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Health & Welfare

Egg saprolegniasis in a commercial sunshine bass hatchery

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By David L. Straus, Ph.D. , Bradley D. Farmer; Cynthia K. Ledbetter; Benjamin H. Beck, Ph.D. and Rick S. Williams; Mike L. Clark; Mike Freeze

Control regime developed using copper sulfate

Most culture operations for hybrid striped bass use the sunshine bass, a cross between a female white bass and a male striped bass.

Most aquaculture operations for production of hybrid striped bass, which began around 30 years ago, now use the sunshine bass, a cross between a female white bass and a male striped bass. Female broodstock are typically injected with human chorionic gonadotropin to induce final maturation and ovulation of eggs; males and females are strip-spawned the following morning to produce fertilized eggs. Eggs are moved to a McDonald hatching jar and exposed to a tannic acid solution for several minutes to inhibit adhesiveness (i.e., clumping). The eggs are incubated in flowing water and typically begin hatching within 48 hours.

Optimal hatchery management procedures are essential to the success of producing sunshine bass larvae. A significant problem during egg incubation is fungal growth (i.e., *Saprolegnia spp.*), which can trap or kill healthy eggs, and fungal problems are exacerbated by the presence of dead or decomposing eggs. The only compounds approved by the U.S. Food and Drug Administration (FDA) for egg fungus control are 37 percent formalin and 35 percent hydrogen peroxide.

Fungal growth is a significant problem during fish egg incubation.

However, copper sulfate ($CuSO_4$) has been used for many years as a waterborne therapeutant to control protozoan parasites and winterkill in commercial and recreational fish ponds, and regulatory action on the use of $CuSO_4$ has been deferred pending the outcome of ongoing research to gain an FDA-approved label for ichthyophthiriasis and saprolegniasis on channel catfish and channel catfish eggs, respectively. Therefore, its use as a therapeutant is currently acceptable.

In our study – here summarized from the original publication (*North American Journal of Aquaculture* 78:243–250, 2016) – we carried out an in vitro experiment to determine the concentration of $CuSO_4$ that would control fungal growth on culture media followed by an acute toxicity experiment to define the safe and lethal concentrations for sunshine bass larvae. We also conducted an experiment at a commercial sunshine bass hatchery to determine the ideal treatment concentration of $CuSO_4$ to control saprolegniasis on sunshine bass eggs.

Hatching experiment at Keo Fish Farms, Inc.

Experimental setup

Experiments were conducted at the Stuttgart National Aquaculture Research Center (SNARC), Stuttgart, Ark., USA, and Keo Fish Farm, a commercial sunshine bass producer located in Keo, Ark. At the beginning of hatching season, fungus for the in vitro experiment was isolated from eggs in McDonald hatching jars (n = 3) at Keo Fish Farm. At SNARC, the fungus was cultured and samples were then identified as *Saprolegnia ferax.* Fresh well water from Keo Fish Farm was also transferred to SNARC to use in the experiments.

For detailed procedures refer to the original publication. For the in vitro experiment, we developed a novel in vitro assay in six-well, flat-bottom, cell culture plates to test the effectiveness of $CuSO_4$. We carried out a second experiment at SNARC to determine the toxicity of $CuSO_4$ to sunshine bass larvae. The bioassays were conducted in 1-liter plastic containers (10 cm top diameter, 9 cm bottom diameter × 13 cm tall) that contained 500 mL of Keo Fish Farm water. Each container held 10 sunshine bass larvae that were ≤24 hours post-hatch. The experiment was conducted under static conditions and mortalities were recorded at intervals up to 48 hours. There were 10 concentrations of $CuSO_4$ and an untreated control (n = 3 replicates per treatment, including the control). The nominal concentrations of $CuSO_4$ ranged from 0.125 to 64 mg/L and were chosen to produce from 0 percent to 100 percent mortality within 48 hours.

