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Effects of sodium and potassium on life history parameters of freshwater cladoceran *Pseudosida ramosa*

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Abstract

In this study, we measured the life history parameters of the tropical cladoceran *Pseudosida ramosa* at sublethal concentrations of sodium and potassium. The results showed that salinity affected its maximum body length at a concentration of 249 mg L⁻¹ of sodium. There was also an increase in the number of days taken by females to reach maturity at a concentration of 11 mg L⁻¹ of potassium, a reduction in the number of neonates produced by primipara at concentrations ranging from 25 to 249 mg L⁻¹ of sodium, reductions in the survival at concentrations of 249 mg L⁻¹ of sodium and 11 mg L⁻¹ of potassium and reductions in the number of total neonates hatched at concentrations ranging from 41 to 249 mg L⁻¹ of sodium and from 3.6 to 11 mg L⁻¹ of potassium. The concentrations of the both salts that affected the reproduction of *P. ramosa* were lower than those that affected other endpoints.

Key words: life history, potassium, *Pseudosida ramosa*, saline stress, sodium.

Efeitos do sódio e potássio sobre parâmetros da história de vida do cladóceros de água doce *Pseudosida ramosa*

Resumo

Neste estudo, nós medimos os parâmetros do ciclo de vida de um cladóceros tropical *Pseudosida ramosa* para concentrações subletais de sódio e potássio. Os resultados mostraram que a salinidade afetou seu comprimento corpóreo máximo em uma concentração de 249 mg L⁻¹ de sódio. Houve também um aumento no número de dias para que as fêmeas atingissem a maturidade em uma concentração de 11 mg L⁻¹ de potássio, uma redução no número de neonatas produzidas pela primípara para concentrações que variaram de 25 a 249 mg L⁻¹ de sódio, reduções na sobrevivência para concentrações de 249 mg L⁻¹ de sódio e 11 mg L⁻¹ de potássio e reduções no número de neonatas total eclodidas para concentrações que variaram de 41 a 249 mg L⁻¹ de sódio e de 3,6 a 11 mg L⁻¹ de potássio. As concentrações dos sais que afetaram a reprodução de *P. ramosa* foram menores do que àquelas que afetaram outros parâmetros.

Palavras-chave: ciclo de vida, potássio, *Pseudosida ramosa*, sódio, stress salino.

INTRODUCTION

The rising salinity in freshwater ecosystems is a problem that is becoming increasingly serious worldwide, but particularly in developing countries, where rapid eutrophication and salinization are occurring (Sarma *et al.*, 2006). Comparative measurements of electrical conductivity in inland freshwater in São Paulo State, Brazil, show that over two decades it increased by more than 200% in many reservoirs (Tundisi *et al.*, 1988).

Several authors (e.g., Hall & Burns, 2003; Schallenberg *et al.*, 2003) have reported a reduction in the abundance and diversity of zooplankton communities in temperate regions exposed to salinity increases. However, more information on how the rise in salinity affects ecosystem integrity is needed, in order to predict the impact and rehabilitation of aquatic systems (Nielsen *et al.*, 2003). Therefore, studies on the effect of salinity stress on freshwater organisms are important to predict possible species losses and replacements, among other types of impact. Among suitable test organisms, zooplankton has long been used to assess the impact of environmental change, owing to their key intermediate position in the trophic food web. Salinity changes in freshwater systems can greatly affect the survival and life history (growth and reproduction) of zooplankton populations and, consequently, have an impact on higher trophic levels. Cladocerans, one of the most abundant primary consumer groups in lentic ecosystems, have varying physiological tolerances to several ionic components (Bos *et al.*, 1996) and are good indicators of environmental changes, especially changes in freshwater salinity (Sanchez-Colomer, 1996; Boronat *et al.*, 2001; Amsinck *et al.*, 2003).

To assess the effects of salinity on tropical freshwater plankton species, we measured life history parameters of *Pseudosida ramosa* (Cladocera, Sididae), a native species in tropical regions (Rey & Vasquez, 1986; Roa & Vasquez, 1991; Korovchinsky, 1992; Morrone & Coscarón, 1998; Sanoamuang, 1998; Elias-Gutierrez *et al.*, 2001; Maiphae *et al.*, 2005; Freitas & Rocha, 2006), at sublethal concentrations of sodium and potassium. The main aim of this study was to determine salinity thresholds that can have adverse effects on *P. ramosa* in chronic exposures and to compare these thresholds with those known for other species.

