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Controlling Mortality Caused by External Columnaris in Largemouth Bass and Bluegill with Chloramine-T or Hydrogen Peroxide

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ARTICLE

Controlling Mortality Caused by External Columnaris in Largemouth Bass and Bluegill with Chloramine-T or Hydrogen Peroxide

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Abstract

Columnaris (causative agent, *Flavobacterium columnare*) is a widespread fish disease of concern among fish culturists in the USA. If left untreated, an entire population of fish may become infected, and morbidity and mortality may reach high levels. In virtually all instances, columnaris outbreaks require intervention to prevent significant losses. A number of sanitizing agents, most notably chloramine-T (CLT) and hydrogen peroxide (HP), have been used to control mortality associated with a variety of bacterial pathogens causing external infections. However, the majority of trials conducted to demonstrate the effectiveness of these chemicals, thereby gaining U.S. Food and Drug Administration approval for their use in treating fish infected with columnaris, have been conducted on salmonids. Accordingly, we conducted seven experiments to evaluate the effectiveness of CLT or HP to control mortality associated with external columnaris in Florida Largemouth Bass *Micropterus salmoides floridanus* and Bluegill *Lepomis macrochirus*. Treatment with CLT or HP significantly reduced cumulative mortality in five of the seven experiments. Cumulative mortality was strongly correlated to pretreatment mortality in treated and control tanks in the five Largemouth Bass experiments, suggesting that intervention at later stages of columnaris progression may result in less favorable outcomes. Odds ratios calculated for individual experiments indicated varying degrees of success in controlling mortality; however, meta-analysis of all experiments indicated treatment with either CLT or HP significantly increased probability of survival, regardless of fish species or test article. These results demonstrate that both chemicals can be effective in controlling mortality associated with external columnaris in Largemouth Bass and Bluegills and that timely treatment of fish will likely result in lower overall mortality.

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Columnaris (causative agent, *Flavobacterium columnare*; formerly *Flexibacter columnaris*) is an acute-to-chronic external or systemic bacterial disease affecting freshwater-reared finfish worldwide (Bullock et al. 1986). Infections begin when *F. columnare* invades epithelial tissues, most commonly affecting the gills and buccal, opercular, dorsal, and caudal surfaces (Post 1987). Lesions form as the infection progresses and are often observed at the base of the dorsal fin or on the caudal fin, lending columnaris its colloquial names, "saddleback" and "fin rot." If left untreated, external lesions may penetrate blood vessels or the body cavity, leading to systemic infections.

Columnaris can be presumptively diagnosed based on clinical signs, including the presence of the aforementioned lesions and long, slender, possibly filamentous, rod-shaped, Gram-negative bacteria (Post 1987) exhibiting the characteristic "haystack" formation and "flexing" behavior of *F. columnare* (Noga 2000). In virtually all instances, columnaris outbreaks require intervention to prevent significant losses. Shedding of bacteria from the epithelial surfaces of infected fish may create a self-perpetuating, population-wide infection (Post 1987). If left untreated, mortalities in overcrowded or unsanitary conditions may reach 70% or higher among young and most susceptible fishes (Post 1987). During outbreaks, fish culturists can often minimize mortality by improving environmental rearing conditions, administering chemotherapeutic bath treatments, or both.

A number of external sanitizing agents, including chloramine-T (CLT) and hydrogen peroxide (HP), have been used to control mortality caused by *Flavobacterium* in a variety of freshwater finfish, including *F. branchiophila* (a causative agent of bacterial gill disease [BGD]) and *F. columnare*. Chloramine-T ($C_7H_7ClNNaO_2S{\cdot}3H_2O$) is a biocide used worldwide as a disinfectant and antiseptic. Although CLT has been used for years under authorization of publically held Investigational New Animal Drug (INAD) exemptions and has been shown to effectively control mortality associated with BGD in freshwater-reared salmonids (From 1980; Speare and Ferguson 1989; Bullock et al. 1991; Thorburn and Moccia 1993; Ostland et al. 1995; Bowker and Erdahl 1998; Bowker et al. 2008) and columnaris in a variety of freshwater finfish, it is not yet approved by the U.S. Food and Drug Administration (FDA) for use on fish.

Hydrogen peroxide (H_2O_2) is used as a bleaching agent in the textile industry, as an antimicrobial agent in cheese production and treatment of drinking water (Marking et al. 1994), and in human healthcare as a first aid antiseptic and oral debriding agent. It has also been used to treat a variety of external fungal, bacterial, and parasite infections and infestations on fish and fish eggs (e.g., Marking et al. 1994; Mitchell and Collins 1997; Speare and Arsenault 1997; Lumsden et al. 1998; Howe et al. 1999; Rach et al. 2000a, 2000b, 2003; Buchmann and Kristensson 2003; Montgomery-Brock et al. 2001, 2004; Sitjà-Bobadilla et al. 2006; Russo et al. 2007; Bravo et al. 2010; Bowker et al. 2012a). The only product approved by the FDA for controlling mortality in fishes associated with *Flavobacterium* is 35% PEROX-AID (active ingredient is 35% HP; Eka Chemical, Marietta, Georgia). Specifically, it can be used to control mortality associated with BGD in freshwater-reared salmonids and external columnaris in coolwater finfish and channel catfish *Ictalurus punctatus* (USFDA 2012), although efficacy has been observed in controlling *Flavobacterium*-related mortality in other fishes (USFWS AADAP 2011).

Although field trials suggest that CLT and HP are effective in controlling *Flavobacterium*-related mortality in a variety of fish (Bonnie Johnson, USFWS, personal communication), relatively few rigorous, experimental trials have been conducted, and therefore the approved uses of these compounds are restricted to the claims previously described for HP. Furthermore, few studies have directly compared the suitability of CLT and HP in controlling external columnaris disease-related mortality. Therefore, we conducted a series of experiments in which Florida Largemouth Bass *Micropterus salmoides floridanus* and Bluegills *Lepomis macrochirus* diagnosed with external columnaris were treated with CLT or HP according to different treatment regimens.

