Available online at www.sciencedirect.com
SCIENCE DIRECT
Aquaculture 219 (2003) 233–240
Aquaculture
www.elsevier.com/locate/aqua-online

The acute toxicity of copper to blue tilapia in dilutions of settled pond water ☆

David L. Straus *

Agricultural Research Service, Harry K. Dupree—Stuttgart National Aquaculture Research Center, U.S. Department of Agriculture, P.O. Box 1050, Stuttgart, AR 72160, USA

Received 27 December 2001; received in revised form 20 May 2002; accepted 30 June 2002

Abstract

Blue tilapia fingerlings (*Oreochromis aureus*) were exposed to copper (Cu) in a series of static toxicity tests. The water used in this study was composed of settled pond water or settled pond water diluted with deionized water. Estimates of mean 96-h LC50 values (median lethal concentration) were 43.06, 6.61, 0.69 and 0.18 mg/l Cu as copper sulfate) in waters having total alkalinities of 225, 112, 57 and 16 mg/l $CaCO_3$, respectively. These data demonstrate the typical acute toxicity response of Cu, in which toxicity to tilapia increases as pH, total alkalinity and total hardness decrease. The results indicate that blue tilapia are relatively tolerant to Cu when compared to other species and that copper sulfate treatments in low-alkalinity waters may be detrimental to the health of blue tilapia.

Keywords: Blue tilapia; Acute toxicity; Copper; Alkalinity; Hardness; pH

1. Introduction

Aquaculture continues to expand and find new niches and markets. The culture of tilapia has exceeded 800,000 metric tons (Popma and Masser, 1999) and is ranked below carps as the second most widely farmed fish group in the world. There are projections that tilapia production will double in the next decade (Stickney, 2001). Popma and Masser (1999) report that 90% of all commercially farmed tilapia outside of Africa are Nile tilapia (*Oreochromis niloticus*) while other farmed species include blue tilapia (*Oreochromis*

☆ Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

* Tel.: +1-870-673-4483; fax: +1-870-673-7710.
E-mail address: dstraus@spa.ars.usda.gov (D.L. Straus).

PII: S0044-8486(02)00350-2

aureus), Mozambique tilapia (*Oreochromis mossambicus*) and the Zanzibar tilapia (*Tilapia urolepis hornorum*). In 1997, blue tilapia culture was estimated to account for 20% of the total tilapia culture in the Americas (Fitzsimmons, 2000).

Copper sulfate is used extensively in aquaculture as a U.S. Environmental Protection Agency-approved algicide (Schnick et al., 1986). It is also used as a therapeutant for protozoan parasites in commercial and recreational fishponds. Copper sulfate is not approved by the U.S. Food and Drug Administration for therapeutic use in aquaculture; however, regulatory action has been deferred pending the outcome of current research.

The effectiveness of copper sulfate as a therapeutant is reduced as the total alkalinity and/or total hardness (as mg/l $CaCO_3$) of waters increase. Additionally, the toxicity of this compound to fish decreases as pH, total alkalinity and total hardness increase, and as copper (Cu) binds to inorganic or organic substrates (Tucker and Robinson, 1990). To account for the reduced efficacy, the current practice for the therapeutic use of copper sulfate is to increase the application rate in direct proportion to the total alkalinity of the water (MacMillan, 1985; Tucker and Robinson, 1990). Copper sulfate is usually not recommended as a therapeutant in waters having less than 50 mg/l alkalinity because of the acute toxicity (Tucker and Robinson, 1990).

The acute toxicity of copper (Cu) to many species has been studied, including other species of tilapia; however, there are no data for blue tilapia. The present study determined the acute toxicity of Cu to blue tilapia in waters composed of settled pond water or settled pond water diluted with deionized water.

2. Materials and methods

Blue tilapia fingerlings were obtained from the Harry K. Dupree—Stuttgart National Aquaculture Research Center (Stuttgart, AR, USA) and acclimated to 20 ± 1 °C for several weeks. A 12-h light/dark cycle was used to minimize any influence on behavior or metabolism. Fish were offered a commercial 36% protein, crumbled feed every other day to maintain body weight. Fish were not fed for 48 h prior to or during toxicity tests.

