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[Article]

Optimizing Copper Sulfate Treatments for Fungus Control on Channel Catfish Eggs

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Abstract.—This range-finding study determined the optimum concentration of copper sulfate ($CuSO_4$) for fungus control on eggs of channel catfish *Ictalurus punctatus*. The study consisted of five $CuSO_4$ concentrations (2.5, 5, 10, 20, and 40 mg/L) and an untreated control in a flow-through system. A single spawn was used for each replication ($N = 4$). Eggs were treated daily until the embryos reached the eyed stage. When hatching was complete for all viable eggs, fry were counted to determine the percent survival in each treatment. Fungal growth was severe in the untreated controls; survival of hatched fry in the control group was approximately 2%. The optimum $CuSO_4$ treatment, as determined by percent survival of hatched fry, was 10 mg/L daily (69% survival); survival for this treatment group was significantly different from that for the controls. Very little fungus was present in treatments receiving 10-mg/L $CuSO_4$ or higher except in one replication that had approximately 40% unfertilized eggs. The average survival rates in the 0-, 2.5-, 5-, 10-, 20-, and 40-mg/L $CuSO_4$ treatments were 2, 34, 50, 69, 59, and 51%, respectively.

Channel catfish *Ictalurus punctatus* are extensively farmed in the southeastern United States; in 2006, approximately 61,270 ha of ponds were in production in Alabama, Arkansas, Louisiana, and Mississippi (NASS 2007a) and 257.3 million kg of catfish were processed (NASS 2007b). Catfish broodstock are routinely spawned in ponds, and the collected eggs are brought into a specialized hatchery, making hatchery management a crucial component of catfish culture (Avery and Steeby 2004). Incubation of channel catfish eggs can range from 5 to 10 d depending on water temperature (Tucker and Robinson 1990). Fungal infections of hatchery-reared channel catfish eggs by *Saprolegnia* spp. frequently cause serious losses among spawns held in hatcheries (Neish and Hughes 1980; Tucker and Robinson 1990; Avery and Steeby 2004; Steeby and Avery 2005). A survey of the catfish industry conducted by the U.S. Department of Agriculture (USDA 2003) cited fungal infections as the leading cause of egg mortality; 8.3% of eggs brought into hatcheries were lost to fungus. Unfertilized eggs ultimately contribute to this loss because these eggs are more easily colonized by fungus than are fertilized eggs. The only U.S. Food and Drug Administration (FDA)-approved compounds for fungus control are 37% formalin and 35% hydrogen peroxide; povidone iodine is on the list of Low Regulatory Priority drugs for aquaculture. There are anecdotal reports of successful use of copper sulfate ($CuSO_4$) for fungus control, but controlled studies have not been reported.

Copper sulfate has been used for many years as a waterborne therapeutant for protozoan parasites and winterkill in commercial and recreational fish ponds, generally for ichthyophthiriasis (MacMillan 1985; Tucker and Robinson 1990; Bly et al. 1992; Noga 1996), and as a U.S. Environmental Protection Agency-approved algicide and molluscicide. Although the effectiveness of $CuSO_4$ as a disease therapeutant in controlling ichthyophthiriasis in channel catfish has been demonstrated (Straus 1993, 2008a; Schlenk et al. 1998), it is not approved by the FDA for therapeutic use in aquaculture. Regulatory action on $CuSO_4$ has been deferred pending the outcome of ongoing research on ichthyophthiriasis and saprolegniasis.

The present study is designed to address approval efforts to control saprolegniasis on channel catfish eggs with $CuSO_4$ because of the effectiveness, history of safe use, and low cost of this compound, especially as compared with the approved alternatives. Copper sulfate is usually administered daily during the egg incubation period (USDA 2003; Steeby and Avery 2005) until the eggs become "eyed" (eye pigment is first seen in the embryos). Treatments are stopped at the eyed stage because embryos within the same

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rearing unit may be hatching and the dose might be toxic to the fry (Straus 2008b). The present study was performed to determine an optimum treatment concentration for $CuSO_4$ to control fungus on channel catfish eggs.

