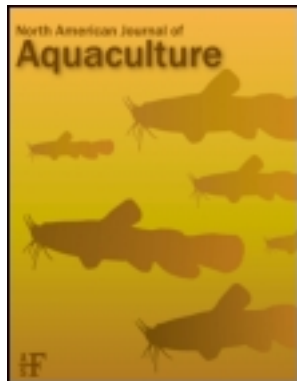


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Efficacy of Aquaflor (50% Florfenicol)-Medicated Feed to Control Mortality Associated with *Flavobacterium columnare* Infection in Florida Largemouth Bass and Bluegill

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ARTICLE

Efficacy of Aquaflor (50% Florfenicol)–Medicated Feed to Control Mortality Associated with *Flavobacterium columnare* Infection in Florida Largemouth Bass and Bluegill

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Abstract

Aquaflor (florfenicol, 50% with/without) is a potent, broad-spectrum, antibacterial agent with bacteriostatic properties that are active against a variety of Gram-positive and Gram-negative bacteria. This product is approved by the U. S. Food and Drug Administration for use on several fish species to control mortality associated with a variety of diseases, including columnaris (causative agent, *Flavobacterium columnare*). Two independent experimental trials were separately conducted to evaluate the effectiveness of Aquaflor to control mortality associated with columnaris disease. Aquaflor was administered in feed at a targeted daily florfenicol dosage of 10 mg/kg of body weight for 10 consecutive days. Test species were fingerling Florida Largemouth Bass *Micropterus salmoides floridanus* and Bluegill *Lepomis macrochirus*. In each trial, 8 or 10 test tanks (4 or 5 treated, 4 or 5 control) were stocked with either approximately 473 bass (mean length = 6.4 mm; mean weight = 3.3 g) or 100 Bluegills (length = 10.3; weight = 25.2 g). At the end of the 14-d posttreatment periods, mean cumulative mortality of bass in treated tanks was 5.7% per tank, which was significantly less than that in control tanks (12.0%). Mean cumulative mortality of Bluegills in treated tanks was 19% per tank, which was significantly less than that in control tanks (38%). Analysis of treated feed samples at the start of each trial verified the initial targeted dose of florfenicol was within 11% of the target dose for both bass and Bluegills. Based on these results, we concluded that Aquaflor-medicated feed homogeneously mixed to provide florfenicol at a daily dose of 10 mg/kg of body weight fed for 10 d was effective in controlling mortality in bass and Bluegill fingerlings, exposed to columnaris disease.

Flavobacterium represent a group of Gram-negative bacteria that can be associated with high levels of mortality in a variety of freshwater fish species. *Flavobacterium columnare* is the causative agent of columnaris disease, which can be a chronic or acute disease that can infect virtually any wild or cultured fish (Plumb 1999). The worldwide distribution of columnaris makes

it one of the most important diseases affecting aquaculture (Morrison et al. 1981; Post 1987; Wagner et al. 2002; Thomas-Jinu and Goodwin 2004). Although columnaris is primarily an external disease, it can become systemic with or without advanced skin and gill necrosis (Noga 2000). There is no difference in the causative Columnaris bacteria between an internal or

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external infection. Differing treatment options dictate the classification of internal or external, not the disease. Establishing and maintaining suitable fish culture conditions and procedures can reduce the occurrence and severity of disease outbreaks (Post 1987; Jeney and Jeney 1995).

Florfenicol is a potent, broad-spectrum, antibacterial agent with bacteriostatic properties and is active against a variety of Gram-positive and Gram-negative bacteria (Horsberg et al. 1996). Because of its palatability to fish and high potency, florfenicol has become an important veterinary therapeutic drug, especially when administered orally in feed (Samuelsen et al. 1998; Gaunt et al. 2003; Wang et al. 2009; T. E. Powers, K. J. Varma, and J. D. Powers [abstract presented at the European Association for Veterinary Pharmacology and Toxicology meeting, 1988]). Aquaflor is an aquaculture feed premix (white powder) containing 50% florfenicol. Worldwide, Aquaflor has been approved in more than 20 countries (e.g., Norway, Japan, Chile, Canada, and the USA) to control disease mortality associated with infectious pathogens in a variety of cultured fish. The U.S. Food and Drug Administration Center for Veterinary Medicine (CVM) has approved Aquaflor as a Veterinary Feed Directive drug for use to control mortality from (1) enteric septicemia (ESC) in catfish associated with *Edwardsiella ictaluri*, (2) cold-water disease in freshwater-reared salmonids associated with *F. psychrophilum*, (3) furunculosis in freshwater-reared salmonids associated with *Aeromonas salmonicida* (post April 2012), (4) columnaris disease in freshwater-reared finfish (including Florida Largemouth Bass *Micropterus salmoides floridanus* and Bluegill *Lepomis macrochirus*) associated with *F. columnare*, and (5) streptococcal septicemia in freshwater-reared warmwater finfish associated with *Streptococcus iniae*. Leading up to approval in April 2012, public and private aquaculture groups in the USA sought to expand the approved Aquaflor label to include additional freshwater-reared finfish species and pathogens susceptible to florfenicol. Florfenicol has been shown to be efficacious against a number of fish pathogens, including *Aeromonas salmonicida* and *Vibrio salmonicida* (Fukui et al. 1987; Inglis and Richards 1991; Nordmo et al. 1998; Samuelsen et al. 1998; Bruun et al. 2000; Schmidt et al. 2001), *Edwardsiella ictaluri* (McGinnis et al. 2003), *F. columnare* (Gaunt et al. 2010b), and *Streptococcus iniae* (Bowker et al. 2010).

