Rev. Mus. Argentino Cienc. Nat., n.s.
6(1): 191-195, 2004
Buenos Aires, ISSN 1514-5158

Acute Cu toxicity, metal bioaccumulation and ion loss in the Neotropical cyprinodontiform *Jenynsia multidentata*

Carlos A. VILLAR [1], Sergio E. GÓMEZ [1,2] & Cristina A. BENTOS [1]

[1] Museo Argentino de Ciencias Naturales «Bernardino Rivadavia», Av. A. Gallardo 470, C1405DJR Buenos Aires, Argentina. [2] Investigador del CONICET. segomez@movi.com.ar.

Abstract: The acute toxicity of Cu, metal bioaccumulation and ion loss in the cyprinodontiform *Jenynsia multidentata*, a highly eurihaline Neotropical freshwater fish of wide distribution in the Río de la Plata basin, was established during a 96 h static exposure. The median lethal concentration at 96 h was 229 μg Cu l^{-1}. The effect of $CuSO_4 5H_2O$ was tested in natural freshwater in two-liter Pyrex glass chambers at controlled temperature, with natural light and artificial aeration. During the first hours of exposure to concentrations above 130 μg Cu l^{-1}, an increase in aquatic surface respiration, air gulping and erratic swimming were observed, showing evidence of the affection of the respiratory system. The concentration of Cu, Na and K in the whole body burden at the end of each experiment showed by correlation analysis Cu bioaccumulation and loss of Na^+ and K^+. The concentration of Cu in water was positively correlated with its bioaccumulation ($r=0.79$, $p=0.06$), and negatively correlated with the whole body burden of K^+ ($r=-0.84$, $p=0.037$). K^+ loss was positively correlated with Na^+ loss ($r=0.88$, $p=0.02$). Cu LC50 for this cyprinodontiform seems rather low when compared to fishes often utilized in toxicity tests.

Key words: copper toxicity, bioaccumulation, ion loss, *Jenynsia multidentata*, Neotropical fish.

With an area of 3 x 10^6 km^2 and a mean discharge of 25000 m^3 s^{-1}, the Río de la Plata basin is the second largest hydrographic system in South America, after the Amazon. Large urbanizations and huge industrial settlements are particularly intense in the lower basin, leading to metal concentrations close to or above levels that could affect biota (Boltovskoy *et al.*, 1997; Villar *et al.*, 1998).

The Neotropical region has more than 2500 fish species (Lowe-McConnel, 1975) in comparison with the around 700 of Holarctic America (Moyle & Cech, 1996). In spite of its evident relevance to environmental impact assessment, information on metal toxicity on Neotropical fish is scarce, and experimental laboratory data on metal bioaccumulation remains unreported.

Cyprinodontids have long been used in toxicity bioassays (Jones, 1964; Heath, 1995). *Jenynsia multidentata*, a widely distributed endemic small fish representative of Neotropical America is the southernmost cyprinodontid of the world, reaching 39° 20' S (Ferriz & López, 1987). This species is observed in at least four countries of Meridional America, suggesting it as a possible bioindicator organism for the region. Gómez (1996) assessed its thermal, salinity, and osmotic pressure resistance, stating that it fulfils requested conditions for laboratory bioassays. *J. multidentata* is highly eurihaline, tolerating long-term exposure to seawater of 35 ‰ (Thörmhalen de Gil, 1949).

In the research presented here we investigated the acute effects of Cu on the survival of wild caught specimens of *J. multidentata* by means of static acute bioassays performed in natural freshwater, and we discussed metal bioaccumulation, ion loss, and the response of this eurihaline fish to Cu toxicity.

MATERIALS AND METHODS

Experimental design

Tests were conducted using 70 adults of *J. multidentata* (not sexed) captured in "La Salada de Monasterio" (35° 47'S, 57° 52'W), a small laguna without any point source contamination. Fishes were acclimated in the laboratory for four weeks prior to experimental use.Water temperature ranged 24-26°C and pH 7.2-8.0. Composition of the water used in the bioassays resembled mean water characteristics of natural environments populated by *J. multidentata*, having a conductivity of 349 μS cm^{-1}, a pH of 7.4 and concentra-

tions of 1.6 mg dissolved organic carbon (DOC) l^{-1}, 26.7 mg Ca^{2+} l^{-1}, 4.4 mg Mg^{2+} l^{-1}, 35.0 mg Na^{+} l^{-1}, 5.3 mg K^{+} l^{-1}, 28.6 mg Cl^{-} l^{-1}, 62.2 mg HCO_3^{-} l^{-1} and 66.3 mg SO_4^{2-} l^{-1}. The concentration of Cu was below 4 μg l^{-1}.

