Monitoring Free Copper to Determine Application Rates of Copper Sulfate: A Case Study

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Abstract: Algae infestations are a common problem in catfish culture ponds. To treat filamentous algae, frequent applications of copper sulfate are necessary. Free copper is toxic to fish, and its concentration is dependent on a variety of factors such as its reaction with carbonate ions, absorption by phytoplankton, and temperature. The Frankfort Fish Hatchery has been using a colorimeter to measure the concentration of free copper ion after the application of copper sulfate to ponds stocked with blue catfish and channel catfish fingerlings. The use of the colorimeter allows managers to determine when the free copper concentration has decreased enough to safely treat the pond again. It has been observed at this hatchery that blue catfish are more sensitive to copper sulfate treatment than channel catfish. During the 2000 production season, the average survival of 3 blue catfish ponds treated with copper sulfate pentahydrate was 75%.

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Aquaculturists have used copper sulfate extensively to control filamentous algae and some species of protozoan parasites of fish (Moore et al. 1984.) In the United States, copper sulfate is labeled by the Food and Drug Administration for use only as an algaecide in food fish ponds, but work is currently being done to obtain approval to use it for the treatment of ichthyophthiriasis in channel catfish (Schlenk et al. 1998). Free copper, Cu^{2+}, is the most toxic form of copper (Stiff 1971). Only a portion of the copper sulfate applied to the water will produce free copper ions. The other portion of the copper sulfate will react with various components of the water or be absorbed by plants (Boyd 1979).

Because of the variation in the composition of different bodies of water, the concentration of free copper after an application of copper sulfate is difficult to predict. Also, the sensitivity of different fish species to free copper can vary. Sometimes fish mortality can occur after using the same application rate of copper sulfate that has been used before in similar water. The ability to measure the concentration of free copper can help the fish pond managers determine the best application rates for copper sulfate. A variety of factors affect the concentration of free copper following the application of copper sulfate. Some of the copper precipitates after it reacts to form tenorite (CuO) and malachite [$Cu_2(OH)_2CO_3$]. Some of the copper forms soluble inorganic complexes $CuOH^+$, $CuCO_3$, and $Cu_2(OH)_2^{2+}$ (Boyd 1979, Stiff 1971). The alkalinity and pH of the water affect the equilibrium of these reactions (Richey and Roseboom 1978, Boyd 1979, Stiff 1971). Copper can bind with muds in a cation exchange reaction. Copper can also be absorbed from the water by phytoplankton and macrophytes (Boyd 1979). Temperature affects the rates of all these reactions. Eventually almost all of the free copper reacts to form nontoxic compounds.

At the Frankfort Fish Hatchery, Frankfort, Kentucky, it had been standard practice since 1990 to apply copper sulfate pentahydrate at 0.58–0.82 mg/liter. The alkalinity of the water was 70–175 mg/liter $CaCO_3$, and the pH generally between 7.5 and 9.0. These treatments were used to control filamentous algae during the spring, summer, and fall. A treatment frequency of 2 to 4 applications per week was necessary for satisfactory control of the filamentous algae. The problem algae were rarely eliminated and control was more effective if the ponds were sprayed even when the filamentous algae had been greatly reduced.

No mortalities believed to be from copper applications were observed in the following fish: channel catfish *(Ictalurus punctatus)* $>$75 mm, bluegill *(Lepomis macrochirus)* $>$20 mm, largemouth bass *(Micropterus salmoides)* $>$20 mm, and hybrid striped bass *(Morone sp.)* $>$25 mm. Ponds with very young fish were not sprayed with copper sulfate. The only mortalities seen were in ponds with channel catfish $<$50 mm. Reduced feeding activity was observed in ponds of channel catfish and pellet-reared largemouth bass when sprayed with copper sulfate at the higher rate.

In 1994 the hatchery started raising blue catfish *(I. furcatus)*. In 1997 we became concerned because they appeared to be more sensitive to copper sulfate treatment than channel catfish. This has not been confirmed, but our routine observations continued to support this idea. Mortality of 50–150 mm fish had been observed that could not be attributed to any disease or parasites, and reduced feeding activity after application of copper sulfate was common even at lower application rates. The standard recommended rate for applying copper sulfate was 0.75–1.0 mg/liter copper sulfate pentahydrate per 100 mg/liter (as $CaCO_3$) alkalinity (Tucker 1985, Moore et al. 1984). According to our experience, this rate was too high for repeated treatments of blue catfish ponds.

