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[Article]

Copper Sulfate Toxicity to Channel Catfish Fry: Yolk Sac versus Swim-Up Fry

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Abstract.—Yolk sac and swim-up fry from five separate spawns of channel catfish *Ictalurus punctatus* were exposed to dissolved copper sulfate ($CuSO_4$) in a series of static toxicity bioassays to observe age sensitivity at 24 and 48 h in waters with two different chemistries at a temperature of 23.1 ± 0.47°C (mean ± SD). The two waters were (1) well water with a total alkalinity (as $CaCO_3$) of 217 mg/L and a total hardness of 126 mg/L and (2) a 1:1 mixture of well water and deionized (WDI) water with a total alkalinity of 112 mg/L and a total hardness of 66 mg/L. Probit median lethal concentration (LC50) values were estimated with PoloPlus using the nominal $CuSO_4$ concentrations. The mean 24-h LC50 values for the yolk sac fry were 62.8 ± 37.1 mg of $CuSO_4$/L in the well water and 10.2 ± 3.4 mg/L in the WDI water; the 24-h LC50 values for the swim-up fry were 13.5 ± 11.5 mg/L in well water and 4.3 ± 4.4 mg/L in WDI water. The mean 48-h LC50 values for the yolk sac fry were 14.9 ± 5.0 mg/L in well water and 3.9 ± 1.6 mg/L in WDI water; the 48-h LC50 values for the swim-up fry were 3.5 ± 1.8 mg/L in well water and 1.5 ± 0.3 mg/L in WDI water. The yolk sac fry were about 4.6 times more tolerant of $CuSO_4$ than the swim-up fry at 24 h and 4.3 times more tolerant at 48 h. Fry were about 4.7 times (at 24 h) and 3.3 times (at 48 h) more sensitive to $CuSO_4$ in water with low alkalinity and hardness (WDI water) than in water with high alkalinity and hardness (well water). The differences in LC50 values between the different spawns of each fry type and time within a water type are attributed to natural variation and channel catfish strain.

Channel catfish *Ictalurus punctatus* (Rafinesque 1818) are extensively farmed in the southeastern USA; approximately 64,000 ha of ponds are in production in Alabama, Arkansas, Louisiana, and Mississippi (Agricultural Statistics Board 2007). Catfish broodstock are routinely spawned in ponds, and the collected eggs are brought into a specialized hatchery; consequently, hatchery management is a crucial component of catfish culture (Avery and Steeby 2004). The incubation time of catfish eggs can range from 5 to 8 d, depending on water temperature (Tucker and Robinson 1990), and fungal infestation of eggs is a commonly occurring disease problem during this period. The hatched yolk sac fry absorb the yolk sac within days; at this point, the fry swim to the surface and look for food and are called swim-up fry. Yolk sac fry do not need to be fed because they receive nourishment from the attached yolk (Tucker and Robinson 1990; Steeby and Avery 2005).

Copper sulfate ($CuSO_4$), an effective algacide approved by the U.S. Environmental Protection Agency, is widely used in pond aquaculture. This compound is also used as a therapeutant for protozoan parasites in commercial and recreational fish ponds, generally for ichthyophthiriasis (MacMillan 1985; Tucker and Robinson 1990), and as a fungicide in catfish hatcheries, mainly for saprolegniasis (Tucker and Robinson 1990; Steeby and Avery 2005). Copper sulfate is usually administered daily during the egg incubation period until the eyed egg stage (when eye pigment is first seen in the embryos). Treatments are stopped at the eyed stage because there may be other embryos within the same rearing unit that are hatching and the dose could be toxic to the fry (J. A. Steeby, Mississippi State University Extension Service, personal communication). Copper sulfate is not approved by the U.S. Food and Drug Administration for therapeutic use in aquaculture; regulatory action has been deferred pending the outcome of ongoing research. A wealth of information exists for this compound, especially for its use in catfish culture (MacMillan 1985; Tucker and Robinson 1990; Avery and Steeby 2004).

The acute toxicity of copper (as $CuSO_4$) to fingerling channel catfish has been studied (Straus and Tucker 1993; Wurts and Perschbacher 1994; Perschbacher and Wurts 1999; Moore 2005; Straus 2006), but no data for channel catfish fry are available. Anecdotal information suggests that catfish fry are much more sensitive than adults; Sprague (1985) corroborates this supposition by stating, "the most sensitive life stages seem to be the embryo–larval and early juvenile stages." The present

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TABLE 1.—Water chemistry parameters for the two types of water, well water and a 1:1 mixture of well water and deionized water (WDI), used in the study of the toxicity of copper sulfate to channel catfish yolk sac and swim-up fry.

