

# Reproduction and survival of *Daphnia magna* Straus, 1820 (Crustacea:Cladocera) under different hardness conditions.

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**ABSTRACT:** Reproduction and survival of *Daphnia magna* Straus, 1820 (Crustacea:Cladocera) under different hardness conditions. **In the state of Rio Grande do Sul, southern Brazil, trials are performed using *Daphnia magna* Straus, 1820, to evaluate the environmental quality of river basins in the state and industrial effluents in the area. Since this species is native to regions where water is hard, while the rivers in this region have soft water, it became necessary to develop a test to evaluate the existence or not of stress in this species, due to changes in hardness. This paper presents the results of chronic tests with *Daphnia magna* Straus, 1820, exposed for 21 days to 10, 40, 80, 120, 160, 200 and 250 mgCaCO<sub>3</sub>/L. During the period in which the organisms were exposed, observations were performed on alternate days to evaluate the survival and reproduction of individuals, considering water hardness variations. The results obtained indicate that there is no significant difference in the reproduction of *D. magna* between the least and most water hardness, as used in the assay. Survival at different harnesses was always within the percentage required for control, and thus considered normal.**

**Key-words:** *Daphnia magna*, hardness, bioassay, Cladocera.

**RESUMO:** Reprodução e sobrevivência de *Daphnia magna* Straus, 1820 (Crustacea:Cladocera) em diferentes durezas. **No estado do Rio Grande do Sul, situado ao sul do Brasil, são realizados ensaios com *Daphnia magna* Straus, 1820, para avaliar a qualidade ambiental das bacias hidrográficas a ele pertencentes e dos efluentes das indústrias nele localizadas. Sendo esta espécie natural de regiões que apresentam água com elevado teor de carbonato, enquanto que as bacias hidrográficas deste Estado apresentam água com baixo teor de carbonato, foi necessário observar a existência ou não de estresse nesta espécie devido a alteração da dureza do meio. Este trabalho apresenta resultados de testes crônicos com *Daphnia magna* Straus, 1820, expostas por 21 dias a 10, 40, 80, 120, 160, 200 e 250mgCaCO<sub>3</sub>/L. Durante o período de exposição dos organismos, a diferentes durezas, foram realizadas observações em dias alternados, para avaliar a sobrevivência e a reprodução dos indivíduos, frente a variação da dureza da água. Os resultados obtidos sugerem não haver diferença significativa na reprodução de *D. magna* entre o valor mais baixo de dureza da água e o mais elevado, utilizados no ensaio. A sobrevivência entre as diferentes durezas esteve sempre dentro da porcentagem exigida para o controle, portanto classificada como normal.**

**Palavras-chave:** *Daphnia magna*, dureza, bioensaios, Cladocera.

## Introduction

Bioassays are ways of evaluating the action of external agents on organisms. These procedures can assess the impact on individuals, due to chemical, physical or even biological agents.

Using chronic tests we can observe what happens in the organism throughout its life cycle and find positive responses to apparently innocuous variables when the organisms

are subject to them for a short time. The expression of many environmental aggressors only become visible when they are present in high doses. However, when they exist in small portions they affect an individual's genetic baggage, interfere in their physiologic functions, change in reproductive frequency or the quality and quantity of organisms generated.

Biological tests covering development phases from the first moments of life until the reproductive period are essential to know whether the quality of the environment is satisfactory to maintain the integrity of aquatic life, both as to diversity and health of indigenous communities. During these two phases (initial and reproductive), the organisms are very fragile and sensitive to environmental changes.

The aim of this study is to evaluate the interference of hardness (Calcium Carbonate) in *Daphnia magna*. Water hardness is a very variable factor depending on which region of the world is involved. The river basins of Rio Grande do Sul, Brazil, usually present low water hardness values. *Daphnia magna* is a microcrustacean from Europe, where the water has a high carbonate content and is thus hard.

In Rio Grande do Sul the Government Environmental Protection Agency (Fundação Estadual de Proteção Ambiental Henrique Luís Roessler/RS-FEPAM) uses this species for acute tests of industrial effluents and chronic tests of environmental quality. Therefore, it became necessary to study the behavior of this microcrustacean with different water hardness values.