Methods

Four experimental hatching troughs were constructed of aluminum and were divided into individual compartments, each compartment holding 35 L of water. Well water was filtered through a 75-μm (pore size) canister filter and flowed through the compartments continuously. Total alkalinity and total hardness (as $CaCO_3$), measured at the beginning and end of the study by titration methods (APHA et al. 2005), were 219.2 ± 0.5 and 88.3 ± 2.8 mg/L (mean ± SD), respectively. The pH (Orion Research 720A Meter; Thermo Electron Corp., Beverly, Massachusetts), which also was measured at the beginning and end of the study, was 7.6 ± 0.1. Dissolved oxygen and water temperature were monitored daily (YSI Model 95; YSI Environmental, Yellow Springs, Ohio) and were 78.0 ± 3.6% saturation and 23.6 ± 0.2°C, respectively.

Troughs were powder-coated light blue to prevent corrosion caused by the $CuSO_4$ and to aid in blinding the experiment for quality assurance and statistical purposes. Each compartment was fitted with a plastic paddle connected to an electric motor via a powder-coated steel shaft for water circulation and aeration; a plastic mesh basket was used to hold eggs until hatching, which enabled hatched fry to descend to the bottom of the trough. Each compartment was also supplied with a separate water inlet and drained through a stand-pipe covered with a deepwater release. Flow rate, set at 1.25 L/min to allow for a 28-min water exchange as in a typical hatchery, was monitored and adjusted daily. The study was maintained under 12 h light : 12 h dark conditions with fluorescent lighting.

Four egg masses less than 24 h old were collected from channel catfish broodstock spawned at the Harry K. Dupree Stuttgart National Aquaculture Research Center (SNARC); egg masses were moved to the laboratory and distributed into the experimental hatching troughs. Each egg mass was gently divided by hand into similar-weight portions (85.1 ± 3.9 g), placed into mesh baskets held in individual compartments of a single hatching trough, and acclimated for 1 h; the adhesive matrix of each egg mass portion remained intact. Two representative samples from each spawn were weighed (14.1 ± 1.5 g), and the adhesive matrix was dissolved with 1.5% sodium sulfite (Sigma Chemical Co., St. Louis, Missouri) to allow for the eggs to be counted. These counts provided an estimate of the number of eggs in each larger portion from the same spawn.

Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) used in this experiment was Triangle Brand Copper Sulfate (Phelps Dodge Refining Corp., El Paso, Texas). A 35-g/L $CuSO_4$ stock solution was prepared with deionized (DI) water before treatment. Eggs were exposed to a control (no $CuSO_4$ added) or to one of five different concentrations of $CuSO_4$ (2.5, 5, 10, 20, and 40 mg/L) in each trough ($N = 4$). A single spawn was used for each replication in the present study because of anecdotal evidence suggesting that hatching success can differ considerably for individual spawns.

To ensure the experiment was blinded, each concentration was prepared as a 40-mL mixture of stock solution and DI water in an amber vial; control treatments were dosed with an aliquot of DI water. After a 1-h acclimation period, each egg mass was treated by pouring the contents of the respective vial into the water column. Treatments were administered at 24-h intervals until the embryos developed eyes. Treatments were discontinued in a trough when any embryo within that trough developed eyes.

Eggs were naturally infested with saprolegniasis; spores of *Saprolegnia* spp. are present in most water sources, and saprolegniasis often infests eggs at the SNARC facility during hatching season. *Saprolegnia* spp. infestation was verified by visual examination of the eggs for a fungal mass, after which a small sample was removed by forceps and examined microscopically for the typical mycelial and hyphal development (presumptive identification) by an American Fisheries Society-certified fish pathologist. Fungus samples from the control treatment of each spawn were morphologically identified to genus and species (when possible) via reproductive structures. A daily log was maintained to record fungal growth in each compartment. A fungal infection ordinal scale (FIOS) was used to indicate total surface area (cm^2) infected with fungus (0 = no fungus, 0.5 = less than 1 cm in diameter, 1 = from 1 cm to less than 2 cm in diameter, 2 = from 2 cm to less than 3 cm in diameter, and so on).