In many cases, when evidence of a pathogen is detected, diagnosis and administration of treatment must be rapid to control mortality and prevent an epizootic (Klontz 1987; Alderman 1988; Plumb 1999). Three oral antibiotics are approved by the U. S. Food and Drug Administration (FDA) for use to control mortality associated with various diseases in cultured fish populations. It is important to clarify that these treatment stipulations pertain to cultured food fish and for stocked recreational species (still considered food fish) when regulations allow for harvest prior to satisfying withdraw periods mandated by the FDA. None of these antibiotics were approved for use on Largemouth Bass *Micropterus salmoides* or Bluegill *Lepomis macrochirus* to control mortality associated with columnaris.

Terramycin 200 for Fish (44% oxytetracycline dihydrate; Phibro Animal Health, Corp., Ridgefield Park, New Jersey) is approved for use on all freshwater-reared rainbow trout *Oncorhynchus mykiss* and Steelhead (anadromous Rainbow Trout) infected with *F. columnare*. Romet 30 (25% sulfadimethoxine, 5% ormetoprim) and Romet TC (16.7% sulfadimethoxine, 3.3% ormetoprim; Aquatic Health Resources, Minnetonka, Minnesota) are approved for salmonids and catfish to treat *Aeromonas salmonicida* and *Edwardsiella ictaluri*. Before April 2012, Aquaflor was conditionally approved for use only on channel catfish *Ictalurus punctatus* to control mortality associated with columnaris disease.

Freshwater sport fish hatchery programs, including those for centrarchid species, continually demand development and approval of new therapeutic treatments for cultured species (Matthews et al. 2012; U.S. Fish and Wildlife Service, unpublished data). To help expand the FDA-approved label for Aquaflor (Lot number 030905, Intervet/Schering-Plow Animal Health Corp., Roseland, New Jersey) to include cultured sport fish of high demand for recreational stocking and restoration, two separate experimental trials were conducted in May and November of 2009 to evaluate the efficacy of Aquaflor-medicated feed to control mortality in freshwater-reared Florida Largemouth Bass and Bluegills diagnosed with columnaris infections. Each trial was conducted under an FDA-accepted research protocol at a targeted daily florfenicol dosage of 10 mg/kg of body weight for 10 d. The goal of the trials was to test the effectiveness of florfenicol to control mortality in Florida Largemouth Bass and Bluegills caused by natural infections of *F. columnare* and to provide evidence to support expanding the Aquaflor label to allow treatment of all freshwater-reared finfish for this claim.

METHODS

Two separate warmwater efficacy trials—one with Florida Largemouth Bass and the second with Bluegill—were conducted at the Florida Bass Conservation Center (FBCC), Richloam Fish Hatchery, Webster, Florida. Each trial lasted 25 d and consisted of a 1-d pretreatment period, a 10-d treatment period, and 14-d posttreatment period. Treatment conditions (Aquaflor-treated versus nontreated control) were allocated among tanks by using a completely randomized design. During the treatment period, Aquaflor-medicated feed was administered to treated tanks, and nonmedicated feed was administered to control tanks. During the posttreatment period, fish in all tanks received nonmedicated feed. The reference populations in both trials consisted of “Phase II” or advanced fingerling production slated for stocking into public assess water bodies. Feed amount for a specific tank was reduced if $\geq 25\%$ mortality occurred in that tank during the treatment period.