MATERIAL AND METHODS

Organism and culture conditions

Individuals of the cladoceran species *Pseudosida ramosa* were originally collected from the Óleo Lake (21°20'–21°55'S by 47°35'–47°55'W), an oxbow lake in the Mogi-Guaçu River flood plain, Jataí Ecological Station, district of Luis Antônio, São Paulo State, Brazil, and were used to establish a culture that has been maintained in our laboratory since 2004.

Neonatal *P. ramosa* were obtained from continuous cultures in 2 L glass beakers containing reconstituted soft

water at $25 \pm 1^\circ\text{C}$, as recommended by the American Society for Testing and Materials (ASTM, 2001). The reconstituted soft water was prepared with $0.03 \text{ g L}^{-1} \text{ CaSO}_4 \cdot 2\text{H}_2\text{O}$, $0.061 \text{ g L}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$, $0.048 \text{ g L}^{-1} \text{ NaHCO}_3$, and $0.002 \text{ g L}^{-1} \text{ KCl}$ dissolved in 1 L of distilled water and had a total hardness equivalent to $40\text{--}48 \text{ mg CaCO}_3 \text{ L}^{-1}$, a pH range of 7.2–7.6 and a conductivity of $160 \mu\text{S cm}^{-1}$.

The culture medium was renewed three times weekly. Individuals of *P. ramosa* were maintained under a light: dark photoperiod of 12:12 h, at a density of below 70 individuals per liter and fed daily with the green algae *Pseudokirchneriella subcapitata* ($10^5 \text{ cells mL}^{-1}$) and a suspension of dried baking yeast given as a food supplement (0.05 mg L^{-1} of dry yeast) as recommended by USEPA (2002). The alga was cultured in CHU-12 medium (Müller, 1972), which was previously autoclaved at 120°C and 1 atm for 20 minutes. The alga was grown at $25 \pm 1^\circ\text{C}$, under a light: dark photoperiod of 12:12 h and the culture medium was gently aerated. Before using *P. subcapitata* as food, it was centrifuged, washed and resuspended in reconstituted soft water to remove culture nutrients.

Chemicals and test solutions

NaCl and KCl of high purity (> 99 %) were purchased from Lafan Química Fina Ltda (Brazil). Stock solutions of NaCl (10 g L^{-1}) and KCl (100 mg L^{-1}) were prepared by dissolving a weighed amount of each compound in distilled water just before the tests. In turn, the nominal tested concentrations of each compound were obtained by diluting the stock solution in reconstituted soft water. In this study, it was taken into account that the water dilution (reconstituted soft water) used in the tests contained ions of Na^+ and K^+ . Their concentrations were added to the final values of the nominal tested concentrations for each ion.

Chemical analysis of test solutions, to confirm the actual concentrations of the toxicants, was not performed, since the salts tested were very unlikely to be lost by evaporation, precipitation, chemical transformation, variations in the oxidation state, or adsorption.

Chronic toxicity tests

The chronic toxicity tests were carried out in accordance with the guidelines of the Organization for Economic Cooperation and Development (OECD, 2008).

The tests were conducted over the whole life cycle, under the same conditions of temperature and photoperiod as described for the culture procedures. Tests were initiated with neonates ($\leq 24 \text{ h}$ old) obtained from the same bulk culture, born between the 3rd and 5th broods.

In total, ten replicate culture vessels were used for each test solution and for control. The neonates used in the chronic assays were individually cultured in 50 mL glass beakers containing 20 mL of the test solution or 20 mL of the

reconstituted soft water (control), totaling 10 test organisms per treatment. The assays were performed at the following nominal concentrations: 25, 41, 72, 131, and 249 mg L⁻¹ for Na⁺ and 1.02, 1.26, 3.6, 6.2, and 11 mg L⁻¹ for K⁺. These concentrations were chosen on the basis of the acute toxicity results (Freitas & Rocha, 2011).

The tests were carried out on a semi-static basis and test solutions were renewed every two days. The individuals were fed daily as previously described. During the experiment, pH, temperature, electrical conductivity, dissolved oxygen, and total hardness were measured every time the test solutions were renewed, to find out whether they were affecting the biological responses or not.

