



U.S. Fish &amp; Wildlife Service

## Aquatic Animal Drug Approval Partnership

**DRUG RESEARCH INFORMATION BULLETIN****Efficacy of Reward® (Diquat Dibromide) to Control Mortality Associated with Columnaris Disease in Walleye *Sander vitreus***Jim Bowker\*, Molly P. Bowman<sup>1</sup>, and J. Alan Johnson<sup>2</sup><sup>1</sup>*U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program  
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Columnaris disease (causative agent, *Flavobacterium columnare*) is a globally distributed acute-to-chronic bacterial infection 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., two antibiotic drugs administered as medicated feed are approved by the U.S. Food and Drug Administration (FDA) to control mortality due to systemic columnaris disease: Aquaflor® (florfenicol; Merck Animal Health, Summit, New Jersey) may be fed to all freshwater-reared finfish, and Terramycin® 200 for Fish (oxytetracycline dihydrate; Phibro Animal Health, Teaneck, New Jersey) may be fed to freshwater-reared *Oncorhynchus mykiss*. In addition, two antimicrobial immersion products are approved by FDA to control mortality due to columnaris disease: Halamid® Aqua (chloramine-T; Axcentive SARL, France; U.S. Distributor, Western Chemical Inc., Ferndale, Washington) may be applied to freshwater-reared salmonids and warmwater finfish and Walleye (WAE, *Sander vitreus*); and 35% Perox-Aid® (35% hydrogen peroxide; Western Chemical, Inc.) may be applied to freshwater-reared coolwater finfish and Channel Catfish (*Ictalurus punctatus*). However, there is anecdotal data that show Reward® (diquat dibromide; diquat) is more effective than either Halamid® Aqua or 35% Perox-Aid® in some instances, particularly when treating populations of WAE to control mortality associated with columnaris disease. Diquat dibromide is used for aquatic weed control in public waters, and its mode of action is to interfere with photosynthesis within green plant tissue. As an antimicrobial, it is characterized as a non-selective sanitizing agent that effectively removes potentially pathogenic material from external fish surfaces, including bacteria that may be present on the skin and gills. Consequently, there is interest within the aquaculture community, particularly those in the Midwest that rear WAE, to obtain approval of diquat as an immersion antimicrobial. To gain 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 diquat to control mortality in WAE fingerlings naturally infected with columnaris disease.

**Methods**

The trial was conducted in 2016 at the Iowa Department of Natural Resources, Rathbun Fish Culture and Research Station (Rathbun FCRS, Moravia, Iowa). Test fish were WAE fingerlings (mean total length and weight, 11.6 cm and 12.1 g) that were progeny of broodstock kept at the Rathbun FCRS. A single production tank of WAE 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 in quadruplicate (n = 8, N = 4). Square (0.51 × 0.55 m), fiberglass test tanks (rearing volume, 94 L) supplied with flow-through water (1 L/min) were impartially stocked with fish from the reference population (85 fish/tank). At the start of the study, fish density in