During acclimation animals were fed with a daily ration of ca. 3 % body weight of commercial fish food Tetra Werke®. Acute static bioassays were performed following Ward & Parrish (1982) and Sprague (1990). The effect of $CuSO_4 5H_2O$ on seven experimental fish groups was tested in two-liter Pyrex glass chambers at controlled temperature, with natural light and artificial aeration. The test was performed with one control chamber and 6 different Cu doses: 2500, 1250, 600, 300, 150 and 5 μg l^{-1} nominal concentrations. Each chamber contained a group of 10 specimens. 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 beginning and at the end of the bioassays. The number of fish alive was registered twice a day during 96 h. All the fishes were measured (standard length) and weighed (wet and dry weight at 60°C) at the end of the bioassays. Average fish load at each chamber was 0.47 ± 0.13 g l^{-1} (wet weight). During the experiment fish were not fed.

Analytical procedures

Water pH (Orion 250 pH meter), conductivity (Luftman conductance meter), and HCO_3^{-} (Gran titration), 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, 1985). DOC was determined after Golterman *et al.* (1978).

Water samples were prepared for metal analysis as follows: 100 ml of water were combined with 3 ml concentrated HNO_3, refluxed in 250 ml flasks until volume was reduced to 10 ml and diluted to 100 ml. Samples with Cu concentrations lower than 40 μg l^{-1} were concentrated in a cation exchange resin (Amberlite IRC50), and later eluted back with 2 M HNO_3. Cu determinations (atomic absorption spectrometry, Buck 200A) were performed following Bettinelli *et al.* (1989).Calibration curves and traceability of the method were assessed using commercially available certified stock solutions. The limit of detection of Cu for water samples was 4 μg l^{-1}.

Fish tissue samples were prepared as follows: a composite sample was digested in 250 ml flasks with the addition of 2 ml H_2SO_4 and 5 ml of HNO_3 until total digestion of organic matter was observed. Cu (atomic absorption spectrometry, Buck 200A), Na and K (flame photometry, Buck 200A) determinations were performed following Bettinelli *et al.* (1989). Traceability of Cu, Na and K results were assessed by the use of bovine liver (SRM 1577b, NIST). The results obtained were in close agreement with the certified values.

Statistical methods

The median lethal concentration LC50 at 24, 48, 72 and 96 h were calculated using the LC50 Calculation Program (Harrass, 1986). Statistical analysis was performed on the average measured concentrations. 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. The arcsine transformation is used with the first two methods. LC50 concentrations obtained by Probit analysis were related with time by means of a decreasing function. Pearson correlation coefficients were calculated between Cu concentrations in water and Cu, K and Na content in the fish tissue.

RESULTS

Table 1 shows the average total Cu concentrations in water and the % mortality at different times. Initial Cu concentrations decreased on average 11 % by the end of the bioassays. No mortality was observed at the control and <4 μg Cu l^{-1} dose. The bioassay with Cu concentration below 4 μg l^{-1} was assigned a value of 2 μg l^{-1} for the statistical analysis. Table 2 shows the LC50 values as a function of exposure time. The average LC50 obtained by the three methods declined during the 96 h test, and was related with time by means of a negative multiplicative function (Fig.1) (time in hours and LC50 in μg Cu l^{-1}):

$$LC50 = 39895 \cdot t^{-1.1208} \quad (R^2 = 0.997)$$

Along the 96 h acute toxicity test the behavioral responses of *J. multidentata* were observed and compared with normal activity. During the first hours of exposure to concentrations above 130 μg Cu l^{-1}, an increase in aquatic surface respiration, air gulping and erratic swimming were observed. After this initial phase, fish behaviour returned to normality.