Animals were checked every day at approximately the same hour for mortality and reproductive state. In this study, a dead individual was defined as one showing no heartbeat when examined under the stereomicroscope (Day *et al.*, 1993). Data were assessed by recording the following parameters during the test: primipara length (mm), primipara age (days), number of eggs (eggs female⁻¹) or neonates (neonates female⁻¹) per primipara, longevity (days), and total number of eggs (eggs female⁻¹) or neonates (neonates female⁻¹) per adult female. When neonates were released, they were counted and discarded.

To monitor individual body length, each female was transferred once a day to a watch glass by means of a widepoint Pasteur pipette, care being taken to avoid damaging the organism. The length of the whole body was measured under a stereoscopic microscope, with the aid of a micrometric ruler, from the top of the head to the rear end of the organism (tip of the carapace). Growth curves were fitted to the data by means of the von Bertalanffy equation (von Bertalanffy, 1938), whose parameters were determined by applying the Ford-Walford transformation (Walford, 1946): $L_t = L_\infty [1 - e^{-K(t-t_0)}]$, where L_t is the body length at time t (in mm), L_∞ its maximum value, to which the curve tends asymptotically (mm), K the growth-rate constant (days⁻¹), e the base of Napierian logarithms and t_0 a time related to the mean length at the moment of birth, L_0 (days).

Data treatment and statistical analysis

The effect of salinity on the life history parameters of *P. ramosa* were expressed as mean values plus standard deviation (SD). Data were first checked for the normality (with the chi-squared test) and homogeneity (with Bartlett's test) of their distribution. To test for significance, a one-way ANOVA was applied to the data. When the ANOVA showed statistical significance, a Tukey test was then applied to the data, to determine which concentrations were significantly different from the control group and of the other treatments. This procedure allowed the determination of the standard NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) values. To detect any significant differences in individual growth, the non-parametric Mann-Whitney test (MW) was used. A value of

$\alpha \leq 0.05$ was taken to show significance in all tests. All data were analyzed using the computer program TOXSTAT 3.3 (Gulley *et al.*, 1991).

RESULTS

During the chronic toxicity tests, the measured pH of the solutions remained within the range 7.2–7.6 and did not vary by more than 1.0 unit in any given test. The temperature ranged from 24.2 to 25.3°C. The electrical conductivity ranged from 154.6 to 1,351 $\mu\text{S cm}^{-1}$ for Na⁺ and from 145.3 to 272.0 $\mu\text{S cm}^{-1}$ for K⁺. The oxygen content of the test solutions ranged from 7.6 to 9.3 mg L⁻¹ and water hardness ranged from 44 to 46 mg CaCO₃ L⁻¹. Thus, all tests met the validity criteria set forth by the OECD guidelines.

The growth results for the *P. ramosa* females are illustrated in Figure 1. In the assay with sodium (a), a modest, but statistically significant decrease in maximum body length was observed between organisms tested with 249 mg L⁻¹ (maximum body length = 2.26 mm) and controls (2.36 mm) (MW = 960.5; $p = 0.03$). No difference was found between controls and organisms treated with lower concentrations of Na⁺ (between control and 25 mg L⁻¹, MW = 1,431 and $p = 0.76$; for 41 mg L⁻¹, MW = 1,283 and $p = 0.85$; for 72 mg L⁻¹, MW = 1,017 and $p = 0.20$; for 131 mg L⁻¹, MW = 997 and $p = 0.08$). No significant difference in maximum body length was found in the chronic toxicity test with potassium (b) (between control and 1.02 mg L⁻¹, MW = 1,466 and $p = 0.18$; for 1.26 mg L⁻¹, MW = 1,615 and $p = 0.30$; for 3.6 mg L⁻¹, MW = 1,999 and $p = 0.95$; for 6.2 mg L⁻¹, MW = 2,014 and $p = 0.46$; for 11 mg L⁻¹, MW = 1,625 and $p = 0.62$).

Figures 2 and 3 showed life history parameters of *P. ramosa* under influence of sodium and potassium, respectively.