Studies have shown that channel catfish is about twice as sensitive as bluegill to soluble copper (Richey and Roseboom 1978). In these studies they also used feeding behavior as an indicator of stress from copper. No comparisons of the copper sensitivity of channel catfish and blue catfish have been found.

Because of concerns about treating blue catfish ponds with copper sulfate, the Frankfort Fish Hatchery in 2000 began using a HACH colorimeter to measure the concentration of free copper. One of our main uses of the colorimeter was to determine if the level of free copper in the pond had decreased enough to safely allow another application. The time required for the free copper to return to baseline level can be determined by measuring the concentration of free copper for several days or weeks after the application.

Copper sulfate cannot be applied as often in cold water as in warm water (Schlenk et al. 1998). The measurement of free copper can help determine what application rate and treatment interval will be effective and tolerated by the fish. Our objectives for using the colorimeter were to determine the application rate of copper sulfate and the frequency of application that could be safely used on blue catfish fingerling ponds at all times of the year. Several things can be learned by using the colorimeter to measure free copper: 1) the concentration of free copper after a measured application of copper sulfate in water of a known alkalinity; 2) the rate of decrease in the concentration of free copper at various water temperatures; 3) the concentrations of free copper that will cause reduced feeding activity, cause mortality, control algae or control parasites; and 4) the treatment regime that will be tolerated by each fish species at various alkalinities and temperatures.

Methods

Free Copper Measurement

The instrument we used was the HACH model DR 890 colorimeter (HACH Co., Loveland, Colo.). It was the least expensive model available that would perform the porphyrin low range copper test. In 2000, the instrument's cost was $899 and cost of the reagents was $0.90/test. Although the instrument is a small hand-held unit, all tests were performed inside the hatchery laboratory rather than pond side. The reagents come in pre-weighed foil packets and the colorimeter has a specific program for running this sensitive free copper test (porphyrin method). The reagents also come with a standard solution. HACH states that the precision when measuring a 100 μg/liter standard was "3.4 μg/liter and the detection limit is 5.4 μg/liter." Our samples were read immediately after collection without any adjustment of the pH. Controls for sample pH and temperature and standards were run to verify the accuracy and precision of our procedure.

A few other supplies were needed. Extra sample vials helped speed the process of running tests on multiple samples. Distilled water and 1:1 nitric acid were needed for washing the glass sample tubes. The tubes get a yellow deposit after a few uses that is easily removed by briefly soaking in the nitric acid solution. The procedure used for the testing at the Frankfort Hatchery was recommended by HACH except for a few minor changes. The procedure calls for 2 sample tubes. One tube is the blank to which a copper masking reagent is added. And the other is the sample to be read. We used a third tube so that 2 readings could be averaged and a procedure error could be easily detected. Three pond samples can be run at the same time. This takes about 20 minutes from start to cleanup.

Sampling Procedure

Post application samples were collected at least 18–24 hours after application of copper sulfate. This time was necessary for adequate mixing to occur so a consistent reading could be obtained. This would not be the highest concentration of free copper to which the fish had been exposed, but can be used to compare results from ponds sampled in the same manner. Samples collected the same day as the application had erratic results that were difficult to interpret. Additional samples were collected over a period of days or weeks to measure the rate of decrease in the concentration of free copper. Sampling continued until it was verified that the concentration of free copper had decreased to near baseline, 6–10 µg/liter. Temperature was routinely measured at 60 cm water depth when oxygen was checked at 0700 hours with a YSI oxygen meter (YSI Inc., Yellow Springs, Ohio). The alkalinities of the ponds were measured before a pond was treated, and again if water was added to the pond or a high dose of copper sulfate was going to be applied. The alkalinity was measured using a HACH digital titrator. Hardness was also measured using a HACH digital titrator. An Orion model 210A pH meter was used (Orion Res., Boston, Mass.).

Application of Copper Sulfate

Applications were made from shore with a tractor drawn sprayer. Usually a sprayer load was split evenly between 3 ponds (0.36–0.5 ha) by using the volume calibrations on the side of the sprayer tank. An aqueous suspension of copper sulfate was sprayed on the pond surface where the algae mats were seen. Only the areas with algae were sprayed, but the application rate was calculated for the whole pond volume. The areas where the catfish were conditioned to feed were not sprayed. An instant powder form of copper sulfate pentahydrate, $CuSO_4$+5H2O, was used. No more than 3 kilograms of powder per 378 liters were mixed in the sprayer tank. The sprayer has a 1,135-liter tank with a PTO driven Hypro model D50 AP-A diaphragm pump (Hypro Corporation, New Brighton, Minn.). The corrosion resistant pump is rated for a maximum output of 50 liters per minute at 568 psi.