METHODS

Test Facility, Test Fish, and Test Articles

Experiments were conducted over a 15-month period from July 2007 to September 2008 at the Florida Bass Conservation Center's Richloam Fish Hatchery, Webster, Florida. Test fish were Bluegills or Florida Largemouth Bass fingerlings collected from onsite reference populations presumptively diagnosed with external columnaris. Test articles were Chloramine-T (100% CLT; H&S Chemical, Covington, Kentucky) and 35% PEROX-AID. Fiberglass, rectangular test tanks (1.8 × 0.45 m, water depth = 0.45 m, rearing volume ≈ 382 L) supplied with degassed, aerated well water (same water source as in reference population tanks) were used for all experiments.

Experimental Design and Procedures

Completely randomized designs (Peterson 1985) were used to allocate fish from reference populations to test tanks and assign treatments to tanks. Each experiment comprised a 1-d pretreatment acclimation period, a treatment period of variable length depending on the drug and treatment regimen, and a 14-d posttreatment period. Static treatments were administered by turning off water to tanks, adding test article or sham water treatments, and mixing tank contents. Water flow was returned to the original rate after 60 min.

Before experiments started, 10 moribund fish were collected from the reference population and evaluated for fish health. The external and internal tissues of all fish examined appeared normal, with the exception of skin lesions characteristic of external columnaris, which were found on all fish. Presumptive diagnoses of columnaris were made via microscopic examination of wet-mount skin-scrapes prepared for each fish, which revealed motile bacteria morphologically similar to

F. columnare in all samples. Pretreatment mortality (i.e., mortality on the day before fish were allocated to test tanks) ranged from 0.1% to 4.7% of the reference population. Except where noted below, mean length of test fish was calculated by collecting and individually measuring 20 reference-population fish, whereas mean weight of test fish was based on sample-counting (Piper et al. 1982) three groups (50 fish/group) of reference population fish.

Experiment 1.—Florida Largemouth Bass were treated with 20 mg/L CLT for 60 min on three consecutive days. The reference population (pretreatment mortality = 0.20%) comprised approximately 21,630 bass fingerlings (mean weight = 4.7 g, mean length = 7.0 cm) held in a single concrete raceway supplied with water to achieve 1.0 exchanges/h. Twelve test tanks (six control tanks and six treated tanks) were stocked with fish from the reference population by weight to achieve target densities 314 fish/tank (3.9 g fish/L). However, at the end of the experiment, when all live fish remaining were hand-counted and mortalities were accounted for, we determined that initial stocking numbers had actually ranged from 292 to 316 fish/tank (3.7–4.0 g fish/L). Water flow to test tanks was 23.7 L/min; thus, water turnover rate was 3.8 exchanges/h. During the experiment, fish were fed a diet formulated for Florida Largemouth Bass at Richloam Fish Hatchery (based on typical trout/salmon feed) and prepared by Nelson and Sons, Inc. (Tooele, Utah) at a daily rate of 5% of estimated body weight delivered every 5 h by Louden-style pneumatic feeders. On day 12 of the posttreatment period, a total of eight moribund fish collected from control tanks were examined for external columnaris. There were no moribund fish in the treated tanks; hence, no treated fish were collected and examined.

Experiment 2.—Florida Largemouth Bass were treated with CLT at 20 mg/L of water for 60 min on three consecutive days. The reference population (pretreatment mortality = 0.08%) comprised approximately 98,800 bass fingerlings (13.6 g, 10.8 cm) held in a single concrete raceway supplied with water to achieve 1.0 exchanges/h. Eight test tanks (four control tanks and four treated tanks) were stocked with fish from the reference population at 800 fish/tank (36.5 g fish/L). Water flow to test tanks was 23.7 L/min, and the water turnover rate was 3.8 exchanges/h. Fish were fed as in experiment 1.

Experiment 3.—Bluegills were treated with CLT at 20 mg/L of water for 60 min on three alternate days. The reference population (pretreatment mortality = 4.40%) comprised approximately 2,400 Bluegill fingerlings (15.8 g, 8.8 cm) held in a single fiberglass rectangular tank supplied with water to achieve 0.9 exchanges/h. Eight test tanks (four control tanks and four treated tanks) were stocked with fish from the reference population at 200 fish/tank (7.6 g fish/L). Water inflow to test tanks was 37.9 L/min, and the water turnover rate was 6.0 exchanges/h. Fish were fed the same diet used in experiment 1, except the daily ration was 3% of estimated body weight. Three moribund fish were collected from each test tank on days 3–5 of the treatment period, and one to three moribund fish were collected from each test tank during the first six posttreatment days; all of these fish were examined for external columnaris.

Experiment 4.—Florida Largemouth Bass were treated with CLT at 20 mg/L of water for 60 min on three alternate days. The reference population (pretreatment mortality = 0.76%) comprised approximately 20,000 bass fingerlings (17.7 g, 12.5 cm) held in a single fiberglass tank supplied with water to achieve 1.1 exchanges/h. Eight test tanks (four control tanks and four treated tanks) were stocked with fish from the reference population at 275 fish/tank (12.2 g fish/L). Water flow to test tanks was 18.9 L/min, and the water turnover rate was 3.0 exchanges/h. Fish were fed in the same manner as described for experiment 1. Three moribund fish were collected from each test tank on days 2–5 of the treatment period, and three fish (most of which were moribund) were sampled from each test tank on posttreatment days 3–5; all of these fish were examined for external columnaris.

Experiment 5.—Florida Largemouth Bass were treated with HP at 150 mg/L of water for 60 min on three consecutive days. The reference population, test tank loading densities, and general experimental conditions were as described for experiment 2 because these two experiments were conducted concurrently.