Tests were conducted in 40-l glass aquaria equipped with airstones to maintain dissolved oxygen levels greater than 75% saturation. Each aquarium contained 10 fish. The study was conducted under static test conditions (APHA, 1998) in a completely randomized design with three replications per treatment. A static test was chosen to imitate conditions during treatment in aquaculture ponds where the compound is applied in a single dose and concentrations of copper decline after application (Boyd, 1990).

A raceway was filled with sand-filtered pond water and was covered with an opaque tarp and remained undisturbed for at least 2 months to allow for complete settling. Aquaria contained 30 l of 100%, 50%, 25% or 6.25% settled pond water diluted with deionized water; these dilutions were used to give a broad range of total alkalinity, total hardness and pH values. Settled pond water was analyzed for total carbon (TC; high-temperature combustion method; APHA, 1998) with a Tekmar–Dohrman High Temperature Total Organic Carbon Analyzer, model DC-190.

During each replication, the fish were exposed to a control (no Cu added) and at least six concentrations of Cu. Copper was added from stock solutions prepared from copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$; 25.45% Cu) purchased from the Sigma Chemical Co. (St. Louis, MO, USA). The concentrations used in each test were chosen to give from 0% to 100% mortality of test animals within 96 h based on the results of preliminary studies. The following water quality variables were monitored daily: total ammonia–nitrogen and nitrite–nitrogen (Hach kit FF-1A), pH (Orion Research 720A Meter), dissolved oxygen and temperature (YSI Model 95). Total alkalinity (standard acid titration method; APHA, 1998), total hardness (EDTA titration method; APHA, 1998) and pH were measured at the beginning of each replication.

A 20-ml water sample was taken immediately (~ 5 s) after each Cu treatment and filtered through a 0.1-μm nylon filter and acidified with nitric acid. Soluble Cu concentrations were measured (APHA, 1998) with a Thermo Jarrell Ash (model AA Scan 1) atomic absorption spectrophotometer equipped with a graphite furnace.

Aquaria were monitored daily and dead fish were removed. Estimates of 96-h LC50 (median lethal concentration) and associated 95% confidence intervals for each treatment were determined by the Trimmed Spearman–Karber method (Hamilton et al., 1977) by using the mean mortality of the fish. Software for the Trimmed Spearman–Karber method (version 1.5) was obtained from the National Exposure Research Laboratory of the U.S. Environmental Protection Agency (http://www.epa.gov/nerleerd).

3. Results and discussion

Total ammonia–nitrogen ranged from 0.1 to 1.2 mg/l and nitrite–nitrogen ranged from 0.0 to 0.3 mg/l during the study. Tilapia are more tolerant to poor water quality than most commonly farmed freshwater fish and these measurements were below concentrations determined to be harmful to tilapia (Popma and Masser, 1999). Mean (± S.D.) fish weight, total lengths and water temperature were 3.7 ± 0.7 g, 6.2 ± 0.4 cm and 20.1 ± 0.4 °C, respectively, and were similar in each replication. Convulsions, lethargy and loss of equilibrium were noticed in many fish prior to death in copper-treated waters. No fish died in the control tanks.

Estimates of 96-h LC50 values are presented in Table 1 along with pH, total alkalinity and total hardness for the settled pond water and each of the three dilutions. Fish in the highest concentration of Cu in each type of water had the greatest mortality. Treatments resulting in high mortality were: 100% settled pond water with 100% mortality at 87.0 mg/l Cu; 50% settled pond water with 97% mortality at 19.3 mg/l Cu; 25% settled pond water with 87% mortality at 0.87 mg/l Cu and 6.25% settled pond water with 93% mortality at 0.29 mg/l Cu. The highest concentration of Cu in each type of water with good survival was: 100% settled pond water with 97% survival at 11.5 mg/l Cu; 50% settled pond water with 80% survival at 2.5 mg/l Cu; 25% settled pond water with 80% survival at 0.59 mg/l Cu and 6.25% settled pond water with 93% mortality at 0.13 mg/l Cu.