The experiment was ended 3 d after hatching began; this allowed sufficient time for all viable eggs to hatch. Healthy yolk sac fry are a golden brown color; they swim to the bottom of the trough after hatching and congregate in the corners of the trough. These fry were siphoned from each compartment and then euthanized in 70% ethanol for later counting. Prematurely hatched (PMH) fry usually hatched 1–2 d early, showed a lack of normal mobility, were a light yellow color, and were scattered in the center of the compartment. Fry designated as PMH were siphoned into a container and live fry were counted immediately. The success of

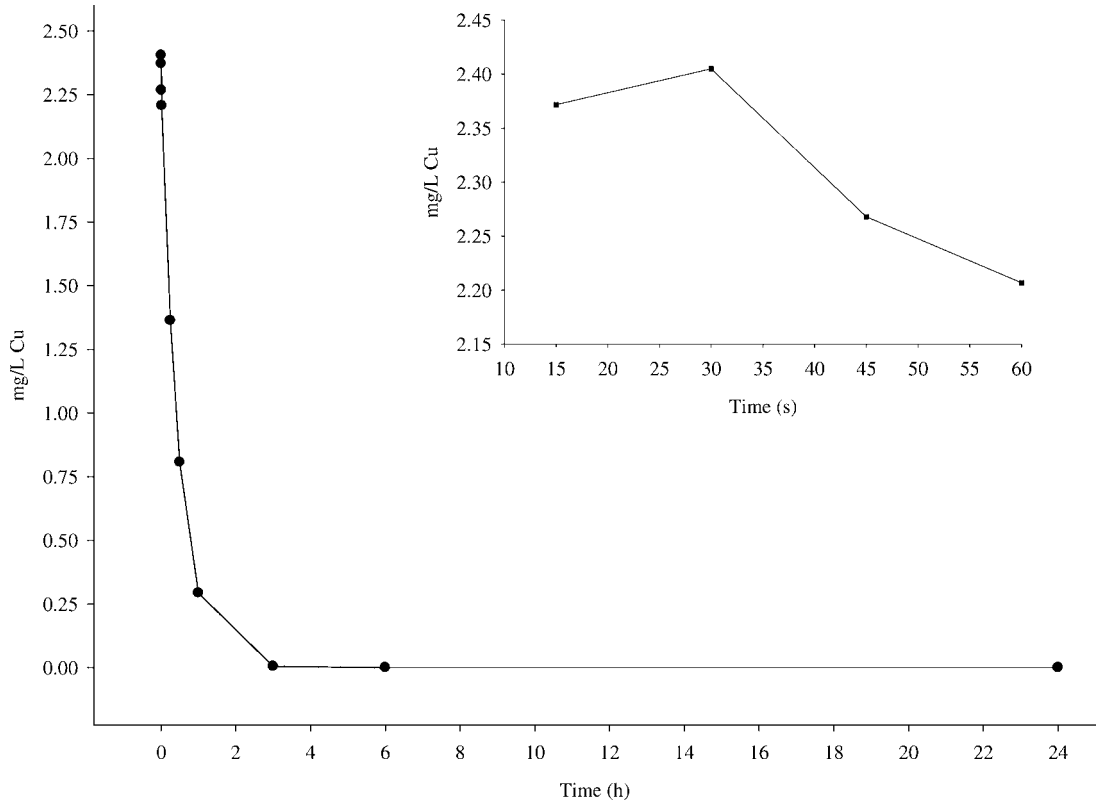

FIGURE 1.—A profile of the rate of elimination of dissolved copper (Cu) from flow-through water (1.25 L/min) after addition of a 10-mg/L copper sulfate ($CuSO_4$) aliquot ($N = 3$). This profile determined the sampling time for the range-finding study on the effectiveness of $CuSO_4$ in controlling fungus on channel catfish eggs. Inset indicates the dissolved Cu concentration at 15, 30, 45, and 60 s.

a treatment was determined by the percent of fry that hatched from the eggs; living fry were counted individually to establish percent survival.

A profile of the rate of elimination during the 24-h period between applications of $CuSO_4$ at 10 mg/L was determined before the present study (Figure 1) with a water flow rate and other conditions as in the present study. To obtain the elimination profile, water was sampled at 15 s, 30 s, 45 s, 60 s, 15 min, 30 min, 1 h, 3 h, 6 h, and 24 h after $CuSO_4$ administration ($N = 3$), filtered through a 0.22-μm nylon filter (Weltje et al. 2003), and acidified to 1% with nitric acid to measure dissolved Cu concentrations. An unfiltered water sample was also taken at these times and acidified to measure total Cu concentrations. Water samples were analyzed for Cu concentration with a Perkin-Elmer inductively coupled plasma–optical emission spectrometer (PerkinElmer, Inc., Waltham, Massachusetts) according to standard methods (APHA et al. 2005). The Cu was completely mixed by 30 s during the rate of elimination profile; consequently, water samples to measure Cu concentration were taken at 30 s in the present study. Water samples were taken at midwater depth. Samples were filtered, acidified, and stored for later analysis (within 1 month). The measured dissolved Cu concentration divided by the calculated total Cu concentration of the treatment equals the percent of Cu recovered, which was used to verify the treatment rate. A well water sample was analyzed for a suite of chemical parameters by the Water Quality Laboratory at the Arkansas Water Resources Center (University of Arkansas, Fayetteville).

