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Copper and Chromium Alter Life Cycle Variables and the Equiproportional Development of the Freshwater Copepod *Notodiaptomus conifer* (SARS)

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Abstract Recent studies have shown that the lower basin of the Salado River is highly polluted with copper and chromium. In order to evaluate the effect of those metals on *Notodiaptomus conifer*, a representative calanoid copepod, we carried out two (acute and chronic) experimental assays. In the first one, the 24- and 48-h EC_{50} values were determined in nauplii and adults. Chronic assays were conducted to evaluate the time of development for nauplii, time of development for each copepodite stage, total development time, growth, number of ovigerous females, fecundity, and time required to produce the first egg sac. Additionally, the effect of those metals on the equiproportional model proposed for copepods was evaluated. Acute experiments reveled that juveniles were more sensible than adults. Although growth was not seriously affected by metal exposition, development time was delayed and reproductive variables were altered with the increase of metal concentrations. The deviation from the equiproportional model proposed for copepods proved to be a useful parameter to provide relevant information on toxicity of both metals along development time. In comparison with other zooplanktonic species, the highest sensitivity of *N. conifer* to copper and chromium makes it a suitable bioindicator in ecotoxicological tests.

Keywords Calanoid copepod · Copper · Chromium · Equiproportional development

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1 Introduction

Heavy metals are considered one of the most harmful aquatic pollutants (Verriopoulos and Moraitu-Apostolopoulou 1982; USEPA 1999). Their effects on aquatic organisms are currently attracting widespread attention, particularly in studies related to industrial pollution. Among heavy metals, copper and chromium are very important since they are essential elements in metabolic processes, but at elevated levels, they become toxic (Sunda et al. 1987; Sullivan et al. 1983; Walker 1990; Flemming and Trevors 1989; Eisler 1986; Gorbi et al. 2004). Recent studies have shown that the lower basin of the Salado River, one of the most important basins in Argentina, is highly polluted with copper and chromium as a consequence of human activities (Ceresoli and Gagneten 2003; Gagneten et al. 2007; Gagneten and Paggi 2009). Copper is used in antifouling paint, in the treatment of fish diseases, and as an algaecide. Chromium is used for a host of purposes: as a leather tanning agent, in electroplating,

in stainless steel production, and in the manufacture of glass, pigments, fungicides, and batteries. Despite the economic, industrial, and social importance of the Salado basin, few studies have been published on the incidence of those heavy metals on its aquatic fauna.

On the other hand, despite the existence of extensive literature on the effects of heavy metals, studies assessing the ecotoxicological effects of those contaminants are not so numerous and are mainly referred to marine organisms. Probably, this may be due to the need for a battery of tests or physiological and chemical analyses which are not yet available for small freshwater and marine invertebrates (Andersen et al. 2001). In this vein, Barata et al. (2004) emphasized the need to develop unambiguous bioassay designs allowing the identification of the main causes of reproductive and developmental impairment.

Among aquatic organisms, copepods are of particular interest in toxicity tests because their ecological importance and their complex life cycle are very useful in obtaining information on different biological variables and, hence, in adopting a better approach to population responses to toxic exposure (Hutchinson 2002). The development of copepod larvae includes 12 stages (six naupli stages and five copepodite stages) with a metamorphic change from naupliar to copepodite morphology.

Therefore, a test based on copepod life cycle and growth models would presumably be a useful tool for detecting alterations along the development time. Indeed, in a recent work, Andersen et al. (2001) found that the development of copepod nauplii to copepodite is an effective variable for chronic toxicity to detect hormonal alterations. The equiproportional model proposed for marine and freshwater copepods has been also widely used to evaluate the effect of natural factors like temperature and food quality on growth and life cycle (Carlotti and Sciandra 1989; Uye 1988; Hart 1994; 1996; 1998). According to this model, each larval stage constitutes a fixed proportion of the total time of development, independently of natural factors that influence metabolism (Corkett and McLaren 1970; Corkett 1984; Landry 1983; Hart 1990). Brown et al. (2002) incorporated this copepod growth model into a life cycle test to obtain information on the toxicity of lindane. However, to this date, there are no studies assessing the stability of the equiproportional model in the presence of heavy metals.

Since it is likely that metals could have multiple effects on individuals' biological traits and that any of them could alter the population growth rate, the aim of this work was to evaluate the effect of copper and chromium on different life history variables of *Notodiaptomus conifer*, a representative calanoid copepod of the South America fauna. Additionally, the effect of those metals on the stability of the equiproportional model was evaluated in order to detect any alterations of the normal pattern of molt.

