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Effect of metals on *Daphnia magna* and cladocerans representatives of the Argentinean Fluvial Littoral

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Abstract

Chronic toxicity tests were conducted to assess the effect of Cu, Cr and Pb on *Moinodaphnia macleayi* and *Ceriodaphnia dubia* -two cladoceran species from the Argentinian Fluvial Littoral Zone (AFLZ)- and *Daphnia magna* -an holarctic species-. The specimens were exposed to three concentrations of each metal. As endpoints, the number of living and dead organisms, molts, neonates released, and the age of first reproduction were recorded. Chronic assays showed that Cu significantly affected the analyzed life history traits in the three species. The lowest Pb and Cr concentrations did not affect survival, molting or fecundity in *D. magna*. Conversely, in *M. macleayi* and *C. dubia*, survival, molting and fecundity showed highly significant differences in all the concentrations tested compared to control assay. The present study stresses the importance of using biological parameters as bioindicators, as well as the study species from the Southern Hemisphere to assess metal pollution.

Key words

Bioindicators, *Ceriodaphnia dubia, Daphnia magna, Moinodaphnia macleayi*, Metal pollution

Introduction

The goal of this study was to assess whether native cladoceran South American species were more sensitive to toxic metals than more commonly used non-native species. In order to achieve this goal, the effect of three metals in two cladoceran species from the Argentinian Fluvial Littoral Zone (AFLZ) (*Moinodaphnia macleayi* and *Ceriodaphnia dubia)* and in an holarctic species (*Daphnia magna*) universally used as test organism were analyzed.

The AFLZ comprises a large area in Northeastern Argentina, including the largest rivers in this country (Paraná, Paraguay, Uruguay and La Plata rivers) belonging to the Cuenca del Plata System – 3,100,000 km^2 – shared by Paraguay, Brazil, Uruguay and Argentina (Bonetto, 1994). The cladoceran fauna of Argentina is especially rich, for it includes 87% of the neotropical genera and 70% of the neotropical fauna species (Paggi, 2004).

In this study, the effect of Cu, Cr and Pb on the following cladoceran biological parameters and life history traits: survival, growth (number of molts produced), age of first reproduction (the day the first neonate is released) and fecundity (number of neonates released) were tested. The selected species were: *Daphnia magna* (Straus, 1820) (Fam. Daphnidae), *Moinodaphnia macleayi* (King, 1853) (Fam. Moinidae) and *Ceriodaphnia dubia* (Richard, 1894) (Fam. Daphnidae). In this way, we aimed to improve the monitoring and assessment of ecological and environmental indicators through the Southern Hemisphere's representative species. Studies comparing the sensitivity of native and non native zooplankton representatives to different toxics are of high ecological relevance, so the results obtained could be used in other countries where these species occur.

In last decades, it became evident that in order to determine the impact of xenobiotics on polluted aquatic ecosystems it is necessary not only to detect and quantify toxic substances through chemical analysis, but also to analyze the impairment of biological parameters of sensitive organisms (bioindicators). These organisms should be abundant, easily and rapidly identified, and well studied in their biological cycles and ecology (Seco Gordillo *et al.,* 1998). As it is well known, each species has certain limits of tolerance between which it can survive, grow and reproduce. In general, narrower the limits are, better is their usefulness as a bioindicator species. According to this, impairment on the survival, age of first reproduction, the number of molts, and fecundity are considered important bioindicators of aquatic ecosystem contamination (Wilhm, 1975; Koivisto, 1995).

Daphnia magna began to be used in toxicity tests during 1940s by Anderson (Anderson, 1944), and since then it has been extensively studied in regulatory testing as well as in basic ecotoxicological research. This species is suitable for laboratory testing because of its large size, high fecundity, short life span, parthenogenetic reproduction, ubiquitous occurrence and easiness of laboratory handling (Adema, 1978; Baudo, 1987). However, other authors have stressed the shortcomings of using it, although it is widely distributed in the Northern Hemisphere, it does not inhabit lakes and ponds of the size of those which are usually effected by pollutants (Koivisto, 1992). According to Rand and Petrocelli (1985), toxicity tests should use species which are indigenous to or representative of ecosystems that receive the pollutants of interest. In this sense, Lynch (1980) and Koivisto (1992 and 1995) suggested that *D. magna* tends to be less sensitive to toxic substances than other cladocerans. *D. magna* does not live in the AFLZ water bodies, which, as was previously mentioned, include the largest rivers in this country. This brings about the need of increasing the knowledge on the sensitivity of some species that represent this important fluvial area.