The recommended dose of copper sulfate as an aquaculture therapeutant is based on total alkalinity; however, earlier literature associated total hardness with Cu toxicity (Spear and Pierce, 1979). Alkalinity and hardness are not directly correlated in all waters. When limestone is responsible for both hardness and alkalinity, the concentrations will be similar; if sodium bicarbonate is responsible for alkalinity, it is possible to have high

Table 1
Water characteristics and 96-h LC50 values (and 95% confidence intervals) for the acute toxicity of copper to blue tilapia

Variable	Settled pond water	Dilution 1	Dilution 2	Dilution 3
Percent settled pond water (%)	100	50	25	6.25
Initial pH[a]	8.7 ± 0.11	8.4 ± 0.08	8.1 ± 0.05	7.4 ± 0.06
pH range during study	8.5–8.8	8.2–8.5	8.0–8.2	7.4–7.8
Initial total alkalinity[a] (mg/l $CaCO_3$)	224.91 ± 2.75	111.84 ± 0.83	57.12 ± 2.09	15.52 ± 0.28
Initial total hardness[a] (mg/l $CaCO_3$)	114.21 ± 2.19	57.46 ± 1.09	28.21 ± 1.09	7.01 ± 0.61
Total Carbon[b] (mg/l C)	53.26 ± 4.79	19.99 ± 2.19	5.74 ± 0.75	0 ± 0
96-h LC50 (mg/l Cu)	43.06 (37.71–49.17)	6.61 (5.31–8.23)	0.69 (0.62–0.77)	0.18 (0.17–0.21)

[a] Mean ± S.D.; $N=6$.
[b] Mean ± S.D.; $N=12$.

alkalinity and low hardness (Wurts and Durborow, 1992). Acidic ground or well water may have low or high hardness and little alkalinity. Other variables that have been shown to influence Cu toxicity are dissolved organic matter, fish size, pH and temperature (Sorenson, 1991). Dissolved organic matter and inorganic or organic carbon have been shown to significantly affect the acute toxicity of metals through complexation (Spear and Pierce, 1979; Sprague, 1985; Welsh et al., 1993; Richards et al., 1999).

Previous research has been carried out on the 96-h acute toxicity of Cu (as $CuCl_2$ or $CuSO_4$) on *O. mossambicus*, but important water characteristics have been omitted from some of these studies so the results cannot be used to relate to the present study (Table 2). The study of Balavenkatasubbaiah et al. (1984) reported a 48-h LC50, and is therefore not included in this table. In the study of Nussey et al. (1996), the experimental design consisted of a flow-through system with constant exposure to Cu; other studies cited in Table 2 used static conditions. The studies of Qureshi and Saksena (1980) and Mukhopadhyay and Konar (1984) included similar influencing characteristics as in the 50% dilution of the present study.

Qureshi and Saksena (1980) used slightly lower alkalinity river water that may have contained a high amount of dissolved organic matter, used larger fish and had a 2-fold higher hardness concentration; all of which would suggest a greater 96-h LC50 value than reported based on results of the present study. Contaminants in the water may have been present to contribute to the high acute toxicity of Cu in their study. The study of Mukhopadhyay and Konar (1984) at pH 8.5 had a similar alkalinity concentration but a 4.7-fold higher hardness concentration to give a 2.7-fold higher 96-h LC50 value as compared with the present study. The higher LC50 estimate of Mukhopadhyay and Konar (1984) was expected based on previous reports concerning the effects of increasing or decreasing hardness while maintaining alkalinity (Chakoumakos et al., 1979; Miller and Mackay, 1980; Wurts and Perschbacher, 1994). The studies of Qureshi and Saksena (1980) and Mukhopadhyay and Konar (1984) did not include measurements of organic matter or other carbon sources that may have been present to affect Cu toxicity through complexation.