Parameter	WDI water	Well water
Initial pH	8.3	8.1
Alkalinity (mg/L)	112	217
Hardness (mg/L)	66	126

study estimated the 24-h and 48-h median lethal concentration (LC50) values for $CuSO_4$ to channel catfish yolk sac fry and swim-up fry in waters of two different chemistries.

Methods

Channel catfish eggs were obtained from the Joe Hogan State Fish Hatchery (Lonoke, Arkansas), Hopper–Stephens Hatcheries, Inc. (Lonoke, Arkansas), and the Harry K. Dupree–Stuttgart National Aquaculture Research Center (SNARC). Eggs were hatched using conventional methods in an indoor system (Tucker and Robinson 1990; Steeby and Avery 2005). Different spawns of eggs or fry were held in separate flow-through troughs with a photoperiod of 12 h light : 12 h dark for hatching and rearing to swim-up fry. Fry were not fed before or during bioassays. The yolk sac fry were less than 24-h posthatch and the swim-up fry were 7–8 d posthatch.

The water used during the study was either well water or well water mixed 1:1 (by volume) with deionized water (WDI water). Well water was filtered through a 75-μM canister filter. Both waters were maintained in an aerated 250-L carboy at room temperature to provide consistent water chemistry during the study. Bioassays were conducted in 1-L round plastic containers (10-cm-diameter top, 9-cm-diameter bottom, 13 cm tall) that contained 500 mL of water. Each container held 10 catfish fry. The study was conducted under static conditions (APHA et al. 1998) in a randomized block design with three replicates per treatment (10 fry/container, for a total of 30 fry), and mortalities were recorded at 24 and 48 h; an individual spawn of yolk sac fry or swim-up fry was exposed concurrently in both test waters.

The $CuSO_4 \cdot 5H_2O$ used in this experiment was 25.4% Cu (Sigma Chemical Co., St. Louis, Missouri). Eight concentrations of $CuSO_4$ were tested: no added $CuSO_4$ (control) and seven different concentrations, from 0.25 to 128 mg/L of water, as calculated by geometric progression (Parrish 1985; APHA et al. 1998). The nominal concentrations were based on the results of preliminary studies and chosen to produce from 0% to 100% mortality within 48 h. It is important to note that nominal concentrations were used to calculate LC50 values so that the results would be useful to hatchery managers and extension personnel and that the copper concentrations were not measured in each bioassay. A stock solution of $CuSO_4$ was prepared immediately before treatment and used to dose the containers.

Total ammonia nitrogen (Hach kit FF-1A; Hach Company, Loveland, Colorado) and dissolved oxygen and temperature (YSI Model 95; YSI Environmental, Yellow Springs, Ohio) were monitored daily. Total alkalinity (standard acid titration method; APHA et al. 1998), total hardness (EDTA titration method; APHA et al. 1998) and pH (Orion Research 720A Meter, Thermo Electron Corporation, Beverly, Massachusetts) were measured at the beginning of each bioassay (Table 1).

The LC50 values and 95% confidence intervals (CIs), estimated with PoloPlus statistical software (LeOra Software Company 2002), were derived from the nominal $CuSO_4$ concentrations and fry mortality of the three replicates. Lethal dose ratios (i.e., LC50 ratio; LDR) were calculated to compare natural variation between the five spawns of each fry type and time within water type (well water or WDI water). The LDR of each LC50 value relative to the lowest LC50 value of that group was determined as described by Robertson et al. (1995). The hypothesis was tested that the value was significantly different from the value of the standard (lowest LC50 value), as described by Robertson and Preisler (1992); significant differences were based on failure of the 95% CI of a ratio to bracket the value 1. The LDR comparisons were also performed as described by Robertson and Preisler (1992) to determine statistical significance between fry type and water type for each individual spawn. The LDRs were considered to be significant ($P < 0.05$) when the confidence intervals did not encompass the value 1.

