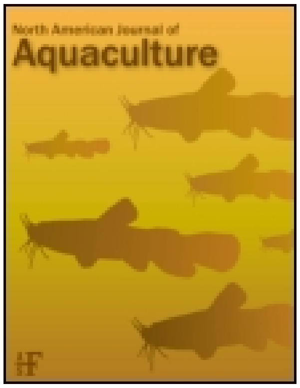

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Abstract.—Formalin is currently the only aquaculture drug approved by the U.S. Food and Drug Administration for the control of fungi on the eggs of all finfish. The efficacy of formalin for disinfecting hybrid catfish (channel catfish *Ictalurus punctatus* × blue catfish *I. furcatus*) eggs was examined and compared with that of three other potential chemotherapeutants. Two experiments were conducted to evaluate treatment dose and frequency on the hatching success of the hybrid catfish. In the first experiment, hybrid catfish egg masses were divided and assigned to 1 of 13 treatments administered as daily 15-min baths. The treatments were as follows: (1) control (no treatment), (2) 50 ppm (mg/L) formalin (F), (3) 100 ppm F, (4) 200 ppm F, (5) 125 ppm hydrogen peroxide (HP), (6) 250 ppm HP, (7) 500 ppm HP, (8) 50 ppm povidone iodine (PI), (9) 100 ppm PI, (10) 200 ppm PI, (11) 2.5 ppm copper sulfate (CS), (12) 5 ppm CS, and (13) 10 ppm CS. Hatching success tended to be highest among eggs treated with 100 ppm F (87.7%), 2.5 ppm CS (87.0%), or 100 ppm PI (85.2%), although these success rates were not significantly ($P > 0.05$) different from that in the control treatment (82.8%). Treatment with 500 ppm HP resulted in a significant ($P < 0.05$) decrease in hatching success (31.5%). In the second experiment, the optimal frequency of formalin treatments was assessed by treating hybrid catfish eggs zero, two, three, or four times daily. Eggs treated three times daily with 100 ppm F had the highest ($P < 0.05$) percentage of hatched eggs. To maximize hatching success, three daily treatments of hybrid catfish eggs with 100 ppm F as a 15-min bath is recommended.

Fungal and bacterial egg infections can be a significant problem in commercial catfish hatcheries. Dead eggs and other organic matter in hatchery culture systems provide excellent substrates for these pathogens. Without chemotherapeutic treatment, these pathogens can quickly overtake the egg mass and significantly reduce embryo survival and hatching success. Several chemical therapeutants have been investigated as potential treatments to control fish egg diseases. Currently, formalin is the only chemotherapeutant designated by the U.S. Food and Drug Administration (FDA) as an approved aquaculture drug for treating all finfish eggs; however, other potential chemotherapeutants are being investigated. Among those chemotherapeutants under investigation, hydrogen peroxide, povidone iodine, and copper sulfate have been suggested as alternatives to reduce channel catfish *Ictalurus punctatus* egg infections and thus improve hatching success (Walser and Phelps 1993; Rach et al. 1998, 2004; Small and Wolters 2003; Small 2004; B. R. Griffen, Harry K. Dupree–Stuttgart National Aquaculture Research Center, unpublished data). Hydrogen peroxide and povidone iodine are currently designated by the FDA as Low Regulatory Priority aquaculture drugs when used as egg surface disinfectants, and the use of copper sulfate is currently under an Investigational New Animal Drug exemption (INAD).

Efforts to develop a viable industry for hybrid catfish (channel catfish × blue catfish *I. furcatus*) have been ongoing for several years. Hybridization of the channel catfish and blue catfish produces a catfish with greater growth potential than either parent (Li et al. 2004); however, limitations in fry production have prevented this fish from being widely cultured. Hybrid production involves stripping ovulating eggs from channel catfish females and fertilizing with sperm from killed blue catfish males. Although fertilization appears to be high, the hatching success for hybrid catfish averages less than 30% (Lambert et al. 1999). Given the degree of gamete handling involved, egg diseases can be a significant factor and probably contribute to poor hatching success. Thus, determining the

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best methods for disinfecting hybrid eggs is of considerable interest. For the research presented here, the efficacy of formalin for treating hybrid catfish eggs was examined and initially compared with the efficacy of treatment with hydrogen peroxide, povidone iodine, and copper sulfate. Two experiments were conducted to evaluate treatment dose and frequency of application on the hatching success of hybrid catfish.