A microcrustacean of the Cladocera order was chosen, specifically *Daphnia magna*, to study environmental evaluation, because these organisms are widely known, easily cultured, held and reproduced in laboratory. They need little space, a small infrastructure, maintenance costs are low and they respond quickly to chronic effects.

The short life cycle and frequency of reproduction (more or less every two days) ensure a sufficient number of young for frequent assays every month of the year. Another advantage of this short life cycle is the possibility of obtaining responses in chronic assays in up to three weeks.

Organisms of this species are widely used in laboratory assays to evaluate the acute action of effluents, or chronic action of rivers, or sediment (Nebeker et al., 1988; Baillieul et al., 1993).

It is important to become acquainted with the behavior of the species under different conditions of hardness, because chronic tests require the exposure of individuals for a long time in their lives to samples that often present a different hardness from the one the organisms has been kept on in the laboratory, or has encountered in its natural habitat. The impact of this factor, or the maintenance of test-individuals, under these conditions may interfere in the response obtained.

Changing the usual hardness of the sample to perform the test may mask the action of toxic materials such as metals or other substances, distorting the final result of the test. Therefore it is important to become acquainted with organism reactions to different harness and thus be sure that this difference will not change the response supplied by the experiment.

*Daphnia magna's* natural environment are places where water is hard. Although it is not native to our environment the species is much used in internationally acknowledged standard tests (Winner, 1981; Le Blanc, 1985; Day & Kaushik, 1987; Kukkonen & Oikari, 1992). Moreover, many responses attributed to *Daphnia magna* are comparable to fishes such as fathead minnows (*Pimephales promelas*) (Gersich & Mayes, 1986; Dillon & Burton, 1991) also used in standard tests in different regions of the world. All these advantages make us choose this crustacean, in our laboratories, in order to evaluate environmental quality in our state.

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## Material and methods

A chronic test was performed with *Daphnia magna*, exposing the species to seven different concentrations of CaCO<sub>3</sub> from lots cultivated at 250mgCaCO<sub>3</sub>/L. The test

lasted 21 days with observations on alternate days regarding the survival and reproduction of these individuals.

Although some authors use 10-day old *Daphnia magna* to perform tests with seven days of exposure, calling these chronic tests short (Lewis & Horning, 1988), we chose to work with longer tests (21 days) in order to obtain more reliable responses regarding the interference of the hardness on the physiologic and reproductive development of this microcrustacean, since the effects could simply occur by prolonged exposure of the microcrustaceans.

Individuals aged from a few hours (less than 24) to 21 days were exposed to seven variations of hardness to cover practically the full ontogenic development and followed from the first offspring (around the 6<sup>th</sup> day) at least to the sixth brood. The organisms used in the tests were born of 28-day old parents, and thus they came from approximately the tenth offspring.

The organisms used in the test belong to Clone A received from the Institut für Wasserboden und Lufthygiene des Bundesgesundheitsamtes, Germany.

The M4 (Eledent & Bias, 1990) culture medium was, used in the experiment and adapted to the reagents responsible for hardness.

Each one of ten beakers per CaCO<sub>3</sub> concentration received a young *Daphnia magna* individual and 25 ml of M4 culture medium according to the defined hardness scale (10, 40, 80, 120, 160, 200 and 250 mgCaCO<sub>3</sub>/L). Then the beakers were covered with Parafilm<sup>®</sup> to avoid the evaporation of the liquid medium and air contamination. The number of *Daphnia magna* used and the form of distribution of an individual per beaker followed the model advocated for semi-static tests (OECD, 1993).

During the days set aside for observations, the old medium (48h) was removed with the help of a wide-tipped pipette, with a 6mm diameter in order to avoid damaging the organisms. Next 25 ml of M4, with a hardness equal to the one removed, is introduced using a wash bottles. The substitution of the culture medium prevented the metabolites of the exposed individuals from changing the quality of the exposure environment and a dispute for space and food, since the young was removed from each beaker.

After this procedure the organism was immediately returned to the beaker to prevent any type of disturbance. It is then fed with *Scenedesmus subspicatus* ad libitum (10<sup>7</sup>cels/cm<sup>3</sup>). The algae are cultivated in the Biology laboratory at FEPAM, from axenic unialgal culture, kept in CHU nutrient medium, under controlled light and temperature conditions.