Test tanks were 378 L (based on stand pipe height) and made of dark green fiberglass (183 × 46 cm, 54 cm deep). Dark colored tanks were used to help calm excitable species like Florida

Largemouth Bass and Bluegill and can be used without tank covers that hinder observation, cleaning, and feed distribution. Aquaflor-medicated feed was administered at a target daily florfenicol dosage of 10 mg/kg of body weight for 10 consecutive days. Using a Marion Mixer, Aquaflor premix (Merck Animal Health, Summit, New Jersey) was top-coated with Menhaden fish oil (Omega Protein, Inc. Houston, Texas; 0.5% with/without) onto Silver Cup #4, 42% protein Salmon/Trout Crumbles (Nelson & Sons, Inc., Murray, Utah) for the bass trial and Silver Cup 2.0 mm, 42% protein Salmon extruded slow-sinking pellets for the Bluegill trial. Medicated and nonmedicated feed samples were collected from which florfenicol concentrations were analytically verified by Eurofins/AvTech Laboratories Inc., Portage, Michigan (Hayes 2005). Analysis of three, 200-g medicated-feed samples collected from the top, middle, and bottom of the feed bag was used to verify actual florfenicol concentration (mg/kg feed) of the mixed feed and to determine whether medicated feed had been mixed homogeneously during top-coating. Daily doses of the top-coated florfenicol feed were not analyzed over the 10-d treatment period. Two, 200-g nonmedicated feed samples were collected to ensure that feed had not been contaminated with florfenicol during manufacturing.

Disease onset in both trials were natural outbreaks. Before each trial began, columnaris was presumptively diagnosed in the reference populations of fingerling bass and Bluegills. Diagnosis was based on observance of skin lesions commonly characterized as "saddleback" in columnaris infections (Noga 2000) and detection of bacteria from posterior kidney tissue streaked on dilute Mueller-Hinton or Shieh's media. All plates were cultured at 26.0°C (Plumb 1999) for 5 d; colonies were visually noticeable between 48 and 72 h. Plates were visually examined every 24 h. Colonies cultured on the media that were yellow, had rhizoid edges, and adhered tightly to the media were examined microscopically. A diagnosis was deemed positive if the bacteria were (1) long, slender rods, (2) exhibited flexing and gliding motility, and (3) aggregated into stacked columns (hay stacks). All cultures meeting these criteria were additionally viewed via hanging drop procedures (Whitman 2004). Cultures from the pretrial evaluations were sent to the U. S. Fish and Wildlife Service (USFWS) La Crosse Fish Health Center (FHC), Onalaska, Wisconsin, for confirmation by polymerase chain reaction (PCR; Bader et al. 2003). During treatment and posttreatment periods, external and internal gross necropsies were performed on selected moribund fish (up to five fish per tank during each period). In addition, skin scrapes and kidney tissue from each fish necropsied were cultured and examined microscopically to presumptively identify cause of mortality via the same protocol used in pretrial diagnosis.

Fish that died during the 1-d pretreatment period were subtracted from the total number of fish transferred to each tank and not used to calculate cumulative mortality at the end of each trial. Mortality, general fish behavior, and fish feeding behavior were recorded daily during the treatment and posttreatment periods. Normal and abnormal behaviors were documented, and feeding

behavior was characterized as aggressive (all feed consumed), semiaggressive (most feed consumed), or nonaggressive (little to no feed consumed). The targeted dose was presumed by the rapid consumption of the feed and lack of residual feed removed daily from the bottom of each tank.

Dissolved oxygen (DO) and water temperature were recorded daily with an Oxyguard Handy Polaris Portable DO meter (Oxyguard International A/S, Birkerød, Denmark). Water alkalinity, hardness, and pH were measured once in water samples collected from each reference population tank and once from a water sample collected from a single test tank during the post-treatment period. These water quality variables were measured with a Hach Advanced portable laboratory CEL 890 digital titrator (Model 16900) and a SensIon1 portable pH meter (Hach Co., Loveland, Colorado). Carbon dioxide was monitored daily with an Oxyguard CO₂ Analyzer.

Each trial focused on the proportional risk of death versus survival. Probability of death was modeled with a mixed-effects logistic model fitted with SAS Proc GLIMMIX (logit link; SAS Institute 2007; Wolfinger and O'Connell 1993). The random effect of a test tank was modeled with an R-side covariance structure. Percent cumulative mortality in each test tank during the treatment and posttreatment periods only was calculated by using the total number of fish in each test tank at the beginning of the treatment period as the denominator (i.e., total mortality in test tank + number of live fish hand-counted from the test tank at end of trial). Mean percent cumulative mortality was compared between treated tanks and control tanks for all treatment and posttreatment days. Treatment levels were considered statistically significant at $\alpha = 0.05$.

Each trial was single-blinded to minimize the potential for data-collection bias. Nonblinded personnel who were aware of treatment conditions, stocked fish into tanks and weighed feed during the treatment period. Blinded participants collected and recorded all other data.

