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Sensitivity of *Cichlasoma facetum* (Cichlidae, Pisces) to Metals

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Although most of the existent information regarding the sensitivity of fish to pollutants has aimed to the study of Holartic species (Ramamoorthy and Baddaloo 1995), contributions on organisms from other types of ecological communities belonging to Neotropical environments have been recently undertaken considering native and introduced species (Domitrovic 1997; de la Torre et al 2000 a, 2000 b; Ronco et al 2000, Carriquiriborde and Ronco 2002). The present study reports data on the acute toxicity to five metals of the widely distributed species *Cichlasoma facetum* in the South Eastern Neotropical region of South America (Ringuelet et al 1967). This species is found in a wide range of habitats, but it is more abundant in ponds, small lakes and streams, with an approximate interval of 30 °C temperature tolerance. Like most other Cichlids, it can be easily kept and reproduced under controlled laboratory conditions. The species spawns every 20 days under favorable conditions, producing between 600 to 1,800 (2 mm of diameter) brown eggs. These characteristics make the species a suitable organism for laboratory toxicity testing.

MATERIALS AND METHODS

Juvenile fish for use as brood stock spawners were obtained from a local pond (Los Talas, Berisso, Buenos Aires) and kept in the laboratory during one year untill spawning took place. Organisms were cultured in a re-circulating system (8 fish per 100 L glass aquarium) with de-chlorinated tap water (conductivity 1.0 mS/cm, hardness 215 mg CO_3Ca/L, alkalinity 183 mg CO_3Ca/L, pH range 7.4 – 7.8) at room temperature and a photoperiod 16/8 l/d and fed with commercial fish food (Ganave®). Water chemical parameters were determined in the laboratory according to standardized methods (APHA 1998). Spawning was achieved simulating appropriate natural environmental conditions (24 ± 2 °C, 500 Lux, 11/13 to 14/10 l/d photoperiod interval, pH 7.5 ± 0.5, saturated DO, in a 100 L glass aquarium). Embryo incubation and larvae maintenance was done at a temperature of 25 ± 0.5 °C with gentle aeration. Fish larvae were fed with *Artemia sp* nauplii from the hatching time until day 10, and from then on with a mixture of *Artemia sp* nauplii and powdered fish food (McShullet® for cold water fish).

The 96 h acute (lethality) toxicity tests were carried out with 15 ± 5 (for all tested

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metals) or 40 ± 6 (only for tests with Cr and Zn) days old (post-hatch) organisms using de-chlorinated tap water. Assay conditions were: 20 ± 1°C and a photoperiod 16/8 l/d, with no feeding. The organisms were acclimated to the testing temperature 5 days before assay performance. Assays were carried out in 0.8 L polyethylene jars filled with 0.5 L of testing solutions containing 10 larvae per jar. Negative controls and at least five dilutions of each toxicant were tested by triplicate. Renewal of solutions was done every 24 h. Solutions after metal adition were allowed to equilibrate during 1 h before organisms were introduced. A test was considered valid if the negative control showed less than 10 % mortality, according to acute toxicity test standardized procedures (USEPA 1993)

The LC_{50} and LC_{10} were estimated by fitting data to a Probit Model (Finney 1971) by using the Probit software from the USEPA (1993). Sensitivity to Cr(VI) and Zn(II) was compared for organisms from two different ages using a two way ANOVA test (Zar 1994) considering the metal and the age as independent variables and the 96 h LC_{50} as the dependent variable.

Toxicant solutions were prepared from analytical grade reagents using $3CdSO_4 \cdot 8H_2O$ (Merck), $Cr_2O_7K_2$ (Analar), $CuSO_4 \cdot 5H_2O$ (Mallinckrodt), $HgCl_2$ (Mallinckrodt), $ZnSO_4 \cdot 7H_2O$ (Baker). Samples of stock solutions used to prepare testing dilutions were collected before starting experiments, acidified (HNO_3 analytical grade, Mallinckrodt) and the total metal concentration was verified by atomic absorption spectrophotometry (Varian Spectra AA, air-acetylene flame for Cd, Cr, Cu and Zn, and cold-vapor for Hg) (APHA 1998) using certified standards (Accu Trace TM, AccuStandard, Inc.). Laboratory quality assessment include routine intercomparison samples.