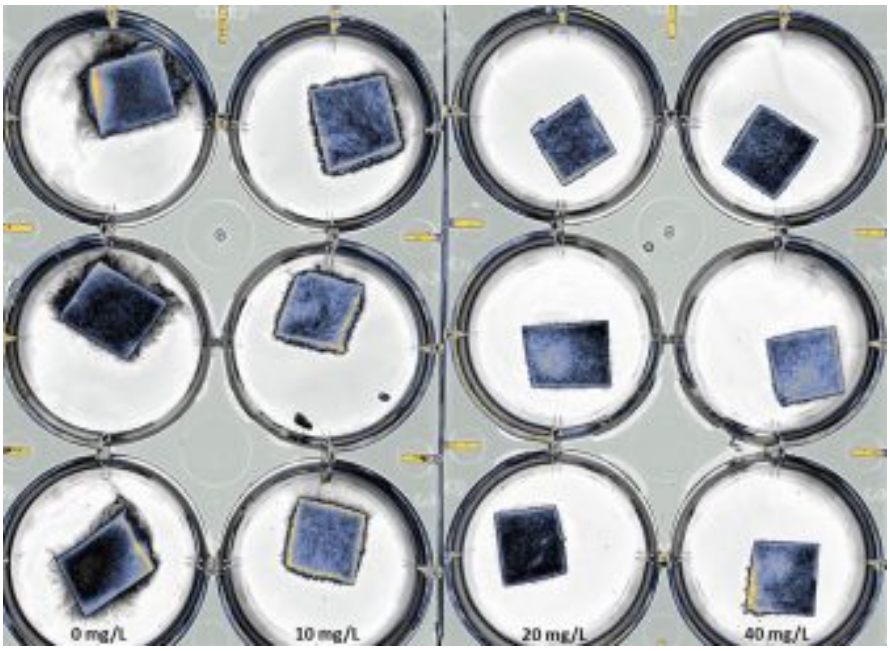
Results of experiments with copper sulfate

Important chemical characteristics for Keo Fish Farm water – essential when Cu toxicity and effectiveness are investigated – included pH = 7.9, alkalinity = 216 mg/L (as $CaCO_3$), hardness = 266 mg/L (as $CaCO_3$), and dissolved organic carbon = 1.05 mg/L.

The in vitro assay used for this study is ideal for use as an initial step to determine the effectiveness of a compound in various waters having different chemistries or organic content; for this reason, Keo Fish Farm water was used. The experiment was conducted at 20 degrees-C, which was the approximate temperature of the effectiveness experiment. The untreated control exhibited normal fungal growth as we see at SNARC.

The 10-mg/L treatment reduced this growth, but not as much as the 20- and 40-mg/L $CuSO_4$ treatments did. Maximum fungal reduction at 48 hours was achieved with 20 mg/L $CuSO_4$. All $CuSO_4$ treatments were significantly different from the control ($P < 0.001$).

The mean LC_{50} values at 24 and 48 hours for sunshine bass larvae were 5.4 (95 percent CI = 4.5–6.4) and 3.9 mg/L $CuSO_4$ (95 percent CI = 3.3–4.6), respectively. The NOEC values were 0.5 mg/L $CuSO_4$ for 24 h and 0.25 mg/L $CuSO_4$ for 48 hours. The mean percent mortality for each treatment is shown in Table 1. Larvae were not fed prior to or during the experiment, because feeding would have a negative effect on water quality and provide a potential binding site for Cu, resulting in lower concentrations available for toxicity. Water quality variables during the toxicity tests were acceptable; total ammonia nitrogen at the end of the experiment was 0.14 ± 0.04 mg/L (mean ± SD); pH was 7.8. Mean DO levels and water temperature were 89.3 ± 4.8 percent and 23.0 ± 0.1°C, respectively.



In vitro experiment – note growth in the 0 and 10 mg/L treatments.

CuSO4 (mg/L)	Mortality (%) 1h	Mortality (%) 2h	Mortality (%) 3h	Mortality (%) 4h	Mortality (%) 8h	Mortality (%) 24h	Mortality (%) 48h
0.125	0	0	0	0	0	0	0
0.25	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	3.3
1.0	0	3.3	3.3	6.7	6.7	10	10
2.0	0	0	3.3	3.3	3.3	10	10
4.0	3.3	3.3	6.7	6.7	10	13.3	30
8.0	3.3	3.3	10	10	16.7	73.3	100
16.0	10	16.7	26.7	30	43.3	100	100
32.0	23.3	40	63.3	80	93.3	100	100
64.0	40	80	100	100	100	100	100

In the effectiveness experiment, the volume of eggs in each hatching chamber was 108.7 ± 4.5 mL (mean ± SD) and yielded a mean of 174,855 ± 19,689 eggs via the XPC enumerator (a commercial device that uses preprogrammed algorithms to correlate the value recorded by the receptor to total number of organisms in the container). The DO and temperature were 93.7 ± 2.7 percent saturation and 19.2 ± 0.1 degrees-C, respectively, and the flow rate through the hatching chambers was 1.15 ± 0.15 L/min.