The mean body length at the first reproduction (primipara) did not differ significantly from the control for either sodium or potassium treatment at any concentration. However, for females cultured in 11 mg L⁻¹ K⁺, there was an increase in the number of days taken to reach maturity (11.2 ± 2.3 days), relative to control (8.75 ± 1.16 days) ($F_{5,47} = 4.29$; $p = 0.003$).

Regarding the number of eggs and neonates produced by the primipara, we found significant differences from the control (2.40 ± 1.43 neonates female⁻¹) in females cultured at the concentrations of 25 (0.80 ± 0.92 neonates female⁻¹), 41 (0.60 ± 0.97 neonates female⁻¹), 72 (0.60 ± 0.70 neonates female⁻¹), 131 (0.58 ± 0.92 neonates female⁻¹), and 249 mg L⁻¹ Na⁺ (0.58 ± 0.93 neonates female⁻¹), in all of which production of neonates was reduced ($F_{5,54} = 4.00$; $p = 0.004$).

Longevities of *P. ramosa* were significantly lower than control longevities (43.6 ± 14.5 days for Na⁺ and 51.0 ± 12.1 days for K⁺) at the concentrations of 249 mg L⁻¹ Na⁺ (23.6 ± 15.2 days) ($F_{5,54} = 2.61$; $p = 0.03$) and 11 mg L⁻¹ K⁺ (28.5 ± 15.6 days) ($F_{5,54} = 2.48$; $p = 0.04$).

The total number of eggs produced during the whole life cycle of *P. ramosa* under the sodium and potassium treatments did not differ significantly from control. In contrast, for the

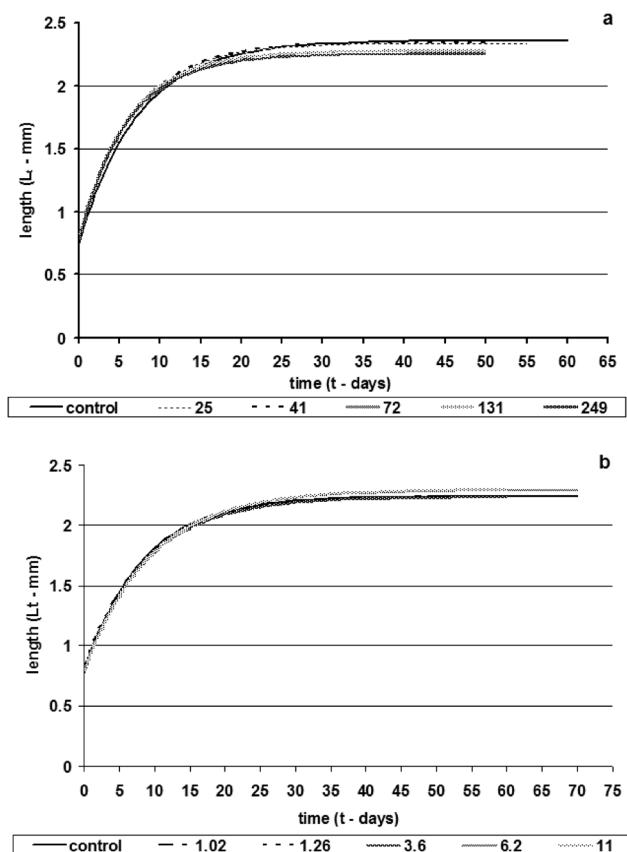


Figure 1 – Growth curves of body length of *P. ramosa* during chronic toxicity tests with (a) sodium (mg L^{-1}) and (b) potassium (mg L^{-1}).

total number of neonates, there was a reduction in the number of individuals hatched at the concentrations of 41 (8.60 ± 7.90 neonates female $^{-1}$), 72 (6.60 ± 4.90 neonates female $^{-1}$), 131 (12.0 ± 11.3 neonates female $^{-1}$) and 249 mg L^{-1} Na^+ (8.89 ± 7.44 neonates female $^{-1}$) ($F_{5,53} = 5.09$; $p = 0.001$), and 3.6 (10.0 ± 4.92 neonates female $^{-1}$), 6.2 (9.89 ± 4.65 neonates female $^{-1}$) and 11 mg L^{-1} K^+ (9.88 ± 5.28 neonates female $^{-1}$) ($F_{5,47} = 4.17$; $p = 0.003$), relative to the control (22.2 ± 8.23 neonates female $^{-1}$ for Na^+ and 18.8 ± 8.22 neonates female $^{-1}$ for K^+).

