INVESTIGATION OF FORMALIN AND HYDROGEN PEROXIDE TREATMENTS TO CONTROL FUNGUS ON FLORIDA LARGEMOUTH BASS EGGS

by

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MANAGEMENT DATA SERIES
No. 216
2003

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ACKNOWLEDGEMENTS

This study was made possible through the hard work of Max Kimmel, Nathan Pence, Nancy Vignali, and Ted Engelhardt of the A. E. Wood fish hatchery. We express our appreciation to Steve Hamby of the Fish Health Lab who performed the treatment concentration verifications, Annette Sudyka who helped with literature procurement, and Loraine Fries and Gerald Kurten for their editorial contributions. This study was funded, in part, through the Federal Aid in Sport Fish Restoration Grant F-101-D of Texas Parks and Wildlife Department.

ABSTRACT

Three chemical treatment regimes and a control (no chemical treatment) were compared for efficacy in treating Florida largemouth bass Micropterus salmoides floridanus eggs to prevent fungal infections and improve hatch rate. The chemical treatments were 500 mg/L of formalin static bath for 60 min, 2,000 mg/L of formalin flow-through bath for 15 min, and 500 mg/L of hydrogen peroxide static bath for 15 min. These treatments were selected according to FDA guidelines. Eggs were treated and hatched in McDonald jars, and fry were harvested prior to swim-up to determine hatch rate. No significant differences in hatch rate were found among treated and untreated eggs ($P > 0.05$). Thus, treatment of Florida largemouth bass eggs with formalin or hydrogen peroxide to improve hatch rate may not be necessary under the conditions of this study.

INTRODUCTION

The Saprolegniacea family of water mold, commonly referred to as fungus (oomycetes), frequently attacks incubating eggs of many species of cultured freshwater fish (Roberts 1989). The zoosporangia of Saprolegnia spp. are capable of discharging large numbers of infectious zoospores into water, so the possibility of infecting eggs or fish is continuous (Post 1987). While the possibility of infection is always present, most epizootics occur when temperatures are lower than optimal for the fish species (Roberts 1989). Fungal infections attack unfertilized or dead eggs, but if left untreated would spread over an entire egg mass and suffocate fertilized eggs to death (Post 1987; Stoskopf 1993; Hoffman 1999). Manual removal of affected eggs by handpicking or siphoning is possible but inefficient and potentially damaging to developing embryos. Conversely, chemical treatments are easy to administer and effective in controlling fungal infections (Bailey and Jeffery 1989). Formalin and hydrogen peroxide are commonly used for prophylactic or therapeutic treatment of fungal infections in various life stages of fish (Barnes et al. 2000; Barnes et al 1998; Froelich and Engelhardt 1996; Waterstrat and Marking 1995; Wright 1976).

The A. E. Wood Fish Hatchery (AEWFH) staff observes a considerable amount of fungus on Florida largemouth bass Micropterus salmoides floridanus eggs each production season during egg incubation. The staff has used formalin for treating fungal infections, and different treatment concentrations have been tried with mixed results that have been poorly documented. A static formalin treatment of 500 mg/L for 60 min was determined anecdotally as effective in preventing fungal infections and has been the standard treatment for Florida largemouth bass eggs. There are well-documented examples of chemical control of fungal infections of salmonids eggs (e.g., Barnes et al. 2000; Barnes et al 1998; Waterstrat and Marking 1995; Wright 1976) but comparatively little information exists for largemouth bass or other cultured warmwater fishes (Gaikowski et al 1999; Rach et al 1997; Froelich and Engelhardt 1996).

Formalin has been used at concentrations of up to 10,000 mg/L for 15 min with no obvious adverse effects on egg hatch rate or fry survival in largemouth bass (Wright 1976). Hydrogen peroxide at a concentration of 500 or 1,000 mg/L for 15 min daily was effective in controlling fungal infections on eggs of salmonids that were intentionally infected with Saprolegnia spp. before the treatments (Marking et al. 1994; Waterstrat and Marking 1995). Marking et al. (1994) also reported that 500 or 1,000 mg/L of hydrogen peroxide for 15 min improved the hatch rate of rainbow trout Oncorhynchus mykiss eggs that were intentionally infected with Saprolegnia spp. before the treatment. However, Barnes et al. (1998) reported that hydrogen peroxide concentrations of 250 and 500 mg/l for 15 min were ineffective in controlling fungal growth on rainbow trout eggs under normal hatchery conditions (i.e., with potentially naturally occurring fungal infections), while 1,000 mg/L for 15 min was effective. Barnes et al. (1998) suggested that the efficacy of hydrogen peroxide may be influenced by water quality and population levels of Saprolegnia spp., so a treatment that works well at one location may not work as well at another location.