RESULTS AND DISCUSSION

Results from toxicity tests (individual LC_{50} and LC_{10} estimation and confidence limits for each test) with all metals at 96 h with 2 week fish are shown in Table 1. When comparing the relative sensitivity of *C. facetum* using LC_{50} values, the following trend, from the most to the least toxic: Cd(II) > Hg(II) > Cu(II) > Zn(II) > Cr(VI), can be seen. This sequence shows a similar behavior for LC_{10} with the exception of Cd and Hg showing a higher sensitivity for the last. It can also be observed (Fig 1,a-e) that sensitivity increases very slightly with exposure time for

Table 1. ***C. facetum*** 96h LC_{50} and LC_{10} (mg/L) and confidence intervals for the studied metals.

Metal (mg/L)	LC_{50}	Lower limit	Upper limit	LC_{10}	Lower limit	Upper limit
Cd(II)	0.091	0.076	0.121	0.047	0.033	0.057
Cr(VI)	20.7	14.4	25.4	10.2	4.2	14.6
Cu(II)	1.4	1.3	1.5	1.0	0.8	1.1
Hg(II)	0.155	0.114	0.216	0.034	0.003	0.062
Zn(II)	4.0	0.1	5.3	2.7	0.0	3.7

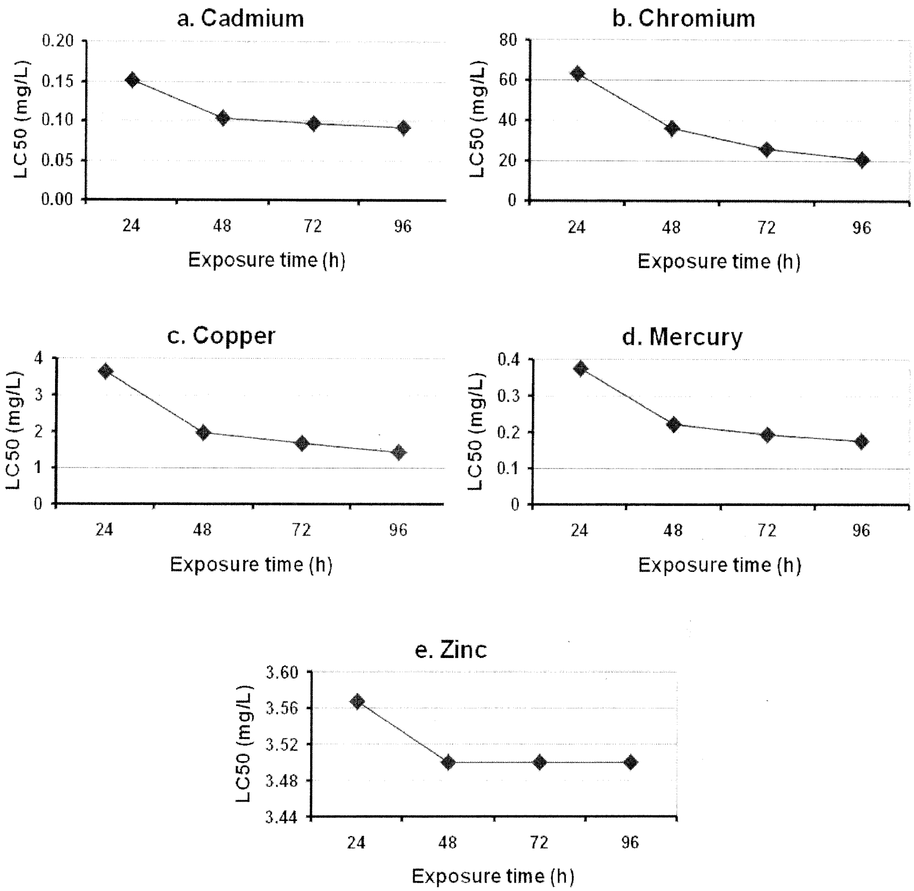

Figure 1. *C. facetum m*etal sensitivity variation with exposure time. Data for Mercury and Zinc correspond to average values from 2 and 3 assays, respectively.