In Argentina, efforts to date have mainly focused on the use of native species to assess the negative effects of organic compounds (Jose de Paggi, 1997; Gagneten and Marchese, 2003). Recently, the toxic effect of metals on indigenous species was addressed using copepods (Gutiérrez *et al.,* 2010 and 2012), but the effect of metals on native South American cladoceran species have been poorly investigated (Andreotti and Gagneten, 2006). Although metals are among the most dangerous toxics of anthropogenic source, studies carried out in the lower basin of the Salado River and some of its tributaries registered high levels of Cu, Cr and Pb compared to the standards, even at the reference site (Gallo *et al.,* 2006; Gagneten *et al.,* 2007; Gagneten and Paggi 2009).

In solution, Cu can occur as a free cation (Cu^{+2}) or form complexes with various organic and inorganic ligands. Contributions from the aquatic environment generally come from copper or bronze corroded pipes, effluents from sewage treatment plants and from the use of copper compounds as pesticides. They can also come from mining, smelting and purification industries, treatment plants for copper wire and from iron and steel industries (Srivastava *et al.,* 2006).

Trivalent chromium (Cr^{+3}) tends to form stable complexes with organic and inorganic molecules, being very soluble when pH of water is neutral. Cr^{+6} is more soluble and considered an oxidizing agent. Major uses of Cr^{+6} compounds include metal plating, manufacturing of pigments, corrosion inhibitors, chemical synthesis, leather tanning and wood preservation (USEPA, 2010).

Lead is almost insoluble, being also associated with suspended matter in aquatic systems. It can enter the aquatic environment through runoff from streets, discharges of industrial wastewater and gas combustion. Mining, processing, and smelting of lead also produce water discharge to the environment, either directly through liquid effluents or by atmospheric inputs (Von Braun *et al.,* 2002).

According to studies of zooplankton assemblages conducted by Gagneten and Paggi (2009) in the lower basin of the Salado River, cladocerans and copepods were most sensitive to Cr, Pb and Cu. These authors found loss of species richness and diversity, as well as changes in community structure, with the proliferation of predominantly opportunistic, *r*-strategist organisms. The authors attributed the impairment of the community to high eutrophication and metal pollution recorded in the system. Through the evaluation of sensitivity of native cladoceran species to metal contaminants, the present study aimed to improve ecological and environmental monitoring and assessment practices in the AFLZ.

Materials and Methods

Test animals and isolation of starter cladoceran clones : Stock cultures of three cladoceran species were carried out at the Ecotoxicology Laboratory of the Faculty of Humanities and Sciences, National University of the Littoral, Santa Fe, Argentina. Size of specimens were as follows: *D. magna*: 5-6 mm total length (TL), *M. macleayi*: 1–1.5 mm TL, and *C. dubia*: 0.5–1.2 mm. The *D. magna* culture belonged to our laboratory's own monoclonal stock culture, which is permanently controlled and maintained under constant environmental conditions. The other two species were collected with a planktonic net (200 µm) from outdoor tanks' cultures (100 l) maintained for more than two years.

Culture conditions : Cladocerans were individually maintained in glass beakers with 30 ml of synthetic medium proposed by the American Public Health Association (APHA, 2005): 2.4 g $MgSO_4$, 3.84 g $NaHCO_3$, 0.16 g KCl and 2.4 g $CaSO_4 2H_2O$ per 20 l distilled water. The major water quality parameters were as follows: pH: 7.6 - 7.75, DO: 7.32 – 7.89 mgl^{-1}, hardness: 160-180 mgl^{-1} $CaCO_3$, temperature and constant photoperiod: 20 ± 1°C and 16 hr light: 8 hr darkness - 800 lux. The culture medium was weekly changed.

Animals were fed three times a week with 40 µl per wheel of suspension of *Chlorella vulgaris* (1.02×10^6 cells ml^{-1}/specimen, according to Porter *et al.* 1982). The strain of *C. vulgaris* (CLV2) was procured from the culture collection of algae at the CICESE (Scientific Research and Superior Education Center of Ensenada, Baja California, Mexico). Algae were cultivated as proposed by Sager and Granick (1953) (Bold Basal Medium, BBM) and centrifuged (3500 r.p.m. during 10 min). The pellet obtained was diluted in ultrapure distilled water. The concentration of algae in the culture was assessed directly using a Neubauer hemocytometer chamber, and indirectly using a spectrophotometric method (absorbance 1.5 λ at 650 nm - Hatch spectrophotometer).

Chemicals : The stock solution was prepared separately by diluting $CuSO_4$ and $K_2Cr_2O_7$ of analytical grade in sterile bi-distilled water in order to obtain a nominal concentration of 10 mgl^{-1}. It was kept in darkness at -4ºC until its analytical determination. The experiments with Pb were conducted using stock analytical grade solutions: $Pb(NO_3)_2$ (Merck 1.19776.0500-NO_3H 0.5 ml^{-1}) at a nominal concentration of 1000 mgl^{-1}.