Two experimental assays were carried out: in the first one, we evaluated the acute toxicity of copepods by exposing them to different concentrations of each metal. In the second one, we evaluated significant variables of their life cycle: from hatch to the time that females produced their first egg sac. Results were used to determine the lowest observed effect concentration (LOEC), the non-observed effect concentration (NOEC), and the subchronic value (SChV).

2 Materials and Methods

2.1 Selection and Rearing of Test Organisms

N. conifer is a species of ecological interest because it is common in South America water bodies. Being one of the principal components of the zooplankton community, it plays an important role in the trophic web of lakes and wetlands in the Neotropical region (Ringuelet 1958; Dussart 1984).

The test animals were collected from 1,000-l tanks from the Instituto Nacional de Limnología (CONICET, UNL) with a planktonic net (200-μm mesh). Adults were used to make a stock culture which was maintained under constant photoperiod (16 L/8 D) and temperature (20±2°C).

Filtered (12 μm) and aerated water from the same tanks was used as culture medium (tank water, TW). Physicochemical characteristics were measured according to the Standard Methods for the Examination of Water and Wastewater: dissolved oxygen, 6.415 (±0.835) ppm; pH 8.39 (±0.24); conductivity, 245.33 (±28.18) μS/cm; nitrates, <0.1 mg l^{-1}; nitrites, 0.01 mg l^{-1}; ammonium, 0.29 mg l^{-1}; chlorides, 3.5 mg l^{-1}; sulfates, 8.3 mg l^{-1}; total alkalinity, 77 mg l^{-1} $CaCO_3$; bicarbonates, 94 mg^{-1}; sodium, 7.7 mg l^{-1}; magnesium, 6.8 mg l^{-1}; calcium, 12.9 mg l^{-1}; potassium, 1.8 mg l^{-1}; DQO,

10 mg l^{-1}; DBO5, 0.08 mg l^{-1}. The organisms were daily fed ad libitum with a *Chlorella* sp. concentrate (algal density, 2.8×105 cells per milliliter).

2.2 Acute Assays

The 24- and 48-h EC_{50} values were determined for two life stages of *N. conifer* for copper and chromium according to the standard static bioassay procedures outlined by the United States Environmental Protection Agency (USEPA 2002). The two life stages selected were: nauplii (<72 h) and adults (1.5±0.05-mm total length).

The stock solutions of copper and chromium were prepared by dissolving in distilled water $CuSO_4$ and $K_2Cr_7O_2$, respectively. These nominal solutions were diluted in TW to make the final test metal concentration prior to each test.

The range of metal concentration tested was established by considering values of other zooplanktonic regional organisms and carrying out preliminary tests. For the determination of each 48-h EC_{50}, nauplii and adult copepods were exposed to six concentrations of each metal. The nominal range of metal concentrations was 29–920 μg Cr per liter and 10–320 μg Cu per liter for nauplii and 30–100 μg Cr l^{-1} and 30–228 μg Cu per liter for adults. Twenty individuals, in groups of five, were exposed to each metal concentration and controls (a total of 120 specimens were used for the determination of 24- and 48-h EC_{50}). No food was added during the experiments. An animal was considered to be dead when it ceased to move and it no longer responded to mechanical stimulation.

Prior to nauplii assay, ovigerous females were isolated in 20-ml glass containers. After egg hatching, the females were removed and 20 nauplii were randomly isolated in groups of five in other 20-ml glass containers with 8 ml of each medium plus treatment solution. In the case of adults, 20 males from the stock culture were randomly sorted and isolated in groups of five in 12 ml of test solution. In both cases, controls were performed with TW without metal.

2.3 Chronic Assays

Chronic assays were conducted to evaluate the following variables of the life cycle: (a) nauplii development time, (b) copepodites (1–5) development time, (c) total development time, (d) body length of each copepodite stage (as measure of growth), (e) number of ovigerous females, (f) fecundity (number of eggs per female), and (g) time required to produce the first egg sac.

Before the experiments, the determination of the final dilutions to be employed was calculated according to Kenaga's (1982) considerations. All test solutions were made by dissolving each toxic compound ($CuSO_4$ or $K_2Cr_7O_2$) in TW. Control was made with TW without any toxic.

Life cycle experiments were initiated with first-instar copepods (naupli, <24 h) which were individually placed in a glass container with 12 ml of each treatment medium. A semi-static design was employed and each solution tests was partially (50%) renewed daily. It employed 25–30 replicates for each treatment. During the experiment, organisms were daily fed with a *Chlorella* sp. concentrate (3.7×10^3cells per milliliter). Along the experiments (19–30 days, depending on the effects of each treatment), each larval stage (one to six naupli or one to five copepodite) was determined under stereoscopic microscope. The copepodite total length was measured from the anterior tip of prosome to the end of caudal rami using a compound microscope with a calibrated ocular micrometer (Nikon 41602). Experiments were conducted under the same temperature and photoperiod regimes as described for the rearing procedures.