Florida Largemouth Bass trial.—The trial was conducted May 20 to June 13, 2009, on fingerling bass (mean length = 6.4 cm [SD = 0.6]; mean weight = 3.3 g [SD not available as only group weight was measured]). Mean weight and length of fish determined before the start of the trial was based on weighing three aliquots of fish (about 100 fish per aliquot) and measuring the total length of 20 fish. Fish were collected from the reference population (76,000 Florida Largemouth Bass fingerlings intensively cultured on pelleted feed in a 45,000-L concrete raceway) and stocked into 10 test tanks, 5 of which were randomly designated as treated and 5 as control. A total of 1,625 g of bass fingerlings (approximately 500 fish) were transferred to each tank to achieve fish densities similar to the reference population. To minimize the potential for bias, the transfer was completed in two rounds. In the first round, approximately 812 g of bass (approximately 250 fish) were weighed and transferred to each test tank; in the second round, approximately 813 g of bass (approximately 250 fish) were weighed and transferred to each test tank. To facilitate fish transfer, fish in

the reference population were crowded to one end of a raceway with a seine and then collected with dip nets. Excess water on fish and net was allowed to drain. For light sedation, the fish in the net were placed into a tarred bucket of water containing tricaine methanesulfonate (Tricaine-S, Western Chemical, Inc., Ferndale, Washington) at 25 mg/L of water and weighed to the nearest 0.1 g on a Denver Instruments balance (Denver MXX-5001 5,000 g, Denver, Colorado). Test tanks were supplied with first-pass well water (mean, 23.9°C) at an inflow of approximately 18.9 L/min (three exchanges/h).

Aquaflor-medicated feed was presumptively administered at a target daily florfenicol dosage of 10 mg/kg of body weight for 10 consecutive days. Actual florfenicol concentrations in the feed were not tested during the 10-d treatment. The medicated feed contained a calculated florfenicol dose of 0.2 g/kg of feed (0.04% florfenicol) and was fed at 5.0% initial mean body weight to achieve the target dose. During the treatment and posttreatment periods, feeding rate was not adjusted for growth. Feed was administered to tanks every 5 h by automatic feeders (Loudon style feeders, EMF Metal Fabrication, Centerville, Iowa), for a daily total of 81.3 g of feed per tank (500 bass/tank \times 3.25 g/bass \times 0.05 body weight). Additionally, medicated feed, florfenicol at 10 mg/kg body weight, was fed daily at 5.0% of body weight to the reference population (76,000 bass; average weight, 3.0 g) in a 45,420-L concrete raceway (U.S. Fish and Wildlife Service Investigational New Animal Drug Identification Number 10-697-09-10) in an effort to reduce mortality of our production bass. The results were used for visual comparison of large-scale field application to the trial tanks for observation of reduced mortality on a production level.

Bluegill trial.—The trial was conducted November 20 to December 14, 2009, on subadult Bluegills (mean length = 10.3 cm [SD = 1.1]; mean weight = 25.2 g [SD not available, only group weight measured]). We were randomly allocated 100 Bluegills to each of eight test tanks (four treated, four control). The remaining Bluegills in the 4,920-L raceway reference population (produced for stocking kids fishing ponds in state managed lakes) numbered less than 150 and were not treated with medicated feed. To minimize the potential for bias, all fish were hand-counted into a bucket and transferred to tanks in two rounds of 50 fish per round. Tanks were supplied with first-pass well water, 22.3°C, at an inflow of approximately 15.1 L/min (2.4 exchanges/h).

Aquaflor-medicated feed was presumptively administered at a daily target florfenicol dosage of 10 mg/kg of body weight for 10 consecutive days. Medicated feed in this trial contained a calculated florfenicol dose of 0.5 g/kg of feed (0.1% florfenicol) that was fed at 2.0% initial mean body weight to achieve the target dose. During the treatment and posttreatment periods, the feeding rate was not adjusted for growth. Feed was administered to tanks every 5 h by automatic feeder, for a total of 50.4 g of feed per tank per day (100 Bluegills/tank \times 25.2 g/Bluegill \times 0.02 body weight).

