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Prevention of *Ichthyophthirius multifiliis* Infestation in Channel Catfish Fingerlings by Copper Sulfate Treatment

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Abstract.—The ability of copper sulfate to control *Ichthyophthirius multifiliis* tomites was determined in pond water and in settled, decanted pond water at 23°C for 7 d. Results indicate that copper concentrations of 0.15 and 0.02 mg/L prevented tomites from infesting channel catfish *Ictalurus punctatus* in pond water (total alkalinity = 366 mg $CaCO_3$/L) and in settled, decanted pond water (total alkalinity = 224 mg $CaCO_3$/L), respectively.

Ichthyophthirius multifiliis is a common ectoparasite that invades the skin and gills of many species of freshwater fish. When fingerlings of channel catfish *Ictalurus punctatus* are raised at high densities, an infestation of *I. multifiliis* can eradicate an entire fish population unless an intensive treatment regime is implemented.

Epizootics occur most frequently in the spring when water temperatures are 20–25°C; however, some outbreaks occur in fall and winter. The life cycle of *I. multifiliis* has been well documented (MacMillan 1985). The infective form (tomite) burrows into the skin or gills, where it matures and feeds on mucus and tissue. The mature parasite (trophozoite) then falls to the pond bottom, where it forms a cyst and undergoes rapid mitotic division; the cyst then bursts, releasing tomites.

Infestations of *I. multifiliis* can be treated effectively during the free-living stages of its life cycle before host invasion (Farley and Heckmann 1980). Recent research by Ewing et al. (1988) suggests that reproduction may also take place within the epithelium of fish. This may help account for difficulties frequently encountered in controlling this parasite.

The U.S. Food and Drug Administration (FDA) recently released a list of drugs approved for use in aquaculture (T. A. Bell, FDA Center for Veterinary Medicine, personal communication); only formalin is legal to use for treatment of parasite infestations of cultured food fish under specific conditions. Use of formalin as a parasiticide is an impractical treatment for an entire pond because of cost and difficulty in handling. Other chemicals have been found to be effective parasiticides, but use of such chemicals violates FDA regulations.

Copper sulfate is legally used as an algicide in waters used to raise fish for human consumption. It is also an effective parasiticide in ponds used to culture channel catfish.

The toxicity of copper sulfate to fish, algae, and presumably parasites is diminished as the total alkalinity, total hardness, and pH of waters increase. Other environmental factors such as inorganic or organic complexation influence the toxicity of copper. To account for the reduced effectiveness, current practice is to increase copper sulfate treatment rates in proportion to the total alkalinity of the water; treatment rates for copper sulfate (mg/L) are calculated by dividing total alkalinity (mg $CaCO_3$/L) by 100 (MacMillan 1985). The relationship between suggested pond treatment rates and toxicity to *I. multifiliis*, and the influence of organic loads encountered in culture ponds, are not known.

The present study was done to determine the ability of copper sulfate to control *I. multifiliis* infestations in pond water and in settled, decanted pond water. Such information may be useful in formulating safe treatment rates for fish culture and in developing new parasiticides.

Methods

Treatment containers were 3.8-L glass jars filled with 3 L of pond water or settled, decanted pond water (4 weeks without light). An air stone in each container maintained dissolved oxygen levels at or greater than 75% saturation level. Water temperature was maintained at 23.0 ± 1.0°C.

The source of water for this study was the same for pond water (pH = 8.4, total alkalinity = 366 mg $CaCO_3$/L, total hardness = 369 mg $CaCO_3$/L) and settled, decanted pond water (pH = 8.4, total alkalinity = 224 mg $CaCO_3$/L, total hardness = 284 mg $CaCO_3$/L) treatments. Lower total alkalinity and hardness in the settled, decanted

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pond water resulted from precipitation of $CaMg(CO_3)_2$ (dolomite) during the settling period.

A local strain of *Ichthyophthirius multifiliis* was obtained from a private catfish farm in the Mississippi Delta and maintained by serial infestation of channel catfish fingerlings in a 38-L glass aquarium provided with an outside biological filter containing conditioned noncarbonate gravel.