Besides its effectiveness in controlling fungal infections on salmonid eggs, hydrogen peroxide has the advantage of being non-carcinogenic, degrading into oxygen and water, and thus of no environmental concern. Thus, assessing the efficacy of hydrogen peroxide for fungus control at a warmwater facility is warranted. The New Animal Drug Application (NADA) label approved by the Food and Drug Administration (FDA) listed formalin as approved at certain concentrations and hydrogen peroxide as a low regulatory priority drug for treatment of finfish eggs. The label for NADA 140-989 allows formalin treatments at concentrations of up to 250 mg/L for 1 h for finfish and 1,000-2,000 mg/L for 15 min for finfish eggs. Low regulatory priority drug guidelines from the FDA allow the use of hydrogen peroxide at rates up to 500 mg/L for 1 h for treating all species and life stages of finfish. The formalin treatment rate of 500 mg/L for 60 min used at the AEWFH does not follow the NADA label; instead, it is used under an Investigational New Animal Drug (INAD) exemption. The objective of this study was to determine which of three prophylactic chemical treatment regimes is effective against fungal infections on Florida largemouth bass eggs while staying within FDA guidelines. Effectiveness was measured by improved hatch rate.

MATERIALS AND METHODS

This experiment was performed at the AEWFH during the 2001 and 2002 Florida largemouth bass production seasons (March through early June). The water source for the hatchery is the upper San Marcos River, with typical mean water quality characteristics as follows: temperature 21° C; pH 7.5; alkalinity 240 mg/L as CaCO3; total hardness 193.5 mg/L as CaCO3. The river water is pumped into a 57,418-m^3 storage reservoir, and then gravity fed into an incubation and raceway building. The study was conducted in three trials. The experimental design for each trial consisted of a control (no chemical treatment) and three chemical treatments, each with three to six replicates depending on the trial. Treatment 1 received 500 mg/L of formalin static bath for 60 min, treatment 2 received 2,000 mg/L of formalin flow-through bath for 15 min, and treatment 3 received 500-mg/L of hydrogen peroxide static bath for 15 min. Treatment concentrations were prepared from hydrogen peroxide of 50% active ingredient and formalin of 100% active ingredient as Parasite-S. Treatment dose verification was performed for the hydrogen peroxide treatments during the 2001 trials and for the formalin treatments in 2002. The dosages were 507 mg/L for the 500 mg/L of hydrogen peroxide, 534 mg/L for the 500 mg/L of formalin, and 1991 mg/L for 2,000 mg/L of formalin.

Eggs for the study were spawned on Spawn-Tex[1] mats in indoor raceways. One egg mass from one female (spawn), large enough to provide 10,000-12,000 eggs, was selected for each trial. The mat with the selected spawn was soaked in a pH-adjusted 1.5% sodium sulfite solution for 30-45 seconds to loosen the eggs. The eggs were then removed from the mat with a gentle stream of fresh water and placed in a 6.5-L McDonald hatching jar for the eggs to water-harden. For the first two trials, eggs were allowed to water-harden for 24 h prior to being enumerated into the experimental

[1] Use of trade or manufacturer's name does not imply endorsement.

McDonald jars using an electronic egg counter. Eggs were counted into 12 groups of 1,000 eggs each. The egg counter was checked for proper calibration by counting a known number of eggs. For the third trial, the eggs were counted by hand after water hardening for 24 h into 24 groups of 20 eggs each.

The McDonald jars for the study were cleaned with detergent and thoroughly rinsed with water, then placed on an incubation rack with a common head-box as the source of water. Water was allowed to flow through the jars at rates of about 2.5-3.0 L/min for two to three days before the study was started. All jars were provided with screens to prevent loss of eggs or fry and small air stones to provide oxygenation to ensure that dissolved oxygen levels were > 5 mg/L. Jars were randomly assigned to the control and chemical treatments. To apply treatments, water flows through the jars were turned off and 2-3 L of the water in each jar was removed by siphoning to allow room for the treatment solution. For each of treatments 1 and 3 replicates, the appropriate amount of chemical required to achieve the target concentration was measured into a 200-mL beaker; water was added to dilute it and then poured into the designated jar. The beaker was rinsed with water and emptied into the jar. Water was added into each jar to achieve the desired treatment concentration for the static bath. The eggs were then transferred into the treatment jars. For treatment 2, a flow-through bath, the eggs were placed in the jars and then a previously prepared 2,000 mg/L of formalin solution was delivered into the jars from an overhead reservoir. The reservoir delivered a flow to the jars at a rate that resulted in a solution exchange of 7.5 L for the 15-min treatment. Immediately following application of the hydrogen peroxide treatment, some eggs floated to the surface of the treatment solution and attached to the screens. This was found to be due to tiny air bubbles that adhered to the eggs. The eggs were gently tapped with a feather to dislodge the air bubbles to allow the eggs to sink. At the end of each treatment period, water flows into replicate jars were returned to 2.5-3.0 L/min to keep the eggs gently rolling to avoid suffocation. The eggs hatched in 36 h, and 48 h later the fry were enumerated with the electronic counter and percent hatch was calculated for each jar.

