



## Efficacy of AQUAFLO<sup>®</sup> (50% Florfenicol) to Control Mortality of Freshwater-Reared Coho Salmon Diagnosed with Furunculosis

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Furunculosis (causative agent, *Aeromonas salmonicida*) is one of the oldest known bacterial fish diseases and is generally considered a disease of salmonids (Plumb 1999). Mortality in affected freshwater-reared salmonid populations and resultant economic losses to producers can be substantial (Clark and Scott 1989). Hence, maintaining healthy rearing conditions, administering preventative vaccines, and administering antimicrobial treatments are strategies routinely used to prevent furunculosis outbreaks or minimize mortality when outbreaks occur (Inglis et al. 1991).

AQUAFLO<sup>®</sup> (50% florfenicol and 50% inert ingredients), a product of Intervet/Schering-Plough Animal Health Corp. (Summit, NJ), is a medicated premix for inclusion into fish feed. Florfenicol is a broad-spectrum antibiotic with both bacteriostatic and bacteriocidal properties. The U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine has approved the use of AQUAFLO<sup>®</sup> as a Veterinary Feed Directive (VFD) drug for a variety of claims, including use to control mortality in freshwater-reared salmonids caused by coldwater disease or furunculosis. A VFD drug can only be administered on the order of a licensed veterinarian, and extra-label prescription use is prohibited. Under the current FDA approvals, AQUAFLO<sup>®</sup> can only be administered in feed at a dosage of 10 mg florfenicol/kg fish/d for 10 consecutive days.

The U.S. Fish and Wildlife Service's (FWS) Aquatic Animal Drug Approval Partnership program conducted controlled field efficacy trials that contributed required data to the salmonid approvals obtained to date. In this bulletin, we summarize the results of a trial that was conducted to evaluate the effectiveness of AQUAFLO<sup>®</sup> to control mortality in freshwater-reared coho salmon *Oncorhynchus kisutch* diagnosed with furunculosis.

### Methods

The trial was conducted July 28 – August 21, 2006, at the FWS's Makah National Fish Hatchery, Neah Bay, WA. AQUAFLO<sup>®</sup> was administered in commercial fish feed at a target dosage of 10 mg florfenicol/kg fish/d for 10 d. Test fish were

coho salmon fingerlings (mean length = 9.9 cm) impartially drawn from a reference population presumptively diagnosed with furunculosis.

Twelve rectangular aluminum test tanks (64.6 L/tank), arranged in two blocks of six tanks each, were used in the trial. Treatment conditions (treated; nontreated controls) were allocated among tanks with a randomized complete block design such that there were three treated tanks and three control tanks per block. Reference population fish held in a single production-scale raceway were allocated among tanks using completely randomized design procedures (N = 151 - 162 fish/tank).

The 25-d trial comprised a 1-d acclimation period, 10-d treatment period, and 14-d posttreatment period. During the trial, feed was administered to test tanks at 1.0% mean fish body weight/tank/d. During the treatment period, AQUAFLO<sup>®</sup>-medicated feed was administered to treated tanks and nonmedicated feed was administered to control tanks. During the post-treatment period, nonmedicated feed was administered to all test tanks.

Mortality, general fish and feeding behaviors, water temperature, and dissolved oxygen concentration data were collected daily during the trial. Moribund fish were necropsied and tissues were sampled during the treatment and posttreatment periods to confirm primary cause of mortality. Florfenicol concentrations in samples of medicated and nonmedicated feed were analytically verified by Eurofins Scientific (Memphis, TN).

In this trial, SAS PROC GLIMMIX (logit link) procedure was used to (a) compare mean cumulative mortality in control tanks to that in treated tanks on each day of the treatment and posttreatment periods and (b) generate odds ratios on each day of the study based on cumulative mortality in control tanks compared to cumulative mortality in treated tanks. An odds ratio >1 indicated that, at a given point in time, the overall odds of mortality in control tanks was greater than the overall odds of mortality in treated tanks. Treatment levels were judged statistically significant if  $P < 0.05$ .

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## Results and Discussion

At the end of the trial, mean cumulative mortality in treated tanks (16.0%; range, 7.6 – 21.4%) was significantly less ( $P = 0.004$ ) than that in control tanks (29.5%; range, 17.3 – 36.0%). Odds ratios (control:treated) ranged from 1.6 to 3.2 during the treatment period and from 2.3 to 3.6 during the posttreatment period (Figure 1), indicating that, during the trial, the odds of mortality in control tanks were always greater than the odds of mortality in treated tanks. *Aeromonas salmonicida* was confirmed as the primary cause of mortality because it was presumptively identified via cultures grown on Brain Heart Infusion Agar and positively identified via polymerase chain reaction assay. No other pathogens were detected that would have negatively impacted the outcome of the trial.

During the trial, treated and control fish appeared to behave normally and were characterized as feeding semi-aggressively or aggressively. Mean ( $\pm$ SD) water temperature ( $18.4^{\circ}\text{C}\pm 0.67$ ) and dissolved oxygen concentration ( $8.9\text{ mg/L}\pm 0.79$ ) were adequate for rearing healthy salmonids. Analytical verification of lorfenicol concentrations in medicated-feed samples revealed treated tanks had received 9.7 mg florfenicol/kg fish/d. No florfenicol was detected in control feeds.

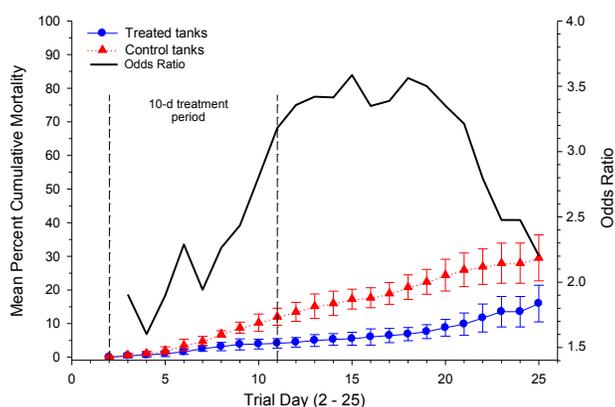
Results from this trial (Final Study Report at: [www.fws.gov/fisheries/aadap/studiesFlorfenicol.htm](http://www.fws.gov/fisheries/aadap/studiesFlorfenicol.htm)) were accepted by FDA as demonstrating the effectiveness of AQUAFLO<sup>®</sup> (administered at a dosage of 10 mg florfenicol/kg fish/d for 10 d) to control mortality caused by furunculosis in freshwater-reared coho salmon.

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**Figure 1.** Mean percent cumulative mortality in treated and control tanks and odds ratios (control:treated tanks; bar =  $\pm$ 1SD).