The study was conducted in a randomized block design with four troughs ($N = 4$); an individual spawn was used in each trough. The fry survival rate was analyzed by the GLIMMIX procedure using Statistical Analysis System software (version 9.1.3; SAS Institute, Cary, North Carolina) for differences between control and treatment groups. The model "treatment" used the fixed effect, and "trough" was the random effect within the model statement. The conclusion of optimum

TABLE 1.—Copper sulfate ($CuSO_4$) treatment concentration, channel catfish fry survival rate (mean ± SD) and statistical *P*-value, percent of survival consisting of prematurely hatched (PMH) fry, and mean highest fungal area (cm^2) in the range-finding study on the effectiveness of $CuSO_4$ in controlling fungus on channel catfish eggs ($N = 4$). Means excluding the replicate with approximately 40% unfertilized eggs are also provided ($N = 3$).

$CuSO_4$ (mg/L)	$N = 4$				$N = 3$			
	% survival	*P*-value[a]	% PMH	Fungus area	% survival	*P*-value[a]	% PMH	Fungus area
0	2.1 ± 2.5		0.3 ± 0.4	7.8 ± 1.7	2.7 ± 2.7		0.4 ± 0.4	7.0 ± 1.0
2.5	34.0 ± 37.9	0.0049	1.0 ± 0.7	4.5 ± 3.4	42.6 ± 41.3	0.0164	0.9 ± 0.7	3.3 ± 3.1
5	49.5 ± 32.2	0.0013	5.4 ± 6.8	2.5 ± 2.6	65.0 ± 10.4	0.0053	6.9 ± 7.4	1.3 ± 1.5
10	69.4 ± 27.9	0.0002	6.6 ± 3.9	1.3 ± 1.9	82.1 ± 13.9	0.0019	7.6 ± 4.1	0.3 ± 0.6
20	58.6 ± 24.5	0.0006	7.1 ± 3.4	0.3 ± 0.5	69.1 ± 15.3	0.0044	7.8 ± 3.8	0.3 ± 0.6
40	51.0 ± 27.6	0.0011	5.5 ± 6.1	1.9 ± 2.8	64.7 ± 4.8	0.0052	6.9 ± 6.6	0.5 ± 0.5

[a] The conclusion of optimum effectiveness was given to the concentration with the lowest *P*-value.

effectiveness was given to the concentration with the lowest *P*-value (<0.05).

Results and Discussion

Once the experiment was set up, eggs were initially treated in the morning and in subsequent mornings. Eye pigment was first seen in the embryos during afternoon observations on day 5, at which time all treatments were stopped. Hatching was complete by day 10.

This is the first controlled study to determine the effectiveness of $CuSO_4$ in controlling fungus on fish eggs. Table 1 displays the results of each treatment of the present study; all treatments gave results that were significantly different from those for the untreated controls. Based on percent survival, a treatment of 10-mg/L $CuSO_4$ daily until embryos developed eyes was optimum. Considerably less fungus was present in treatments receiving 10-mg/L $CuSO_4$ or higher, except in one replicate (spawn) that had numerous unfertilized eggs (~40%); these eggs were clearly identified because pigmentation does not develop as in normal eggs. About 2–5% of eggs in the other replicates were unfertilized. Mean data excluding the replicate with numerous unfertilized eggs are also provided in Table 1.

Fungus samples from all troughs were identified as *Saprolegnia* spp. Species-level determination could not be made because of a lack of oogonial development. Common genera that have been implicated in saprolegniasis are *Saprolegnia* spp. and *Achlya* spp. (Tucker and Robinson 1990; Noga 1993; Hawke and Khoo 2004). *Aphanomyces* spp. have been identified in other recent studies at SNARC, but *Achlya* spp. were not found during those studies. This suggests that species of fungus causing saprolegniasis on channel catfish eggs are more diverse than previously considered and probably are defined by locality and other environmental factors.