Experiment 6.—Florida Largemouth Bass were treated with HP at 50 mg/L of water for 60 min on three alternate days. The reference population, test tanks loading densities, diet and feeding rate, and general experimental conditions were as described for experiment 4 because these two experiments were conducted concurrently.

Experiment 7.—Bluegills were treated with HP at 50 mg/L of water for 60 min on three alternate days. The reference population (pretreatment mortality = 3.65%) comprised approximately 1,700 Bluegill fingerlings (16.5 g, 10.8 cm) held in a single fiberglass tank supplied with water to achieve 0.7 exchanges/h. Twelve test tanks (six control tanks and six treated tanks) were stocked with fish from the reference population at 100 fish/tank (4.6 g fish/L). Water flow to test tanks was 11.4 L/min, and the water turnover rate was 1.8 exchanges/h. Twice daily, Bluegills were hand fed the same diet used in experiment 1 at a daily rate of 2% of estimated body weight. Three moribund fish were collected from each test tank on days 2–5 of the treatment period, and three fish were sampled from each test tank on posttreatment days 4–7; all of these fish were examined for external columnaris. Fish sampled from treated tanks appeared healthy, whereas eight of the fish sampled from controls tanks appeared healthy and the remaining 10 fish were moribund.

Data Collection

Mortalities were recorded daily. Fish collected and examined for external columnaris were examined grossly to assess external and internal organs: skin and gills were examined for presence of lesions and tissue necrosis, and wet mounts of skin scrapes and gill filaments were examined microscopically for the presence of motile bacteria presumptively identified as *F. columnare*.

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TABLE 1. Mean (ranges in parentheses) water quality metrics and analytically verified concentrations of drugs, as measured during experiments conducted to evaluate the effectiveness of chloramine-T or hydrogen peroxide to control mortality associated with columnaris in Largemouth Bass and Bluegills.

Experiment	Water temperature (°C)	Dissolved oxygen (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)	pH	Verified concentration (mg/L)	Deviation from target dose (%)
1	25.2 (24.1–26.4)	15.8 (10.7–17.7)	245	373	8.1	19.5 (16.7–21.6)	2.5
2	24.8 (24.1–25.4)	15.2 (10.2–19.2)	264	381	7.9	20.5 (18.5–22.9)	2.5
3	22.2 (19.0–24.8)	11.9 (8.5–16.3)	317	337	7.6	22.1 (21.3–23.6)	10.5
4	24.8 (22.5–27.2)	13.3 (8.8–17.6)	320	345	7.6	20.5 (20.0–21.1)	2.5
5	24.8 (24.1–25.4)	15.2 (10.2–19.2)	364	382	7.9	153.8 (136.0–165.8)	2.5
6	24.8 (22.5–27.2)	13.9 (8.8–17.7)	320	350	7.6	50.3 (46.8–53.1)	0.6
7	24.9 (23.8–26.6)	15.2 (10.6–18.1)	345	370	7.6	48.6 (46.3–51.4)	2.8

General fish behavior was assessed daily and characterized as normal or abnormal for each tank, and any abnormal behaviors (e.g., lethargy, hyperactivity, flashing, piping at the surface) were described. Feeding behavior was also assessed daily and characterized as "2 = aggressive" (fish appeared to be actively feeding and that nearly all feed offered was consumed), "1 = semiaggressive" (some fish appeared to be feeding actively but some fish did not appear to be feeding), or "0 = nonaggressive" (none of the fish appeared to be feeding).

Water temperature and dissolved oxygen (DO) concentration were measured at least once daily during each experiment, whereas water hardness, alkalinity, and pH were measured at the beginning and end of each experiment from water samples collected from one randomly selected test tank. Water temperature and DO concentration were measured with an OxyGuard Handy Polaris Portable DO Meter (OxyGuard International A/S, Birkerød, Denmark). Water hardness and alkalinity were measured with a Hach titrator and reagents (Hach Co., Loveland, Colorado), and pH was measured with a Hach sensION1 portable pH meter. All water quality parameters measured were within ranges considered adequate for rearing Largemouth Bass and Bluegill (Table 1).

Water samples were collected from each test tank during each treatment period and analyzed to verify CLT or HP dose. The titrimetric method described by Jeffery et al. (1989) was used to determine HP concentrations. A Hach DR/890 Colorimeter was used to measure free and total chlorine, and these values were used to calculate CLT concentrations as described by Dawson et al. (2003).

Data Analysis

Mortality data analysis was done using the PROC GLIMMIX (logit link; Wolfinger and O'Connell 1993) function of the Statistical Analysis System software (version 9.2, SAS Institute, Cary, North Carolina), comparing treated fish to control fish on each day of the treatment and posttreatment periods.

Individual experiment odds ratios and associated asymmetric 95% confidence intervals (CIs) were calculated (PROC GLIMMIX); 95% CIs > 1 indicated that treatment significantly improved the probability of survival. An overall odds ratio and associated asymmetric 95% CI were calculated using the meta-analysis function of MedCalc software (version 12.3.0.0, MedCalc Software, Mariakerke, Belgium). The meta-analysis test for heterogeneity revealed significant variation among the experiments; thus, the random effects model (DerSimonian and Laird 1986) was used to generate a more conservative estimate of the overall odds ratio.

For analysis of individual experiments, tank was considered the experimental unit ($N =$ 4 or 6, depending on the experiment); for the meta-analysis, experiments were considered the experimental unit. All hypotheses were tested at an $\alpha = 0.05$ (two-tailed tests).

Microsoft Excel software (Microsoft Corporation 2010), SYSTAT 12 software (SYSTAT 2007), and SigmaPlot 12 software (SYSTAT 2011) were used to generate summary statistics (e.g., mean, standard deviation, range) for water temperature, dissolved oxygen concentration, other water chemistry measurements, and mean daily mortality. Linear regressions (SYSTAT 2011) were used to determine if there was a relation between pretreatment mortality of Florida Largemouth Bass in the reference population and the cumulative mortality among (1) treated fish or (2) control fish. For each regression, pretreatment mortality was the independent variable, and cumulative mortality was the dependent variable.