Methods

Hybrid catfish embryos were produced from channel catfish eggs fertilized with blue catfish sperm. Mature female catfish (3–5 years old) were injected with either carp pituitary extract (Stoller Fisheries, Spirit Lake, Iowa, USA) or LHRHa (Syndel International, Inc., Vancouver, British Columbia, Canada). Injection regimes were 2 mg/kg female body weight (BW) initial injection and 8 mg/kg BW 20 h later for carp pituitary extract, or 40 μg/kg BW initial injection followed by 80 μg/kg BW 20 h later for LHRHa. Twenty-four hours after the second injection, eggs were dry-stripped from ovulating females, held in Hank's balanced salt solution (HBS), and fertilized within 15 min of stripping. Blue catfish sperm was prepared by macerating testes from sacrificed blue catfish males and pooling the sperm in HBS. Fertilized eggs were water-hardened and allowed to develop an adhesive matrix, after which they were divided according to treatment and placed in mesh baskets suspended in individual incubation troughs supplied with well water (temperature, 26–28°C; pH, 8.6–8.8; total hardness, 20–60 ppm). Numbers of sac fry at hatch were estimated volumetrically, and hatching success was calculated as the percentage of eggs hatched.

In the first experiment, the efficacy of formalin (formalin-F; Natchez Animal Supply, Natchez, Mississippi) was compared with that of hydrogen peroxide (Natchez Animal Supply), povidone iodine (Aquadine; Aquacenter, Leland, Mississippi), and copper sulfate (Sigma-Aldrich, St. Louis, Missouri). Three hybrid catfish spawns of approximately 300 g each were subdivided into 13 approximately equal masses (19.3 ± 0.3 g; [mean ± SE]) and randomly assigned to thirty-nine 76-L incubation troughs equipped with a wire hatching basket and an air stone. The number of eggs per mass was calculated after determining eggs per gram. Each trough was supplied with flowing well water (26°C) at a rate of one complete water exchange every 20 min. Chemotherapeutic treatments were randomly assigned such that eggs from each spawn (n = 3) received the following treatments: (1) control (no treatment), (2) 50 ppm (mg/L) formalin (F), (3) 100 ppm F, (4) 200 ppm F, (5) 125 ppm hydrogen peroxide (HP), (6) 250 ppm HP, (7) 500 ppm HP, (8) 50 ppm povidone iodine (PI), (9) 100 ppm PI, (10) 200 ppm PI, (11) 2.5 ppm copper sulfate (CS), (12) 5 ppm CS, and (13) 10 ppm CS. Chemotherapeutic treatments were administered once daily as a 15-min bath, during which the flow of water to the trough was stopped and then restored. Egg masses were allowed to hatch to completion within individual troughs. When hatching was complete, the fry were siphoned into a graduated cylinder and the volume of fry was recorded. The total number of fry was calculated after determining the number of fry in 1 mL and multiplying this times the total volume of fry collected.

In the second experiment, the effect of the frequency of formalin treatment on hatching success was examined. Four hybrid catfish spawns of approximately 400 g each were divided into four approximately equal egg masses (86.5 ± 4.2 g) and the number of eggs per mass was determined. Each egg mass was randomly assigned to sixteen 13-L incubation troughs equipped with a wire hatching basket and an air stone. Each trough was supplied with flowing well water (28°C), adjusted so that the entire water in the troughs was exchanged every 20 min. Formalin was administered at a concentration of 100 ppm, during which flow of water to the trough was stopped for 15 min and then restored. The effect of treatment frequency was examined by treating the egg masses zero, two, three, or four times daily until hatching began. Treatments were equally spaced from the time of egg fertilization. Egg masses from each spawn received all four treatment regimes. When hatching was complete within each individual trough, the fry from that trough were siphoned into a graduated cylinder and the volume of fry was recorded for determining hatching success as previously described. This experiment was repeated four times within a 1-month period, using four new egg masses each time.