The average survival in the 10-mg/L $CuSO_4$ treatment was 69%, of which 7% were considered PMH fry. There was no apparent correlation of PMH fry to Cu concentration. The lower percent of PMH fry in treatments at lower $CuSO_4$ concentrations is attributed to higher fungal growth and hence lower survival. Preliminary observations at SNARC suggest that a portion of PMH fry may recover and appear normal. Future studies should investigate the survival of PMH fry for longer durations.

Mean survival of the 10-, 20-, and 40-mg/L $CuSO_4$ treatments was 60% ($N = 4$; Table 1). The replicate with numerous unfertilized eggs contributed greatly to fungal development as is evident by the large standard deviations of percent survival; mean survival at 10-, 20-, and 40-mg/L $CuSO_4$ without this replicate ($N = 3$) was 72%. The mean egg survival in the channel catfish industry has been estimated to be approximately 60% (Wolters 2001) when formalin and iodine are used as needed, along with other best management practices. Mean survival of the 10-, 20-, and 40-mg/L $CuSO_4$ treatments for the replicate with numerous unfertilized eggs was 23%.

The highest mean fungal area (cm^2) for all replications of each treatment is given in Table 1. The replicate with approximately 40% unfertilized eggs contributed greatly to fungal development, as is evident by the large standard deviations of the four-replicate analyses versus those of the three-replicate analyses. This is particularly true in treatments of 10- and 40-mg/L $CuSO_4$, given that other replicates of these treatments either had no fungus or had a FIOS score of 1.0 or 0.5. The highest mean fungal area calculated while excluding the replicate with 40% unfertilized eggs was 0.3 and 0.5 cm^2 for the 10- and 40-mg/L $CuSO_4$ treatments, respectively (Table 1). Because unfertilized eggs provide a substrate for fungus to grow, the higher percent of unfertilized eggs in this replicate suggests that the greater amount of fungus provided an

TABLE 2.—Copper sulfate ($CuSO_4$) treatment concentration, calculated Cu, measured dissolved Cu (mean ± SD), and percent recovery (measured Cu/calculated Cu) in the range-finding study on the effectiveness of $CuSO_4$ in controlling fungus on channel catfish eggs ($N = 4$).

$CuSO_4$ (mg/L)	Calculated Cu (mg/L)	Measured dissolved Cu (mg/L)	Cu recovery (%)
0	0.00	0.00	0.0
2.5	0.64	0.70 ± 0.08	110.1
5	1.27	1.36 ± 0.09	106.8
10	2.54	2.56 ± 0.24	100.7
20	5.09	4.55 ± 0.34	89.5
40	10.18	6.55 ± 0.46	64.4

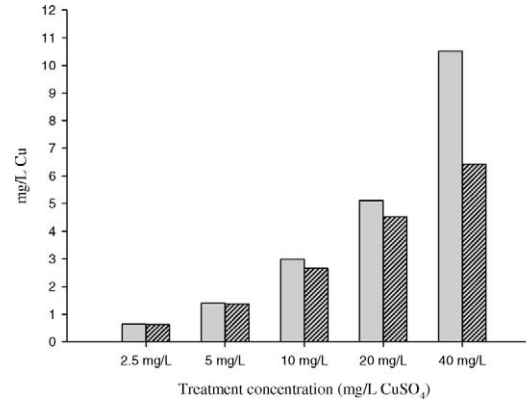

FIGURE 2.—The 30-s mean concentrations of total Cu (solid bars) and dissolved Cu (hash-marked bars) when treated in flow-through water (1.25 L/min) with a 2.5-, 5-, 10-, 20-, or 40-mg/L aliquot of copper sulfate ($CuSO_4$; $N = 3$) in an experimental hatching trough containing channel catfish eggs; the calculated Cu concentration of each treatment was 0.64, 1.27, 2.54, 5.09, and 10.18 mg/L, respectively. This experiment provided additional data for the range-finding study on the effectiveness of $CuSO_4$ in controlling fungus on channel catfish eggs. Total Cu concentrations were determined from unfiltered samples.

abundance of binding sites for Cu, which reduced the effectiveness or toxicity of $CuSO_4$.