The concentrations of Cu, Na and K in the whole body burden at the end of each experiment showed Cu accumulation and loss of Na^{+} and K^{+} (Table 3). The amount of cation loss was related with the Cu dose. Cu concentration in water was positively correlated with Cu ($r=0.79$, $p=0.06$) and negatively with K^{+} ($r= -0.93$, $p=0.01$) content in fish tissue. K^{+} content in fish tissue was positively correlated with its Na^{+} content ($r= 0.88$, $p= 0.04$) , and nega-

Table 1. Average Cu concentrations in water, water temperature, average standard length and average weight of fish, and mortality (M) at different times (h) in each Cu dose assayed.

Cu conc. $\mu g\ l^{-1}$	T °C	Standard length mm	Weight g	M(24) %	M(48) %	M(72) %	M(96) %
2123	23.0-26.8	17.2	0.06	90	100	100	100
1252	24.0-25.7	18.6	0.07	70	100	100	100
536	22.9-26.8	16.4	0.04	0	40	70	80
250	22.9-26.8	16.3	0.04	0	10	30	40
131	22.7-26.3	18.3	0.05	0	10	20	30
<4	24.3-26.0	16.8	0.04	0	0	0	0
control	24.0-26.0	16.3	0.06	0	0	0	0

Table 2. LC50 of Cu ($\mu g\ l^{-1}$) at different exposure times, calculated by three different methods. In each case lower and upper confidence limits are indicated (LL and UL).

Time (h)	24	48	72	96
Probit				
LC50	1136	528	313	248
LL	797	359	203	144
UL	1470	774	460	369
Moving average				
LC50	1092	417	291	205
LL	811	249	149	87
UL	1615	706	569	397
Spearman-Karber				
LC50	1018	858	379	234
LL	785	610	187	99
UL	1320	1205	769	557
Average	1082	601	328	229
Standard deviation	60	229	46	22

Table 3. Content of Cu, Na and K (DW) and Na/K molar ratio of the whole body burden of *J. multidentata* at the end of the bioassay.

Cu conc. $\mu g\ l^{-1}$	Cu $mg\ kg^{-1}$	Na $g\ kg^{-1}$	K $g\ kg^{-1}$	Na/K
2123	73.1	2.64	4.3	1.35
1252	16.9	3.03	7.8	0.63
536	30.8	4.24	8.2	0.88
250	29.8	4.37	10.0	0.74
131	21.4	4.04	9.3	0.74
<4	13.8	7.19	12.2	1.0
control	13.7	7.20	12.1	1.0

tively correlated with Cu bioaccumulation ($r = -0.84$, $p = 0.04$). Cu uptake by the fish was rapid, increasing 5.3 times in less than 24 h in the higher Cu concentration assayed. The molar ratio of Na and K in fishes not exposed to Cu was 1.0. This relation decreased in fishes exposed to Cu, excepting in the highest concentration assayed. Care must be taken when comparing Cu, Na and K in the whole body burden as a function of time because exposure time was not similar for each Cu concentration.

DISCUSSION

The time-related development of the LC50 values (24, 48, 72, and 96 h) did not completely reach a stabilizing concentration within the 96 h of the bioassay. LC50 of *J. multidentata* was 26 % lower at 96 than at 72 h, showing that acute mortality had not reached an expected asymptotic value yet, as is most generally observed within 96 h (Ward & Parrish, 1982). LC50 24 h of this fish was 3.6 times higher than that of *Cnesterodon decemmaculatus*, another sympatric cyprinodontid that shares a similar life history (Villar *et al.*, 2000), but the difference between both species decreased multiplicatively with time ($R^2 = 0.998$), being only 1.6 times higher for *J. multidentata* at 96 h (Fig.1). Higher resistance of *J. multidentata* during the first hours of exposition may be related with the enhanced capacity of osmoregulation of eurihaline fishes, which have the ability to reverse the active movement of Na^+ and Cl^- across the gill tissue, to change the amount and composition of urine excreted, and to alter epithelial permeability, all within a matter of hours. Nevertheless, Cu toxicity seems to impair this preliminary enhanced tolerance of *J.multidentata* to acute Cu exposition, leading to figures of LC50 similar to those of non eurihaline fishes at longer exposition times.