Once copepods reached adult stage (C6), females were transferred to 20-ml glass containers with the respective treatment medium together with five to eight males in order to induce reproduction. In this phase, we registered time to produce the first egg sac (as first reproduction time), number of eggs per female, and number of ovigerous females.

2.3.1 Statistical Analysis

The EC_{50} values and their 95% confidence limits for 24 and 48 h to nauplii and adults were estimated with the standard method of Probit analysis as described by Finney (1971).

To test the significance of each concentration of both metals on *N. conifer* life history traits in chronic assays (development time, total length, first reproduction time, and fecundity), a one-way ANOVA was employed followed by a post hoc Dunnett's test. Differences were considered significant at $p<0.05$. Prior to ANOVA, data were tested for normality and homogeneity of variances using the Kolmogorov–

Smirnov test. All statistical analyses were carried out using the package GraphPad InStat (2004).

The specific proportion of every stage of copepods in relation with the total development time was calculated. The chi-square goodness-of-fit test was used to evaluate the effect of both metals on the equiproportional model proposed for copepods.

Metal effect on length increment was examined by linear regression:

$$TL_{t+1}(\text{postmolt}) = a + b(TL)\ (\text{premolt})$$

where a is the intercept in TL_{t+1} and b is the constant of growth. A Student's t test was employed in order to compare the calculated slop of the regression lines (Zar 1984).

The LOEC (lowest concentration that produced a significant toxic response) and the NOEC (highest concentration that did not produce a significant toxic response) were determined by testing the responses in each concentration group and comparing responses with those of the control group (Dunnett's test). The subchronic value (SChV) was computed as the geometric mean of the highest NOEC and the lowest LOEC values. The SChV is an estimate of the chronic value and represents the hypothetical toxic threshold between the NOEC and LOEC for a given endpoint (Mount and Stephan 1967; Norber-King 1989; Hutchinson et al. 1994).

3 Results

3.1 Acute Assays

During the experiments, there were no dead copepods in the control groups. Table 1 shows EC_{50} 24- and 48-h values for nauplii and copepodites. In both cases, nauplii were more sensitive than adults. For copper, the EC_{50} to nauplii was 90.5% and 66.8% of adults value at 24 and 48 h, respectively. For chromium, the EC_{50} of nauplii was 63.27% and 73.21% of adult values at 24 and 48 h, respectively.

3.2 Chronic Assays

3.2.1 Development Time

Figure 1 shows total development times of copepods exposed to copper (a) and chromium (b). In the case of copper, total development was significantly longer than the control group at the highest concentration (3 μg l^{-1}; ANOVA: F=4.065, df=70, p<0.01). Nauplii development time at the two highest concentrations (1.6 and 3 μg Cu per liter) was significantly longer than the control (ANOVA: F=21.258, df=90, p<0.05). There were no significant differences between copepodites exposed to treatments and control (ANOVA: F=1.710, df=70, p>0.05).

In the case of chromium, development time of copepods exposed to 60 μg l^{-1} could not be calculated because no organisms reached the third copepodite stage. In the case of 30 μg l^{-1}, only three organisms reached the adult stage. Total development time of copepods exposed to 7.5 μg Cr per liter and 15 μg Cr per liter was significantly longer than that of control (ANOVA: df=41, p<0.05). Nauplii development time was significantly different in 7.5, 15, 30, and 60 μg Cr per liter (ANOVA: df=40, p<0.05). Nevertheless, though at the lowest concentration (3.75 μg Cr per liter) the nauplii development time was slightly shorter than in control, there was not a significant statistical difference (Dunnet: F=1.375, df=40, p>0.05). Copepodite development time showed significant differences between 7.5 and 15 μg Cr per liter and the control group (ANOVA: F=4.822, df=40, p<0.05).