RESULTS

Florida Largemouth Bass

At trial completion, mean percent cumulative mortality in treated tanks (5.7% [SD = 1.6] per tank) was significantly ($P = 0.0004$) less than that in control tanks (12.0% [SD = 2.2]; Figure 1). A significant difference in mean percent cumulative mortality between treated and control tanks was detected and remained in effect from treatment day 4 through the end of the trial (posttreatment day 14). Mean cumulative mortality in control tanks (255 fish) was about twice that of treated tanks (126) during the 10 d treatment period, and nearly six times that during the 14 d posttreatment period (35 versus 6 fish). Results of PCR assay of cultured bacterial isolates (pretrial) positively confirmed the presence of *F. columnare* on three of three plates tested. Necropsy results were also consistent with columnaris and indicated no concomitant pathogens were present, but no generalized screening on TSA with blood was completed to verify. *Tetrahymena* and *Ichthyobodo* were found in low prevalence externally on several of the control bass, but we judged these parasites to be secondary invaders targeting the lesions initially caused by *F. columnare*. Mortality decreased concurrently in the treated reference population over the 10-day treatment period, 977 mortalities counted on day 2 and 32 on day 10. Mean percent cumulative mortality closely mimicked the trial tanks (Figure 1).

Predetermined sampling protocols utilized for this trial did not mandate large numbers of bacterial cultures during any phase of the trial. During the 10-d treatment period, 12 treatment and 16 moribund bass from control tanks (range, 1–4 per tank) were examined for columnaris, of which 6 treated and 11 control fish were plated. There were 11 fish (5 treated and 6

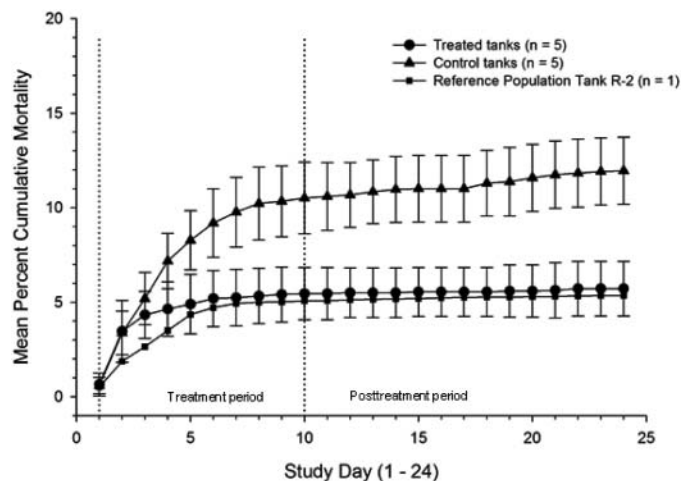


FIGURE 1. Mean (\pm 1 SD) percent cumulative mortality of Florida Largemouth Bass in florfenicol-treated and control tanks, as well as in the reference population tank that was similarly treated with florfenicol. Treatments were to assess efficacy in control of mortality caused by columnaris.

control, posttreatment) examined for columnaris, of which two treated and one control were plated. Plates that exhibited no growth after 5 d were considered not infected with *F. columnare* and discarded.

General behavior of fish in treated and control tanks appeared to improve during the treatment period. Normal behavior was characterized by constant mid-level swimming with adequate response to stimuli such as feed addition and tank cleaning. The predominant abnormal behavior observed during this period was lethargy (resting near or on the bottom). Hyperactivity (elevated gilling and fin movements and repeated swimming end to end in the tank not resulting from stimulus) was noted among fish in one treated tank during the first few days of the treatment period but was not observed thereafter. During the posttreatment period, fish behavior in all tanks was characterized as normal. Throughout the trial, feeding behavior was characterized as aggressive (all food consumed) in all control tanks and in four of five treated tanks. Fish in one treated tank exhibited semi-aggressive (majority of feed consumed) feeding behavior during the first 7 d of the treatment period. Feed analysis confirmed the mean florfenicol concentration from three samples was homogeneously mixed at 10.5 mg/kg of body weight per day, and no florfenicol contamination was measured in the control feed. Florfenicol dose consumed by each fish was presumptively based on the initial verified dose and total feed consumption. Actual amount of feed consumed per fish was unknown. No visual difference between treatment and control fish feeding behavior was observed during the 10-d treatment period, indicating no effects concerning florfenicol on feed consumption. At the end of trial, we discovered that about 473 fish had been stocked into each tank at the beginning of the trial in contrast with our assumption of 500 fish/tank. Consequently, the initial daily dose of florfenicol administered to fish in treated tanks was 11.1 mg/kg of body weight (111% of target).

Mean water temperature during the trial was 23.9°C (range, 22.6–24.9°C), and dissolved oxygen concentration was 13.7 mg/L (range, 12.0–14.6 mg/L). Carbon dioxide levels averaged 9 mg/L (range, 8–11 mg/L). Ranges depict daily measurements observed over the 25-d trial period. Mean water hardness (353 mg/L as CaCO₃), alkalinity (380 mg/L as CaCO₃), and pH 7.8 were within ranges suitable for rearing bass at the hatchery. Each of these three water chemistry metrics were recorded twice.