Water temperature, pH, and dissolved oxygen were monitored twice daily. The egg hatch rate and water quality data were analyzed using ANOVA of SYSTAT (SPSS, Inc. 2001), and differences in mean values were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

There were no significant differences in hatch rate among treatments and the control ($P = 0.06$; Figure 1), and none of the chemical treatments completely prevented growth of fungus. Fungus and dead eggs were observed in all jars; however, neither was quantified. Water quality was consistent among treatments and the control in each trial due to the use of a common headbox as the source of water for all the jars, although there was some variability in water quality among trials (Table 1). Temperatures ranged from 22.2 to 25.9° C, dissolved oxygen ranged from 7.7 to 9.4 mg/L, and pH ranged from 7.8 to 8.1. Water quality variables were within ranges considered suitable for the spawning of largemouth bass (Piper et al. 1983) and did not seem to have affected the results.

Dose verifications of the hydrogen peroxide and formalin solutions were performed once in each case during the study. The dose verification revealed a concentration of 507 mg/L of hydrogen peroxide for the 500-mg/L treatment (i.e., 0.01% stronger), 534 mg/L of formalin for the 500-mg/L treatment (i.e., 0.06 % stronger), and 1,991 mg/L of formalin for the 2,000-mg/L treatment (i.e., 0.005 % weaker). The hydrogen peroxide treatment dosage was within the range of accuracy (± 5%) for the standard test, whereas the dosage for the 500 mg/L of formalin treatment was just outside that range. The 2,000-mg/L of formalin was a flow-through treatment and the dosage concentration may have been diluted during the treatment as suggested by Rach et al. (2000). Dose verifications are not commonly reported for studies investigating fungal control with chemicals. The differences between targeted and achieved concentrations in this study demonstrate the usefulness of dose verification in determining the reliability of study results, as well as to allow comparison of different study results.

The results of this study revealed that treatment of Florida largemouth bass eggs did not significantly improve hatch rate (Table 2) and suggested that such treatments may be unnecessary. These results are similar to those of several previous studies (Table 3). Barnes et al. (1998) reported that prophylactic treatments using 250-1,000 mg/L of hydrogen peroxide and 1,667 mg/L of formalin were ineffective in preventing fungal growth on rainbow trout Oncorhynchus mykiss eyed eggs. However, they found that therapeutic treatments using hydrogen peroxide at 250, 500, and 1,000 mg/L and formalin at 1,667 mg/L on rainbow trout eyed-eggs were effective in improving hatch rate. Froelich and Engelhardt (1996), studying a strain of common carp Cyprinus carpio, found no significant differences in hatch rates between the untreated control and prophylactic treatments with 250 mg/L and 1,000 mg/L of formalin at temperatures of 23-26°C. Waterstrat et al. (1995) found prophylactic formalin treatments to be effective in preventing fungal growth without affecting the hatch rate of Chinook salmon Oncorhynchus tshawytscha.

High hatch rates of eggs incubated at colder temperatures may be more an indication of light fungus populations in the incubation waters than successful prophylactic chemical treatments. Hatch rates in cold incubation waters have been high for several fish species in several studies (e.g., Marking et al 1994, 1998; Waterstrat et al. 1995; Schreier et al.1996; Barnes et al. 2000), and usually these hatch rates did not significantly differ between prophylactically treated eggs and untreated eggs. Marking et al. (1994) found hatch rates of infected verse uninfected eggs that were therapeutically treated with formalin or hydrogen peroxide to exceed 83% regardless of the degree of infection. However, the hatch rate for uninfected and untreated eggs was also 83% (Marking et al. 1994), suggesting that the treatment was ineffective. Barnes et al. (1998) in a study using a mean temperature of 11°C found hatch rates of 40.6-50.2% among prophylactic treatments using formalin and hydrogen peroxide, but without a true control it is difficult to determine if these treatments were effective.

