Life table demography of *Ceriodaphnia dubia* (Cladocera) exposed to copper at different levels and periods

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**Abstract:** We studied the demographic responses of *Ceriodaphnia dubia* exposed to copper at 3 different levels (0.1, 0.2 and 0.4 mg l⁻¹) and at 4 exposure periods (3, 6, 12 and 24 hr) in addition to controls (without the heavy metal). The tested levels of Cu concentration and the chosen periods of exposure affected some demographic variables (average lifespan, gross and net reproductive rates and generation time). Depending on the heavy metal concentration and the period of exposure, the average lifespan of *C. dubia* varied from 18 to 24 day. Net reproductive rates (NRR) ranged from 50 to 80 offspring per female per lifespan, while the rate of population increase (r) varied from 0.30 to 0.34 per day. Though gross reproductive rates (especially at low and intermediate copper levels and shorter duration of exposure) showed significantly higher values than controls, both NRR and r were either unaffected or decreased. It is therefore necessary to regulate the release of untreated industrial effluents containing copper into freshwater ecosystems. Our study thus highlights the importance of including, not only toxicant concentrations but also exposure time, in ecotoxicological evaluations.

**Key words:** Cladocera, Heavy metal, Zooplankton, Demography

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**Introduction**

Life table demographic responses of cladocerans are used for the assessment of water quality, including metal toxicity (Stark and Banks, 2003). Cladoceran genera such as, *Ceriodaphnia*, *Diaphanosoma*, *Daphnia* and *Moina* are the common components of freshwaters (Dodson and Frey, 2001; Sharma and Cyril, 2007) and are often affected by industrial effluents. While *Daphnia*, mainly *D. magna* has received considerable importance as a test organism in ecotoxicological evaluations (Sarma and Nandini, 2006), in terms of sensitivity, *Ceriodaphnia* is comparable to daphnids (Versteeg et al., 1997). A large set of information is available on the life history of variables of *Ceriodaphnia* sp using different food types and concentrations (Sarma et al., 2005). However, when exposed to toxic substances, not all demographic variables are adversely affected (Kammenga and Laskosky, 2000). For example, variables related to survivorship such as the average lifespan or the life expectancy at birth may not be affected with the same magnitude as those of reproduction such as the rate of population increase (r) (Forbes and Calow, 1999).

Copper is one of the heavy metals, that is required at low concentrations but at higher levels it becomes toxic to zooplankton (Bossuyt and Janssen, 2004). Its role at intermediate concentrations is of considerable interest because it stimulates reproductive output of zooplankton possibly at the cost of an organism’s body size or duration of life (Calabrese and Baldwing, 2003). Industrial effluents in Mexico contain copper at levels higher than those prescribed by the national laws (Cervantes and Moreno-Sánchez, 1999). Most toxicity tests are limited to derivation of median lethal concentration (LC₅₀) and only in some cases, demographic or population level impact is assessed (Chapman et al., 2003). Demographic responses are particularly appropriate for evaluating the impact of toxic substances including heavy metals since the life history variables are sensitive and easy to quantify based on two main components: age-specific survival and reproduction (Barata et al., 2002). These are not easy to obtain from population growth studies of zooplankton (Krebs, 1985).

Most ecotoxicological works have considered continuous exposure of the test species to toxicants (Sharma and Agrawal, 2005). However, since the release of industrial effluents into natural waterbodies is pulsed and subsequently diluted, their impact on aquatic organisms remains mostly unknown (Pascoe and Shazlie, 1986). Consequently, zooplankton that predominate in these altered systems, are in a constant physiological challenge as well as subjected to different levels of stress during their life cycle (Nandini et al., 2004; Park and Shin, 2007). Recovery of cladocerans exposed to a given toxicant may be evaluated by growing the one-time or repeatedly exposed populations in the toxicant free medium. Some information is available in the former case (Mangas-Ramirez et al., 2004), while for the latter, there is a need to obtain it. Therefore, the aim of this study was to estimate the impact of copper concentration and exposure time on the demography of *Ceriodaphnia dubia*. 