Bluegill

At the end of this trial, mean percent cumulative Bluegill mortality in treated tanks (18.5% [SD = 6.6] per tank) was significantly ($P = 0.0098$) less than that in control tanks, (37.5% [SD = 7.2] per tank; Figure 2). A significant difference in mean cumulative mortality between treated and control tanks was detected and remained in effect from treatment day 8 through the end of the trial. Mean cumulative mortality in control tanks (129 fish) was nearly double that of the treated tanks (71) during the treatment period and was three times that of treated tanks (21 ver-

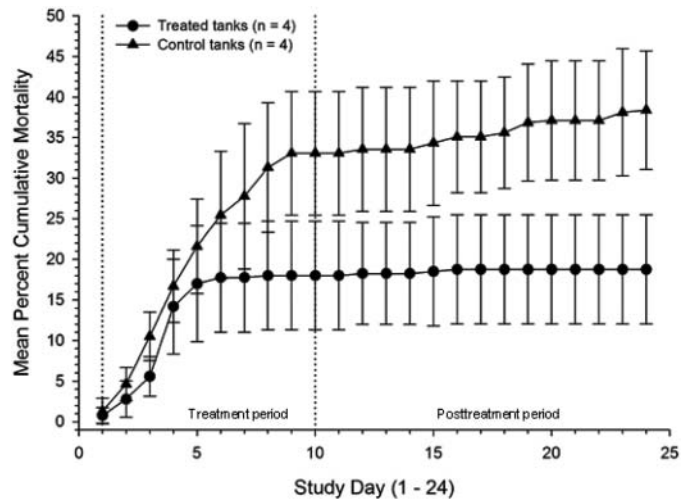


FIGURE 2. Mean (± 1 SD) percent cumulative mortality of Bluegills diagnosed with columnaris disease in florfenicol-treated and control tanks. Treatments were to assess efficacy in control of mortality caused by columnaris.

sus 7 fish) during the posttreatment period. Approximately 86% of the total mortality in control tanks and approximately 96% of total mortality in treated tanks occurred during the 10-d treatment period. Results of PCR assay of cultured bacterial isolates (pretrial) positively confirmed the pathogen as *F. columnare* on six of nine plates tested.

During the 10-d treatment period 32 moribund Bluegills, 16 treated fish (4/tank) and 16 control fish (4/tank) were examined. Kidney tissue streaked on Shieh's media produced *F. columnare* in 15 of the 16 cultures from the treated tanks and 16 of the 16 cultures from the control tanks. Results from 11 fish sampled during the posttreatment period showed no confirmed *F. columnare* cultures (posterior kidney) from three moribund Bluegills collected from three separate treatment tanks. However, cultured kidney tissue from seven of the eight moribund Bluegills from control tanks (1–3 fish/tank) showed an *F. columnare* infection. Low sample size during the posttreatment period reflects low numbers of moribund fish available to sample. Plates that exhibited no growth after 5 d were considered negative for *F. columnare* infection and discarded. Colonies not identified as columnaris bacteria were randomly examined specifically for *Aeromonas hydrophilla* on Tryptic soy agar with blood, which resulted in no positive identification. Colony identification methods performed were hanging drop slide, cytochrome oxidase test, 3% KOH test, and gram stain. No other bacterial identifications were completed or required by FDA protocol at the time of these trials.

General feeding behavior in treatment and control tanks ranged from nonaggressive (small amounts of feed consumed) to aggressive during the first 6 d of the treatment period and was aggressive thereafter. Throughout the trial, general fish behavior was characterized as normal. The analytically verified initial daily dose of florfenicol administered to fish in treated tanks was 10.0 mg/kg of body weight (100% of target), and no

florfenicol contamination was measured in the control feed. Florfenicol dose consumed by each fish was presumptively based on the initial verified dose and total feed consumption. Actual amount of feed consumed per fish was unknown. No visual difference between treatment and control fish feeding behavior was observed during the 10-d treatment period, indicating no effects concerning florfenicol on feed consumption.

Mean water temperature during the trial was 22.3°C (range, 20.3–23.8°C), and dissolved oxygen concentration was 10.5 mg/L (range, 9.1–11.1 mg/L). Carbon dioxide levels averaged 11 mg/L (range, 8–15 mg/L). Ranges depict differences observed over the 25-d trial period. Mean water hardness (365 as mg/L CaCO₃), alkalinity (340 mg/L as CaCO₃), and pH 7.9 were within ranges suitable for rearing Bluegills at the hatchery. Each of these three water chemistry metrics were recorded twice, as protocol requested.