Table 2

Summary of research on the 96-h acute toxicity of Cu to tilapia including the present study (bottom)

LC50 (mg/l)	Assay setup	Cu source	Water source	Alkalinity (mg/l)	Hardness (mg/l)	Organic matter (mg/l C)	Temperature (°C)	pH	Fish weight (g)	Reference
1.7	Static	$CuSO_4$	tap	ND	93	ND	27–29	7.2–7.5	30–50	de Vera and Pocsidio (1998)
5.0	Static-renewal	$CuSO_4$	tap	ND	165	ND	26–28	7.5	6–20	Jafri and Shaikh (1998)
1.5	ND	ND	tap	ND	ND	ND	ND	ND	ND	Lam et al. (1998)
2.6	Flow-through	$CuCl_2$	borehole	77	80	ND	29	7.7–7.8	5–23.6	Nussey et al. (1996)
2.8	Flow-through	$CuCl_2$	borehole	76	79	ND	19	7.8–7.9	5–23.6	Nussey et al. (1996)
1.5	Static	$CuSO_4$	river	98	115	ND	25	8.5	18	Qureshi and Saksena (1980)
133.5	Static	$CuSO_4$	borehole	115	268	ND	27	6.5	6.0	Mukhopadhyay and Konar (1984)
34.75	Static	$CuSO_4$	borehole	115	268	ND	27	7.0	6.0	Mukhopadhyay and Konar (1984)
18.0	Static	$CuSO_4$	borehole	115	268	ND	27	8.5	6.0	Mukhopadhyay and Konar (1984)
43.1	Static	$CuSO_4$	settled pond	224.9	114.2	TC=53.3	20.1	8.7	3.7	Present study
6.6	Static	$CuSO_4$	settled pond	111.8	57.5	TC=20.0	20.1	8.4	3.7	Present study
0.7	Static	$CuSO_4$	settled pond	57.1	28.2	TC=5.7	20.1	8.1	3.7	Present study
0.2	Static	$CuSO_4$	settled pond	15.5	7.0	TC=0	20.1	7.4	3.7	Present study

ND indicates no data reported for the characteristic.

All studies were with *O. mossambicus* except the present study that used *O. aureus*.

The total carbon (TC) content is a measure of organic and inorganic carbon (Table 1). As with the other measured water characteristics, TC decreased as the settled pond water was diluted. As mentioned previously, sources of carbon are important in Cu complexation by rendering a significant portion of the total Cu content unavailable. Spear and Pierce (1979) estimated that 58–98% of Cu may be associated with organic ligands.

Previous research by Nussey et al. (1996) reported the total copper concentrations for each treatment by acidifying the unfiltered sample prior to measurement by atomic absorption spectroscopy, and found that the measured concentrations were very similar to the amount added. Table 3 presents Cu information for each dilution of the present study. Soluble Cu was defined as the amount of Cu that would pass through a 0.1-μm nylon filter; the sample was then acidified with nitric acid prior to measurement by atomic absorption spectroscopy. The measured soluble Cu divided by the calculated Cu concen-

Table 3
Treatment rate for copper sulfate ($CuSO_4$), calculated and actual soluble Cu concentrations for the acute toxicity of copper to blue tilapia

Percent settled pond water (%)	$CuSO_4$ treatment (mg/l)	Calculated Cu (mg/l)	Measured soluble Cu (mg/l)	Measured/calculated Cu (%)
100	0	0	0.01 ± 0.02[a]	
	30	7.63	3.13 ± 0.17	41.0
	45	11.45	4.81 ± 1.65	42.0
	67	17.04	5.76 ± 2.71	33.8
	101	25.69	10.18 ± 0.88	39.6
	152	38.67	15.30 ± 3.74	39.6
	228	58.00	23.42 ± 2.06	40.4
	342	87.00	33.61 ± 4.49[b]	38.6
50	0	0	0.01 ± 0.00	
	10	2.54	2.11 ± 0.41	83.1
	15	3.82	3.04 ± 0.52	79.7
	22	5.60	5.35 ± 5.56[b]	95.7
	34	8.65	4.54 ± 1.16	52.5
	50	12.72	7.52 ± 2.05	59.1
	76	19.33	11.98 ± 1.86	61.9
	114	29.00	14.89 ± 3.51	51.4
25	0	0	0.00 ± 0.00	
	0.45	0.11	0.11 ± 0.03	96.2
	0.70	0.18	0.17 ± 0.03[b]	96.4
	1.00	0.25	0.20 ± 0.06	80.1
	1.50	0.38	0.27 ± 0.07[b]	71.0
	2.30	0.59	0.57 ± 0.27	96.9
	3.40	0.86	0.67 ± 0.09[b]	77.0
6.25	0	0	0.00 ± 0.00	
	0.20	0.05	0.04 ± 0.01	70.9
	0.35	0.09	0.07 ± 0.01	74.7
	0.50	0.13	0.08 ± 0.01	66.7
	0.75	0.19	0.16 ± 0.06	85.7
	1.15	0.29	0.18 ± 0.08	61.0
	1.70	0.43	0.33 ± 0.04	76.7