Materials and Methods

The test species _C. dubia_ was originally isolated from a local waterbody in the State of Mexico and was cultured using the single celled green alga _Chlorella vulgaris_ and moderately hardwater (EPA medium) as the medium. The EPA medium was prepared by dissolving 96 mg NaHCO$_3$, 60 mg CaSO$_4$, 2H$_2$O, 60 mg MgSO$_4$, and 4 mg KCl in one litre of distilled water (Weber, 1993). The alga was batch-cultured using Bold’s basal medium. Log phase alga was harvested, centrifuged at 4000 rpm for 5 min. and resuspended in distilled water. The algal concentrate was stored at 4°C in dark until use. For _C. dubia_ stock cultures and the life table experiments, we used _Chlorella_ of 0.5 x 10$^6$ cells ml$^{-1}$, offered once every 24 hr. Test conditions were: pH: 7.0-7.2, continuous but diffused fluorescent illumination and temperature: 23±1°C and the medium was 100% replaced daily.

Analytical grade anhydrous copper sulphate was dissolved (500 mg l$^{-1}$) in distilled water. From this stock solution, through serial dilution, we chose, based on preliminary toxicity tests, 3 sublethal concentrations (0.1, 0.2 and 0.4 mg l$^{-1}$) with 4 durations of exposure (3, 6, 12 and 24 hr). A total of 52 (= 3 toxic levels x 4 exposure periods x 4 replicates + 4 controls) 50-ml transparent glass vessels containing 25 ml medium with alga (and the heavy metal only for 24 hr exposure treatments) served as test jars. Cohort dynamic life table demography experiments were initiated with neonate (<24 hr age) _C. dubia_. Into each test jar, we introduced 20 individuals using wide-bore Pasteur pipette. To expose the test populations for different durations, we transferred _C. dubia_ individuals to the chosen heavy metal concentrations (containing alga at the same density as in test jars) and retained for 3, 6 and 12 hr before returning to test jars. Medium for treatments containing zero hr and 24 hr exposure periods was changed after 24 hr. Following initiation of the experiments, we quantified and eliminated the number of dead individuals of the original cohort population and the neonates produced. The surviving adults were transferred to the corresponding fresh jars with medium.

Based on the data collected, we calculated age-specific survival and fecundity, life expectancy at birth, gross and net reproductive rates, generation time and the rate of population increase per day. The following formulae were used (Krebs, 1985):

- Life expectancy: \( e_x = \frac{T_x}{n_x} \)
- Gross reproductive rate: \( \sum_{x=0}^{\infty} m_x \)
- Net reproductive rate: \( R_o = \sum_{x=0}^{\infty} l_x \cdot m_x \)

Generation time: \( T = \frac{\sum_{x=0}^{\infty} l_x \cdot m_x \cdot x}{R_o} \)

Rate of population increase, Euler-Lotka equation (solved iteratively)

\[
\sum_{x=0}^{\infty} e^{-rx} \cdot l_x \cdot m_x = 1
\]

where \( r \) = rate of population increase per day, \( w \) = age at maturity (days).

Two-way analysis of variance (ANOVA) and post hoc analysis (Tukey test) were used to quantify the differences among different treatments of selected variables (average lifespan, life expectancy at birth, gross and net reproductive rates, generation time and the rate of population increase) (Sokal and Rohlf, 2000; Statistica Ver. 5, StatSoft Inc., USA).

Results and Discussion

Data on the age specific survivorship of _C. dubia_ under different concentrations of Cu and with different durations of exposure are presented in Fig. 1. The general pattern of survivorship curves of _C. dubia_ was that there was higher mortality during the first 10 days, less for the next 20 days and steep thereafter. However, this trend was evident in the control and in the treatments containing 0.1 mg l$^{-1}$ of Cu concentration and the shorter exposure periods (3 hr). At the copper concentration of 0.4 mg l$^{-1}$ and with exposure periods of >12 hr, the mortality was not only steep but also continuous. Age specific fecundity curves indicated nearly continuous reproduction throughout the lifespan for the test treatments (Fig. 2). The general pattern of offspring production (\( m_x \)) appeared to be that in the control and in the highest Cu concentration (0.4 mg l$^{-1}$) and 24 hr exposure period, the reproductive output was similar. However, at the low and the intermediate Cu concentrations and the shorter exposure periods (3 or 6 hr), offspring production was higher compared with the control. The highest offspring production on a given day _i.e._, the clutch size, was nearly 30 neonates per female.