The metal concentrations were measured using a Perkin Elmer atomic absorption spectrophotometer (model PE 8000 Analyst) at the INTEC-CONICET (Parque Tecnológico Litoral Centro, Santa Fe, Argentina), with a standard addition technique for calibration. The detection limits for heavy metals were <4 and <3 µgl^{-1} for Pb and Cu, respectively. All glassware and materials were cleaned before metal analyses. Certified analytical grade reagents were used throughout the study. Blanks were run with all experiments. The calibration blank was checked at the beginning and end of the analysis for each group of samples to ensure that the instrument calibration had not drifted. The measured heavy metal real concentrations in the stock solutions were 10.14 (± 0.03), 8.91 (± 0.02) and 1001 (± 2) mgl^{-1} for total Cr, Cu and Pb, respectively. These stock solutions were used to prepare the test toxic solutions for the experiments with three cladoceran species.

Chronic assays. Life history traits experiments : A neonate (24-hr-old) of each species was transferred into a 50 ml beaker containing 30 ml of culture medium in treatments and in control, exposing them to sublethal levels of Cu, Cr and Pb, with 10 replicates each. The assay lasted for 15 days, under identical conditions to those used for stock culture. Animals were fed three times a week with 40 µl per wheel of a suspension of *C. vulgaris* (absorbance = 1.5 λ, 650 nm). pH values and oxygen concentration were recorded at the beginning and at the end of each assay, taking into account the aforementioned limits established by APHA (2005).

Metal concentrations used in bioassays were established taking as reference data obtained by the authors, both from acute tests and from recent field studies in the Argentinian Fluvial Littoral Zone. In this sense, the concentrations used in the bioassays are environmentally realistic.

Concentrations were different depending on the sensitivity of the species and were prepared prior to each test by dissolving the stock solutions in the same medium for tests and controls. Metals were diluted in synthetic medium (APHA, 2005). For *D. magna*, *M. macleayi* and *C. dubia*, the following nominal Cu concentrations were obtained: 20 (T_1), 40 (T_2) and 60 (T_3) µgl^{-1}; 5 (T_1), 15 (T_2) and 25 (T_3) µgl^{-1}; 2.5 (T_1), 5 (T_2) and 10 (T_3) µgl^{-1}, respectively. Nominal Cr and Pb concentrations were: 5 (T_1), 15 (T_2) and 25 (T_3) µgl^{-1}, and 30 (T_1), 90 (T_2) and 270 (T_3) µgl^{-1}, respectively, for the three species tested. Four endpoints were registered three times a week: survival, number of molts produced and released in the beakers (as indirect measure of growth, since the number of molts increased with body size), age of first reproduction, and fecundity (number of neonates produced).

In order to quantify possible significant differences in each of the mentioned endpoints between control and treatments, one factor ANOVA was carried out, followed by a Tukey-Kramer Multiple Comparisons post Test at a 95% confidence level (Sokal and Rohlf, 1969). The dependent variables (number of living organisms, number of molts, and number of neonates released) were $\log_n(x+1)$ transformed when necessary. Prior to each analysis, the normality (Kolmogorov-Smirnov's test) of the data obtained was verified. Kruskal-Wallis Test and Dunnet's Multiple Comparisons post Test were done to evaluate the possible significant differences in age of first reproduction between control and treatments. All statistical analyses were carried out using the package GraphPad InStat (2004).

Results and Discussion

Stress effect of metals on life history traits of *D. magna*, *M. macleayi* and *C. dubia* after being exposed to three concentrations of Cu, Cr and Pb during 15 days are shown in Fig. 1 to 3. Table 1 shows the level of significance obtained for survival, fecundity (number of neonates) and growth (number of molts) after comparing the control values to the ones obtained in all heavy metal treatments. Analyses of variance with Tukey-Kramer post tests were performed. Table 2 shows the number of females reaching sexual maturity and the age of first reproduction in each one of the bioassays performed.

In Cu chronic tests, all life history traits analyzed in *D. magna* showed significant differences when compared to controls, even at lowest concentration (20 µgl^{-1}) (Table 1). Unterstein *et al.* (2003) found alterations in the swimming patterns after exposing *D. magna* to 10 µgl^{-1} Cu. In contrast, *D. magna*´s age of first reproduction showed some tolerance to the lowest concentrations of chromium and lead (5 and 30 µgL^{-1}, respectively). No significant differences were found in survival, number of neonates, and molts in the treatments compared to control (Table 1). Similarly, Munzinger and Monicelli (1992) found that neonates of *D. magna* exposed to 5 μgl^{-1} Cr were more tolerant, showing higher survival and fecundity than control.