DISCUSSION

In both trials, the targeted concentration of Aquaflor top-coated feed (florfenicol at 10 mg/kg of fish body weight) was effective in controlling mortality caused by confirmed columnaris infections in two representative warmwater fish populations. No further analysis of the medicated feed was collected during the 10-d treatment period. The lack of monitoring florfenicol dose concentration over the treatment period allows for changes in medication concentrations, if any, to go unreported. Evaluation of florfenicol concentrations at three doses (10, 30, and 50 mg/kg of body weight per day) in channel catfish feed showed no significant change in medication concentrations at any level after a 19-d interval (Gaikowski et al. 2003). Initial florfenicol concentrations were analyzed in both FBCC trials and homogeneously mixed feed concentrations were established. Gaunt et al. (2003) reported florfenicol remained stable in homogeneously mixed catfish feeds. Concern of reduced florfenicol concentration leaching from the medicated feed was minimized based on pharmacokinetic studies that showed considerable loss of florfenicol from uneaten feed in water after 5 min (Yanong et al. 2005). In both of our trials, the majority of the feed was consumed in the treated tanks within seconds of the feed hitting the water. Uneaten feed was removed along with waste products after each feeding.

Prevalence of the infection was only visually observed and not statistically evaluated in the bass and Bluegill reference populations. Daily mortality counts from the bass reference population showed a 97% decline in mortality from day 2 to day 10 of the treatment period. Data were not reported from the Bluegill reference population due to the diminished population size after stocking the trial tanks. To our knowledge, there is no other published information reporting the effectiveness of florfenicol treatments on Florida Largemouth Bass and Bluegills for this claim. However, there is considerable evidence showing that florfenicol is effective against *F. columnare* in other fish species, as well as against other bacterial pathogens. Gaunt et al. (2010b)

reported that florfenicol was effective in controlling mortality caused by columnaris in channel catfish (mean weight range, 6.8–9.2 g) when administered at the same dosage used in our trials. The authors reported that mean cumulative mortality following a 14-d posttreatment period was 8% in treated tanks and 54% in control tanks. Others have reported that florfenicol treatments reduced mortality in catfish caused by ESC associated with *Edwardsiella ictaluri* (Gaunt et al. 2003, 2004, 2006), in Atlantic Salmon *Salmo solar* caused by furunculosis associated with *Aeromonas salmonicida* (Samuelsen et al. 1998), and in sunshine bass (female White Bass *Morone chrysops* × male Striped Bass *M. saxatilis*) associated with *Streptococcus iniae* (Darwish 2007; Bowker et al. 2010). Bowker et al. (2010) reported that the analytically verified florfenicol dose administered was 8.3 mg/kg body weight per day. Regardless, these authors reported that mean percent cumulative mortality in treated tanks (19%) was significantly different from that in control tanks (52%).

Animal safety studies, dose determination, and residue depletion have not been conducted on Largemouth Bass or Bluegill but have been conducted on other warmwater and coolwater species. Gaikowski et al. (2003) reported that no detrimental histological effects were detected in channel catfish fed medicated feed at daily florfenicol dosages of 10, 30 or 50 mg/kg of body weight for 20 d. Straus et al. (2012) reported that no significant clinical histological lesions were detected when sunshine bass were fed florfenicol-medicated feed at daily dosages of 15, 45, or 75 mg/kg of body weight for 20 d. Similar results have been observed in Yellow Perch *Perca flavescens* (J. Bowker, unpublished) and tilapia (*M. Gaikowski*, USGS personal communication). Gaunt et al. (2004, 2010a) reported that Aquaflor was palatable, safe, and efficacious for controlling mortality caused by ESC in Channel Catfish and *Streptococcus iniae* in Nile Tilapia *Oreochromis niloticus* at florfenicol concentrations of 10–15 mg/kg of body weight per day. Florfenicol administered daily at 10 mg/kg of body weight to cultured Atlantic Salmon was also reported efficacious for the treatment of furunculosis (Inglis et al. 1991; Nordmo et al. 1994; Samuelsen et al. 1998).

