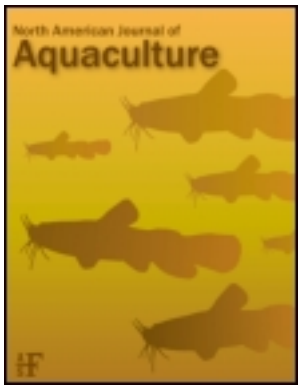


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# Accounting for Water Temperature during Hydrogen Peroxide Treatment of Channel Catfish Eggs

Brian C. Small [a]

[a] U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, Post Office Box 38, Stoneville, Mississippi, 38776, USA

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# Accounting for Water Temperature during Hydrogen Peroxide Treatment of Channel Catfish Eggs

BRIAN C. SMALL*

*U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, Post Office Box 38, Stoneville, Mississippi 38776, USA*

*Abstract.*—The effect of water temperature on the efficacy of hydrogen peroxide ($H_2O_2$) as a disinfectant for channel catfish *Ictalurus punctatus* eggs was examined at 24°C and 28°C. Eggs at each temperature were treated with $H_2O_2$ at 0, 100, 250, 500, or 1,000 mg/L as a daily 15-min bath. Mean hatching success at 24°C was greatest ($P > 0.05$) for eggs treated with 250 and 500 mg/L $H_2O_2$, hatching success tending to increase with increasing treatment concentration between 100 and 500 mg/L. The opposite effect was observed at 28°C: hatching success was greatest ($P < 0.05$) for eggs treated with 100 and 250 mg/L $H_2O_2$, hatching success tending to decrease with increasing $H_2O_2$ concentration between 100 and 500 mg/L. The results of this research, together with previously published results, demonstrate that there is a negative correlation between $H_2O_2$ concentration and water temperature with respect to the optimal efficiency of $H_2O_2$ as an egg disinfectant and suggest that there is an increased toxicity of $H_2O_2$ at higher temperatures. Careful consideration of temperature is necessary when disinfecting channel catfish eggs with $H_2O_2$.



Hydrogen peroxide ($H_2O_2$) was initially reported by Marking et al. (1994) to be effective for controlling fungal (*Saprolegnia* sp.) infections of fish eggs; it has since been investigated as an alternative treatment to control fungal infections of eggs of several additional fish species (Waterstrat and Marking 1995; Schreier et al. 1996; Rach et al. 1998; Arndt et al. 2001; Barnes et al. 2003). Currently, $H_2O_2$ is designated by the U.S. Food and Drug Administration (FDA) as an aquaculture drug of low regulatory priority (LRP) when administered at concentrations as great as 500 mg/L. Small and Wolters (2003) investigated the effectiveness of $H_2O_2$ as a treatment for disinfecting channel catfish *Ictalurus punctatus* eggs, considering hatching success and embryo survival in various $H_2O_2$ treatment regimes ranging in concentration from 0 to 500 mg/L at a water temperature of 26°C. They found a once-daily 15-min bath in $H_2O_2$ at 250 mg/L significantly improved hatching success and yielded the greatest embryo survival. In contrast, Rach et al. (1998) reported using treatment regimes at concentrations as great as 3,000 mg/L to improve channel catfish hatching success at a water temperature of 22°C.

The effect of temperature on $H_2O_2$ efficacy as a chemotherapeutic for aquaculture was first demonstrated when treating gill ectoparasites that affected coldwater fishes (Bruno and Raynard 1994; Kiemer and Black 1997; Treasurer and Grant 1997). Those studies, which showed increased mortality associated with $H_2O_2$ treatment at increasing temperatures, led to management recommendations to reduce treatment times at higher temperatures. Toxicity of $H_2O_2$ also appears to increase with temperature for warm- and coolwater species at postlarval life stages (Rach et al. 1997); however, little information exists regarding the effect of temperature on eggs and embryo survival during $H_2O_2$ treatment. Differences in reported optimal $H_2O_2$ concentrations for treating channel catfish eggs at 22°C (1,000 mg/L; Rach et al. 1998) and 26°C (250 mg/L; Small and Wolters 2003) suggest temperature-related toxicity trends are similar whether treating postlarval fish or eggs. In the present study, channel catfish eggs incubated at 24°C and 28°C were treated with increasing concentrations of $H_2O_2$ to determine what concentration yielded the greatest hatching success. These findings on the effect of temperature are discussed with consideration of the results observed by Rach et al. (1998) and Small and Wolters (2003).

## Methods

Eggs from USDA203 strain channel catfish were collected from ponds of the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Catfish Genetics Research Unit (CGRU), in Stoneville, Mississippi, during July 2003. Spawning containers were checked in the morning every 2 d for egg masses, and microscopically staged to determine embryo development (Makeeva and Emel'yanova 1993). Only fertilized eggs less than 24 h old were used at the start of these studies. No visible fungal growth was

* Corresponding author: bsmall@ars.usda.gov

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observed by gross observation in any of the egg masses before the start of the trial.