RESULTS

A significant difference was detected in mortality between treated and control groups at some point during each experiment. However, at the end of the posttreatment periods, cumulative mortality of treated fish was significantly less than that of control fish in five of the seven experiments (experiments 1, 3, 4, 6, and 7). Odds ratios calculated for individual experiments indicated significantly greater probabilities of survival among treated fish compared with control fish in the same five experiments for which significant differences in cumulative mortality were observed (range = 1.25–3.03). However, the overall odds ratio resulting from the meta-analysis was 1.83 (95% CI = 1.43–2.35), indicating a significant beneficial effect of treatment, regardless of fish species or test article. Cumulative mortality was strongly and positively related to pretreatment mortality in treated ($r^2 = 0.98$) and control ($r^2 = 0.96$) tanks in the five Florida Largemouth Bass experiments. In both treated

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and control groups, higher mortality rates in the reference population before the start of the experiments were associated with higher cumulative mortality rates.

Dose verification levels were within ±20% of the target dose in each experiment (Table 1), and no CLT or HP was detected in samples collected from control tanks. Mean water temperature for each experiment ranged from 22.2°C to 25.2°C, and individual measurements ranged from 19.0°C to 27.2°C (Table 1). Mean DO concentrations for each experiment ranged from 11.9 to 15.8 mg/L, and individual measurements ranged from 8.5 to 19.2 mg/L. Except where noted below, fish behavior was considered normal in all test tanks during each experiment, and fish fed at least semiaggressively on the first day of the experiment and aggressively thereafter.

Experiment 1

At the end of this experiment, mean cumulative mortality of Florida Largemouth Bass treated with CLT was 26.8% (SD = 5.4, range = 20.5–34.0%), which was significantly ($P = 0.0100$) less than the mean of 35.3% for control fish (SD = 3.4, range = 32.8–41.9%). A significant difference between groups was evident after experiment day 2 (Figure 1). The odds ratio for this experiment was 1.49 (95% CI, 1.12–1.97). Active *F. columnare* were observed on all skin scrapes taken from fish sampled from control tanks during the posttreatment period.

Experiment 2

At the end of this experiment, mean cumulative mortality of Florida Largemouth Bass treated with CLT was 17.9% (SD = 6.6, range = 13.0–27.6%), which was not significantly ($P = 0.4842$) different from the control fish mean of 21.9% (SD = 8.9, range = 15.8–33.9%), However, significant differences were detected on experiment days 3–12 (Figure 2). The odds ratio for this experiment was 1.29 (95% CI, 0.56–2.94). General fish behavior was characterized as abnormal (hyperactive) in seven of eight tanks during the treatment period but normal in all tanks during the posttreatment period.

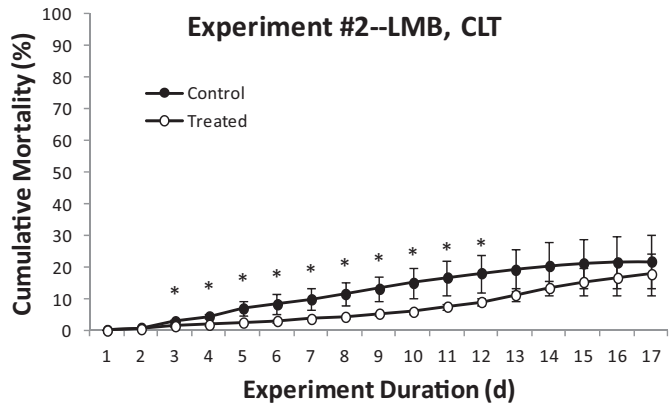

FIGURE 2. Mortality of Florida Largemouth Bass (LMB) observed in experiment 2. Treated fish were exposed to chloramine-T (CLT) at 20 mg/L of water for 60 min/d on three consecutive days, whereas control fish received sham treatments (four replicate tanks/treatment). Asterisks indicate days on which a significant difference ($P < 0.05$) was detected between treated and control groups; at the end of the trial, cumulative mortality of treated fish was not significantly ($P = 0.4842$) different from that of control fish.

Experiment 3

At the end of this experiment, mean cumulative mortality of Bluegills treated with CLT was 12.9% (SD = 2.3, range = 9.8–15.5%), which was significantly ($P = 0.0304$) less than the control fish mean of 26.9% (SD = 10.3, range = 18.7–41.5%). In addition, a significant difference in mortality was detected between treated and control groups from day 10 through the end of the experiment (Figure 3). The odds ratio for this

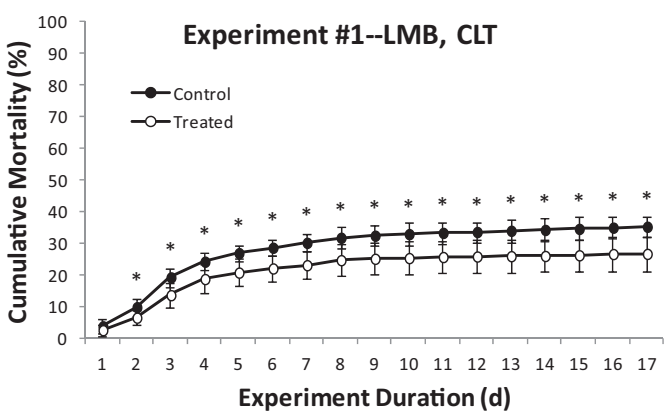

FIGURE 1. Mortality of Florida Largemouth Bass (LMB) observed in experiment 1. Treated fish were exposed to chloramine-T (CLT) at 20 mg/L of water for 60 min/d on 3 consecutive days, whereas control fish received sham treatments (six replicate tanks/treatment). Asterisks indicate days on which a significant difference ($P < 0.05$) was detected between treated and control groups; at the end of the trial, cumulative mortality of treated fish was significantly ($P = 0.0100$) less than that of control fish.