Average lifespan and life expectancy at birth ranged from 16 to 25 days depending on the concentration of copper and the duration of exposure period. Overall, both these variables decreased with increasing copper concentration and the duration of exposure. Gross and net reproductive rates ranged from 190 to 290 and 50 to 80 offspring per female per lifespan, respectively. Regardless of Cu concentration, increase in exposure time decreased net reproductive output. Gross reproductive rate, especially at the low and the intermediate copper levels and the shorter durations of exposure, showed significantly (p<0.05, Tukey test) higher values than either control or those under higher metal concentration (0.4 mg l$^{-1}$) and the longer exposure period (24 hr). Generation time of _C. dubia_ varied from 21 to 25 days, while the rate of population increase (\( r \))
The concentrations of copper used in this study are not uncommon in waterbodies since CuSO₄ at levels as high as 1.7 mg l⁻¹ is used to eradicate algae in reservoirs (Hawkins, 1988). Many studies have assessed the acute and chronic effects of heavy metals including copper on zooplankton (Bouwuyt and Janssen, 2004; Gama-Flores et al., 2005). Effect of heavy metals on demographic variables have also received considerable attention (Kammenga and Laskosky, 2000). For instance, Forbes and Calow (1999) showed that lifespan, life expectancy and generation time are

ranged from 0.30 to 0.33 d⁻¹ depending on the heavy metal concentration and the duration of exposure (Table 1).

Copper concentration and the period of exposure, separately and together in an interactive way, affected significantly nearly all the derived demographic variables of *Ceriodaphnia dubia* (p<0.05, Two-way ANOVA). For the r, neither the copper concentration nor its interaction with duration of exposure was significant (p>0.05). Only duration of exposure was significant (p<0.01).

**Fig. 1:** Age-specific survivorship curves (l) of *Ceriodaphnia dubia* under different concentrations of copper exposed to different durations. Shown are the mean ± SE based on four replicates (cohorts).
generally less sensitive than reproductive variables. Nandini et al. (2004) studied the demographic responses of *Moina macrocopa* subjected to different stresses including the sublethal levels of cadmium. They have noticed that gross reproductive rate and the rate of population increase were less sensitive to the toxicant stress. It appears that heavy metals have different modes of action depending on their properties and the test species (Roex et al., 2000; Bossuyt and Janssen, 2004). It also implies that sensitivity depends on the taxa and that not all life history variables are equally and consistently sensitive to a given stress. For example, copper concentration as low as 0.03 mg l\(^{-1}\) had an adverse effect on the population growth rates of rotifers and levels higher than 0.12 mg l\(^{-1}\), reproduction was inhibited (Gama-Flores et al., 2005). On the other hand, median lethal concentrations for a 48 hr bioassay for *Daphnia longispina* varied from 0.06 to 0.37 mg l\(^{-1}\) (Lopes et al., 2004).

Age-specific survivorship (*l_*\(j\)) and reproduction (*m_*\(j\)) curves yield information on how a given population experiences mortality and offspring production in relation to age-class. Under ideal conditions, most zooplankton species are expected to have little mortality during the early stages but experience steep death rates.
towards the end of life (Type I survivorship) (Steams, 1992). With stress from toxicants, mortality rates may increase especially at the early stages thereby the expected trends deviate considerably (Kammenga and Laskosky, 2000). In our study though even in the controls the Type I curve was not obtained, in the presence of higher concentrations of Cu and under longer exposure time, the mortality of C. dubia was higher during the early age-classes. Previous works have shown similar responses in zooplankton under stress (Mangas-Ramírez et al., 2004; Nandini et al., 2004).

Offspring production in cladocerans is through distinct clutches. Clutch size and the inter-clutch duration vary among different taxa. Generally long-lived taxa such as Daphnia and Ceriodaphnia have extended reproductive period over several weeks compared to short-lived taxa such as Moina (Sarma et al., 2005). In the present work, the reproductive period of C. dubia lasted for about 40 days. Nandini and Sarma (2002) have also reported similar duration for the same species. They also reported peak clutch size of about 14 offspring female⁻¹, as was also observed here.