Results and Discussion

Eggs were hatched and fry were reared in separate flow-through troughs at 23.5°C; the total alkalinity of this water was 217 mg/L and the total hardness was 132 mg/L. Measures of water quality during the bioassays were acceptable for catfish fry (Tucker and Robinson 1990; Avery and Steeby 2004). Dissolved oxygen levels were greater than 75% saturation in all containers. Total ammonia nitrogen ranged from 0.2 to 1.0 mg/L (calculated un-ionized ammonia was 0.01–0.05 mg/L). The water temperature during the bioassays was 23.1 ± 0.47°C (mean ± SD), with a range of 22.2–23.6°C.

Visual observation of the bioassay containers indicated that copper in the higher $CuSO_4$ concentrations in well water precipitated out of solution by forming various

TABLE 2.—Acute toxicity of copper sulfate to channel catfish yolk sac and swim-up fry in two types of water (see Table 1) at 24 and 48 h, as determined by median lethal concentration (LC50) value. The LC50 values and 95% CIs (parentheses) were calculated by PoloPlus (Robertson et al. 1995) using nominal $CuSO_4$ concentrations. The lowest LC50 value for each fry type, water type (WDI is deionized water), and exposure period was used to calculate lethal dose ratios to indicate significant differences and is given in bold italics; different lowercase letters denote significant differences.

Fry	Spawn	Mean weight (g)[a]	24 h		48 h	
			Well water	WDI water	Well water	WDI water
Yolk sac	1	0.018	51.9 (36.8–89.3) y	***8.0 (6.2–10.3) z***	16.1 (12.7–20.7) y	4.3 (3.5–5.3) y
	2	0.015	88.7 (38.5–1,169.9) y	16.0 (11.5–29.1) y	13.4 (7.5–29.2) z	6.2 (4.7–8.7) y
	3	0.013	113.4 (49.0–946.3) y	10.8 (7.4–16.6) y	***7.1 (3.8–12.7) z***	***1.9 (1.2–3.0) z***
	4	0.013	33.6 (28.3–40.0) z	8.3 (7.0–10.0) y	19.0 (16.4–22.0) y	4.0 (3.1–5.2) y
	5	0.015	***26.6 (14.9–58.6) z***	8.1 (6.1–10.7) y	19.0 (11.9–32.3) y	3.1 (2.2–4.4) y
Swim-up	1	0.031	7.4 (6.1–9.1) y	2.5 (2.1–3.0) y	5.3 (3.9–7.2) y	2.0 (1.7–2.3) y
	2[b]	0.027	33.3 y	12.0 y		
	3	0.022	13.2 (10.0–17.4) y	3.4 (2.5–4.6) y	4.7 (2.7–7.4) y	1.5 (1.1–2.0) y
	4	0.019	***4.7 (3.2–6.7) z***	***1.5 (1.2–1.8) z***	***1.6 (0.8–2.6) z***	***1.2 (1.0–1.4) z***
	5	0.024	8.8 (6.9– 11.2) y	2.0 (1.6–2.5) y	2.2 (1.7–2.7) z	1.4 (1.0–1.9) y

[a] $n = 30$.
[b] The 24-h LC50 values are based on one replicate each, so that 95% CIs could not be calculated; no data were available for calculating 48-h LC50 values.

carbonate complexes. This is common during $CuSO_4$ application to ponds because copper quickly forms insoluble precipitates in water of high alkalinity, with the result that treatment efficacy is considerably reduced in high-alkalinity water (Tucker and Robinson 1990). The precipitation noted above would obviously affect the concentrations of the toxic form of copper and therefore result in a much different LC50 value for free copper.

The 24-h LC50 values for the yolk sac fry were 62.8 ± 37.1 mg of $CuSO_4$/L in the well water and 10.2 ± 3.4 mg/L in the WDI water. The 24-h LC50 values for the swim-up fry were 13.5 ± 11.5 mg/L in well water and 4.3 ± 4.4 mg/L in WDI water. The 48-h LC50 values for the yolk sac fry were 14.9 ± 5.0 mg/L in well water and 3.9 ± 1.6 mg/L in WDI water; the 48-h LC50 values for the swim-up fry were 3.5 ± 1.8 mg/L in well water and 1.5 ± 0.3 mg/L in WDI water. Table 2 presents the 24- and 48-h LC50 values for the individual spawns of yolk sac and swim-up fry. The LC50 values in Table 2 differ by as much as eightfold among groups (i.e., fry type × time × water type). A certain amount of natural variation is present when any bioassay is repeated (Robertson et al. 1995). In this study, part of the variation may be attributed to the particular origin of each spawn and strain of channel catfish (spawn 1 being obtained from the Joe Hogan State Fish Hatchery, spawn 2 from the SNARC, and spawns 3–5 from Hopper-Stephens Hatcheries, Inc.). The spawns were from unspecified strains of channel catfish.