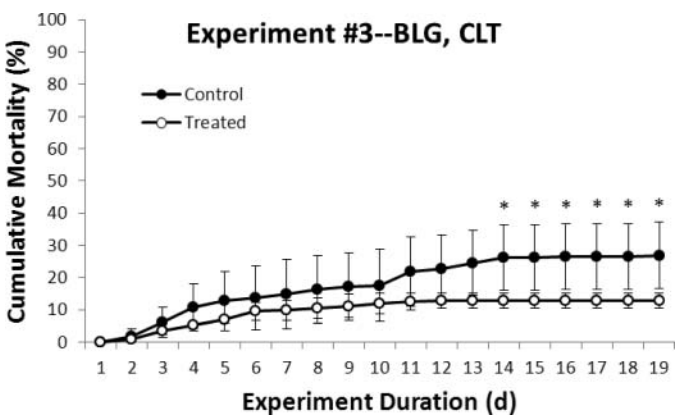

FIGURE 3. Mortality of Bluegills (BLG) observed in experiment 3. Treated fish were exposed to chloramine-T (CLT) at 20 mg/L of water for 60 min/d on three alternate days, whereas control fish received sham treatments (four replicate tanks/treatment). Asterisks indicate days on which a significant difference ($P < 0.05$) was detected between treated and control groups; at the end of the trial, cumulative mortality of treated fish was significantly ($P = 0.0304$) less than that of control fish.

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experiment was 2.50 (95% CI, 1.13–5.54). Feeding behavior was characterized as nonaggressive during the treatment period and from nonaggressive to aggressive during the posttreatment period. Bacteria presumptively identified as *F. columnare* were observed on skin scrapes from 7 fish sampled from treated tanks and 11 fish sampled from control tanks during the treatment period. Bacteria presumptively identified as *F. columnare* were observed on skin scrapes from all fish sampled from control tanks but not on the samples collected from treated tanks during the posttreatment period.

Experiment 4

At the end of this experiment, mean cumulative mortality of Florida Largemouth Bass treated with CLT was 45.5% (SD = 6.3, range = 41.0–54.9%), which was significantly (P = 0.0034) less than the control fish mean of 62.6% (SD = 2.9, range = 59.5–66.2%). In addition, a significant difference in mortality was detected between treated and control groups for the last 10 d of the experiment (Figure 4). The odds ratio for this experiment was 1.97 (95% CI, 1.38–2.80). Bacteria presumptively identified as *F. columnare* were observed on skin scrapes from most (20 of 24) fish sampled during the treatment period, and from all 12 fish sampled from control tanks and from 1 fish sampled from treated tanks during the posttreatment period.

Experiment 5

At the end of this experiment, mean cumulative mortality of Florida Largemouth Bass treated with HP was 17.6% (SD = 4.9, range = 12.1–22.8%), which was not significantly (P = 0.3779) different from the control fish mean of 21.1% (SD = 5.47, range = 13.3–22.8%). However, a significant difference in mortality was detected between treated and control groups on experiment days 9–13 (Figure 5). The odds ratio for this experiment was 1.25 (95% CI, 0.70–2.23).

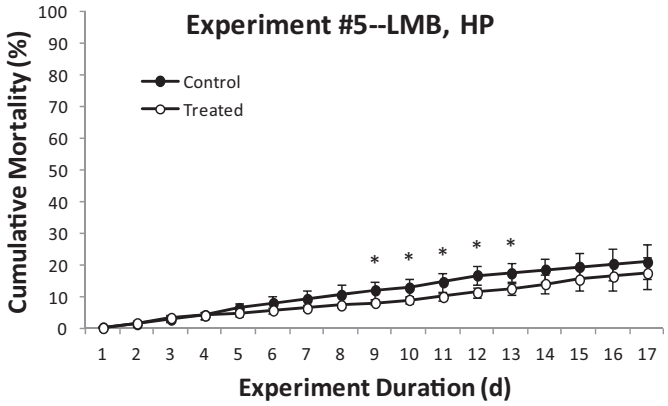

FIGURE 5. Mortality of Florida Largemouth Bass (LMB) observed in experiment 5. Treated fish were exposed to chloramine-T (CLT) at 150 mg/L of water for 60 min/d on three consecutive days, whereas control fish received sham treatments (four replicate tanks/treatment). Asterisks indicate days on which a significant difference (P < 0.05) was detected between treated and control groups; at the end of the trial, cumulative mortality of treated fish was not significantly (P = 0.3779) different from that of control fish.

Experiment 6

At the end of this experiment, cumulative mortality of Florida Largemouth Bass treated with HP was 49.0% (SD = 9.3, range = 36.8–57.4%), which was significantly (P = 0.0085) less than the control fish mean of 74.5% (SD = 8.3, range = 67.6–85.2%). In addition, a significant difference in mortality was detected between treated and control groups on day 3 through the end of the experiment (Figure 6). The odds ratio for this experiment was 3.03 (95% CI, 1.50–6.14). Virtually all fish sampled during the treatment period were presumptively

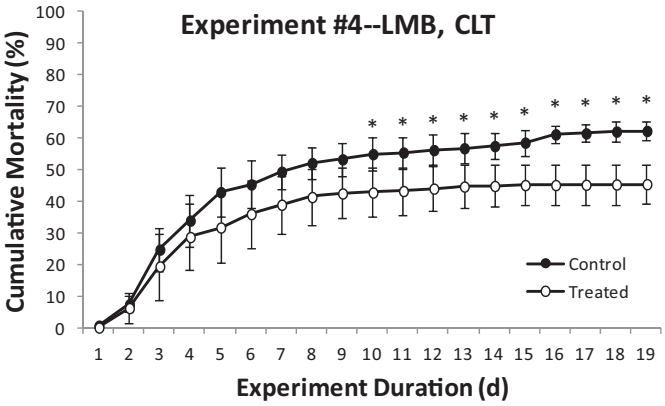

FIGURE 4. Mortality of Florida Largemouth Bass (LMB) observed in experiment 4. Treated fish were exposed to chloramine-T (CLT) at 20 mg/L of water for 60 min/d on three alternate days, whereas control fish received sham treatments (four replicate tanks/treatment). Asterisks indicate days on which a significant difference (P < 0.05) was detected between treated and control groups; at the end of the trial, cumulative mortality of treated fish was significantly (P = 0.0034) less than that of control fish.