The profile of the rate of elimination shows that approximately 93% of total Cu was recovered before 3 h, and the concentration of dissolved Cu had decreased to 0.005 mg/L by 3 h (Figure 1). This indicates that a very low concentration of Cu for a short duration is needed to control saprolegniasis. The Cu concentration of untreated well water was less than 0.001 mg/L. The mean dissolved Cu concentration of all treatments was 94% of the calculated concentration (Table 2). The low concentration of dissolved Cu in the 20- and 40-mg/L $CuSO_4$ treatments suggested that total mixing did not occur here, which warranted further research. To provide data verifying that a 40-mg/L concentration was introduced into these treatments, another profile of the rate of elimination during the 24-h period for each $CuSO_4$ concentration was carried out. The 30-s sample analyses from this latter profile demonstrates that much of the total Cu (unfiltered sample) is bound and removed by filtering to obtain the dissolved Cu concentration at the two highest treatment concentrations (Figure 2). This agrees with the dissolved Cu concentrations in Table 2.

Standard methods (APHA et al. 2005) recommend passing water through a 0.45-μm membrane filter for analysis of dissolved metals. Use of a filter of this pore size has led to erratic Cu measurements during previous studies with water obtained from ponds in this laboratory (Straus 2008a). Weltje et al. (2003) advocated the use of 0.2-μm membrane filters to reduce the amount of bacteria and colloids and to prevent any microbiological alterations to the sample. Because studies in this laboratory have confirmed the interpretation of Weltje et al. (2003), 0.22-μm nylon filters are used in studies measuring dissolved Cu concentrations.

The results of the initial water analyses for chemical characteristics are presented in Table 3. A detailed account through a certified testing laboratory is warranted for studies involving metal toxicity or efficacy to reflect the water composition. Much of this information is missing from earlier toxicity references yet is required in current research for computer modeling of metal toxicity and speciation.

In all waters, the toxicity or effectiveness of $CuSO_4$ is reduced as the total alkalinity and total hardness increase and as copper binds to inorganic or organic substrates (Tucker and Robinson 1990; Straus and Tucker 1993; Hargreaves and Tomasso 2004). To account for reduced effectiveness, current practice for control of protozoan parasites in static pond applications is to increase the rate of $CuSO_4$ in direct proportion to the total alkalinity of the water (MacMillan 1985). Pond applications typically consist of treatments at 1–2-mg/L $CuSO_4$ (higher treatment concentrations may be toxic to fish); alkalinity, hardness, and so forth will affect treatment rates because a treatment dose may require an hour or more to apply. Alkalinity and hardness are not factors when treating eggs for fungus control because the treatment rate for eggs is much greater and the application is accomplished in less than 1 min.

Copper sulfate is recommended by extension personnel for use in channel catfish hatcheries as a fungicide because of its effectiveness, history of safe use, and low cost. The results of the present study will help hatchery managers and extension personnel formulate safer and more effective application rates

TABLE 3.—Water characteristics (determined by the Water Quality Laboratory, Arkansas Water Resources Center) of the range-finding study on the effectiveness of copper sulfate in controlling fungus on channel catfish eggs.

Characteristic	Concentration[a]	Characteristic	Concentration[a]
Aggressive index[b]	12.32	Nitrate-nitrogen	0.93
Bicarbonate[c]	217.4	Nonpurgeable organic carbon[e]	0.50
Calcium	27.38	Phosphate	0.18
Carbonate[c]	0.0	Potassium	7.01
Chloride	33.40	Sodium	90.89
Conductivity[d]	530	Sulfate	0.03
Copper	0.00	Total dissolved solids	339.20
Fluoride	0.10	Dissolved organic carbon[e]	6.9
Iron	0.02	Total suspended solids	0.10
Magnesium	6.71	Zinc	0.01
Manganese	0.00		

[a] Units are mg/L unless noted otherwise.
[b] A general indicator of the tendency for corrosion to occur; no units for concentration.
[c] As $CaCO_3$.
[d] Units of μmhos/cm.
[e] As carbon; nonpurgeable organic carbon was determined on an unfiltered sample; dissolved organic carbon was determined on a 0.45-μm filtered sample.

for $CuSO_4$. These results demonstrate that 10-mg/L $CuSO_4$ was sufficient to control fungus on eggs, whereas higher concentrations did not increase the effectiveness. One should note that catfish fry may suffer mortalities at the rates of $CuSO_4$ used to treat egg masses; accordingly, the continued prohibition of treatment of eggs once the eyed stage has been reached is recommended. In few instances would $CuSO_4$ be administered to channel catfish yolk sac or swim-up fry unless they are located in a hatching trough that also contains eggs being treated to control saprolegniasis. Copper toxicity to fry (Straus 2008b) indicates that such a scenario should be avoided. Future studies required for FDA approval will investigate the safety of $CuSO_4$ to channel catfish eggs and to the environment.

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