Results

When the colorimeter was first purchased, the free copper measurements (Table 1) were taken in some ponds being treated with copper sulfate and ponds where mortalities or reduced feeding activity had been observed. Note that mortalities of >75 mm blue catfish were observed, but not for >75 mm channel catfish exposed to a higher concentration of free copper. Hardness and pH data were not routinely collected, but they are presented (Table 2) to show the general water quality of ponds at the Frankfort Hatchery.

An example (Table 3) shows data of free copper and alkalinity measurements from 2 ponds. The duplicate readings for the free copper have a small coefficient of variation indicating an accurate procedure. The residual free copper before treatment was low. A safe application rate of copper sulfate was calculated based on the alkalinity and the pond volume. The measurements taken after treatment show the concentration of free copper resulting from the application and the decrease in concentration over time.

Most of the data was collected for the purpose of making decisions on the timing and quantity of the application of copper sulfate. The ponds were not set up as replicates and some data necessary for further analysis was not collected. The data was

Table 1. Species of fish, minimum size, water temperature, alkalinity, application rates, and frequencies, and concentration 24 hours after treatment for fish treated with copper sulfate at the Frankfort Fish Hatchery, Kentucky Department of Fish and Wildlife Resources.

Species	Minimum size (mm)	Temperature (°C)	Alkalinity mg/liter[a]	Application rate[b] mg/liter	Frequency[c]	Cu^{+2}mg/liter morning[d]	Observations
Bluegill	20	12.8–27.0	75–175	0.82	3/5	~175	No Mortality
Channel catfish	50	25.5	125	0.58	2/5	75.7	~100 dead
	75	12.8–27.0	75–175	0.82	3/5	~175	No Mortality
Blue catfish	75	25.5	74	0.38	2/3	82.9	~20 dead
	100	18.3	135	0.38	1/9	19.8	Poor Feeding Response

a. Alkalinity mg/liter of $CaCO_3$.

b. Application rate mg/liter $CuSO_4 \cdot 5H_2O$.

c. Frequency is number of applications/number of days

d. Concentration in µg/liter Cu^{2+} the morning after the application.

Table 2. Early September 2000 values for alkalinity (mg/liter $CaCO_3$), hardness (mg/liter $CaCO_3$), and pH (morning and afternoon readings) in catfish and bluegill fingerlings ponds at the Frankfort Fish Hatchery, Frankfort, Kentucky. SEM is the standard error of the mean.

Parameter	Species	Time	*N*	Mean	SEM	Range
Alkalinity			24	134	5.10	72–174
Hardness			9	167	7.60	131–195
pH	Catfish	Morning	9	7.87	0.14	7.4–8.8
pH	Catfish	Afternoon	9	8.74	0.16	7.8–9.5
pH	Bluegill	Morning	5	9.01	0.10	8.8–9.4
pH	Bluegill	Afternoon	5	9.52	0.16	9.2–10.1

Table 3. Example of the decrease in the concentration of free copper (Cu^{2+}) after application of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) into 2 ponds. The water quality parameters were measured at 0730, and the pond treated at 0800 hours.

Parameter (unit)	Time after application (hours)	Pond 1	Pond 3
Free Cu^{2+}(µg/liter)	0	11.7	8.5
Alkalinity (mg/liter $CaCO_3$)	0	91	139
Temperature (°C)	0	26.7	26.7
Concentration of $CuSO_4 \cdot 5H_2O$ applied (ppm)	0	0.70	1.05
Free Cu^{2+}(µg/liter)	24	69.5	70.9
Free Cu^{2+} (µg/liter)	52	26.7	32.3
Rate of decrease in free Cu^{2+} (%)	52	61.6	54.4

Table 4. Rate of decrease in free copper per day at different temperatures (°C) and alkalinity (mg/liter $CaCO_3$) values. Values are mean ± standard error.

