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Lethal Concentration of Cu in the Neotropical Fish *Cnesterodon decemmaculatus* (Pisces, Cyprinodontiformes)

C. A. Villar,[1] S. E. Gómez,[1,2] C. A. Bentos[2]

[1] Limnology Institute "Dr. R. A. Ringuelet" (UNLP-CONICET), C. C. 712 (1900), La Plata, Argentina
[2] Argentine Museum of Natural Sciences "Bernardino Rivadavia," Av. Angel Gallardo 470 (1405), Buenos Aires, Argentina

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Copper is a classic limiting factor of fishes, as it is both essential and toxic. In small amounts the element is a vital micronutrient needed for haemoglobin synthesis and a major component of cytochrome oxidase. Indeed, most fishes will selectively accumulate copper from surrounding water independent of actual concentrations. As concentrations exceed metabolic requirements, however, copper becomes injurious to fishes, and ultimately reaches lethal levels if concentrations continue to increase.

Most research on fish toxicity was carried out on the holartic fauna, while metal toxicity on neotropical fish remains mainly unreported. *Cnesterodon decemmaculatus*, a widely distributed endemic small fish of neotropical America, which fulfils requested conditions for toxicity tests, has been used in water quality assays (De la Torre et al. 1997; Garcia et al. 1998), in Zn toxicity tests (Gómez et al. 1998), and to evaluate its resistance to changes in water pH (Gómez and Toresani 1998). The present contribution assesses Cu lethal concentration (LC50) on *C. decemmaculatus* by means of static acute bioassays in natural water. Obtained results will be used to stablish sublethal concentrations for chronic bioassays.

MATERIALS AND METHODS

Acute static bioassays were performed following Ward and Parrish (1982) and Sprague (1990). Lethal tests were conducted using 150 adults of *C. decemmaculatus* (not sexed) captured in a small lake without any point source contamination. Fishes were acclimated in the laboratory with test water (24-26°C, pH of 7.2-8.0) for four weeks prior to experimental use. Composition of the water used in the bioassays (Table 1) resembles mean water characteristics of natural environments populated by *C. decemmaculatus*.

During acclimation animals were fed with a daily ration of ca. 3% body weight of commercial fish food Tetra Werke®. The fish were split up into seven groups. The effect of $CuSO_45H_2O$ on fish was tested in 2 L Pyrex glass chambers at controlled temperature, with natural light and artificial aeration. The test was

Correspondence to: C. A. Villar

Table 1. Physical and chemical composition of the test water.

	Cond $\mu S\ cm^{-1}$	pH	DOC	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	HCO_3^{-}	$SO_4^{=}$
						$mg\ L^{-1}$				
Test water	312	7.7	1.3	21.0	3.7	29.5	3.9	25.0	59.5	48.6

performed with one control chamber and 7 different Cu doses: 600, 300, 100, 50, 30, 5 and 1 $\mu g\ L^{-1}$ nominal concentrations. Each chamber contained a group of 10 specimens. Bioassays with 50 and 30 μg Cu L^{-1} were tested in 20 L chambers containing 60 and 40 specimens, respectively, as they were part of a chronic Cu exposure experience. Cu concentration at each chamber was attained by pipetting from a stock solution of 1 g Cu L^{-1}. Total Cu concentrations were measured at the begining and at the end of the bioassays. Final Cu concentrations were roughly 5.5-8.9 % lower. Average total Cu concentrations were 469, 251, 110, 49, 30, 3 and < 2 $\mu g\ L^{-1}$, respectively. Statistical analysis was performed on the average measured concentrations. The number of fish alive was registered twice a day during 96 hr. All the fishes were measured and weighed at the end of the bioassays. Average fish load at each chamber was 0.48 g L^{-1}. During the experiment fish were not fed.

The median lethal concentration (LC50) at 24, 48, 72 and 96 hr were calculated using the LC50 Calculation Program (Harrass 1986). This program calculates an LC50 estimate with confidence limits based on data from toxicity tests using three methods: Probit analysis, Moving Average, and trimmed Spearman-Karber. LC50 concentrations obtained by Probit analysis were related with time by means of an asymptotic function to obtain an estimate of LC50 at infinite time. The bioassay with Cu concentration below 2 $\mu g\ L^{-1}$ was asigned a value of 1 $\mu g\ L^{-1}$ for the statistical analysis.

Water pH (Orion 250 pH meter), conductivity (Luftman conductance meter), and HCO_3^{-} (Gran titration), Na^{+} and K^{+} (flame photometry), Cl^{-} (silver nitrate titration), SO_4^{2-} (turbidimetry), Ca^{2+} and Mg^{2+} (flame atomic absorption) concentrations were determined at the beginning of the test (APHA 1998). Dissolved organic carbon (DOC) was determined after GOLTERMAN et al. (1978). Cu concentrations were assessed by flame atomic absorption (Buck 200A) following Bettinelli et al. (1989). Samples with Cu concentrations lower than 40 $\mu g\ L^{-1}$ were concentrated in a cation exchange resin (Amberlite IRC50), and latter eluted back with 2*M* HNO_3. The limit of detection for Cu was 2 $\mu g\ L^{-1}$.

RESULTS AND DISCUSSION

Table 2 shows the measured Cu concentrations in water and the % mortality at different times. No mortality was observed at the control, 3 and <2 μg Cu L^{-1} doses. Table 3 shows the LC50 values as a function of exposure time.