Regardless of Cu concentration and the exposure period, the data on the life history variables obtained here are within the range reported for the same species in literature (Sarma et al., 2005). For example, C. dubia has lifespan which varies from 17 to 43 days depending on the race and the test conditions. Our values here are closer to those (20-25 days) reported in Nandini and Sarma (2002). A wide range of net reproductive rates (8 to >40 offspring per female per lifespan) have been reported for C. dubia under different food concentrations (Sarma et al., 2005). The population growth rates recorded here differed by about 12% from control to the treatments under the highest toxicant concentrations. In literature Ceriodaphnia with r varying from 0.03 to 1.61 𝑑⁻¹ has been documented (Forbes and Calow, 1999; Sarma et al., 2005).

Information related to toxic levels and exposure time that could permit recuperation of the stressed organisms is limited. Mangas-Ramírez et al. (2004) have found some recovery of stressed populations previously exposed to low levels of cadmium. For gross reproductive rate, the performance of Cu-exposed populations of C. dubia was comparable to or even higher than controls. Stimulated responses (morphology, survivorship- and reproduction-related variables) are known to occur when exposed to sublethal levels of certain heavy metals including copper (Hunter and Pyle, 2004; De Schampheelaere and Janssen, 2004). This phenomenon, known as hormetic response to stress, is due to homeostatic overcompensation optimizing the ability of an organism to meet challenges beyond the limits of normal adaptation (Calabrese and Baldwin, 2003). Hormesis is probably responsible for the enhanced values in the gross reproductive rate, which showed some kind of biphasic trend, i.e., an inverted “U” (with higher values at intermediate toxic concentrations and exposure periods) known in Daphnia under copper treatments (Calabrese and Baldwin, 2003).

For responding to stress, organisms handle a fixed budget of available resources, and any change in distribution, allocation to one process incurs a cost of other (trade offs) (Tessier et al., 2000). One of them appears between resources allocated to growth versus resources allocated to reproduction (Walls and Ketola, 1989), or survival versus reproduction (Sarma et al., 2002). In this context, our results suggest that Ceriodaphnia dubia exposed to the combination of copper level and exposure period reduces its