Temperature (°C) [range]	Alkalinity (mg/liter $CaCO_3$)	*N*	Mean % decrease in free Cu^{2+}/day	SEM
6.7	84	3	10.0	1.50
[3.3–8.9]	109	6	12.5	1.44
	135	3	14.9	1.47
16.7	72	4	17.9	5.15
[12.8–18.3]	99	7	20.3	3.20
	135	3	23.5	3.15
26.8	78	4	53.3	5.12
[23.9–29.4]	96	6	54.7	3.48
	132	2	57.7	3.30

pooled according to 2 alkalinity ranges and 3 temperature ranges to obtain mean values. The alkalinity ranges were 75–125 mg/liter and 125–175 mg/liter. The temperature ranges were 3.3–8.9 C, 12.8–18.3 C and 23.9–29.4 C. The measurement of free copper clearly shows the effect temperature has on the rate of decrease in the concentration of free copper. The data is expressed as a percent decrease in the concentration of free copper in a day (Table 4). The rate of decrease is slower at lower temperatures. In the summer free copper will return to base line in 2–3 days. In the winter it takes 7–10 days. Base line concentration in our ponds is 6–10 µg/liter free copper. Water with higher alkalinity has a faster decline in the concentration of free copper.

Discussion

We observed mortalities in our ponds after application of copper sulfate that could not be attributed to disease and were not seen in a period without applications. We sometimes also observed poor response to feeding in the afternoon when copper sulfate was sprayed on a pond in the morning. Many applications, however, were made at frequent intervals without any observations of mortalities or altered behavior. Ponds with >20 mm bluegill or 75–150 mm channel catfish that were sprayed 3 times a week would have free copper concentration of 175 µg/liter and show no adverse effect on the fish. A few times ponds with 38–50 mm channel catfish or 75–100 mm blue catfish would have some mortalities after spraying 0.58 mg/liter copper sulfate pentahydrate and in most cases the free copper would be 75–85 µg/liter the morning after the application.

To stay below the concentration of free copper that we believe caused mortality, the application rate was reduced and the frequency of the application varied according to the water temperature. The frequency of application was about every 3 days at ~27 %C and every 10 days ~5 %C. Before spraying, a free copper reading was taken to insure that the concentration was close to background.

Three ponds of blue catfish fingerlings were harvested in April 2001 and the average survival of the fry was 75%. No losses were observed that were attributed to the application of copper sulfate. Reduced feeding activity was often observed the day of the copper sulfate applications. The ponds were treated to control filamentous algae throughout the whole year. The colorimeter was used to monitor the concentration of free copper. The ponds were not treated until the concentration of free copper had decreased to less than 15 µg/liter. And a low application rate of 0.12 mg/liter of copper sulfate pentahydrate was used so that the free copper measurement the morning after application was no greater than 75 µg/liter.

The most useful purpose for the instrument was to monitor copper sulfate applications made in water of different temperature. At lower temperatures the concentration of free copper does not decrease as fast over time. Even a cold period in the summer could significantly affect the rate of decrease. To spray at the same rate as the preceding week could have adverse effects on the fish. The cold part of the year is the time to be most cautious about spraying copper sulfate. The data shows that in our ponds copper sulfate should not be applied more frequently than once a week when the water temperature is below 8.9 C. In ponds with different water quality the period of time between treatments may need to be even longer. The complexity of the chemical reactions of copper in pond water is why the ability to monitor free copper is so beneficial.

Most of the observations of adverse effects of copper sulfate on fish were made before the hatchery started using the colorimeter. We sprayed copper sulfate as frequently as possible to control algae in bluegill and catfish fingerling ponds. The rate was always below the recommendation of applying copper sulfate at one hundredth of the alkalinity.

The colorimeter can be used to document the concentration of free copper that causes the observed effects. With that information the hatchery manager can refine the treatment rates, so the fish are not adversely affected. By using the colorimeter to guide our treatment schedule, we have been able to safely spray blue catfish ponds with copper sulfate year round without having to guess at the safe interval between treatments. This study should be repeated in a variety of different waters and with more complete data collection, so that better guidelines can be developed for the use of copper sulfate in aquaculture. Also a controlled study is needed to confirm that blue catfish are more sensitive to copper than channel catfish. The colorimeter would be good for measuring the actual concentrations of free copper in the study tanks.

Lessons Learned

The high sensitivity porphyrin method free copper assay and the HACH model 890 colorimeter can be a useful tool when treating fish ponds with copper sulfate. Copper sulfate can be applied safely at all times of year if time is allowed between treatments for the concentration of free copper to decrease to near background level.

Blue catfish seem to be less tolerant of free copper than channel catfish.

After a learning period, there is no need to monitor after every application of copper sulfate.

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