The number of females reaching the age of first reproduction within control and Cu, Cr and Pb assays was reduced with the increment in the concentration of metals, although no significant differences were found between treatments and control at the significance level of α=95% (Table 2).

On the other hand, chronic tests showed that *M. macleayi* was more sensitive to Cr and Cu than *D. magna*. Fecundity and number of molts were significantly affected after exposing *M. macleayi* to 5 μgl^{-1} Cr, while *D. magna* was not affected by this concentration. In a similar manner, when *M. macleayi* was exposed to 5 μgl^{-1} Cu, fecundity and number of molts were also impaired, and survival was negatively affected when this species was exposed to 15 $\mu g\ l^{-1}$ Cu. Possibly, at low metal concentrations, *M. macleayi* allocated energy resources to survival –as shown in Cu and Cr chronic assays-, but not to reproduction or growth (Table 1), which can be understood as a possible trade-off (Forbes and Calow, 1996) between the ability to survive to the toxic and the rate of growth and fecundity.

The age of first reproduction was also delayed in the medium and higher concentrations of Cu and Cr. After being

Table 1 : Results of analysis of variance (ANOVA) with Tukey-Kramer post test, for survival, fecundity and number of molts for the tree tested species after being exposed to copper (Cu: 20 (T_1), 40 (T_2) and 60 (T_3) μgl^{-1}; 5 (T_1), 15 (T_2) and 25 (T_3) μgl^{-1}; 2.5 (T_1), 5 (T_2) and 10 (T_3) μgl^{-1} for *D. magna*, *M. macleayi* and *C. dubia* respectively); chromium (Cr: 5 (T_1), 15 (T_2) and 25 (T_3) μgl^{-1} for the three species) and lead (Pb: 30 (T_1), 90 (T_2) and 270 (T_3) μgl^{-1} for the three species) during 15 days. ns: No significant; (*) Significant differences (p<0.05); (**) Highly significant differences (p<0.01); (***) Extremely significant differences (p< 0.001) (n=10)

	Survival			Fecundity			Number of molts		
D. magna	Cu	Cr	Pb	Cu	Cr	Pb	Cu	Cr	Pb
Control vs T_1	***	ns	ns	***	ns	ns	**	ns	ns
Control vs T_2	***	ns	***	***	***	*	**	*	*
Control vs T_3	***	***	***	***	***	***	***	***	***
M. macleayi	Cu	Cr	Pb	Cu	Cr	Pb	Cu	Cr	Pb
Control vs T_1	ns	ns	***	**	***	***	**	*	***
Control vs T_2	***	***	***	***	***	***	***	***	***
Control vs T_3	***	*	***	***	***	***	***	***	***
C. dubia	Cu	Cr	Pb	Cu	Cr	Pb	Cu	Cr	Pb
Control vs T_1	ns	***	*	ns	***	***	ns	***	**
Control vs T_2	***	***	**	ns	***	***	**	***	***
Control vs T_3	***	***	***	**	***	***	***	***	***

Table 2 : Age of the first reproduction (days) of *Daphnia magna*, *Ceriodaphnia dubia* and *Moinodaphnia macleayi* after being exposed to copper (Cu: 20 (T_1), 40 (T_2) and 60 (T_3) μgl^{-1}; 5 (T_1), 15 (T_2) and 25 (T_3) μgl^{-1}; 2.5 (T_1), 5 (T_2) and 10 (T_3) μgl^{-1} for *D. magna*, *M. macleayi* and *C. dubia* respectively); chromium (Cr: 5 (T_1), 15 (T_2) and 25 (T_3) μgl^{-1} for the three species) and lead (Pb: 30 (T_1), 90 (T_2) and 270 (T_3) μgl^{-1} for the three species) and the control during 15 days. (n= number of females reaching sexual maturity). ns: No significant; (*) Significant differences (p<0.05); (**) Highly significant differences (p<0.01); (***) Extremely significant differences (p< 0.001) (n=10). - : no reproduction