One concern of fish culturists, fish health biologists, and veterinarians involved in fish culture is the withdrawal period, which is the number of days treated fish must be held before they can be harvested for market or released into the wild. For economic and logistic reasons, shorter withdrawal times are desirable. The tolerance for florfenicol has been established by the FDA at 1,000 parts/10⁹ (or 1 µg/g) in muscle tissue and skin for Channel Catfish and salmonids (Bowser et al. 2009). This information, in part, was used by FDA to establish a withdrawal period of 12 d for the initial approval of Aquaflor for approved use in Channel Catfish (before 2009). This withdrawal period is considerably shorter than the withdrawal periods established for Terramycin 200 for Fish (21 d) and Romet30 and RometTC on salmonids (42 d) but considerably longer than for Romet30 and RometTC (3 d) on Channel Catfish (USFWS 2011). Recent trials conducted to evaluate depletion of florfenicol residues over

time in a variety of coolwater and warmwater fish species fed at a daily target dosage of 10–15 mg/kg of body weight for 10 d showed that water temperature and fish size are factors affecting the rate of drug residue depletion from fish tissues. Wrzesinski et al. (2006) reported for Channel Catfish (mean weight, about 900 g) florfenicol residue levels in muscle tissue remained below tolerance levels at 4 d following a 12-d treatment at a daily dose of 9.3 mg/kg of body weight and remained below tolerance levels thereafter. Kosoff et al. (2009) reported that florfenicol residues reached the 1,000 parts/10⁹ (1 µg/g) tolerance level in (1) Nile Tilapia between 4.1 and 6.1 d when fish were tested at a water temperature of 30°C, (2) Walleye *Sander vitreus* between 9.7 and 12.6 d when tested at 25°C, and (3) sunshine bass between 0.7 and 2.6 d when tested at 25°C and 20°C. Bowser et al. (2009) reported that when florfenicol was administered at a daily dose of 15 mg/kg body weight to three different sizes of Nile Tilapia that elimination was slightly quicker for smaller fish: 9.2 d for 100-g fish, 8.6 d for 250-g fish, and 12.7 d for 500-g fish. Based on this information, the FDA added 3 d to the withdrawal period, making it 15-d. This ensured that florfenicol residues declined to acceptable FDA standards for freshwater species (including Largemouth Bass and Bluegills treated at daily florfenicol doses up to 15 mg/kg of body weight reared or held at water temperatures ≥20°C. As a result of our trials and other associated literature, Aquaflor use in the USA has been expanded and approved (April 9th, 2012) to treat all freshwater-reared finfish to control mortality caused by columnaris; fish may be treated at a florfenicol dose of 10–15 mg/kg of body weight per day with a withdrawal period of 15 d (still under the Veterinary Feed Directive).

Evaluating efficacy is not only dependent on proper conduct of a trial, but perhaps more importantly on the early diagnosis of the disease, timely administration of treatment, and definitive confirmation of the causative infectious fish pathogen. In the Florida Largemouth Bass trial, the actual initial florfenicol dose administered to fish in treated tanks was 11.1 mg/kg of body weight per day. Despite evidence that the bass were initially slightly overmedicated, the therapeutic effects of treatment became evident midway through the treatment period when mortality in treated tanks decreased to negligible levels. Further support of treatment efficacy was evident by the pattern of mortality observed in the bass reference population, where mean percent cumulative mortality mimicked that observed in test tanks treated with Aquaflor in our trial.

Fish disease management strategies, such as those described by Piper et al. (1982), emphasize that it is incumbent upon the fish culturist to maintain the best possible environmental conditions and minimize stress. However, even under the best environmental conditions, disease outbreaks can occur. In such situations, it is imperative for fish culturists to have legal access to antibiotics such as florfenicol to help control disease-related mortality. In the Bluegill trial, cultured posterior kidney tissue identified *F. columnare* in 94% and 100% of the samples for both treatment and control tanks during the treatment pe-

riod and 0% and 88% during the posttreatment period. Similar patterns were found for the bass trial, but small numbers of plate cultures made comparisons difficult. Reduced mortality in treated tanks restricted numbers of moribund fish sampled during the posttreatment period in both trials. Control tanks continued to experience columnaris-related mortality during the posttreatment period, while no bass or Bluegill mortalities from a treated tank were diagnosed positive for columnaris during the same time. Based on these trial results, we consider Aquaflor-medicated feed effective in controlling columnaris-caused mortality in Largemouth Bass and Bluegill fingerlings. Both trials were initiated in an attempt to supply supporting evidence to gain approval for the use of Florfenicol on all freshwater species. Our efforts successfully aided in the approval process, and the now-approved use of florfenicol will help culturists reduce mortality levels caused by columnaris infections on freshwater species in the USA. Our trials were not designed to address the susceptibility of *F. columnare* to florfenicol. Minimum inhibitory concentrations (MIC) of 0.5–1.0 µg/mL were reported from *F. columnare* isolates against florfenicol from infected Channel Catfish (Gaunt et al. 2010b). Future *F. columnare* studies that include MIC testing should utilize methods detailed by Darwish et al. (2008).

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