Development time of the copepods increased with the increase of copper (r=0.4125, df=70, p=0.0004) and chromium (r=0.3510, df=41, p=0.0227) concentrations (Fig. 2). The tendency curve for copper was

Table 1 EC_{50} 24 and 48 h to nauplii and adults

	EC_{50} (μg l^{-1})			
	24 h		48 h	
	Nauplii	Adults	Nauplii	Adults
Chromium	380 (368–1641)	599 (250–698)	170 (126–243)	230 (131–338)
Copper	109 (77–167)	120.5 (95–166)	42 (31–56)	62.9 (51–76)

The 95% confidence interval is shown between parenthesis

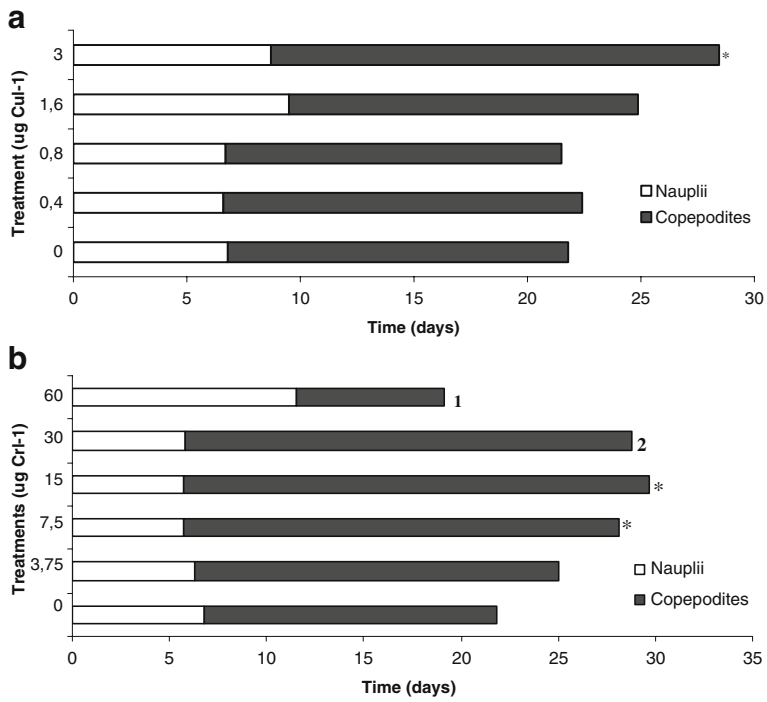

Fig. 1 Mean development time of *N. conifer* exposed to copper (**a**) and chromium (**b**). *White areas* are related to nauplii development and *black areas* are related to copepodite development. *1* No copepod reached the third copepodite stage. *2* Only three copepods reached the adult stage. *$p<0.05$ for total development time

linear, indicating that the development time of the copepods increased with the increase of copper concentration. In contrast, the curve obtained for chromium was increasing in the first part, but it became constantly asymptotic at the highest concentrations (15 and 30 μg Cr per liter). The values of development time to copepods exposed to 60 μg Cr per liter were not included because no copepod reached the third copepodite stage.

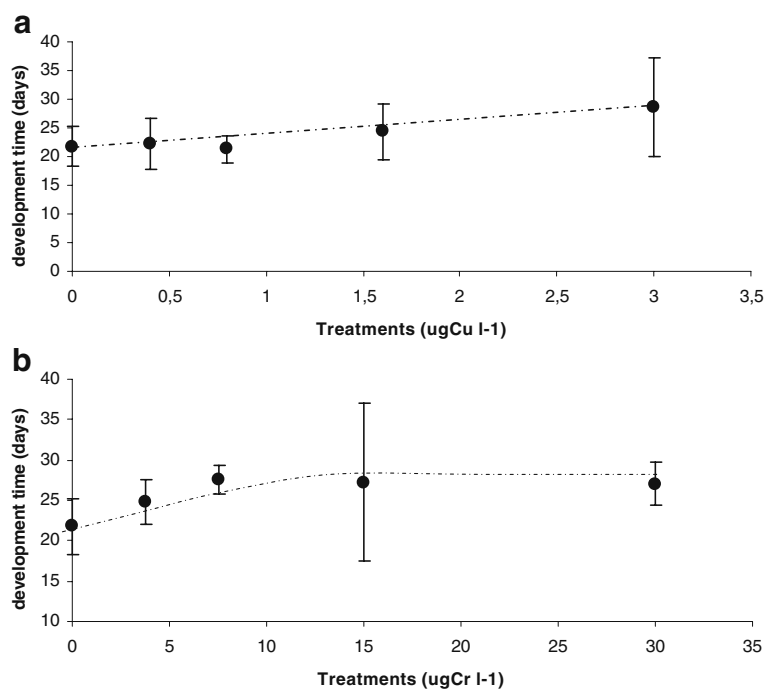

Fig. 2 Relation between development time and all treatments of copper (**a**) and chromium (**b**)

The mean length of the intermolt period of each larval stage was similar in the control and the lowest three concentrations of copper (ANOVA, $p<0.05$). The highest concentration caused an increase of the latest larval stage (Dunnet: $F=9.8$, $df=71$, $p>0.05$). On the other hand, copepods exposed to 15 and 30 μg Cr per liter increased the intermolt period of the C4 stage (Dunnett: $F=5.92$ and $F=3.06$, $df=43$, $p>0.05$) and the intermolt period of the C5 stage to 7.5, 15, and 30 μg Cr per liter (Dunnett: $F=4.44$ and $F=3.91$ and $F=4.12$ respectively, $df = 41$, $p>0.05$). The highest concentration (60 μg Cr per liter) was not considered.