		Age of first reproduction (Days)		
D. magna	Control	12 (± 2.1) (n:8)	12 (± 2.1) (n:8)	12 (± 2.1) (n:8)
	Metals	Cu	Cr	Pb
	T_1	11.3 (± 3) (n:6)	11.7 (± 1.5) (n:10)	11.6 (± 1) (n:10)
	T_2	12.7 (± 2.3) (n:3)	13.2 (± 3) (n:5)	11 (± 0) (n:5)
	T_3	12 (± 3.5) (n:3)	10 (± 0) (n:1)*	- *
M. macleayi	Control	12.5 (± 0.9) (n:10)	12.5 (± 0.9) (n:10)	12.5 (± 0.9) (n:10)
	Metals	Cu	Cr	Pb
	T_1	12.3 (± 2) (n:6)	14 (± 1.1) (n:4)	13 (± 0) (n:2)*
	T_2	15 (± 0) (n:1)**	14.3 (± 1.1) (n:3)*	11.7 (± 2.3) (n:3)*
	T_3	15 (± 0) (n:1)**	15 (± 0) (n:3)*	12 (± 0) (n:3)*
C. dubia	Control	11.6 (± 1.5) (n:9)	11.6 (± 1.5) (n:9)	11.6 (± 1.5) (n:9)
	Metals	Cu	Cr	Pb
	T_1	11.9 (± 1.1) (n:7)	12 (± 1.4) (n:2)*	15 (± 0) (n:1)***
	T_2	11.2 (± 1.6) (n:5)	14 (± 1.4) (n:2)*	- ***
	T_3	- **	12 (± 1.4) (n:2)*	- ***

exposed to Pb, although *M. macleayi* produced very few females, it reached the age of first reproduction at a similar time in control than in all the concentrations tested (Table 2). In contrast, highly significant differences were registered between treatments and control when comparing survival, fecundity and number of molts. Similar results were obtained in the chronic tests conducted with *C. dubia* exposed to Cr and Pb (Table 1).

Chromium and lead affected negatively all the attributes of *C. dubia* (Table 1). Even lower concentrations of Cu were used in *C. dubia* assays (2.5, 5 and 10 μgl^{-1}). In this case, there were significant differences in the survival and number of molts when comparing control to T_2 and T_3, and in the number of neonates between control and T_3 (Table 1). 20 μg l^{-1} Cu negatively affected the survival, fecundity and growth of *D. magna*, although sexual maturity was not delayed. Lower concentrations -e.g. 15 μg l^{-1}- affected negatively all the life history traits of *M. macleayi*, even its sexual maturity. Even lower Cu concentrations -10 μg l^{-1}- significantly impaired the fecundity and sexual maturity of *C. dubia* (Tables 1 and 2).

Regarding the effects of chromium, 25 μg l^{-1} impaired *D. magna*´s survival and sexual maturity, while lower concentrations -15 μg l^{-1}- negatively affected its fecundity and number of molts. This latter concentration also affected *M. macleayi*´s survival and sexual maturity, while when this species was exposed to lower Cr concentrations - 5 μg l^{-1} – fecundity and growth were negatively affected; this concentration also affected negatively all the studied attributes of *C. dubia* (Tables 1 and 2).

Similarly, Pd significantly affected *D. magna*´s attributes after being exposed to 90 μg l^{-1} Pb. Lower concentrations -30 μg l^{-1}