Barnes et al. (2000) found yearly differences in the efficacy of formalin during a multi-year study involving rainbow trout eggs and attributed those differences to

broodfish age, spawning frequency, and sample size. They also concluded that egg quality negated the influence of formalin and hypothesized that larger eggs with consequently greater outer egg membrane thickness may provide more resistance to degradation or infection (Barnes et al. 2000). This hypothesis appears to be supported by other authors (e.g., Millenbach 1950; Buss and McCreary 1960; Pitman 1979) and if this is true then treatments may differ among eggs of different sizes. Historically at the AEWFH, as broodfish grows older, egg quality appears to increase because hatch rate increases. It seems probable that egg quality is an important factor in hatching success; however, we did not see improvement in hatch rates over the two years of this study. It appears from the present study that prophylactic treatment of Florida largemouth bass eggs for fungal infections within FDA guidelines under the conditions in this study, where the eggs were allowed to gently roll in the incubation jars, may be unwarranted.

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Table 1. Mean values (ranges in parentheses) of water quality variables for the water used to incubate Florida largemouth bass eggs or prepare chemical solutions for treating the eggs.

Trial	Morning temperature (° C)	Morning dissolved oxygen (mg/L)	Morning pH	Afternoon temperature (°C)	Afternoon dissolved oxygen (mg/L)	Afternoon pH
1	22.2 (20.4 – 23.7)	9.4 (7.8 – 11.8)	7.9 (7.3 – 8.3)	22.3 (20.7 - 24.6)	8.5 (7.6 -10.0)	8 (7.6 - 8.1)
2	25.1 (24.8 – 25.3)	7.7 (7.5 – 8.0)	8.1 (8.0 – 8.1)	24.5 (23.7 – 25.5)	8 (7.8 – 8.2)	8 (7.6 – 8.3)
3	22.5 (21.7 – 23.7)	9.1 (8.4 – 9.6)	7.9 (7.7 – 8.2)	25.9 (25.7 – 26.2)	8.7 (8.7 – 8.8)	7.8 (7.8 – 7.8)
Overall mean	22.5	9.1	7.9	25.9	8.7	7.8
SD	1.6	0.9	0.1	1.8	0.4	0.1

Table 2. Mean percent hatch of Florida largemouth bass eggs subjected to three chemical treatments and a control (no chemical treatment) and incubated in McDonald hatching jars receiving a common source of water. Chemical treatments were 500 mg/L of formalin static bath for 60 min, 2,000 mg/L of formalin flow-through bath for 15 min, and 500 mg/L of hydrogen peroxide static bath for 15 min. No significant differences in hatch rates among treatments and the control ($P > 0.5$)

Trial	Control (no chemical treatment)	Formalin 500 mg/L	Formalin 2,000 mg/L	Hydrogen peroxide 500 mg/L
1	71.4 (68.1 - 75.1)	58.3 (48.4 - 65.5)	56.9 (38.9 - 80.3)	44.6 (29.5 - 54.8)
2	66.2 (44.9 - 84.7)	73.8 (57.4 - 99.5)	70 (51.6 - 88.7)	68.2 (61.8 - 88.7)
3	78.3 (65.0 - 90.0)	65 (50.0 - 75.0)	65 (50.0 - 80.0)	33.7 (25.0 - 40.0)
Overall mean	71.9	65.7	63.9	48.8
SD	0.13	0.14	0.17	0.18