Figure 3a, b shows the mean proportion of each larval stage for the control copper and chromium. In the case of copper, there was a clear significant change in the proportion of the total development time spent in each copepod molt stage at the highest concentration (3 μg Cu per liter; $\chi^2=54.32$, $p<0.001$). Copepodites exposed to chromium showed a significant change in the proportion of the total development time spent in each copepodite molt stage at 7.5, 15, and 30 μg Cr per liter ($\chi^2=49.29$, $\chi^2=18.2$, $\chi^2=48.11$, respectively; $p<0.001$).

3.2.2 Growth

There were no significant differences on body length at each copepodite stage between the control and all treatments with copper and chromium (ANOVA, $p>0.05$; Fig. 4).

A significant relation was found between premolt and postmolt length in the control (ANOVA: $df=40$, $p<0.05$), copper (ANOVA: $df=34$, $p<0.05$), and chromium treatments (ANOVA: $df=38$, $p<0.05$). Nevertheless, no differences were found in the size increase of control and copper (Student's t: $df=72$, $t=0.3599$, $p=0.7199$), control and chromium (Student's t: $df=91$, $t=0.7177$, $p=0.4748$), or between both metals (Student's t: $df=135$, $t=1.775$, $p=0.0782$).

3.2.3 Reproduction

Table 2 summarizes the reproductive traits measured for both metals. There were no significant differences on fecundity between the control and the three lowest treatments with copper (ANOVA: $F=2.047$, $df=26$, $p>0.05$) and time to produce the first egg sac (ANOVA:

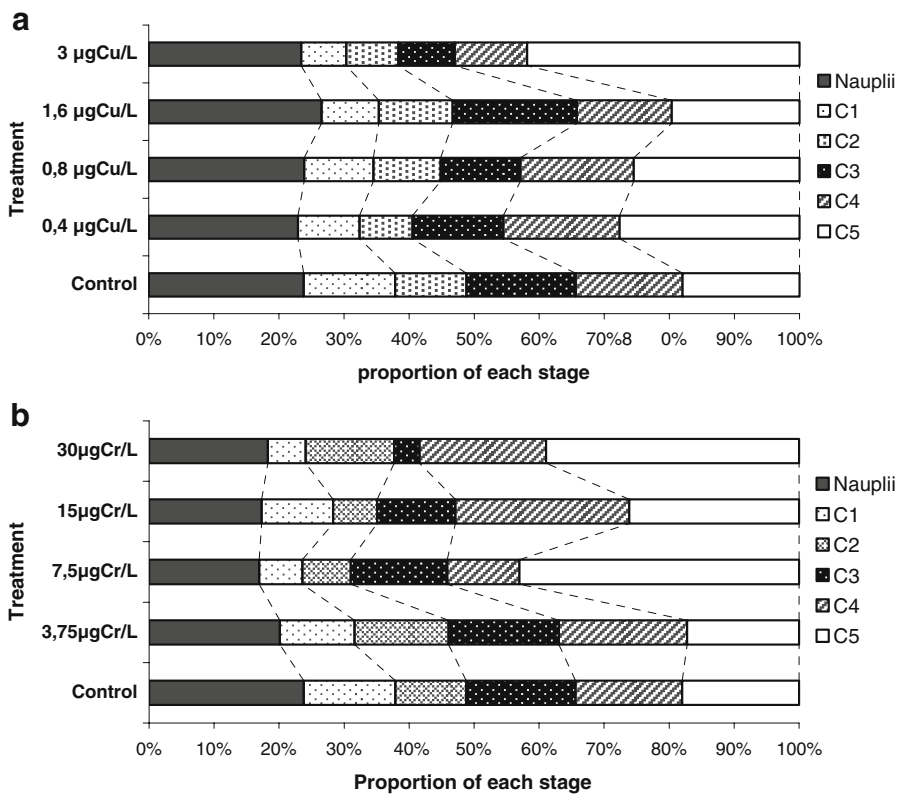

Fig. 3 Mean proportion of each larval stage for the control, copper (**a**), and chromium (**b**) treatments