Tomites of *I. multifiliis* were obtained by a modification of the method described by Dickerson et al. (1981). Fish with mature trophozoites were placed in a small beaker of test water and gently rubbed to dislodge the parasites. Trophozoites were incubated at 23°C for 18–24 h, during which time they underwent repeated mitotic divisions that produced infestive tomites. Tomites were counted with a Whipple grid and a Sedgwick-Rafter counting cell containing 1.0 mL of the incubated solution, which had been transferred to the chamber by a wide bore pipette. Field counting (APHA et al. 1985) was used on the sample and counts were taken from 25 fields. Dose of the parasite was set at 2.0×10^4 tomites/fish.

Channel catfish fingerlings weighing 8–12 g were obtained from the Delta Research and Extension Center and fed a commercial, 35% protein, crumbled feed every other day. One week prior to the beginning of an experiment, fish were acclimated to test temperature. Fish were not fed for 48 h before or during the bioassays (APHA et al. 1985). Total ammonia-nitrogen concentration, nitrite concentration, and pH were monitored during the toxicity test (<1.0 mg/L, <0.04 mg/L, and 8.4, respectively). These concentrations were below the levels that have been determined to cause stress to channel catfish; therefore, water quality was not considered to affect the ability of copper to control the parasite.

Treatments consisted of two replications with one fish per container. A negative control was run with each replication. In each replication, the fish and dose of tomites were added; a copper sulfate solution was then added to each container within 1 min to expose tomites to a nominal copper concentration determined in preliminary experiments. Doses for pond water were 0.05, 0.10, 0.15, 0.20, and 0.25 mg Cu/L; doses for settled, decanted pond water were 0.02, 0.04, 0.06, 0.08, and 0.10 mg Cu/L.

After 7 d, skin scrapings of the entire body and gill tissues from one side of the fish were examined under a microscope at 100× magnification for visual signs of the parasite.

TABLE 1.—Ability of *Ichthyophthirius multifiliis* to infest channel catfish fingerlings in water treated with Cu (as copper sulfate).

Pond water		Settled, decanted pond water	
Dose (mg Cu/L)	Response[a]	Dose (mg Cu/L)	Response[a]
Control	++ ++	Control	++ ++
0.05	++ ++	0.02	– –
0.10	+ –	0.04	– –
0.15	– –	0.06	– –
0.20	– –	0.08	– –
0.25	– –	0.10	– –

[a] Symbols: –, no parasites were observed, +, less than 10 parasites were observed, ++, more than 10 parasites were observed. Two replications were carried out for each treatment.

Results and Discussion

In pond water, a concentration of 0.15 mg Cu/L prevented tomites from attaching to the fish (Table 1). In settled, decanted pond water, no trophozoites developed at the lowest copper concentration (0.02 mg/L).

These experiments suggest that copper is toxic to tomites. It is possible that increased mucus production, as a result of $CuSO_4$ treatment, reduced the possibility of tomite infestation; however, the low copper concentrations used in this experiment made this an unlikely factor.

Elder and Horne (1978) listed five pathways that copper may follow in the aquatic environment: (1) movement of dissolved copper to living cells, (2) complexation of dissolved copper by organic and inorganic ligands, (3) conversion of dissolved copper into suspended particulate forms, (4) fallout into the sediments, and (5) solubilization of copper in the sediments.

In the present study, settled, decanted pond water (lacking sediment and organic matter) showed the true toxicity of copper to *I. multifiliis* at a total alkalinity of 224 mg/L. However, sediment and organic matter must be considered major factors contributing to copper toxicity in ponds, because the organic load will vary between ponds. Williams (1969) demonstrated that organic material was associated with 5–28% of the total soluble copper in seawater. In the pond water treatment of the present study, complexation of copper with organic matter apparently resulted in decreased toxicity to the organism.

In pond water (total alkalinity = 366 mg/L), the copper treatment required to prevent tomites from attaching to the host was a single dose of 0.15 mg Cu/L. However, the current recommendation (MacMillan 1985) for an active infestation in a pond situation at this alkalinity would be to treat the water with 0.93 mg Cu/L every other day for 3–5 treatments to control the parasite. Other than plankton, environmental factors and sediments responsible for complexation in a pond situation were not present in the laboratory experiment. Because of the notable difference in application rates, further investigation of the recommended rate could be economically beneficial to the farmer.

Copper toxicity to aquatic animals has been widely studied (USEPA 1980); physiological effects on these animals and chemical characteristics of the metal in the aquatic environment have been well documented (Boyd 1990). Further experiments should be done to determine the effectiveness of copper sulfate at various alkalinity and pH values (because alkalinity or pH, or both, may dictate toxicity) and to investigate the toxicity of other copper compounds to parasites that affect cultured species.

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