6,500 TIM fingerlings was used as the reference population.

Reference population fish were presumptively diagnosed with external columnaris and cultures from skin tissue of 10 moribund fish from this population was streaked on Hsu-Shotts media and shipped to the USFWS La Crosse Fish Health Center (FHC; La Crosse, WI) where Polymerase Chain Reaction (PCR) was used to confirm that cultures were *F. Columnare*. Completely randomized design procedures were used to assign fish and treatment conditions (treated vs. nontreated control) to test tanks. Rectangular, aluminum test tanks (rearing volume, 82.3 L), plumbed with water from the Otselic River, were stocked with fish from the reference population. Each treatment condition was replicated four times (n = 8 test tanks at approximately 165 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 four 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 four 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 Pocket Chlorine Colorimeter (HACH Co., Loveland, Colorado). Note that samples collected during the first treatment were measured with a faulty colorimeter and were not included in the results. During the treatment and post-treatment periods, moribund fish were sampled from each tank and cultures from skin tissue streaked on Hsu-Shotts media were confirmed by PCR as *F. Columnare* by staff at the La Crosse FHC.

The SAS PROC GLIMMIX (logit link) procedure was used to

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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, 12.6 ± 13.35 , was significantly less ($P = 0.0145$) than mean percent cumulative mortality (\pm SD) in control tanks 81.9 ± 22.92 .

The overall mean analytically verified CLT concentration administered to treated tanks was 20.6 mg/L (1.2% from the target dose). Chloramine-T was not detected in control tanks.

Mean water temperature (16.0°C ; range, $13.6 - 18.9^{\circ}\text{C}$) and mean dissolved oxygen concentration (6.6 mg/L; range, $3.8 - 8.8$ mg/L) during the study were suitable for rearing healthy TIM.

General fish behavior was considered mostly normal in treated tanks and mostly abnormal in control tanks. Abnormal behavior consisted of fish positioned high in the water column and lethargy. During the treatment period, all fish were characterized as feeding semi-aggressively. During the posttreatment period, feeding characterization ranged from non-aggressive to semi-aggressive for all fish, and fish in control tanks fed less aggressively than those in treated tanks.

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 TIM for a period up to 14-d posttreatment. A Final Study Report has been prepared and will be 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 coolwater finfish, and we will encourage the sponsor to expand the label to allow use to control mortality of all non-salmonids caused by columnaris.

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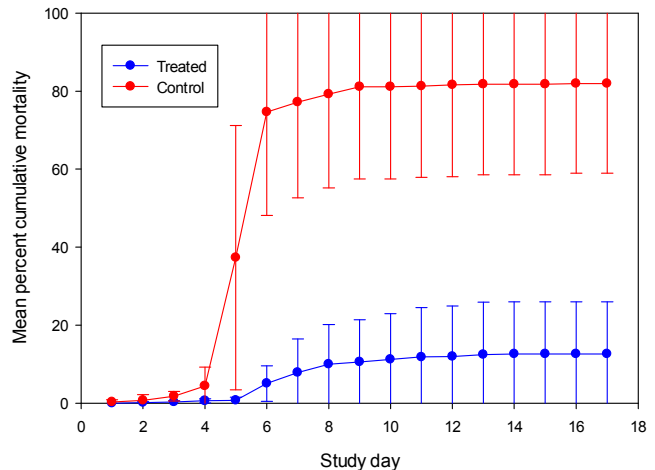


Figure 1. Mean percent cumulative mortality of TIM in treated and control tanks during the study (error bars = ± 1 SD). Test tanks were treated on study days 1- 3.

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