The LC50 decreased exponentially with time ($p<0.05$), towards an asymptotic

value attained at about 96 hr, as is normally observed (Ward and Parrish 1982; Sprague 1990). Mean LC50 (96hr) obtained by the three methods was 155 μg Cu L^{-1}. Figures obtained by Probit analysis were related with time (t in hr) leading to the following equation:

$$LC50 = 101.6 + 5309 \cdot t^{-1} \quad R^2 = 0.97$$

resulting in an estimated $LC50_{(\infty)}$ of 102 μg L^{-1} (Fig.1).

Table 2. Average Cu concentrations (Cu in μg L^{-1}), water temperature (T in °C), number (N), average standard length (STL in mm) and average weight (W in g, range between brackets) of fish and percent mortality (M in %) at different times (hr) in each Cu dose assayed.

Cu	T	N	STL	W	M(24hr)	M(48hr)	M(72hr)	M(96hr)
469	24.2-27.0	10	20.6	0.4 (0.36-0.42)	80	80	100	100
251	24.0-26.3	10	18.8	0.1 (0.06-0.14)	20	50	50	50
110	24.2-27.0	10	19.2	0.1 (0.06-0.12)	20	30	50	50
49	24.5-26.0	60	16.3	0.07 (0.05-0.13)	1.7	1.7	5	6.7
30	24.3-26.9	40	15.0	0.05 (0.04-0.07)	2.5	2.5	2.5	2.5
3	24.5-25.5	10	20.2	0.13 (0.10-0.15)	0	0	0	0
<2	24.5-25.5	10	19.8	0.12 (0.09-0.14)	0	0	0	0

During the first hours of exposition above 100 μg Cu L^{-1}, fish increased the use of aquatic surface respiration, showing evidence of the affection of the respiratory system. High doses of Cu cause death by the combination of hypoxemia due to impaired oxygen uptake by the gills, and dysfunctions in ionorregulation (Heath 1995). After this initial phase, fish activity returned to normality.

Table 3. Median lethal concentration (LC50, μg L^{-1}) at different exposure time, calculated by three different methods. In each case lower and upper confidence limits are indicated (LL and UL).

Time (hr)		24	48	72	96
Probit					
	LC50	318	231	160	157
	LL	209	165	121	118
	UL	659	390	242	238
Moving average					
	LC50	342	219	154	151
	LL	214	155	122	119
	UL	1296	365	214	210
Spearman-Karber					
	LC50	139	96	157	156
	LL	98	62	103	102
	UL	198	148	241	240

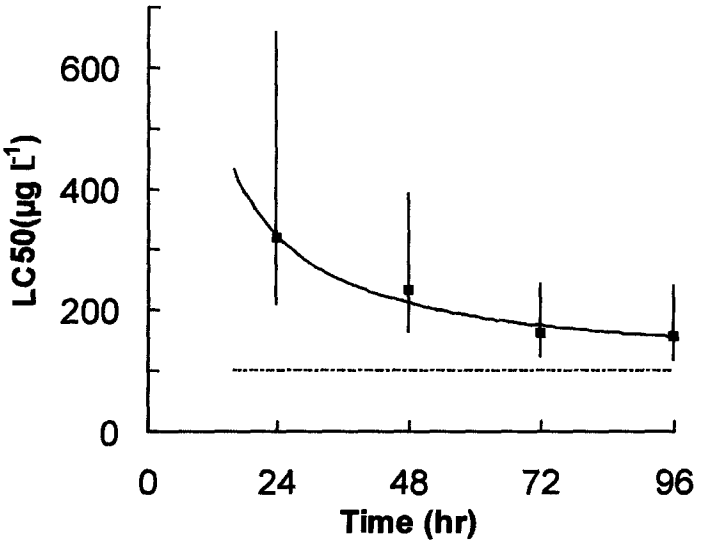

Figure 1. LC50 values calculated by Probit analysis (dots) and confidence limits (vertical bars) plotted as a function of exposure time (hr). Continuous line represent the fitted asymptotic function. Dotted line represent asymptotic value of LC50(∝).

Because Cu toxicity in water is influenced by water hardness, alkalinity, pH, and dissolved organic matter, and experiments on fish toxicity are carried out at a wide range of different experimental conditions, caution should be taken when comparing LC50 data from the literature. LC50s of *C. decemmaculatus* resemble rather low when compared, at similar experimental conditions, to fishes often utilised in toxicity tests. In the upper range of the literature data, fingerlings of *Ictalurus punctatus* (catfish) showed a LC50 96 hr of 730 µg Cu L^{-1} (Straus and Tucker 1993), and adults of the same specie a LC50 48 hr of 28 mg Cu L^{-1} (Stouthart et al. 1996), while juveniles of *Tilapia nilotica* showed LC50 72 hr of 58.3 mg Cu L^{-1} (Somsiri 1982). In the lower range of the literature data, *Noemacheilus rupicola* showed a LC50 96 hr of 230 µg Cu L^{-1} (Joshi and Prakash-Semwal 1990), and *Acrossocheilus paradoxus* a LC50 96hr of 26 µg Cu L^{-1}, being the sensitivity of the last specie similar to that of *Salmo sp.* (Chen and Yuan 1994).

Gómez et al. (1998) observed that *C. decemmaculatus* showed a comparatively large resistance to zinc injury, being the LC50 96 hr of 87.8 mg Zn L^{-1}, and attributed it to the remarkable euritopic characteristic of this fish. Nevertheless, the apparent resistance to zinc injury is not shared with Cu toxicity. Present results show that Cu is much more toxic to *C. decemmaculatus* than Zn, in coincidence with previous works with other fish species (Chen and Yuan 1994).

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