The LC50 values of different species or distinct populations are often examined statistically via mean separation tests or by determining whether the 95% CIs overlap; however, these methods are statistically inadequate for the various reasons described by Robertson and Preisler (1992), Payton et al. (2003), and Wheeler et al. (2006). Determining LDRs as described by Robertson and Preisler (1992) has been demonstrated to be the best procedure for comparing LC50 values; this procedure is not available in many software packages, such as SAS (Payton et al. 2003), but is in PoloPlus software.

The LC50 values of the yolk sac fry in the 24-h bioassays (Table 2) were variable, as demonstrated by their 95% CIs. At 48 h, the yolk sac fry from spawn 3 had the lowest LC50 values in both water types, whereas the lowest values at 24 h were split between spawns 1 and 5. Of the yolk sac fry LC50 values, 69% were significantly different from the lowest value. Swim-up fry from spawn 4 had the lowest LC50 values in all bioassays, and this spawn was evidently more sensitive to copper than other spawns. Of the swim-up fry LC50 values, 71% were significantly different from spawn 4.

To compare LC50 values for fry of a specific spawn in different water types, the LDR comparisons at 24 and 48 h were performed by dividing the LC50 value of the well water bioassay for a given fry type and spawn by the corresponding LC50 value of the WDI water bioassay (Table 3). All LDRs at both 24 and 48 h indicated statistically significant differences between the water types except for spawn 4 of the swim-up fry at 48 h, which we attributed to the low LC50 value and relatively large 95% CI for the well water bioassay. As mentioned above, the LC50 values of the yolk sac fry in the 24-h bioassays were variable, as demonstrated by their 95% CIs, and this would greatly contribute to the range of the LDRs. The data in Table 3 demonstrate that fry in the WDI water were 2.8–10.5 times more sensitive to $CuSO_4$ than those in well water at 24 h and 1.4–6.2

TABLE 3.—Lethal dose ratios and 95% CIs (parentheses) of channel catfish fry for well water and WDI (deionized water) at 24 and 48 h. Each value is the ratio of the LC50 for fish in well water to the corresponding LC50 for fish in WDI water (Robertson and Preisler 1992). The LC50 values are provided in Table 2. Asterisks indicate significantly different ($P < 0.05$) LC50 values between water types.

Fry	Spawn	24 h	48 h
Yolk sac	1	6.5 (4.1–10.3)*	3.7 (2.9–4.8)*
	2	5.5 (2.3–13.1)*	2.2 (1.1–4.3)*
	3	10.5 (5.4–20.4)*	3.7 (2.2–6.1)*
	4	4.0 (3.2–5.2)*	4.7 (3.7–6.0)*
	5	3.3 (2.2–4.9)*	6.2 (4.1–9.3)*
Swim-up	1	2.9 (2.3–3.8)*	2.7 (2.1–3.5)*
	2	2.8 (1.9–4.1)*	[a]
	3	3.9 (2.9–5.3)*	3.2 (2.2–4.6)*
	4	3.2 (2.3–4.4)*	1.4 (0.9–2.2)
	5	4.5 (3.3–6.1)*	1.6 (1.2–2.2)*

[a] Data not available.

TABLE 4.—Lethal dose ratios and 95% CIs (parentheses) of channel catfish yolk sac versus swim-up fry at 24 and 48 h by water type. Asterisks indicate significantly different ($P < 0.05$) LC50 values between fry types. See Table 3 for further details; DNI = deionized water.