Statistical comparisons for both experiments were conducted using the SAS software system (SAS Institute, Inc. 1996). Assumptions for homogeneity of variance and normality of the data were tested by examination of correlation between absolute residuals and predicted values and by the Shapiro–Wilk test for normality. Hatching success data, expressed on a percentage basis, were arcsin-transformed before analysis of variance (ANOVA)

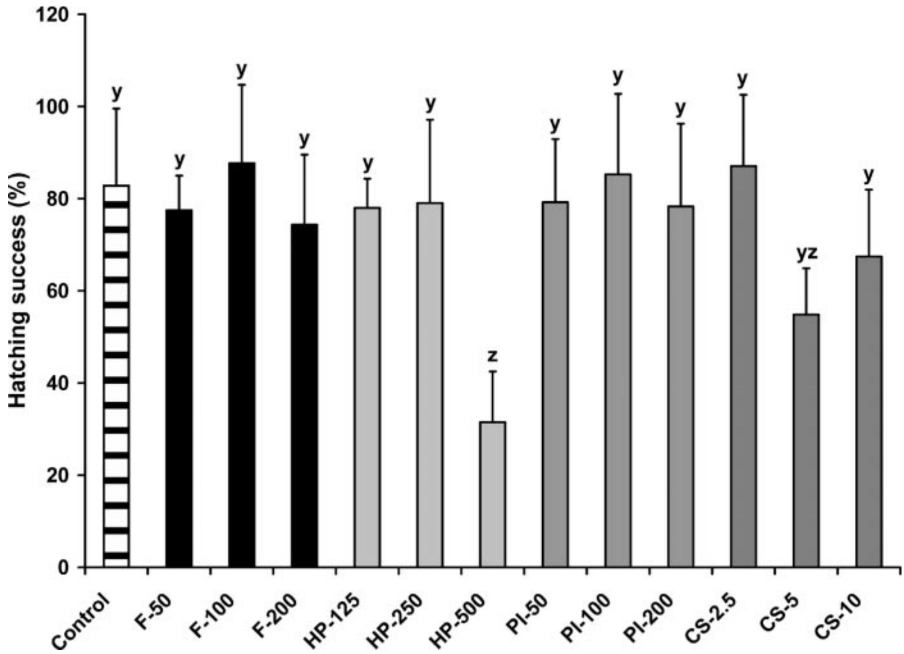

FIGURE 1.—Mean + SE (n = 3) hatching success for hybrid catfish eggs treated once daily as a 15-min bath with formalin (F), hydrogen peroxide (HP), povidone iodine (PI), or copper sulfate (CS) at increasing concentrations (see text for details). Significant ($P < 0.05$) differences are indicated by different letters.

in which mixed-model procedures with treatment was the fixed effect and spawns within treatment was the random effect. For the second experiment, the time of the month in which the experiment was repeated was not significant ($P > 0.05$). When significant differences were found by ANOVA, we used pairwise contrasts with using Fisher's least significant difference test to identify differences at the 5% level.

Results and Discussion

In the first experiment, hybrid catfish hatching success was higher than the average published values (Lambert et al. 1999). Hatch rates were also highly variable between spawns, probably because of differences in gamete quality inherent to the hybridization process. Hatching success of untreated eggs (controls) in this experiment was 82.8 $\pm$ 16.7% with no significant ($P > 0.05$) improvement in percent hatch that could be attributed to chemotherapeutic treatment (Figure 1). Although the increase was not significant, formalin at a concentration of 100 ppm yielded the highest average hatching success (87.7 $\pm$ 17.0%). Eggs treated with 500 ppm HP had significantly lower ($P < 0.05$) hatching success (31.5 $\pm$ 11.0%) than untreated eggs. Channel catfish eggs are reportedly sensitive to high levels of hydrogen peroxide. Although Small and Wolters (2003) reported poor embryo survival related to chorion deterioration and premature hatching of channel catfish eggs treated with 500 ppm HP, chorion deterioration was not obvious in the present experiment. Reduced hatching success during treatment with high levels of hydrogen peroxide may also be the result of toxicity (Rach et al. 1998).