[a] Determined by atomic absorption spectroscopy. Mean ± S.D.; $N=3$, unless noted.
[b] $N=2$.

tration indicates the amount of complexation that took place in each of the dilutions. Visual observation of the aquaria indicated that much of the copper quickly precipitated out of solution by forming various carbonate complexes; this was most prevalent in the 100% settled pond water treatment. It is interesting to note that the amount of soluble Cu in the 100% settled pond water averaged about 39.3% of the total calculated Cu concentration. With only 39.3% of the Cu added to the 100% settled pond water treatments being soluble, the LC50 value would be lowered to 16.9 mg/l Cu. As expected, the percent of soluble Cu increased as the water was diluted further (50% dilution averaged 69.1% and the 25% dilution averaged 86.3% soluble Cu); this trend was not apparent in the 6.25% dilution (averaged 72.6% soluble Cu).

The present study, along with the studies of Qureshi and Saksena (1980) and Mukhopadhyay and Konar (1984), indicate that tilapia are much more tolerant to Cu than channel catfish (*Ictalurus punctatus*; Straus and Tucker, 1993), cutthroat (*Salmo clarki)* and rainbow trout (*Oncorhynchus mykiss*; Chakoumakos et al., 1979) and fathead minnow (*Pimephales promelas*; Welsh et al., 1993). Sensitivity to Cu between *O. mossambicus* and *O. aureus* could not be made based on the data in Table 2. Differences between water characteristics and the associated acute toxicity demonstrate the complexities involved in copper toxicity.

4. Conclusion

Copper sulfate is the compound of choice as an ectoparasite therapeutant because of its effectiveness and low cost, but it can be extremely toxic to fish in water of low alkalinity. This information will help extension personnel formulate safer and more effective application rates for copper sulfate in blue tilapia farming. These results indicate that blue tilapia are relatively tolerant to copper sulfate in comparison to other species and that copper sulfate treatments in low alkalinity waters may be detrimental to the health of blue tilapia.

Acknowledgements

This research was supported in part by a grant from the International Association of Fish and Wildlife Agencies. Special thanks to Jan Simpson and Kelly Gill for technical help throughout the studies. Drs. Kenneth B. Davis, Andy Goodwin and Billy Griffin provided critical reviews of the manuscript.

References

APHA (American Public Health Association), American Water Works Association, Water Pollution Control Federation, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. Washington, DC, USA.

Balavenkatasubbaiah, M., Rani, A.U., Geethanjali, K., Purushotham, K.R., Ramamurthi, R., 1984. Effect of cupric chloride on oxidative metabolism in the freshwater teleost, *Tilapia mossambica*. Ecotoxicol. Environ. Saf. 8, 289–293.

Boyd, C.E., 1990. Water Quality in Ponds for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, AL, USA.

Chakoumakos, C., Russo, R.C., Thurston, R.V., 1979. Toxicity of copper to cutthroat trout (*Salmo clarki*) under different conditions of alkalinity, pH, and hardness. Environ. Sci. Technol. 13, 213–219.

de Vera, M.P., Pocsidio, G.N., 1998. Potential protective effect of calcium carbonate as liming agent against copper toxicity in the African tilapia *Oreochromis mossambicus*. Sci. Total Environ. 214, 193–202.

Fitzsimmons, K., 2000. Future trends of tilapia aquaculture in the Americas. In: Costa-Pierce, B.A., Rakocy, J.E. (Eds.), Tilapia Aquaculture in the Americas, vol. 2. The World Aquaculture Society, Baton Rouge, LA, USA, pp. 252–264.