Catfish eggs from three spawns of approximately 250–300 g each, representing three distinct families, were each subdivided into 10 approximately equal masses (26.0 ± 0.6 g each; mean ± SE) and individually assigned to small, wire hatching baskets (23 cm × 10 cm × 8.5 cm) lined with 1.6-mm mesh for containment of hatching sac-fry. Five small hatching baskets per distinct spawn were then randomly hung inside five larger, standard hatching baskets (i.e., three small baskets per standard basket), and assigned to one of two living stream raceways (LS-510; Frigid Units Inc., Toledo, Ohio). The design was such that five submasses from each of the three distinct spawns were represented in both raceways. The number of eggs per mass was approximately 914 ± 27 (mean ± SE), determined by calculating the number of eggs per gram and multiplying by the number of grams per mass. The raceways were equipped with separate heater/chiller units to maintain water temperature at 24°C and 28°C, respectively. Each raceway contained four air stones placed between the five standard hatching baskets. Well water (pH, 8.8; total hardness, 97 mg/L; alkalinity, 400 mg/L) was supplied at a rate of 7.6 L/min.

Each $H_2O_2$ (AquaCenter, Leland, Mississippi) treatment (0, 100, 250, 500, and 1,000 mg/L) was randomly assigned to one small basket per spawn per raceway. Treatments were administered every afternoon until embryonic eye pigmentation became apparent. During treatment, the small hatching baskets were transferred for 15 min to aquaria containing water at 24°C or 28°C, respectively, and one of the tested $H_2O_2$ concentrations and then were returned to the raceways. Egg masses were allowed to hatch to completion within individual hatching baskets. 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 then multiplying this number by the total volume (in milliliters) of fry collected. Hatching success was calculated as the percentage of eggs that hatched.

Hatching success data, expressed on a percentage basis, were arcsine transformed before statistical analysis. Statistical significance was determined by analysis of variance (ANOVA) using mixed-model procedures (SAS software system version 8.00; SAS Institute, Inc. 1996). When significant differences were found by ANOVA, I made pairwise contrasts by using a least significant difference test to identify significant differences between treatments at the 5% level.

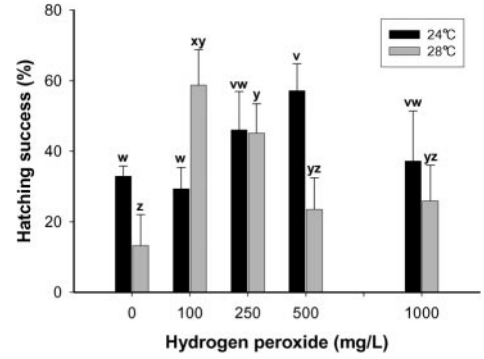


FIGURE 1.—Mean (±SE) hatching success for channel catfish eggs treated once daily for 15 min at 24°C and 28°C with increasing concentrations of hydrogen peroxide until the fish developed eyes. Significant differences ($P < 0.05$) within temperature are indicated by different letters.

## Results and Discussion

Temperature had a significant ($P < 0.05$) effect on hatching success between the control treatments. From gross observation, eggs incubated at 28°C without $H_2O_2$ treatment appeared to be overwhelmed with fungus; the average hatching success of these eggs was 13.2%, compared with 32.9% for untreated eggs at 24°C. The optimal temperature for rearing channel catfish embryos to hatch is reported to be 26–28°C (Small 2002); however, those study conclusions were based on the fact that eggs used in the study were treated with chemotherapeutic agents throughout the study to prevent infection. Although in the present study the mean hatching success at 24°C was not significantly ($P > 0.05$) different between eggs treated with $H_2O_2$ at 250 and 500 mg/L, there was a trend toward increased hatching success with increasing treatment concentration between 100 and 500 mg/L (Figure 1). The opposite was observed at 28°C, in which hatching success was highest ($P < 0.05$) for eggs treated with $H_2O_2$ at 100 and 250 mg/L, with a trend toward decreasing hatching success with increasing $H_2O_2$ concentration between 100 and 500 mg/L.

Rach et al. (1998) evaluated the efficacy and toxicity of $H_2O_2$ as a 15-min bath of the eggs of several warm- and coolwater fishes. In their studies, hatching success of channel catfish eggs incubated at 22°C was greatest relative to untreated controls when treated with $H_2O_2$ at 1,000 mg /L. In a separate study, Small and Wolters (2003) re-

ported the optimal concentration of $H_2O_2$ for disinfecting channel catfish eggs at 26°C in a 15-min bath was 250 mg/L. Optimal dosing regimes from these two studies, combined with results from the present study, clearly show a temperature effect on $H_2O_2$ efficacy and toxicity for channel catfish eggs. Similarly, Kiemer and Black (1997), evaluating the efficacy of $H_2O_2$ to treat ectoparasites of Atlantic salmon *Salmo salar*, found a linear relationship between $H_2O_2$ efficacy and temperature, demonstrating a significant correlation between the level of $H_2O_2$ exposure and the degree of gill damage in smolts.

The recommended $H_2O_2$ treatments for optimal catfish egg hatching success found in the literature differ greatly, which suggests that incubation temperature may have affected the efficacy and toxicity of $H_2O_2$ treatments. Results from the present study, together with previously published results, demonstrate a negative correlation between $H_2O_2$ concentration for optimal efficacy as an egg disinfectant and water temperature, and suggest increased toxicity of $H_2O_2$ at higher temperatures. Given the possibility that even a 2°C difference in water temperature could greatly affect the efficacy and toxicity of $H_2O_2$ as a disinfectant for channel catfish eggs, it is recommended that a pilot study be conducted under user conditions before one implements a hatchery-wide $H_2O_2$ dosing therapy to improve egg hatching by controlling *Saprolegnia* infections.



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