Water type	Spawn	24 h	48 h
Well	1	7.0 (4.5–11.0)*	3.1 (2.4–4.0)*
	2	2.7 (1.2–5.9)*	[a]
	3	8.6 (4.5–16.5)*	1.5 (0.9–2.5)
	4	7.1 (5.2–9.8)*	11.5 (7.5–17.8)*
	5	3.0 (2.0–4.6)*	8.8 (6.0–12.8)*
WDI	1	3.2 (2.4–4.2)*	2.2 (1.7–2.8)*
	2	1.3 (0.8–2.2)	[a]
	3	3.2 (2.3–4.4)*	1.3 (0.9–1.9)
	4	5.6 (4.4–7.3)*	3.4 (2.6–4.5)*
	5	4.1 (3.1–5.4)*	2.3 (1.6–3.2)*

[a] Data not available.

times more sensitive at 48 h. These data are consistent with the current understanding of $CuSO_4$ toxicity, as demonstrated in channel catfish fingerlings by Straus and Tucker (1993), in cutthroat trout *Salmo clarkii* by Chakoumakos et al. (1979), in juvenile rainbow trout *Oncorhynchus mykiss* by Laurén and McDonald (1986), and others as listed by Sprague (1985).

To compare LC50 values for fry at different ages, the LDR comparisons at 24 and 48 h were performed by dividing the LC50 value of yolk sac fry for a given water type and spawn by the corresponding LC50 value of the swim-up fry (Table 4). At 24 h, all LDRs were significantly different (i.e., the toxicity of $CuSO_4$ differed between yolk and swim-up fry) except for the fry comparison of spawn 2 in WDI water; at 48 h, the LDRs were significantly different except for spawn 3 in both types of water. The data in Table 4 demonstrate that swim-up fry were 1.3–8.6 times more sensitive to $CuSO_4$ than yolk sac fry at 24 h and 1.3–11.5 times more sensitive at 48 h. These results are contrary to previous findings (Sprague 1985); however, past studies have focused on the chronic toxicity to embryo–larval stages or on the acute toxicity to juveniles rather than on the differences between newly hatched (yolk sac fry) and 7–8-d-old swim-up fry (as in this study). Sprague (1985) suggests that no overall relation can be applied across species, toxicant, and size range and that any stress resulting from the toxicant would probably be exacerbated by stress from other sources. In addition, Rand et al. (1995) suggests rates and patterns of organism metabolism and excretion can affect toxicity, as can genetic and dietary factors and the degree of development of detoxification mechanisms.

The yolk sac provides nourishment until the catfish fry become swim-up fry, and it is reasonable to assume that when this yolk sac is absorbed the fry will have a weakened system with respect to any toxicant until they regain input from an energy source (i.e., nutrition). Fry were not fed before or during the study because yolk sac fry do not eat; also, feeding would have had a negative effect on water quality in the static containers and would have provided a potential binding site for copper, lowering the copper concentrations contributing to toxicity and thereby confounding the results. Other physiological possibilities for the difference in toxicity between fry type could be the increased metabolic rate and increased vascularized gill surface area of the swim-up fry (E. L. Torrans, Thad Cochran National Warm-water Aquaculture Center, personal communication) or the lack of intestinal absorption in the yolk sac fry.

As discussed by Straus and Tucker (1993), the toxic form of copper is thought to be the cupric ion (Cu^{+2}); when added to water, Cu^{+2} reacts to form precipitates of tenorite (CuO) and additional dissolved complexes. Research has indicated that the major controlling water quality variables are hardness, pH, and alkalinity (Chakoumakos et al. 1979; Laurén and McDonald 1986; Straus and Tucker 1993; Wurts and Perschbacher 1994). Hardness (Ca and Mg ions) moderates copper toxicity through competitive binding to exchange sites on the gill, whereas alkalinity affects copper solubility directly through the formation of inorganic complexes and indirectly through the effect on equilibrium pH (Hargreaves and Tomasso 2004). Tucker and Robinson (1990) suggest that calcium hardness may protect fry from ammonia and metal toxicosis at higher concentrations. Other variables that have been shown to influence Cu toxicity are dissolved organic matter in the water column, fish size, and temperature (Sorenson 1991).

Copper sulfate is recommended by extension personnel for use in catfish hatcheries as a fungicide because of its effectiveness and low cost. Under routine hatchery practices, $CuSO_4$ would not be administered

to catfish swim-up fry unless they were located in a hatching trough that also contained eggs being treated to control saprolegniasis. Data provided from this study indicate that this scenario should be avoided. The results of this study will help hatchery managers and extension personnel formulate safer and more effective application rates for $CuSO_4$. These results demonstrate that channel catfish swim-up fry are less tolerant to $CuSO_4$ concentrations than yolk sac fry in waters from the same source. Because yolk sac fry and swim-up fry may suffer mortalities at rates of $CuSO_4$ used to treat egg masses, the continued prohibition of treatment of eggs as they approach hatching is recommended.

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