



**Efficacy of Pennox 343<sup>®</sup> (Oxytetracycline hydrochloride) to Control Mortality Associated with Columnaris Disease in Bluegill *Lepomis macrochirus***

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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 2010). *Flavobacterium columnare* is more pathogenic at temperatures >15°C, and both mortality and acuteness of disease increase with temperature (Noga 2000). In the U.S., Aquaflo<sup>®</sup> (florfenicol; Merck Animal Health, Summit, New Jersey) is approved by the U.S. Food and Drug Administration (FDA) as an antibiotic that can be administered in feed to all freshwater-reared fin fish to control mortality due to systemic columnaris disease, and Terramycin<sup>®</sup> 200 for Fish (oxytetracycline dihydrate; Phibro Animal Health, Teaneck, New Jersey) is approved by FDA to control mortality in freshwater-reared *Oncorhynchus mykiss* due to systemic columnaris. In addition, two antimicrobial products are also approved by FDA for use to control mortality due to columnaris disease in select non-salmonid fish species: Halamid<sup>®</sup> Aqua (chloramine-T; Axcentive SARL, France; U.S. Distributor, Western Chemical Inc., Ferndale, Washington); and 35% Perox-Aid<sup>®</sup> (35% hydrogen peroxide; Western Chemical, Inc.). In spite of the approvals of these drugs, there is interest by the aquaculture community to obtain approval of Pennox 343<sup>®</sup> (76.5% oxytetracycline hydrochloride, OTC-HCl), an antibiotic that can be administered as a bath solution. To gain U.S. Food and Drug Administration (FDA) approval, it must be demonstrated that the product is safe and effective.

In this bulletin, we summarize the results of a trial conducted to demonstrate the effectiveness of OTC-HCl to control mortality in bluegill (BLG) *Lepomis macrochirus* fingerlings naturally infected with columnaris disease.

### Methods

The trial was conducted in 2014 at the State of Florida Bass Conservation Center's Richloom Fish Hatchery (RFH) in Webster, Florida. Test fish were BLG fingerlings (mean length, 8.3 cm). A single production tank of BLG fingerlings was used

as the reference population.

After reference population fish were diagnosed with external columnaris, completely randomized design procedures were used to assign fish and treatment conditions (treated vs. nontreated control) to test tanks. Rectangular, fiberglass test tanks (rearing volume, 360 L) were stocked with fish impartially collected from the reference population. Each treatment condition was replicated four times (n = 8 test tanks at 50 fish/tank). At the start of the study, the initial test-tank loading rate (0.79 g/L) was lower than the loading rate in the reference population tank (9.4 g/L). Each trial comprised a 1-d acclimation period, 3-d treatment period, and 14-d post-treatment observation period. During the treatment period, OTC-HCl was administered to the four treated tanks at a target concentration of 40 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; aeration was not used during the treatment period. Mortality, general fish behavior, feeding behavior (i.e., non-aggressive, semi-aggressive, or aggressive), water temperature, and dissolved oxygen concentration data were collected daily throughout the trial. Water hardness (360 mg/L as CaCO<sub>3</sub>), alkalinity (330 mg/L as CaCO<sub>3</sub>), and pH (7.8) were measured twice during the study. A water sample was collected for OTC-HCl dose verification at 45-55 min into the 60 min treatment period from one randomly selected treated tank and one randomly selected control tank on each of the three treatment days. Analytical dose verification was conducted at the U.S. Geological Survey Upper Midwest Environmental Science Center and measured for OTC-HCl by high performance liquid chromatography.

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  $P < 0.05$ .

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## Results

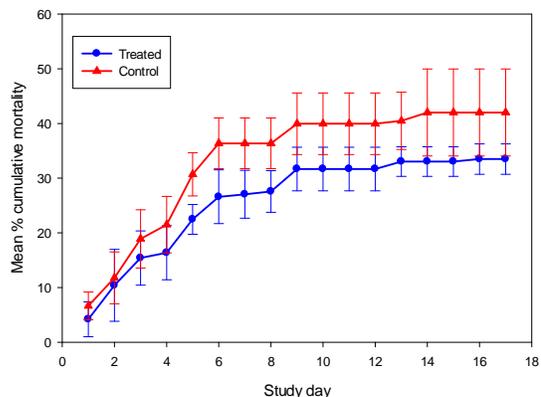
At the end of the trial (Figure 1), mean percent cumulative mortality ( $\pm$  SD) in treated tanks ( $33.5 \pm 2.78$ ) was lower than that in control tanks ( $42.0 \pm 7.9$ ), but the difference was not significant ( $P = 0.0849$ ). A significant difference was detected between mean relative mortality in treated and control tanks on study days 5-13.

Based on dose verification samples collected ( $n = 3$  treated;  $n = 3$  control), the overall mean OTC-HCl concentration administered to treated tanks was 48.8 mg/L. The mean dose administered was 22% from the target dose, which was not within the FDA-acceptable limit ( $\pm 20\%$ ). The drug was not detected in samples collected from control tanks.

Mean water temperature ( $24.7^{\circ}\text{C}$ ; range,  $24.0 - 26.4^{\circ}\text{C}$ ) and mean dissolved oxygen concentration ( $14.0$  mg/L; range,  $8.3 - 18.3$  mg/L) during the trial were suitable for rearing healthy BLG. General fish behavior was considered normal, and no behavioral differences were noted between treated and control tanks. During the treatment period, all fish were characterized as feeding non-aggressively. During the post-treatment period, feeding characterization ranged from non-aggressive (on post-treatment day 1 only) to semi-aggressive for all fish.

## Discussion

In this trial, OTC-HCl administered at 49 mg/L for 60 min daily in a static bath on three consecutive days was not effective in controlling mortality caused by external columnaris in fingerling BLG at the end of the 14 d post-treatment period. Treatment did appear effective over a 9-d period during the middle of the study. Mortality in both treated and control tanks returned to near-zero starting on about day 7 of the study. We speculate that had fish been stocked in test tanks at a density that matched the density in the reference population tank, mortality among control tanks would have remained elevated. Alternatively, many antibiotics chelate divalent cations such as magnesium and calcium, causing their inactivation. This is particularly the case with all tetracyclines, and use of higher doses should be considered when treating fish in hard water. Results have been submitted to FDA for review, but we anticipate that this study will have to be repeated and assurances made to stock test tanks at a density that more closely matches that of the reference population tank.



**Figure 1.** Mean percent cumulative mortality of BLG in treated and control tanks during the trial (error bars =  $\pm 1$ SD).

## Acknowledgments

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## References

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