Hamilton, M.A., Russo, R.C., Thurston, R.V., 1997. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11 (7), 714–719. correction, 12(4), 417 (1978).

Jafri, S.I.H., Shaikh, S.A., 1998. Toxicity of copper to tilapia, *Oreochromis mossambicus* (Teleostei): histopathology of liver and testis. Pak. J. Zool. 30, 167–171.

Lam, K.C., Ko, P.W., Wong, J.K.Y., Chan, K.M., 1998. Metal toxicity and metallothionein gene expression studies in common carp and tilapia. Mar. Environ. Res. 46, 563–566.

MacMillan, J.R., 1985. Infectious diseases. In: Tucker, C.S. (Ed.), Channel Catfish Culture. Elsevier, The Netherlands, pp. 405–496.

Miller, T.G., Mackay, W.C., 1980. The effects of hardness, alkalinity and pH of test water on the toxicity of copper to rainbow trout (*Salmo gairdneri*). Water Res. 14, 129–133.

Mukhopadhyay, M.K., Konar, S.K., 1984. Toxicity of copper, zinc and iron to fish, plankton and worm. Geobios (Jodhpur) 11, 204–207.

Nussey, G., van Vuren, J.H.J., du Preez, H.H., 1996. Acute toxicity tests of copper on juvenile Mozambique tilapia, *Oreochromis mossambicus* (Cichlidae), at different temperatures. S. Afr. J. Wildl. Res. 26, 47–55.

Popma, T., Masser, M., 1999. Tilapia. Life History and Biology. Southern Regional Aquaculture Center, publication no. 283.

Qureshi, S.A., Saksena, A.B., 1980. The acute toxicity of some heavy metals to *Tilapia mossambica* (Peters). Aqua 1, 19–20.

Richards, J.G., Burnison, B.K., Playle, R.C., 1999. Natural and commercial dissolved organic matter protects against the physiological effects of a combined cadmium and copper exposure on rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 56 (3), 407–418.

Schnick, R.A., Meyer, F.P., Walsh, D.F., 1986. Status of fishery chemicals in 1985. Prog. Fish-Cult. 48, 1–17.

Sorenson, E.M., 1991. Metal Poisoning in Fish. CRC Press, Boca Raton, FL, USA, pp. 235–283.

Spear, P.A., Pierce, R.C., 1979. Copper in the aquatic environment: chemistry, distribution and toxicology. Natl. Res. Counc. Can., Environ. Secr. Publ. 16454.

Sprague, J.B., 1985. Factors that modify toxicity. In: Rand, G.M., Petrocelli, S.R. (Eds.), Fundamentals of Aquatic Toxicology: Methods and Applications. Hemisphere Publishing, New York, NY, USA, pp. 124–163.

Stickney, R.R., 2001. Tilapia update 2000. World Aquac. 32 (3), 4–7.

Straus, D.L., Tucker, C.S., 1993. Acute toxicity of copper sulfate and chelated copper to channel catfish *Ictalurus punctatus*. J. World Aquac. Soc. 24, 390–395.

Tucker, C.S., Robinson, E.H., 1990. Channel Catfish Farming Handbook. Van Nostrand-Reinhold, New York, NY, USA.

Welsh, P.G., Skidmore, J.F., Spry, D.J., Dixon, D.G., Hodson, P.V., Hutchinson, N.J., Hickie, B.E., 1993. Effect of pH and dissolved organic carbon on the toxicity of copper to larval fathead minnow (*Pimephales promelas*) in natural lake waters of low alkalinity. Can. J. Fish. Aquat. Sci. 50, 1356–1362.

Wurts, W.A., Durborow, R.M., 1992. Interactions of pH, carbon dioxide, alkalinity and hardness in fishponds. South. Reg. Aquac. Cent. Publ. 464.

Wurts, W.A., Perschbacher, P.W., 1994. Effects of bicarbonate alkalinity and calcium on the acute toxicity of copper to juvenile channel catfish (*Ictalurus punctatus*). Aquaculture 125, 73–79.