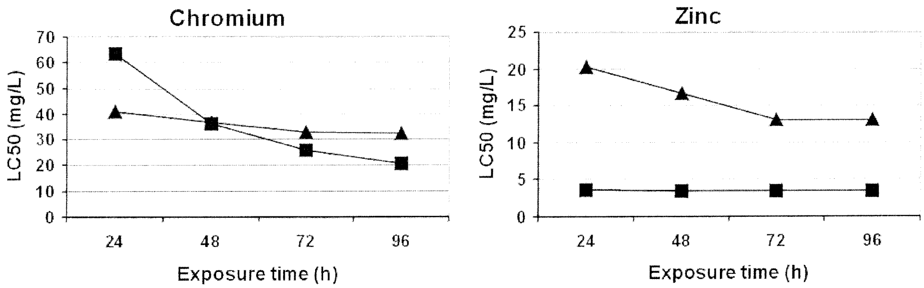

Figure 2. *C. facetum m*etal sensitivity variation with exposure time and age of the organisms. Plotted Zinc data for organisms correspond to average values from 3 assays. ■ and ▲ corresponds to 15 and 40 days old organisms, respectively.

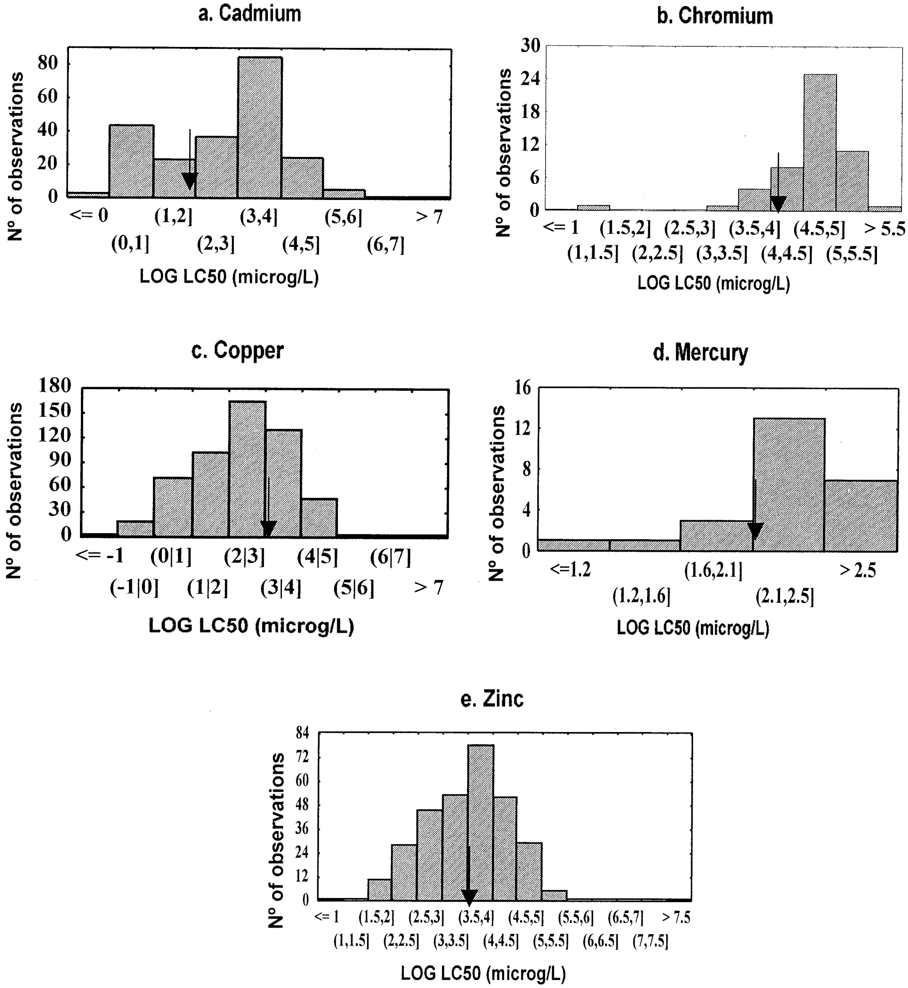

Figure 3. Metal sensitivity distribution for the log 96h LC_{50} for different fish species (from USEPA ECOTOX electronic data base). The arrow points to the log 96h LC_{50} for *C. facetum*