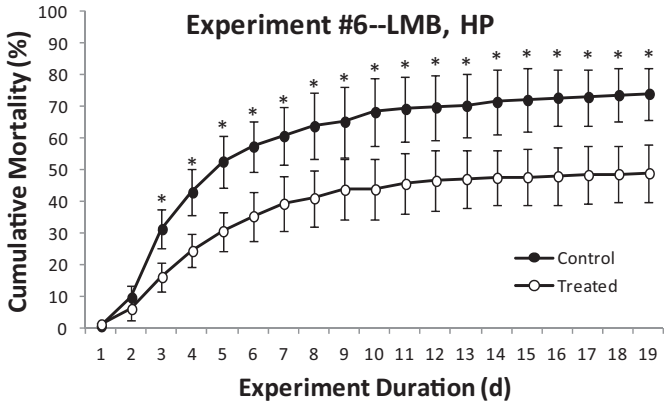

FIGURE 6. Mortality of Florida Largemouth Bass (LMB) observed in experiment 6. Treated fish were exposed to hydrogen peroxide (HP) at 50 mg/L of water for 60 min/d on three alternate days, whereas control fish received sham treatments (four replicate tanks/treatment). Asterisks indicate days on which a significant difference (P < 0.05) was detected between treated and control groups; at the end of the trial, cumulative mortality of treated fish was significantly (P = 0.0085) less than that of control fish.

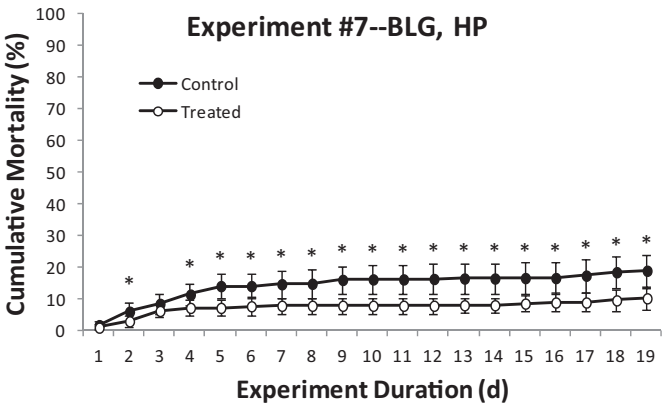

FIGURE 7. Mortality of Bluegills observed in experiment 7. Treated fish were exposed to hydrogen peroxide (HP) at 50 mg/L of water for 60 min/d on three alternate days, whereas control fish received sham treatments (six replicate tanks/treatment). Asterisks indicate days on which a significant difference ($P < 0.05$) was detected between treated and control groups; at the end of the trial, cumulative mortality of treated fish was significantly ($P = 0.0051$) less than that of control fish.

diagnosed with columnaris, and bacteria presumptively identified as *F. columnare* were observed on skin scrapes from 1 fish sampled from treated tanks and all 12 fish sampled from control tanks during the posttreatment period.

Experiment 7

At the end of this experiment, cumulative mortality of Bluegills treated with HP was 10.3% (SD = 3.4, range = 5.8–15.3%), which was significantly ($P = 0.0051$) less than the control fish mean of 19.0% (SD = 5.2, range = 13.4–24.6%). In addition, a significant difference in mortality was detected between treated and control groups on experiment day 2 and day 4 through the end of the experiment (Figure 7). The odds ratio for this experiment was 2.21 (95% CI, 1.37–3.59). Nearly all (34 of 36) fish sampled during the treatment period were presumptively diagnosed with external columnaris. During the posttreatment period, none of the fish sampled from the treated tanks exhibited clinical signs of columnaris; however, at least one fish sampled from each control tank was presumptively diagnosed with the disease.

DISCUSSION

Our results demonstrate that CLT and HP can be effective in controlling mortality associated with external columnaris disease in Florida Largemouth Bass and Bluegills. Treatments successfully reduced mortality associated with columnaris in five of seven experiments (experiments 1, 3, 4, 6, and 7). Although a significant reduction in mortality was detected at some point in the other two experiments (experiments 2 and 5, which were conducted concurrently), mortality did not vary significantly on the last 4–5 d of either experiment. However, the treatment protocols we employed are not completely consistent with the likely disease management approach used in a hatchery setting. The constraints of effectiveness-testing experiments (as generally accepted by FDA) limit treatment to a single treatment cycle; however, if reinfection was suspected, most fisheries professionals would have confirmed this suspicion and considered retreating the affected population. We speculate some degree of reinfection occurred in experiments 2 and 5 and contributed to the loss of statistical significance during the course of the experiments. If our experimental designs had allowed for treatments to be reapplied, it seems likely that the increases in mortality observed in experiments 2 and 5 could have been curbed, perhaps resulting in statistically significant differences between treated and control groups. Comparing our results to previous research is challenging because there is relatively little peer-reviewed information about the effectiveness of CLT or HP in controlling mortality associated with *Flavobacterium* infections in finfish other than salmonids (Speare and Arsenault 1997; Bowker and Erdahl 1998; Rach et al. 2000a). Nonetheless, our results are broadly consistent with other reports of using CLT and HP in the treatment of columnaris. For example, Rach et al. (2003) showed that survival of fingerling Walleyes *Sander vitreus* infected with columnaris was significantly improved when treated with HP at 50 mg/L of water for 60 min/d on three alternate days compared with untreated fish; however, exposure to higher concentrations of HP (75 or 100 mg/L) resulted in reduced survival. Similarly, Altinok (2004) reported higher survival in *F. columnare*-infected Goldfish *Carassius auratus* following a single 60-min treatment with CLT at 5, 10, 15, or 20 mg/L of water compared with untreated fish, but increasing the dose to 25 mg/L was less beneficial. Survival of fingerling Channel Catfish *Ictalurus punctatus* with columnaris was significantly improved after being treated with HP at 50, 75, or 100 mg/L of water for 60 min/d on three alternate days, but these fish did not exhibit sensitivity to the higher doses (Rach et al. 2003). Results from these studies suggest varying sensitivity to CLT and HP and a "Goldilocks paradigm" (i.e., CLT and HP doses that are too low or too high may yield less desirable results than one that is just right for the species or lifestage; Gaikowski et al. 1999; Russo et al. 2007; Gaikowski et al. 2008; Bowker et al. 2011).