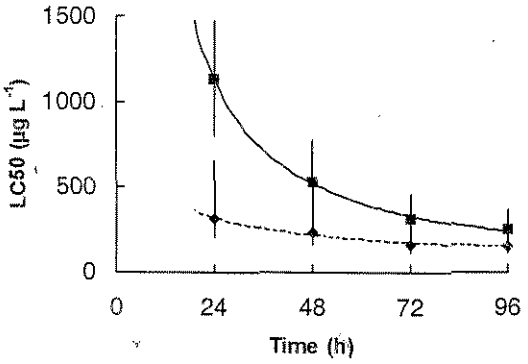

Fig. 1. LC50 values calculated by Probit analysis for *J. multidentata* (filled line) and *C. decemmaculatus* (dotted line, from Villar *et al.*, 2000) and confidence limits (vertical bars) plotted as a function of exposure time (hr).

Cu causes a comparatively large upset in osmoregulation in freshwater fishes; exposed fish show a rather rapid decrease in plasma electrolytes and/or osmolality, coincident with the observed loss of Na and K in the body burden in the present research. The variation of the Na/K ratio observed suggested that the processes related with Na^+ regulation were more affected than those of K^+. The mechanism of the Cu effect on osmoregulation in freshwater fishes appears to be an inhibition of Na^+ and Cl^- uptake by the gills probably due to an inhibition of the enzyme Na^+, K^+ ATPase in the gill tissue. High doses of Cu cause death by the combination of hypoxemia due to impaired oxygen uptake by the gills, and dysfunctions in ionoregulation (Heath, 1995).

Because Cu toxicity in water is influenced by water hardness, alkalinity, pH, and dissolved organic matter, and because 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. Cu LC50 for *J. multidentata* seems rather low when compared to fishes often utilized in toxicity tests. Sorensen (1991) compiled LC50 96 h of 11 fish species, ranging between 60 µg Cu l^{-1} for *Oncorhynchus kisutch* and 2400 µg Cu l^{-1} for *Lepomis macrochirus*, with an average value of 561 µg Cu l^{-1} (SE = 144). In the upper range of the literature data, fingerlings of *Ictalurus punctatus* (catfish) showed a LC50 96 h of 730 µg Cu l^{-1} (Straus & Tucher, 1993), and adults of the same species a LC50 48 h of 28 mg Cu l^{-1} (Stouthart *et al.*, 1996), while juveniles of *Tilapia nilotica* showed LC50 72 h of 58.3 mg Cu l^{-1} (Somsiri, 1982). In the lower range of the literature data, *Noemacheilus rupicola* showed a LC50 96 h of 230 µg Cu l^{-1} (Joshi & Prakash-Semwal, 1990), and *Acrossocheilus paradoxus* a LC50 96 h of 26 µg Cu l^{-1}, being the sensitivity of the last species similar to that of *Salmo sp.* (Chen & Yuan, 1994).

The sensitivity to Cu of *J. multidentata* was in a range comparable to that of the commonly used US Environmental Protection Agency (U.S.EPA) standard test organisms maintained in continuous culture: *Daphnia magna* and *Ceriodaphnia dubia* 48 h EC50 27 and 52 µg Cu l^{-1}, respectively; *Selenastrum capricornutum* 96 h EC50 40 µg Cu l^{-1}; *Mysidopsis bahia* 96 h LC50 160 µg Cu l^{-1}; and *Pimephales promelas* 96 h LC50 480 µg Cu l^{-1} (Shedd *et al.*, 1999).

ACKNOWLEDGEMENTS

This work was supported by grants from IFS, CONICET and Pablo Cassará Foundation.

BIBLIOGRAPHY

APHA. 1985. *Standard Methods for the examination of water and wastewater.* Amer. Publ. Health Assoc., Washington. 1134 pp.

Bettinelli, M., U. Baroni & N. Pastorelli. 1989. Determinazione diretta di elementi in traccia in matrici ambientali mediante imprego dell´ ETA-AAS Zeeman e midificazione di matrice. In: Minoia C. & S. Caroli (eds.) *Applicazioni dell´ETA-AAS Zeeman nel laboratorio chimico e tossicologico.* Vol 1. pp. 49: Eddizioni Libreria Cortina. Padova.

Boltovskoy, D., N. Correa, D. Cataldo, J. Stripeikis & M. Tudino. 1997. Environmental stress on *Corbicula fluminea* (Bivalvia) in the Paraná River delta (Argentina): complex pollution-related disruption of population structures. *Arch. Hydrobiol.* 138(4): 483-507.