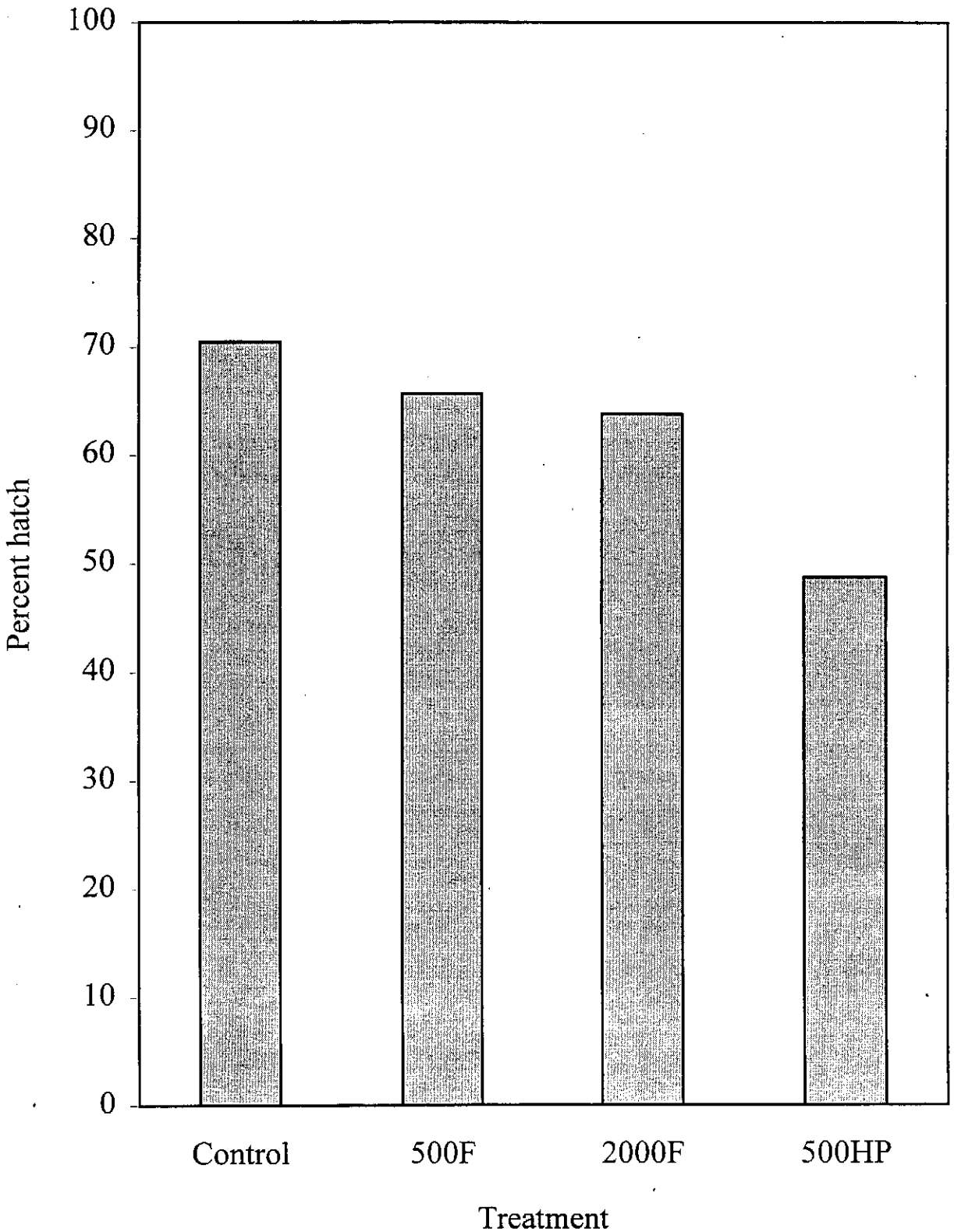
Table 3. Mean percent hatch of eggs treated with different prophylactic chemical regimes to prevent fungal infection. There were no significant differences in hatch rates among treatments.

	Temperature	Formalin						Hand -	Hydrogen peroxide					
Species	(°C)	0	250	500	1,000	1,500	1,667	picked	0	100	250	500	1,000	Source
														Barnes et al. 2000
Rainbow trout	11						85.4	85.7						1995
	11						86.0	84.6						1996
	11						74.9	71.3						1997
	11						84.4	81.4						1998
Koi carp	21	69.6	98.8		58.1									Froelich et al 1996
Rainbow trout														Barnes et al 1998
														Kamloops strain *
	11						50.2				42.3	43.2	40.6	Low density
							46.2				33.1	43.0	38.6	High density
														Fish Lake strain **
	11						81.9				83.3	80.9	82.1	Low density
							82.2				84.1	82.0	83.2	High density
Rainbow trout	N/A	89.1		88.8	90.2	89.6			95.6	95.1	95.5	95.3		Schreier et al 1996
	N/A	90.6				88.9							90.6	

* fertilized eggs; 32-d hatch

** eyed eggs; 14-d hatch

Figure 1. Mean hatch rates for three chemical treatments and a control to inhibit fungal infections on Florida largemouth bass eggs ($N = 48$, $P = 0.06$). The 500F represents 500 mg/L of formalin static bath for 60 min; the 2000F represents 2,000 mg/L of formalin flow-through bath for 15 min; the 500HP represents 500 mg/L of hydrogen peroxide static bath for 15 min; and the control represents no chemical treatment. No significant differences in hatch rate among chemical treatments and control.


100
90
80
70
60
50
40
30
20
10
0
Percent hatch
Control
500F
2000F
500HP
Treatment