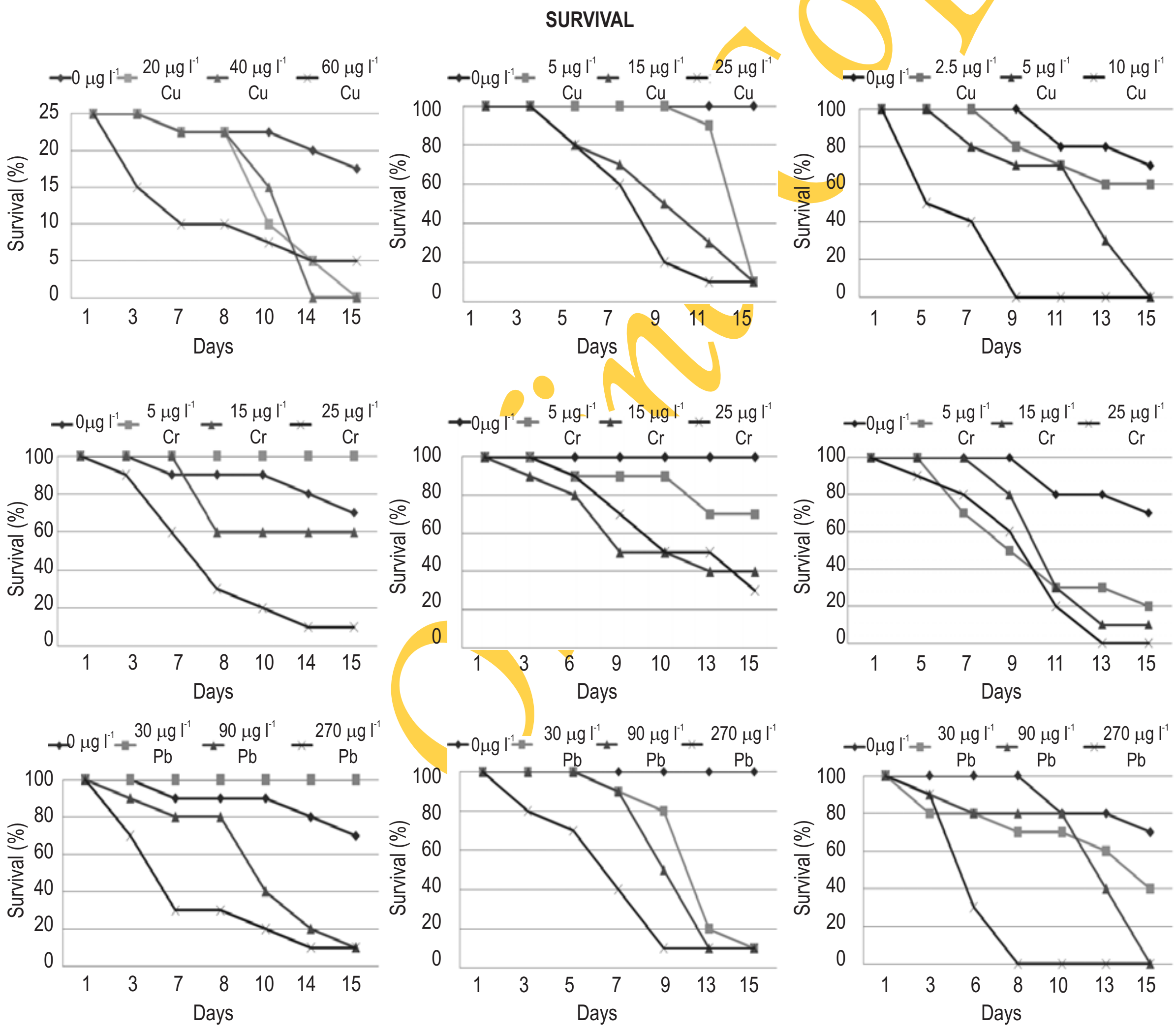

Fig. 1: Survival percent of *Daphnia magna*, *Ceriodaphnia dubia* and *Moinodaphnia macleayi* after being exposed to copper (Cu), chromium (Cr) and lead (Pb) for a period of 15 days. (10 replicates per treatment)

- affected all the studied traits in two species belonging to the ALFZ (Tables 1 and 2).

Fig. 2 and 3 show that fecundity of *D. magna* exposed to Cu increased between day 10 and 12 (in T_1), while the number of molts declined. Similarly, in *M. macleayi*, fecundity increased between day 11 and 15, when the number of molts also declined, and in *C. dubia*, the same situation was observed between day 11 and 13 (T_2).

D. magna fecundity in the lowest Cr concentration (T_1) progressively increased from day 8 to the end of the test, but there was a decline in the number of molts from day 8 to day 12. In *M. macleayi*, fecundity increased at all Cr concentrations tested from day 13 to day 15, when the number of molts either decreased or remained constant (T_2); similar results were obtained with *C. dubia* in T_2 assays. Moreover, a similar behavior was observed with *M. macleayi* from day 9 to day 13 in the lowest and highest concentrations of Pb, while *C. dubia* exposed to Pb could reproduce only at the lowest concentration at the end of assay, when the number of molts also decreased. These results may be interpreted within the allocation theory, as proposed by Forbes and Forbes (1994): the organisms can maintain reproductive functions at the expense of the investment in growth. *D. magna* exposed to Cu (T_2 and T_3) and Pb (T_1), and *C. dubia* exposed to Pb (T_2) allocated significantly more resources for maintenance and growth (evidenced by the higher number of molts produced) than for their reproduction.

According to Beyers *et al.* (1999), when an organism lives in an aquatic environment with adverse conditions, additional metabolic costs cause a reallocation of energy resources to