Chen, H. & Y. Yuan. 1994. Acute toxicity of copper, cadmium and zinc to freshwater fish *Acrossocheilus paradoxus*. *Acta Zool. Taiwan* 5(2): 45-60.

Ferriz, R.A. & G.R. López.1987. *Jenynsia lineata lineata* (Jenyns) (Teleostei, Jenynsiidae). Nueva cita para el norte de Patagonia. *Rev. Mus. Arg. Cienc. Nat. Hydrobiol.* 6(4): 23-27.

Golterman, H., R. Clymo & M. Ohnstad.1978. *Methods for the physical and chemical examination of freshwaters.* Blackwell Sci. Pub., Oxford, 166 pp.

Gómez, S.E. 1996. Resistenza alla temperatura e salinitá in pesci della Provincia di Buenos Aires (Argentina), con implicazioni zoogeografiche. In AIIAD (ed.) *Atti Congressuali, IV Convegno Nazionale Associazione Italiana Ittiologi Aque Dolci*, Trento, 532 pp.

Harrass, M. 1986. LC50 calculation program. version 2.0.

Heath, A.G. 1995. *Water pollution and fish physiology.* Lewis Publishers, New York, 359 pp.

Jones, J.R.E. 1964. *Fish and river pollution.* Butterworth Inc., Washington, 203 pp.

Joshi, S.N. & V. Prakash-Semwal. 1990. Toxicity of zinc

sulphate and copper sulphate to a hill-stream cobitid fish *Noemacheilus rupicola*. *Indian J. Ecol.* 17(2): 148-150.

Lowe-McConnel, R.H. 1975. *Fish communities in tropical freshwater. Their distribution, ecology and evolution*. Longman, London, 337 pp.

Moyle, P. B. & J.J. Cech. 1996. *Fishes, an introduction to icthyology*. Prentice Hall, Atlantic City, 590 pp.

Shedd, T.R., M.W. Widder, M.W. Toussaint, M.C. Sunkel & E. Hull. 1999. Evaluation of the annual killfish *Notobranchius guentheri* as a tool for rapid acute toxicity screening. *Environmental Toxicology and Chemistry* 18(10): 2258-2261.

Somsiri, C. 1982. Acute toxicity of mercury, copper and zinc to the Nile tilapia. *Thai. Fish. Gaz.* 35(3): 313-318.

Sorensen, E.M. 1991. *Metal poisoning in fish*. CRC Press, Boston, 374 pp.

Sprague, J.B. 1990. Aquatic toxicology. In: Schreck C. B. & P. B. Moyle (eds.) *Methods for fish biology* C 15. American Fisheries Society, Maryland, 684 pp.

Stouthart, X.J., J.L.M. Haans, R.A.C. Lock & S.E.W. Bonga. 1996. Effects of water pH on copper toxicity to early life stages of the common carp (*Cyprinus carpio*). *Environ. Toxicol. Chem.* 15(3): 376-383.

Straus, D.L. & C.S. Tucker. 1993. Acute toxicity of copper sulphate and chelated copper to channel catfish *Ictalurus punctatus*. *J. World Aquacult. Soc.* 24(3): 390-395.

Thörmhalen de Gil, A.L. 1949. Estudio biológico y experimental de las adaptaciones (Eurihalinidad) del pez vivíparo *Jenynsia lineata*. *Revista del Museo de la Plata (n.s.) Zool.* V(35bis): 441-540.

Villar, C.A., S.E. Gómez & C.A. Bentos. 2000. Lethal concentration of Cu in the neotropical fish *Cnesterodon decemmaculatus* (Pisces, Cyprinodontiformes) *Bull. Environ. Contam. Toxicol.* 65(4): 465-469.

Villar, C.A , M. Tudino, C. Bonetto, L. de Cabo, J. Stripeikis, L. D'hucque & O. Troccoli. 1998. Heavy metal concentrations in the Lower Paraná and right margin of the Río de la Plata Estuary. *Verh. Internat. Verein. Limnol.* 26(3): 963-966.

Ward, G.S. & P.R. Parrish. 1982. Manual de métodos de investigación del medio ambiente acuático. Parte 6. Ensayos de toxicidad. *FAO, Doc. Téc. Pesca* (185) 25pp.

Recibido: 18-I-2004
Aceptado: 30-III-2004