Formalin has long been recommended to treat diseases associated with channel catfish eggs (Clemens and Sneed 1958; Piper et al. 1982; Jensen et al. 1983; Rogers 1985; Walser and Phelps 1993) and is widely used by catfish hatchery managers to control egg diseases (personal communications). Both recommended and actual formalin concentrations used in channel catfish hatcheries vary greatly, as does the frequency of treatments. Walser and Phelps (1993) recommended 400 ppm flush treatments, whereas Clemens and Sneed (1958) recommended 1-h formalin treatments at 250 ppm. The treatment time used in the present experiment of 15 min was based on FDA guidelines for formalin-F (NADA 137–687). Even so, published concentration recommendations for treating channel catfish eggs for 15 min vary greatly, ranging from 100 ppm (Jensen et al. 1983) to

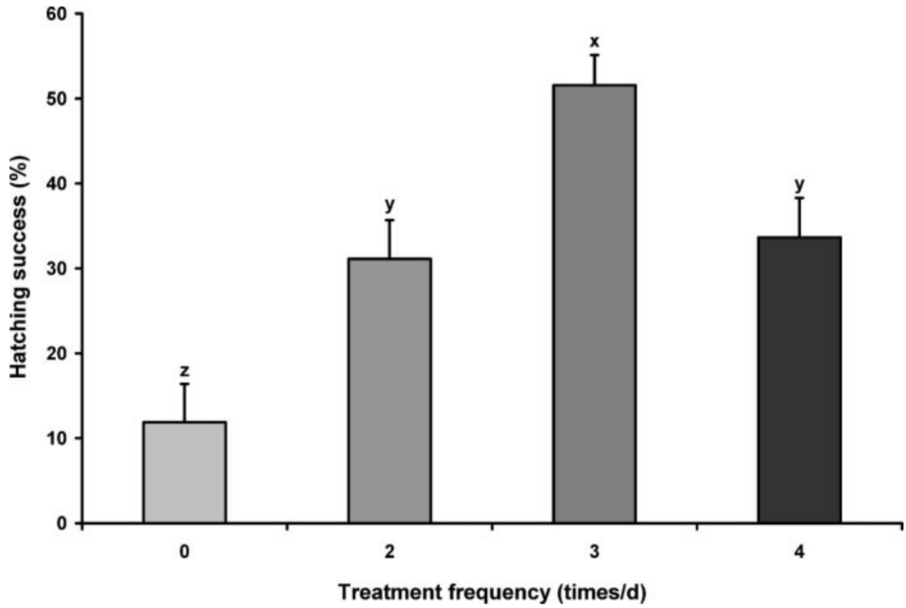

FIGURE 2.—Mean + SE (n = 16) hatching success for hybrid catfish eggs treated with 100 ppm formalin at a frequency of zero, two, three, or four times daily as a 15-min bath. Significant ($P < 0.05$) differences are indicated by different letters.

1,600 ppm (Rogers 1985). No published recommendations could be found for blue or hybrid catfish. In the present study, 100 ppm formalin had no negative effects on hybrid catfish hatching success in the first experiment and was selected for determining the effect of treatment frequency in the second experiment.

In the second experiment, the frequency of formalin treatments was found to significantly affect hybrid hatching success in a classic dose–response manner (Figure 2). As the frequency of treatments increased to three times daily, hatching success also increased, followed by a decrease in hatching success when treatments were administered four times daily. Although no studies comparing formalin treatment frequency have been published for hybrid catfish, Lambert et al. (1999) report treating hybrid egg masses with 100 ppm formalin three times per day as part of their husbandry procedures. Improvements in hatching success of channel catfish relative to that of untreated eggs have been reported when formalin was administered twice daily as a flush treatment at concentrations of 100, 200, or 400 ppm, the 400-ppm treatment being the most effective (Walser and Phelps 1993). Taken together with the hybrid results presented here, these data suggest a possible dose × frequency interaction. Further, the many differing published formalin treatment recommendations for channel catfish eggs suggest that more than one treatment regime might be effective for improving hatching success.

In the present study, the treatment regime most effective for improving hybrid catfish hatching success was the administration of 15-min formalin baths three times daily at a concentration of 100 ppm. Compared with the untreated controls, hatching success increased more than fourfold in the second experiment, from 12.7 to 51.6%, supporting a treatment schedule of every 8 h after fertilization. This study suggests that highly variable hatching success of hybrid catfish can be substantially improved through proper disease control, which can be successfully managed with formalin.

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