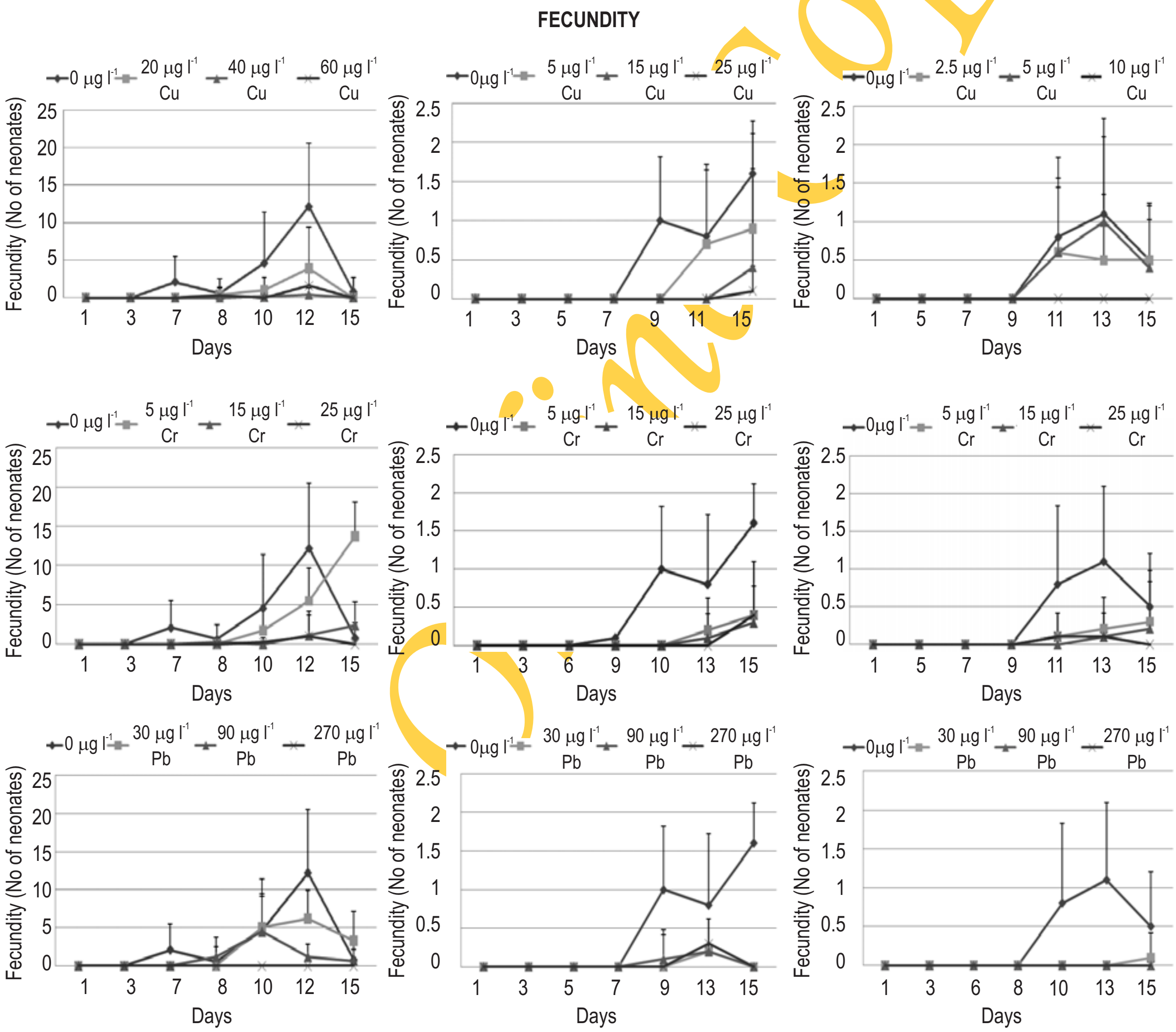

Fig. 2 : Fecundity (Number of neonates) of *Daphnia magna*, *Ceriodaphnia dubia* and *Moinodaphnia macleayi* after being exposed to copper (Cu), chromium (Cr) and lead (Pb) for a period of 15 days. (10 replicates per treatment. Bars indicate S.D.)

processes involved in survival and population growth. In a similar way, our results show that increasing concentrations of Cu, Cr and Pb negatively affected the survival, fecundity, growth, and age of first reproduction of *D. magna*, *M. macleayi* and *C. dubia*.

The results of this study provide useful and relevant information on the effects of heavy metals detected in AFLZ aquatic ecosystems on cladoceran species from these environments. All the metals studied in this work showed significant differences in their toxic effects between treatments and controls, mainly in the AFLZ species. These findings prevent against the direct extrapolation of results obtained from the Northern Hemisphere to the Southern Hemisphere: *D. magna* was less sensitive to some metals (e.g., Cr and Pb) in sublethal exposures than two native species. Considerable differences exist among species in relation to uptake, distribution, interaction with biomolecules, storage, or elimination of metals. A metal may also affect an organism in more than one way (Vaal *et al.,* 1997). Additionally, other factors such as taxonomic relationships and acclimation/adaptation to a certain environment may explain the difference in sensitivity between species (Bossuyt and Janssen 2005). In this sense, Vasela and Vijverberg (2007) established that smaller the size of daphnid, higher the sensitivity to heavy metal toxicity.