Excluding circumstances when treatment concentrations exceed a fish's tolerance threshold, available data suggest that both CLT and HP are effective in controlling or reducing mortality associated with columnaris. Results of the side-by-side experiments evaluating CLT and HP underscore this point. Experiments 2 (CLT) and 5 (HP) were conducted concurrently with fish from the same reference population of Florida Largemouth Bass, and the mortality patterns (Figure 8) and odds ratios observed in these experiments were nearly identical. Experiments 3 (CLT) and 7 (HP) were not conducted concurrently, but were conducted with Bluegills from reference populations with similar pretreatment mortality; mortality and odds ratios (Figure 9) reported for these experiments were also similar. Chloramine-T and HP are characterized as microbicides with broad-spectrum antiseptic properties (McDonnell and Russell 1999); thus, it is perhaps not surprising they yield similar results when used to

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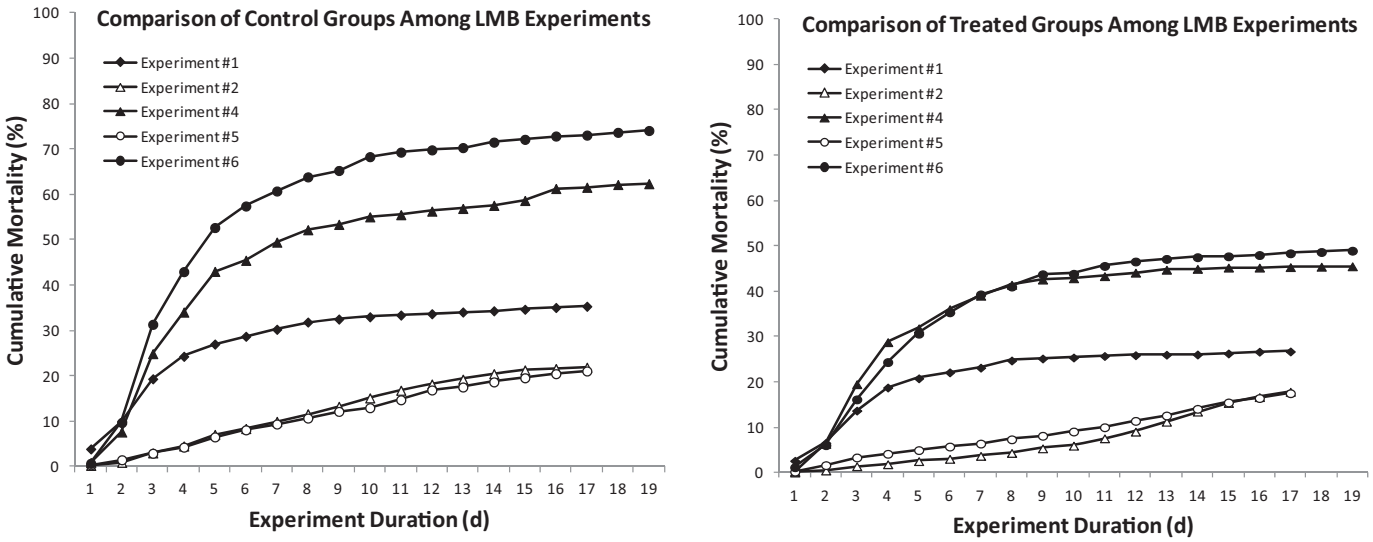

FIGURE 8. Mortality curves for treated and untreated groups of Florida Largemouth Bass (LMB) in experiments 1, 2, 4, 5, and 6.

treat external infections such as columnaris. Both compounds act via nonselective, oxidative processes that damage peptide linkages, proteins, and other cellular constituents and disrupt essential cellular processes (Maris 1995). Microorganisms, such as bacteria, are especially susceptible to these oxidative processes, which kill the organism quickly, often by disrupting their cellular membrane (Maris 1995). Because of the nonspecificity of this mode of action, microorganisms do not develop resistance to CLT or HP as can occur with antibiotics with specific cellular targets (Masten and Haneke 2002). However, chemicals such as CLT and HP provide virtually no prolonged therapeutic effect after treatment has been terminated and the chemical has been flushed from the rearing system

Our side-by-side comparison experiments indicate that CLT and HP are equally effective in controlling or reducing mortality associated with columnaris in Florida Largemouth Bass and Bluegills, which is also supported by the results of the meta-analysis. However, the significance and magnitude of mortality reduction varied considerably among our experiments. In experiments 1, 2, 5, and 7 the difference in mortality between treated and control groups was less than 10%, whereas in experiment 6, mortality was reduced by more than 25%. Many factors can affect the outcome of experiments conducted to evaluate the effectiveness of a drug to control mortality caused by an infectious fish pathogen. First, the effectiveness of a waterborne treatment is determined by mode of action and may be