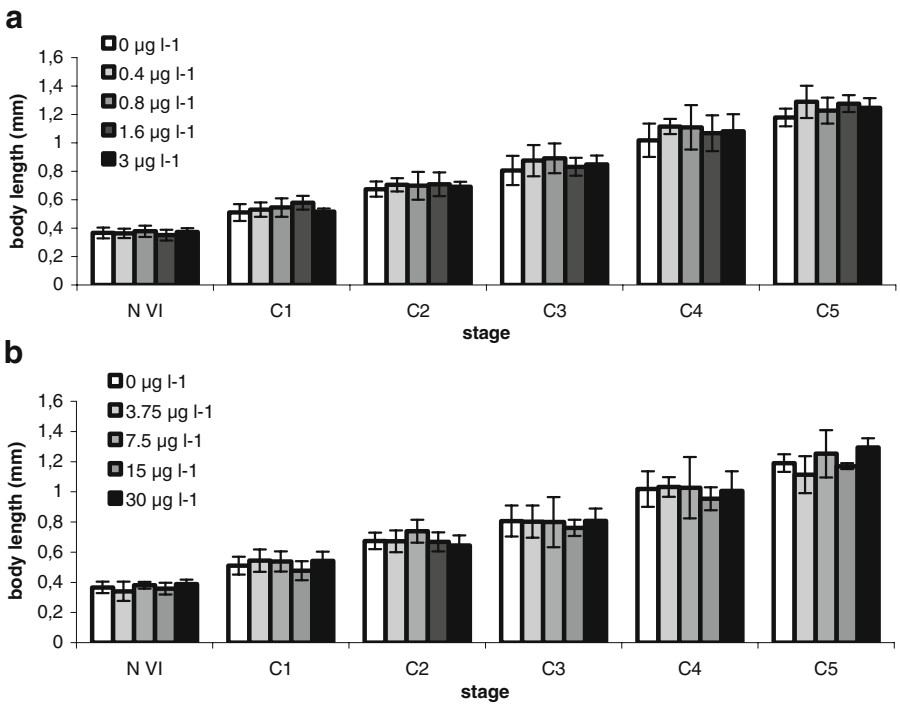

Fig. 4 Mean body length at each larval stage to control and all treatments with copper (**a**) and chromium (**b**)

F=0.9201, df=16, p=0.4584). No females exposed to the highest concentration of copper (3 µg l^{-1}) and the three higher concentrations of chromium (7.5, 15, and 30 µg l^{-1}) produce egg sacs. Females exposed to 7.5 µg Cr per liter produced fewer eggs than those of the control group (ANOVA: F=3.231, df=12, p< 0.05). Time to produce the first egg sac was longer in all organisms exposed to chromium (ANOVA: F= 6.190, df=7, p=0.0011).

The NOEC, the LOEC, and the SChV based upon all life history traits analyzed are summarized in Table 3.

Table 2 Reproductive parameters of *N. conifer* in the control, copper, and chromium treatments

Treatment	Ovigerous females (%)	First egg sac (days)	No. of eggs/female (mean)	No. of eggs/total female
Control	71.43	3.8 (±0.83)	5.67 (±1.14)	4 (±2.9)
Copper				
0.4 µg l^{-1}	100	4.8 (±1.64)	7(±1.26)	7(±1.26)
0.8 µg l^{-1}	62.5	4.2 (±0.83)	11.2(±0.84)	7 (±0.84)
1.6 µg l^{-1}	33.3	5	7.5(±0.7)	2.5 (±3.9)
3 µg l^{-1}	0	–	–	–
Chromium				
	3.75 µg l^{-1}		9.66 (±2.08)*	4.66(±0.58)
		60		
2.8 (±2.56)				
7.5 µg l^{-1}	33.3	10*	3*	1 (±1.7)*
15 µg l^{-1}	0	–	–	–
30 µg l^{-1}	0	–	–	–
60 µg l^{-1}	0	–	–	–

(–) no ovigerous sac was produced

*p<0.05

Table 3 Toxicity of copper and chromium to *N. conifer*

	LOEC ($\mu g\ l^{-1}$)	NOEC ($\mu g\ l^{-1}$)	SChV ($\mu g\ l^{-1}$)
Copper			
Survival nauplii	115	57	80.9
Survival adults	45	30	36.7
Total development time	3	1.6	2.2
Nauplii development time	1.6	0.8	1.2
Copepodite development time	>3	3	–
Equiproportional development	3	1.6	
Growth	>3	3	–
Eggs/females	>3	3	–
First reproduction time	>3	3	–
Chromium			
Survival nauplii	40	20	28.3
Survival adults	125	60	86.6
Total development time	7.5	3.75	5.3
Nauplii development time	7.5	3.75	5.3
Copepodite development time	7.5	3.75	5.3
Equiproportional development	7.5	3.75	5.3
Growth	>60	60	–
Eggs/female	7.5	3.75	5.3
First reproduction time	3.75	<3.75	3.7