Eggs hatched within 48 h as expected. Fungus was severe in the untreated controls, with visually less fungus in treatments receiving 10 mg/L $CuSO_4$ or higher. Table 2 shows the least-squares mean hatch rate with four replications versus three replications. Linear regression was used to explain $CuSO_4$ treatment rates on egg survival; the equation for percent survival for n = 4 is 22.55 + 0.597 × treatment rate (F = 22.47, P = 0.0008) and for n = 3 is 28.71 + 0.659 × treatment rate (F = 18.39, P = 0.0034). Predicted values for each treatment rate based on these equations are shown in Table 2 as well. The best survival was at 40 mg/L $CuSO_4$ (50.5 percent survival); however, the 20-mg/L $CuSO_4$ treatment (45.8 percent survival) was statistically similar and thus allows for a greater margin of safety when using $CuSO_4$.

Eggs infected with fungus (left) and normal, healthy eggs (right) in McDonald hatching jars.

In a separate experiment, sunshine bass eggs were treated with 20 mg/L $CuSO_4$ and sampled for Cu content at 15 and 30 min and 1, 2, 4, and 12 h (n = 2 replicates per treatment). Analyses of the water samples (n = 2) before and 30 s after treatment indicated concentrations of 0.0 and 3.6 ± 0.49 mg/L Cu, respectively. The lower concentration of Cu in the water after treatment (70.7 percent of the calculated Cu) was attributed to immediate uptake of Cu by the eggs. The concentration in eggs prior to treatment was 2.95 µg/g Cu; the range during treatment was 54.0–67.3 µg/g Cu. This demonstrates that the Cu is taken up into the chorion and might give an extended effect when controlling fungus on eggs. Future experiments should be designed to address this.

CuSO4 (mg/L)	% Actual survival n=4	% Actual survival n=3	% Predicted survival n=4	% Predicted survival n=3
0	24.0 ± 9.0 y	27.7 ± 6.4 x	22.6	28.7
10	31.2 ± 9.0 y	31.4 ± 9.0 yx	29.1	34.7

CuSO4 (mg/L)	% Actual survival n=4	% Actual survival n=3	% Predicted survival n=4	% Predicted survival n=3
20	36.6 ± 21.1 zy	45.8 ± 13.0 zy	35.7	40.6
40	49.1 ± 3.9 z	50.5 ± 3.3 z	48.9	52.6

As a cautionary measure, sunshine bass eggs and larvae that had been treated with $CuSO_4$ were observed periodically for deformities or other possible health issues during growth up to 7.6 cm (3 in); the fish were determined to be healthy compared with fry that had not been treated. Therefore, a 20- mg/L $CuSO_4$ treatment of sunshine bass eggs should be safe.

Perspectives

These results demonstrate that 20 mg/L $CuSO_4$ was sufficient to control fungus on sunshine bass eggs. Results from this study demonstrate the effectiveness of $CuSO_4$ in controlling saprolegniasis and increasing sunshine bass larval survival in flow-through hatching jars. A reasonable assumption could be made that $CuSO_4$ may be effective on other eggs hatched in such systems; e.g., Ictaluridae (e.g., catfish), Percidae (e.g., walleye), Centrarchidae (e.g., largemouth bass) and Salmonidae (e.g., rainbow trout and other salmonids). Sunshine bass larvae may suffer mortalities at rates of $CuSO_4$ used to treat eggs for saprolegniasis; therefore, treatments should cease when hatching begins. Results of this study verify that $CuSO_4$ can be an economical and valuable tool in the control of saprolegniasis on sunshine bass eggs during hatchery operations.

The first author (Straus) gave a presentation on use of copper sulfate in aquaculture earlier this year. He stated, “Copper is very cheap for hatcheries to treat fish eggs.” For example, treating a catfish egg-hatching trough with the recommended concentration of hydrogen peroxide is $0.89, treating a trough with formalin is 73 cents, and treating a trough with copper sulfate is 2 cents. Straus has found that the egg shell protects channel catfish, largemouth bass and rainbow trout up to 100 ppm copper sulfate. However, the culturist needs to be aware of fish in waters leaving the hatchery and the water chemistry, especially alkalinity, as copper sulfate is very toxic in low alkalinity waters.

Authors

DAVID L. STRAUS, PH.D.

U.S. Department of Agriculture
Agricultural Research Service
Harry K. Dupree Stuttgart National Aquaculture Research Center
Post Office Box 1050
Stuttgart, Arkansas 72160 USA

Dave.Straus@ARS.USDA.GOV (mailto:Dave.Straus@ARS.USDA.GOV)

BRADLEY D. FARMER; CYNTHIA K. LEDBETTER; BENJAMIN H. BECK, PH.D.

U.S. Department of Agriculture
Agricultural Research Service
Harry K. Dupree Stuttgart National Aquaculture Research Center
Post Office Box 1050
Stuttgart, Arkansas 72160 USA

RICK S. WILLIAMS; MIKE L. CLARK; MIKE FREEZE

Keo Fish Farms, Inc.
Post Office Box 166
Keo, Arkansas 72083 USA