The tests were performed in a programmed incubator for a 16h/8h (light/dark) photoperiod and at a temperature of 21° C±1°C.

The frequency of mortality and the comparison between the offspring were examined through the Duncan Test using the SPSS 8.0 for Windows (1997) program.

The week before the assays begin sensitivity tests are performed accepting only the lots in which LC<sub>50</sub> 24h is around 1.00mg/L K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, a value calculated using the Trimmed Spearman-Kärber Method (Toxstat, version 1.5).

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## Results and discussion

The different hardness values calculated in the form of a CaCO<sub>3</sub> concentration, did not present variations in the response between the least hardness (10) and the highest one (250) once the births and adult survival have been taken into account.

Cowgill & Milazzo (1991a), did not also observe any significant effect on survival when the hardness between 198mgCaCO<sub>3</sub>/L and zero were tested.

The mean time of survival of *D.magna* exposed to soft, medium-hard and hard water, indicated that in up to 50 days of exposure, survival was not affected by different types of hardness (Paulauskis & Winner,1988). The same authors not find any difference as to the day of first reproduction, although there was an effect on brood size, the largest litters being found in the hardest water (197 mgCaCO<sub>3</sub>/l).

Evaluating the neonates present at each concentration, 1,516; 1,770; 1,654; 1,595; 1,596; 1,787 and 1,770 births were found for hardness of 10, 40, 80, 120, 160, 200 and 250mgCaCO<sub>3</sub>/L, respectively (Fig. 1).

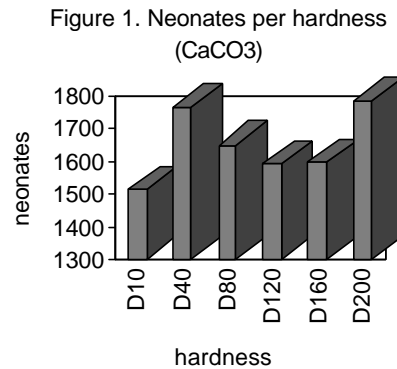


Figure 1: Neonates per hardness

Cowgill & Milazzo (1991b), using hardnesses which ranged from 1,928mgCaCO<sub>3</sub>/L to zero, found a significant depletion of births only in the first brood at a greater hardness. Same observations were found in our data.

Like Paulauskis & Winner (1988), no difference between the variables at the beginning of reproductive age was recorded, since at all hardnesses the individuals began reproducing on the 7<sup>th</sup> day of life. Between the seventh and twenty-first day around six offspring were born at all hardness values, although with differences among none replicas. The offspring varied from 5 to 6 most times, and only two episodes occurred in one of the replicas at hardness 40, where an individual died on the 15<sup>th</sup> day of the test; four in one of the hardness 250 replicas and seven in one of the 80 and 200 hardness replicas.

According to the hardness, the mean number of individuals per offspring ranged from 20 to 51; the mean neonates per treatment were lower than 34 individuals and the coefficients of variation did not exceed 25% (Tab.I).

The disturbance caused by the different hardnesses did not affect the development

Table 1: Mean number per replica (r) of neonates per offspring, according to hardness (CaCO<sub>3</sub>).

mg/L \ r	10	40	80	120	160	200	250
1	25	29	25	27	29	34	32
2	30	36	33	32	25	36	35
3	32	33	22	31	32	32	33
4	26	30	30	23	23	28	30
5	25	30	22	30	24	34	27
6	20	36	27	32	25	36	32
7	21	36	28	26	30	35	30
8	25	51	34	23	28	32	26
9	30	24	28	25	28	28	30
10	28	34	30	26	33	31	39
X	26.2	33.9	27.9	27.5	27.7	32.6	31.4
SD	3.88	7.14	4.09	3.50	3.40	2.95	3.78
CV%	15	21	15	13	12	9	12

of *Daphnia magna*, since the organisms transferred from high hardness (hard or very hard water) to a low hardness (soft water), presented no stress. For instance, organisms that had been kept at a hardness of 250mgCaCO<sub>3</sub>/L, when transferred to lower values such as

10mgCaCO<sub>3</sub>/L did not undergo any change in behavior or in reproduction.

These results suggest that *Daphnia magna* can be used to evaluate environments where water has lower values for hardness, even if they are being cultured in very hard water.

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