(–) Value not determined

4 Discussion

The results obtained in this work demonstrate that copper and chromium are toxic to *N. conifer* and adversely affect different life history traits. In agreement with other investigations, the acute toxicity tests carried out in this study indicate that copper resulted the most toxic element (Bellavere and Gorbi 1981; Verriopoulos and Moraitu-Apostolopoulou, 1982; Sullivan et al. 1983; Sunda et al. 1987; Wong and Pak 2004). Nevertheless, although *N. conifer* was relatively less sensitive to chromium, the effect between those metals in life cycle toxicity tests was different. Mainly, the thresholds of *N. conifer* exposed to copper were lower than those of chromium. This indicates that metal accumulation patterns and toxicity mechanisms are different for the two metals (Rainbow 2002).

Acute assays showed that nauplii were more sensitive than adults. This result may be associated with the thinness of their body exoskeleton and their higher metabolic rate (Vasela and Vijverberg 2007). In the absence of food, the main route by which animals take up the toxicants is by contact with their body surfaces. The body covering diminishes the entrance of metal, increasing the resistance of adults. Moreover, the detoxification mechanisms are probably less effective in young individuals than in adults (Bryan and Hummerstone 1971; Koivisto 1995). On the other hand, the metabolic rate of smaller organisms is higher than those of larger ones; as a consequence, the influx rate of metals increases with decreasing body size. According to Verriopoulos and Moraitu-Apostolopoulou (1982), this result demonstrates that research including various life stages of an organism provides more complete information about the responses of an animal to pollutants in nature.

Among all the variables analyzed on chronic assays, the body size of *N. conifer* was not affected by metal exposition, indicating that this trait was not a sensitive indicator of toxicity. However, development time, the equiproportional development, and the number of ovigerous females were severely affected by both metals at relative low concentrations. Nevertheless, each metal influenced *N. conifer* life history traits in different ways.

The development time of copepods was altered at the highest concentration of copper but at low

concentrations of chromium. In the former case, it increased linearly with an increase of the concentration, but the development time of copepods exposed to chromium increased linearly until reaching a maximum threshold at 15 μ l^{-1}; then there was a tendency to maintain a constant level. This reflects that in spite of chromium toxicity, organisms could not postpone their sexual maturation more than 27–30 days because it would require a higher energetic cost that may affect the individual performance and therefore the population fitness (Fox 2000; Fox and Czesak 2000). Our results indicate that chromium was more toxic and could alter molt physiological mechanisms in a stronger manner than copper. The reasons may be that accumulation patterns and detoxification processes were different. An important consequence of retarding development is that generational time increases. Allan and Daniels (1982) and Gentile et al. (1982) have demonstrated through population models that the effect of the increment in generational time is an important reduction in size of populations exposed to contaminants. The ecological relevance of this reduction lies in the fact that the population would—in the long run—be able to become extinct generating serious consequences at community level (Forbes and Calow 1996; Sibly and Calow 1986).

The proportional stability of each larval stage was also altered in different ways. Only copepods exposed to the highest concentration of copper modified their equiproportionality in relation to that of control; in the case of chromium, this was observed in all three highest concentrations analyzed. According to the equiproportional model proposed for copepods, each larval stage, which is mediated by complex physiological processes, constitutes a fixed proportion of the total development time, independently of factors that influence metabolism (Hart 1990, 1996, 1998). As a consequence, if any factor alters this proportionality, it is reasonable to think that both metals could alter any of the physiological mechanisms responsible for molting processes. In addition, considering that there would be a developmental constraint that makes the molting pattern so constant and inflexible along the evolutionary history of copepods, alterations in this pattern would be an important indicator of toxicity and variables of ecological relevance to be analyzed. These results reflect the utility of this growth model to screen for chemicals suspected of altering molting processes (Brown et al. 2002) and constitute an important contribution to the knowledge of copper and chromium, particularly on sensitive life stages during copepod development.