The choice of a suitable autochthonous cladoceran species for toxicity testing is influenced by the performance of organisms in relation with: a low sensitivity to handling; a large number of neonates produced per female and a small associated variability among replicate females to increase precision and discriminatory power; a frequent reproduction and a short life-cycle to minimize time and cost; a large body to facilitate handling

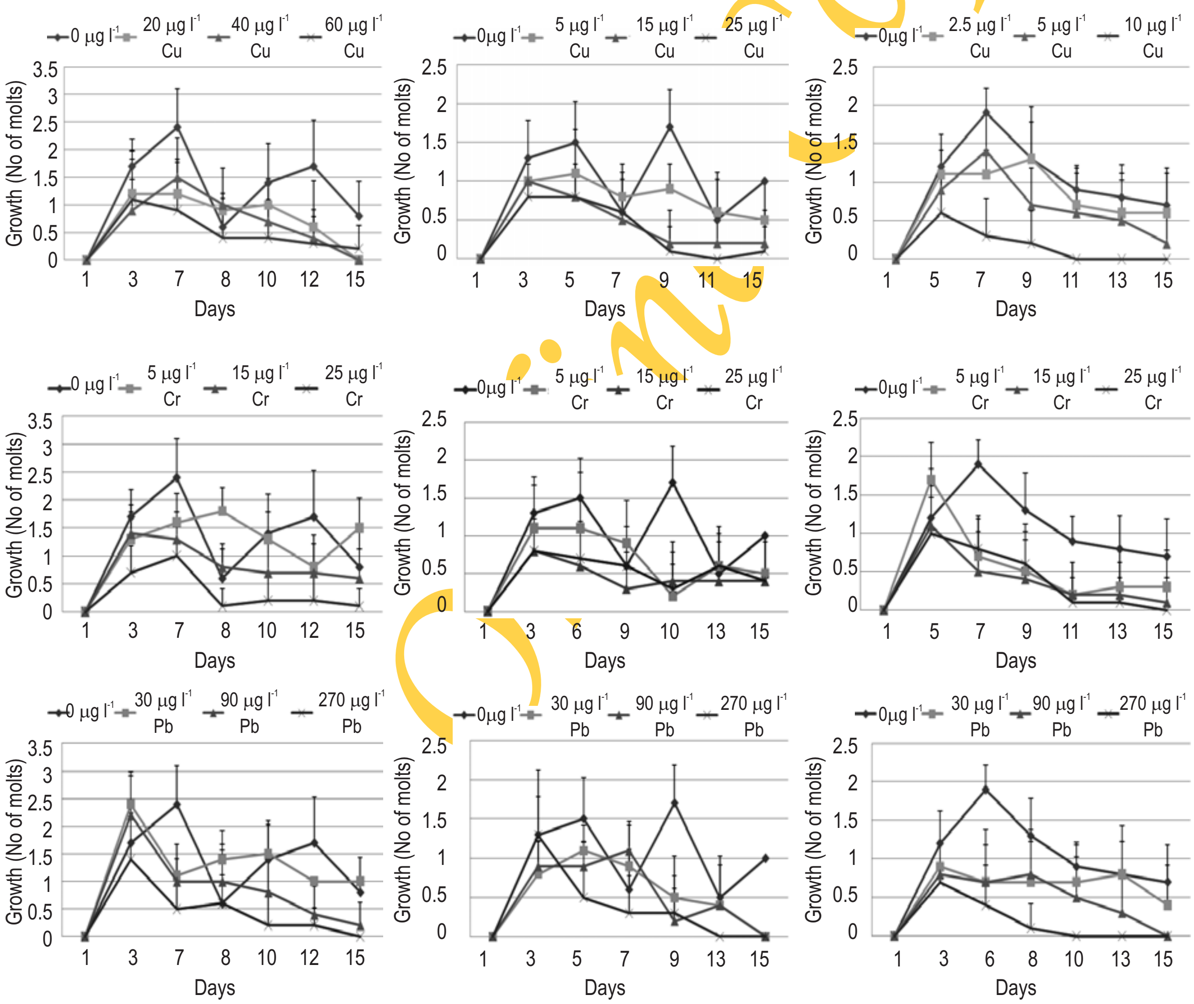

Fig. 3 : Number of molts of *Daphnia magna*, *Ceriodaphnia dubia* and *Moinodaphnia macleayi* after being exposed to copper (Cu), chromium (Cr) and lead (Pb) for a period of 15 days. 10 replicates per treatment.Bars indicate Standard Deviation (S.D.)

(at assay initiation and during medium renewal) and neonates' and dead organisms' counting; a high tolerance to physical and chemical parameters (e.g. water hardness), and a high sensitivity to the pollutants of interest (Lopes, 2011). All these traits were fulfilled by *M. macleayi*, therefore the present study emphasis on the importance of working with native species to detect metal pollution and this native species was proposed for future metal pollution monitoring in the Argentinian Fluvial Littoral Zone and other South American areas.

Through this study we confirm toxic effects caused by exposure to heavy metals on the life cycle of Northern and Southern cladocerans and we promote biological monitoring, which proved to be an essential complement to the classic physico-chemical monitoring of contaminated water ecosystems.

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