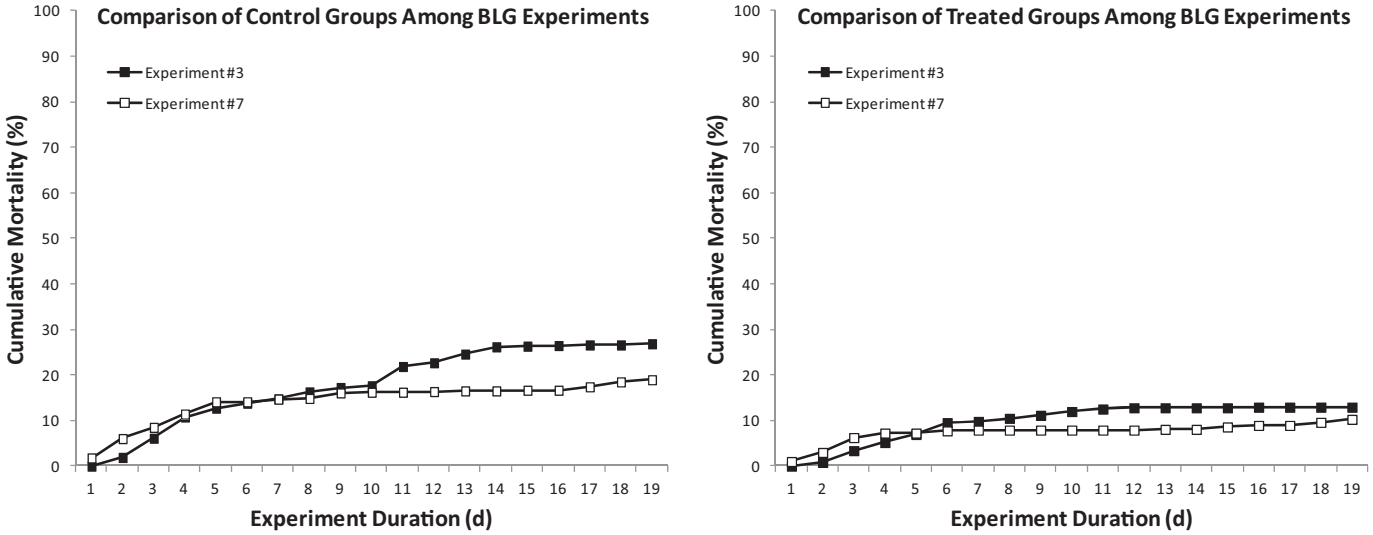

FIGURE 9. Mortality curves for treated and untreated groups of Bluegills (BLG) in experiments 3 and 7.

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influenced by water chemistry and other environmental variables. For example, Thomas-Jinu and Goodwin (2004) observed copper sulfate, pyrazinedium dibromide (diquat), potassium permanganate, CLT, and HP, to vary in their ability to control mortality of channel catfish infected with different strains of *F. columnare*; pyrazinedium bromide was extremely effective, copper sulfate was not, and the various oxidizers yielded intermediate results. These authors also emphasized that the effectiveness of oxidizers could be strongly influenced by the oxidizable organic load in the system, which could neutralize a significant amount of the antiseptic, effectively reducing the treatment dose. The effectiveness of a drug, particularly an oral antibiotic, may also be affected by water temperature, especially if the temperature is on the lower or upper end of the fish's optimal thermal range. Second, as results from our Largemouth Bass experiments show, the extent of a disease's progression before intervention (i.e., pretreatment mortality) has a substantial influence on cumulative mortality in both treated and control groups during an experiment: the lower the mortality at the start of the study, the lower the cumulative mortality in the treated and control tanks and the smaller the difference between the two. Clearly, water quality, pretreatment mortality, and disease progression should be taken into consideration when assessing the relative merits of waterborne treatment options.

Microbicidal treatment should not be considered the principal means of disease management. Although relatively inexpensive, such treatments add to overall production costs. Assuming a 3,780 L (1,000 gal) culture tank and current pricing for the test articles used in this study, treating with CLT (3 treatments, 20 mg/L) would cost US$4.53, and treating with HP (3 treatments, 50 mg/L) would cost $9.03. Rather, a comprehensive disease management plan should be based primarily on procedures to minimize the likelihood of a disease outbreak, with treatment protocols as a secondary approach should these prevention measures prove insufficient. Such strategies may include using groundwater sources to minimize introduction of bacteria and other disease vectors, disinfecting incoming surface water with ultraviolet light or ozone, avoiding or minimizing the effects of chronic (e.g., poor water quality, improper fish densities) and acute stressors (e.g, handling, transport), and administering effective vaccines (Bowker et al. 2012b). Improving culture conditions by increasing water flow is another strategy to consider; however, in our studies, increased water exchange rates in test tanks ($\geq$2.5 times that of reference population tanks) was insufficient to control mortality without some other intervention. Regardless of the specific strategies used, any disease management plan should include routine inspection of fish for signs of distressed or diseased fish and protocols to ensure corrective steps are taken at the first sign of disease. In the case of columnaris, if treatment is recommended, CLT and HP will probably yield similar results; however, it is also likely that either microbicide will be more effective in controlling mortality if administered during the early stages of disease progression. Furthermore, we speculate that other nonspecific oxidizing microbicides similar to CLT and HP would yield similar results in this context.

In conclusion, our results indicate that either CLT administered at 20 mg/L or HP at 50 mg/L for 60 min/d on three alternate or consecutive days can be effective in controlling mortality associated with external columnaris disease in Florida Largemouth Bass and Bluegills. Future research should focus on evaluating the efficacy of these two drugs for the control of external columnaris-induced mortality in other warmwater finfishes.

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