



## The Efficacy of Aquaflor<sup>®</sup> to Control Mortality in Freshwater-Reared Salmonids Naturally Infected with Coldwater Disease

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Bacterial coldwater disease (CWD; causative agent, *Flavobacterium psychrophilum*) causes high mortality in cultured freshwater-reared salmonids (LaFrentz et al. 2003). Salmonids ranging from yolk-sac fry to yearlings are susceptible to CWD; typically, the younger the fish, the more severe the disease (Leek 1987). To control mortality, systemic infections require antibiotic treatment (Noga 2000). In the U.S., Aquaflor<sup>®</sup> (50% florfenicol; Schering Plough Animal Health Corp., Summit, NJ) used under a U.S. Fish and Wildlife Service (FWS)-sponsored Investigational New Animal Drug exemption (INAD No. 10-697) has been shown to be effective in controlling mortality caused by CWD in a variety of freshwater-reared salmonids. To gain U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) approval, it must be demonstrated that the product is safe and effective.

In this bulletin, we summarize the results of two independent trials conducted by the FWS in 2002 to demonstrate the effectiveness of Aquaflor<sup>®</sup> to control mortality caused by CWD in two salmonids species.

### Methods

Trial 1 was conducted at the Makah National Fish Hatchery, Neah Bay, WA. Trial 2 was conducted at Washoe Park Trout Hatchery, Anaconda, MT. Aquaflor<sup>®</sup>-medicated feed was administered to hatchery-reared steelhead trout *Oncorhynchus mykiss* in Trial 1 (mean length, 5.8 cm) and westslope cutthroat trout *O. clarki lewisi* in Trial 2 (mean length, 2.6 cm). In both trials, the target dosage was 10 mg florfenicol/kg fish/d for 10 d.

In each trial, a reference population of fish was presumptively diagnosed with CWD. Fish were impartially collected from the reference population, and were assigned to treatment conditions (treated vs. non-treated control) and test tanks (Trial 1 = 66.5 L/tank, n = 220 fish/tank; Trial 2 = 59.2 L/tank, n = 667 fish/tank) by using completely randomized design procedures. Each trial comprised a 1-d acclimation period, a 10-d treatment period, and a 13 or 14-d post-treatment period. During the treatment period, fish in treated tanks (n = 6) were fed

Aquaflor<sup>®</sup>-medicated feed, and fish in control tanks (n = 6) were fed nonmedicated feed. Exclusive of the florfenicol, *per se*, all tanks of fish received the same feed formulation.

During each trial, mortality, general fish behavior, water temperature, and dissolved oxygen concentration data were collected daily. Moribund fish were collected to confirm cause of morbidity and probable cause of mortality. Florfenicol concentration in feed was determined analytically to confirm actual dose.

For each trial, SAS PROC GLIMMIX (logit link) was used to compare mean cumulative mortality in control tanks to that in treated tanks on each day of the trial and to generate daily odds ratios (odds of mortality in control tanks:odds of mortality in treated tanks). Treatment levels were judged statistically significant if  $P < 0.05$ .

### Results

#### Trial 1 (steelhead trout)

At the end of the trial, mean cumulative mortality in treated tanks (2.0%; range 1 – 3%) was significantly less ( $P = 0.0212$ ) than mortality in control tanks (5.0%; range 1 – 9%). Daily odds ratios (control:treated) ranged from 0.9 to 2.1 during the treatment period and from 2.2 to 2.7 during the post-treatment period (Figure 1).

#### Trial 2 (westslope cutthroat trout)

At the end of the trial, mean cumulative mortality in treated tanks (75%; range 69 – 79%) was significantly less ( $P < 0.0001$ ) than mortality in control tanks (94%; range 92 – 96%). Daily odds ratios (control:treated) ranged from 0.8 to 1.4 during the treatment period and from 1.6 to 5.6 during the post-treatment period (Figure 2).

*Flavobacterium psychrophilum* was confirmed as the pathogen causing mortality and morbidity in Trial 1 by fluorescent antibody test and in Trial 2 by polymerase chain reaction assay. Mean water temperature and dissolved oxygen concentration in Trial 1 (14.2°C and 9.5 mg/L) and Trial 2 (9.3°C and 9.2 mg/L) were considered adequate for rearing healthy salmonids.

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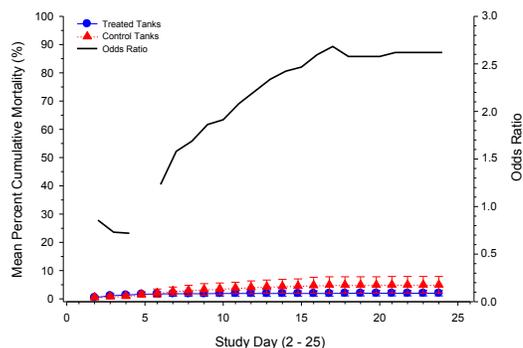
Analytical verification of florfenicol concentration in feed confirmed fish in treated tanks received 10.2 and 9.7 mg florfenicol/kg fish/d in Trials 1 and 2, respectively. No florfenicol was detected in control feed. In both trials, all fish appeared to behave normally and fed either aggressively (Trial 1) or semi-aggressively (Trial 2).

### Discussion

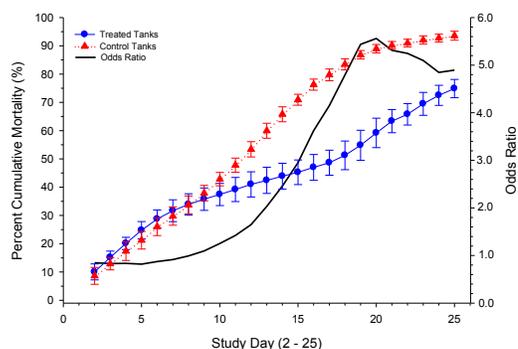
Results from these trials (found online at: <http://www.fws.gov/fisheries/aadap/studiesFlorfenicol.htm>) were accepted by FDA/CVM as demonstrating the efficacy of Aquaflor® (10 mg florfenicol/kg fish/d for 10 d) to control mortality in hatchery-reared steelhead and westslope cutthroat trout caused by CWD. As such, both trials contributed to the FDA/CVM approval (March 2007) of Aquaflor® for use to control mortality in all freshwater-reared salmonids caused by CWD.

### References

- LaFrentz, B. R., S. E. LaPatra, G. R. Jones, and K. D. Cain. 2003. Passive immunization of rainbow trout, *Oncorhynchus mykiss* (Walbaum), against *Flavobacterium psychrophilum*, causative agent of bacterial coldwater disease and rainbow trout fry syndrome. *Journal of Fish Diseases* 26:377 – 384.
- Leek, S. L. 1987. Viral erythrocytic inclusion body syndrome (EIBS) occurring in juvenile spring Chinook salmon (*Oncorhynchus tshawytscha*) reared in fresh water. *Canadian Journal of Fisheries and Aquatic Science* 44:685 – 688.
- Noga, E. J. 2000. *Fish disease: Diagnosis and treatment*. Iowa State University Press, Ames, Iowa.



**Figure 1.** Trial 1: Mean percent cumulative mortality of the treated and control tanks and odds ratio (control:treated tanks; bar = ±1SD).



**Figure 2.** Trial 2: Mean percent cumulative mortality of the treated and control tanks and the odds ratio (control:treated tanks; bar = ±1SD).

### Acknowledgments

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