all the studied metals. For the case of zinc, no changes were detected after 48 h exposure. Results shown in Table 1 indicate that a reduction of 40% of fish mortality (difference in the effects between LC_{50} and LC_{10} values) corresponds to a reduction of the lethal concentration between 30% (for Cu(II)) and 70% (for Hg(II)).

The relationship between exposure time, age of the organisms and individual LC_{50} estimations for Zn(II) and Cr(VI) can be seen in Figure 2. The observed difference between organisms of two different ages was corroborated by a two way ANOVA test ($p<0.0096$). Significant differences were also observed between the two metals ($p<0.002$). Results show that the use of 15 days old organisms when testing for sensitivity, should be recommended. At the assay exposure conditions, recently hatched organisms (less than 10 days post-hatch) were not used in tests due to a higher background death level in negative controls after 48 h.

When comparing the sensitivity of *C. facetum* to the studied metals with respect to existent fish data (Figure 3 a-e), the decimal logarithm of the 96 h LC_{50} for the species is in the central sector of the frequency distribution, coinciding with the maximum frequency interval for the case of Hg(II) and Zn(II), and being slightly more sensitive to Cr(VI) and Cd(II), and slightly less to Cu(II).

The results of sensitivity to metals reported here for *C. facetum*, together with the easiness for maintenance, growth and reproduction in the laboratory, and small space requirements, show the potential of this species as a reference organism in ecotoxicological studies for the Neotropical region.

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REFERENCES

APHA, AWWA, WEF (1998) Standard methods for the examination of water and wastewater. 20th Edition, American Public Health Association, Washington DC

Carriquiriborde P, Ronco AE (2002) Acute effect of metals on the neotropical species *Odonthestes bonariensis* (Pisces, Atherinidae). Bull Environ Contam Toxicol 69:294-301

De la Torre FR, Salibián A, Ferrari L (2000a) Biomarkers assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium. Environ Pollut 109:277-282

De la Torre FR, Ferrari L, Salibián A (2000b) Long-term in situ toxicity bioassays of the Reconquista River (Argentina) water with *Cyprinus carpio* as sentinel organism. Water Air Soil Pollut 121:205-215

Domitrovic HA (1997) El empleo de peces autóctonos para la realización de ensayos de toxicidad: evaluación de la especie *Aequidens portalegrensis* (Hensel, 1870). Ictiología 5:37-42

Finney DJ (1971) Probit analysis. Third edition. Cambridge University Press, Cambridge

Ramamoorthy S, Baddaloo EG (1995) Handbook of chemical toxicity profiles of biological species. Lewis Publishers, Boca Raton

Ringuelet RA, Aramburu RH, Alonso de Aramburu A (1967) Los peces argentinos de agua dulce. Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, La Plata

Ronco AE, Castillo G, Díaz MC (2000) Development and application of microbioassays for routine testing and biomonitoring in Argentina, Chile and Colombia. In: Persoone G, Janseen C, De Coen W (eds) New microbiotests

for routine toxicity screening and biomonitoring. Kluwer Academic/Plenum Publishers, London, p 49
U.S. Environmental Protection Agency (1993) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth edition. EPA/600/4-90/027F. Environmental Monitoring Systems Laboratory, Cincinati, Ohio, USA
USEPA ECOTOX Data Bank, AQUIRE (Aquatic toxicity Information Retrieval) database. U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, Duluth, Minnesota, USA. http/www.epa.gov/ecotox
Zar JH (1994) Biostatistical analysis. Third edition. Prentice Hall, Upper Saddle River