Reproductive variables were also affected by both metals, but with few exceptions, copper resulted less toxic than chromium. Fecundity and time to produce the first egg sac was affected by copper only in the highest concentration, but chromium resulted highly toxic to that variables in all concentrations tested. According to ecophysiological theories in which resource allocation is the choice to save precious resources, it is probable that organisms exposed to chromium have established trade-offs between conflicting energy demands (Stearns and Kawecki 1994). The need of neutralizing the toxicants required an energetic cost that was evidenced in the lowered number of eggs and the longer time to produce the first egg sac. In natural water bodies, in the long run, these consequences could affect evolutionary processes at population and community levels (Forbes and Calow 1996). In relation to the number of ovigerous females, it was low at highest concentrations of both metals. Previous works have demonstrated that copulatory behaviors are extremely complex in copepods and require a huge spending of energy (Blades and Youngbluth 1980; Maier 1995). Probably, the presence of copper and chromium in the water would affect the interactions between mates by altering pre- and post-copulatory behavior (Yen et al. 1991).

According to the National Hidric Resources (2003), the acute guide levels for the protection of local aquatic biota was established in 19 μg l^{-1} for copper and 15 μg l^{-1} for chromium. These values are lower than those obtained on acute assays in this work for *N. conifer*. Nevertheless, when the sublethal variables (LOEC, NOEC, and SChV) were considered, it became evident that this species is highly sensitive.

Many studies have reported the sensitivity of *Daphnia magna* to metal contamination, an appropriate species to establish water quality standards (Dodson and Hanazato 1995). In the present study, adults of *N. conifer* were affected by chromium and copper at higher concentrations than values indicated for *D. magna* (from 60 to 210 μg l^{-1} for chromium and from 1.4 to 18.5 μg l^{-1} for copper; Khangarot 1989; Persoone et al. 1989; Fargasová 1994; Ewell et al. 1986; Dave 1984; Winner and Farrell 1976). These data indicate that *D. magna* is more sensitive than the

copepod. Nevertheless, according to Koivisto (1995) who emphasized the problems in extrapolating results from *D. magna* toxicity tests to other zooplanktonic species, we suggest that this cladocera is not a good species to predict the effect of xenobiotic in neotropical freshwater ecosystems. To gain more information about the potential for ecological damage, we must study the responses of species that are representative and play essential roles in these communities. Among these species, copepods are one of the most sensitive groups in the zooplankton community (José de Paggi 1997); native copepod species can accumulate chromium (Gagneten et al. 2009) and their complex life cycle allows researchers to obtain information on different biological variables. In contrast, a comparison to other related species indicates that *N. conifer* is highly sensitive to the toxicity of both metals. The LC_{50} determined at 48 h to *Ceriodaphnia dubia* for chromium was 144 μg l^{-1} (Spehar and Fiandt 1986). In the case of copper, Belanger et al. (1989) found values ranging from 35 to 79 μg l^{-1} at different water hardness, but Gagneten and Vila (2001) established lower limits (between 5 and 20 μg l^{-1}) at different levels of pH. Compared to other freshwater copepods, values are also higher, e.g., the LC_{50} to 48 h recorded to nauplii of *Mesocyclops pehpeiensis* was 75 μg l^{-1} to copper and 510 μg l^{-1} to chromium (Wong and Pak 2004). Adults of *Cyclops abyssorum* registered LC_{50} 48 h to chromium and copper of 10,000 and 2,500 μgl^{-1}, respectively, and *Eudiaptomus padanus* registered LC_{50} 48-h values to Cr and Cu of 10,100 and 500 μgl^{-1}, respectively (Baudouin and Scoppa 1974).

Another aspect that should be considered about the effect of xenobiotics in neotropical freshwater ecosystems is the influence of exposition time and the presence of biotic and abiotic factors in the natural environments. Numerous studies have been conducted on the incidence of those factors and metal bioavailability on the intake and toxicity of metals to aquatic organisms (Walker 1990; Castañé et al. 2003). For that reason, although direct extrapolation of the results of the present experiments to natural ecosystems cannot be made, studies such as the present one, in which information is obtained by means of controlled conditions using a similar culture medium of the regional ecosystems water, are valuable as their results can be compared to other similar studies conducted with other regional zooplanktonic species.

In summary, our results demonstrate that copper and chromium altered different *N. conifer* life history traits. Time to reach the adult stage, number of ovigerous females, time of first reproduction, and fecundity were particularly sensitive indicators of sublethal copper and chromium stress. These variables are not only sensitive indicators of pollutant stress but are traits of considerable ecological significance with implications at the population level. Additionally, testing the deviation from the equiproportionality also helped us understand any possible alteration during development time caused by both metals. Due to the sensitivity of *N. conifer*, it is suggested that this copepod be used as bioindicator to provide relevant information in ecotoxicological tests.

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