### Table 1: Selected life history variables (ALS = Average lifespan (days), LEB = Life expectancy at birth (days), GRR = Gross reproductive rate (offspring per female per lifespan), NRR = Net reproductive rate (survival-weighted offspring per female per lifespan), GT = Generation time (days) and r = Rate of population increase (d⁻¹)) of C. dubia exposed to different durations and concentrations (mg l⁻¹) of copper. Values present mean ± standard error based on 4 replicates (cohorts). For a given variable under a chosen Cu concentration, data containing same letters are not significant (p>0.05, Tukey test); presence of * = Significantly different (p<0.05) from controls; rest = non-significant (p>0.05)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALS</th>
<th>LEB</th>
<th>GRR</th>
<th>NRR</th>
<th>GT</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.6±1.2</td>
<td>24.0±1.3</td>
<td>185.7±13.1</td>
<td>67.4±1.9</td>
<td>24.7±0.3</td>
<td>0.342±0.006</td>
</tr>
<tr>
<td>0.1 mg l⁻¹</td>
<td>20.1±0.4ʰ</td>
<td>19.6±0.4ʰ</td>
<td>254.0±18.3ʰ</td>
<td>75.0±3.4ʰ</td>
<td>22.9±0.2ʰ</td>
<td>0.310±0.014ʰ</td>
</tr>
<tr>
<td>0.2 mg l⁻¹</td>
<td>20.5±0.5ʰ</td>
<td>20.0±0.4ʰ</td>
<td>262.7±9.4ʰ</td>
<td>75.9±1.5ʰ</td>
<td>23.2±0.5ʰ</td>
<td>0.304±0.012ʰ</td>
</tr>
<tr>
<td>3 hr</td>
<td>19.1±0.3ʰ</td>
<td>18.5±0.3ᵃ</td>
<td>289.2±8.1ᵃ</td>
<td>76.8±1.7ᵃ</td>
<td>21.9±0.1ᵇ</td>
<td>0.322±0.010ᵃ</td>
</tr>
<tr>
<td>6 hr</td>
<td>23.1±0.1ᵃ</td>
<td>22.6±0.1ᵃ</td>
<td>165.1±6.7ᵇ</td>
<td>60.2±1.3ᵇ</td>
<td>24.3±0.5ᵇ</td>
<td>0.309±0.006ᵇ</td>
</tr>
<tr>
<td>12 hr</td>
<td>20.5±0.5ʰ</td>
<td>20.0±0.4ʰ</td>
<td>268.6±5.8ᵃ</td>
<td>80.4±4.5ᵃ</td>
<td>22.3±0.3ᵃ</td>
<td>0.317±0.007ᵃ</td>
</tr>
<tr>
<td>24 hr</td>
<td>19.4±0.2ᵃ</td>
<td>18.9±0.1ᵃ</td>
<td>290.3±29.4ᵃ</td>
<td>72.4±4.1ᵇ</td>
<td>22.4±1.2ᵇ</td>
<td>0.314±0.011ᵇ</td>
</tr>
<tr>
<td>3 hr</td>
<td>18.4±0.4ᵃ</td>
<td>17.9±0.4ᵃ</td>
<td>271.5±18.2ᵃ</td>
<td>71.7±5.2ᵇ</td>
<td>21.1±0.7ᵇ</td>
<td>0.329±0.003ᵇ</td>
</tr>
<tr>
<td>6 hr</td>
<td>21.5±0.3ᵃ</td>
<td>21.0±0.2ᵇ</td>
<td>190.3±13.3ᵇ</td>
<td>58.2±1.1ᵇ</td>
<td>23.7±0.4ᵇ</td>
<td>0.313±0.004ᵇ</td>
</tr>
<tr>
<td>12 hr</td>
<td>18.6±0.5ᵃ</td>
<td>18.1±0.5ᵃ</td>
<td>270.9±17.9ᵃ</td>
<td>66.3±3.9ᵃ</td>
<td>21.0±0.5ᵃ</td>
<td>0.318±0.008ᵃ</td>
</tr>
<tr>
<td>24 hr</td>
<td>18.1±0.5ᵃ</td>
<td>17.6±0.5ᵃ</td>
<td>266.0±20.9ᵃ</td>
<td>64.8±3.7ᵃ</td>
<td>21.1±0.3ᵇ</td>
<td>0.307±0.004ᵇ</td>
</tr>
<tr>
<td>3 hr</td>
<td>16.0±0.2ᵇ</td>
<td>15.5±0.2ᵇ</td>
<td>237.9±4.2ᵇ</td>
<td>48.9±1.5ᵇ</td>
<td>21.0±0.2ᵇ</td>
<td>0.308±0.007ᵇ</td>
</tr>
<tr>
<td>6 hr</td>
<td>18.9±0.3ᵇ</td>
<td>18.4±0.3ᵇ</td>
<td>220.9±12.3ᵇ</td>
<td>55.5±1.1ᵇ</td>
<td>23.1±0.2ᵇ</td>
<td>0.309±0.007ᵇ</td>
</tr>
</tbody>
</table>
lifespan in favour of higher gross reproductive output. However, this by itself does not necessarily lead to higher \( r \) since age-specific mortality influences the survival-weighted net reproductive rate. For example, gross reproductive rates in many copper treatments (about 70% cases) were significantly higher than those in the control, while net reproductive rate was affected in only one case and none for population growth rate. This implies that elevated reproduction due to stress from sublethal levels of copper actually did not contribute to increase in \( r \), which is one of the most important variables in life history theory (Steams, 1992). Since gross reproductive rate is not survival-weighted, its enhanced values gain importance only with the higher survival rates of the reproducing mothers (Kreb's, 1985).

In our study though Cu had a positive influence on the gross reproductive rate, this was nullified by the low survival rate of the reproducing mothers. Our data showed that duration of lifespan and life expectancy at birth decreased with increasing concentration of copper and duration of exposure. Hence, industrial effluents containing copper must be treated before releasing into freshwater ecosystems, regardless of the duration of effluent release.

In conclusion, since Cladocera has a functional role in structuring phytoplankton populations (Dodson and Frey, 2001), the combined impact of copper levels and exposure time on \textit{C. dubia} is a matter of concern, because in lakes and ponds, it could lead to a series of ecological processes. It is therefore necessary to regulate the release of untreated industrial effluents containing copper into freshwater ecosystems. Our study thus highlights the importance of including, not only heavy metal concentrations but also exposure time, for ecotoxicological evaluations.

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References