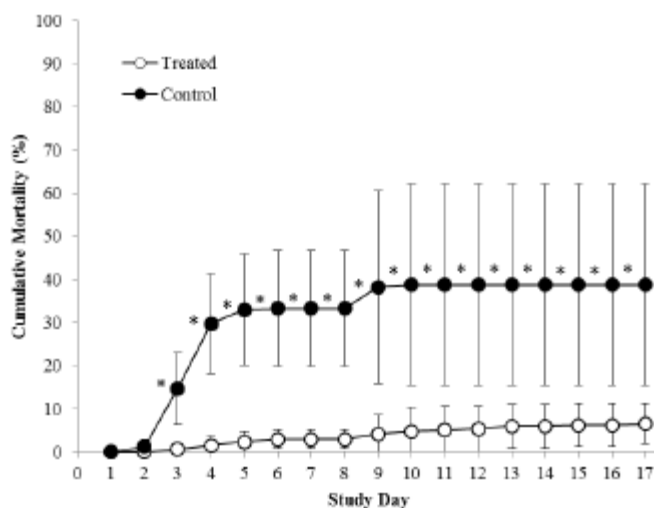
the test tanks (10.9 g/L) was comparable to density in the reference population tank (10.6 g/L). The trial comprised a 3-d treatment period and 14-d post-treatment observation period. Due to the rapid onset of disease and associated mortality in the reference population tank, fish were transferred into and acclimated in their test tanks for about 1 h before the first treatment was administered. During the treatment period, diquat was administered to the four treated tanks at a target dosage of 18 mg/L in a static bath for 120 min per day on three consecutive days; the four control tanks received a hatchery water sham treatment under similar static-bath conditions. Briefly, water flow was halted, 4.5 g diquat dissolved in 150 mL hatchery water (for treated tanks) or 150 mL hatchery water sham (for control tanks) was added to the test tank, and the water was mixed to ensure homogenous distribution. After 120 min elapsed, water flow was resumed. Supplemental aeration was not provided during normal rearing or during the treatment period.

Mortality, general fish behavior, feeding behavior (i.e., non-aggressive, semi-aggressive, or aggressive), water temperature, and dissolved oxygen (DO) concentration data were collected daily throughout the trial. Water hardness (120 mg/L as  $\text{CaCO}_3$ ), alkalinity (120 mg/L as  $\text{CaCO}_3$ ), and pH (7.6) were not measured but information was collected from historical records. Water samples were not collected for diquat dose verification during the study. Moribund fish were collected from treated ( $n=2$  fish) and control tanks ( $n=9$  fish) on days 3 and 4 of the study for necropsy and microscopic examination of skin scrapes.

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 trial (Figure 1), mean percent cumulative mortality ( $\pm$  SD) in treated tanks ( $6.5 \pm 4.7\%$ ) was significantly less ( $P = 0.0376$ ) than that in control tanks ( $38.8 \pm 23.4\%$ ). In addition, on study days 03 – 17, mean cumulative mortality was significantly less in treated tanks than in control tanks.



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

During the study, general fish behavior was generally characterized as normal in all eight test tanks during the treatment and post-treatment periods of the study. Fish appetite behavior throughout the study was described as primarily non-aggressive or semi-aggressive (124 of 128 observations).

Necropsies of moribund fish sampled during the study showed that no lesions were present and no bacteria were observed microscopically on skin scrapes from the two fish sampled from a treated tank. Conversely, lesions were detected and bacteria presumptively identified as *F. columnare* were observed microscopically on skin scrapes prepared from the nine fish sampled from control tanks.

Mean water temperature ( $26.5^{\circ}\text{C}$ ; range,  $25.3 - 27.4^{\circ}\text{C}$ ) and mean DO concentration ( $9.0\text{ mg/L}$ ; range,  $1.6 - 12.6\text{ mg/L}$ ) during the trial were suitable for rearing healthy WAE. Dissolved oxygen concentration was  $< 2\text{ mg/L}$  in two tanks on day 6 of the post-treatment period due to low water flow and fish behavior in these two tanks was characterized as lethargic. The low DO concentration problem was resolved immediately by clearing the water line and normal fish behavior was observed thereafter.

## **Discussion**

In this trial, diquat administered at 18 mg/L for 120 min daily in a static bath on three consecutive days was effective in controlling mortality caused by external columnaris in fingerling WAE. Mortality in treated tanks was reduced compared to that in control tanks before the end of the treatment period and remained at near-zero levels throughout the treatment period. Results have been submitted to FDA for review and we anticipate that FDA will agree that treatment was efficacious. The data may be accepted, but the future of diquat is uncertain given that there is no sponsor for the product at this time. In addition, there is currently no FDA-accepted method available to measure diquat in water to confirm the dose administered. Regardless of the lack of a sponsor and dose verification method, results from this study indicate the diquat would be a valuable addition to the fish culture medicine chest.

## **Acknowledgments**

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## **References**

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