The values calculated for NOEC and LOEC in the chronic toxicity tests with Na^+ and K^+ are presented in Table 1.

DISCUSSION

The cladoceran crustaceans are aquatic invertebrates widely used to assess environmental quality (ISO, 1996; USEPA, 2002). However, certain regions, most cladocerans used to monitor the environment are exotic species. This often generates results that do not reflect the real situation in the local area. In this study, the idea was to use *Pseudosida ramosa*, a species native to the tropics, to assess the possible effects of salinization on freshwater cladocerans.

With respect to the growth curves, a modest but statistically significant decrease in maximum body length was observed between the control group and the group exposed to the highest

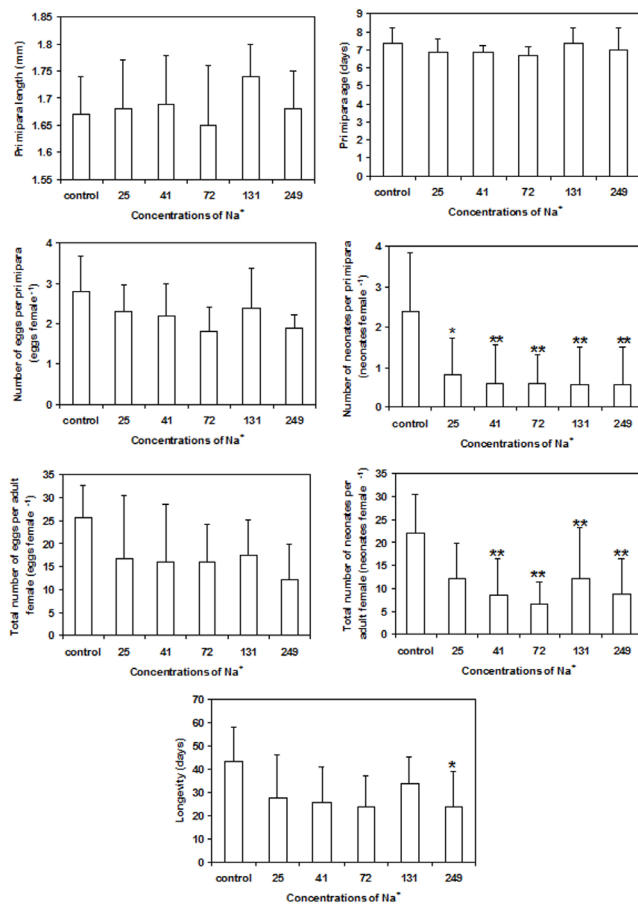


Figure 2 – Life history parameters of *P. ramosa* under influence of various concentrations of sodium (mg L^{-1}). Values are mean of 10 replicates (\pm SD). Statistically significant differences in relation to the control (Tukey's test) are indicated by * ($p \leq 0.05$) or ** ($p \leq 0.01$).

concentration (249 mg L^{-1}) of sodium. Even though there was no significant difference between any other treatment group (for Na^+ or K^+) and the control animals, there was a tendency for the maximum body length (L_{∞}) to be shorter in the organisms subjected to chronic toxicity tests with sodium. Such a tendency might be due to physiological stress caused by raised salinity, in that the animal would have to allocate energy to osmoregulation that would normally be employed in growth of the body. It may be of interest that cladocerans found successfully colonizing brackish habitats were smaller than those in freshwater populations and showed a reduced breeding rate (Arne'r & Koivisto, 1993). No size-reduction tendency was observed in the group of *P. ramosa* subjected to chronic toxicity tests with up to 11 mg L^{-1} of K^+ . However, Utz & Bohrer (2001) found significant reductions in the growth of *D. similis* when exposed to potassium chloride concentrations higher than 80 mg L^{-1} .

Life history traits of *P. ramosa* were affected by the increase of salinity in our study. The most dramatic change caused by sodium was in the number of neonates. Concentrations higher than 25 mg L^{-1} were sufficient to prevent the normal development of eggs in the incubation chamber of the females. Several authors (e.g., Baird *et al.*, 1991; Guilhermino *et al.*,

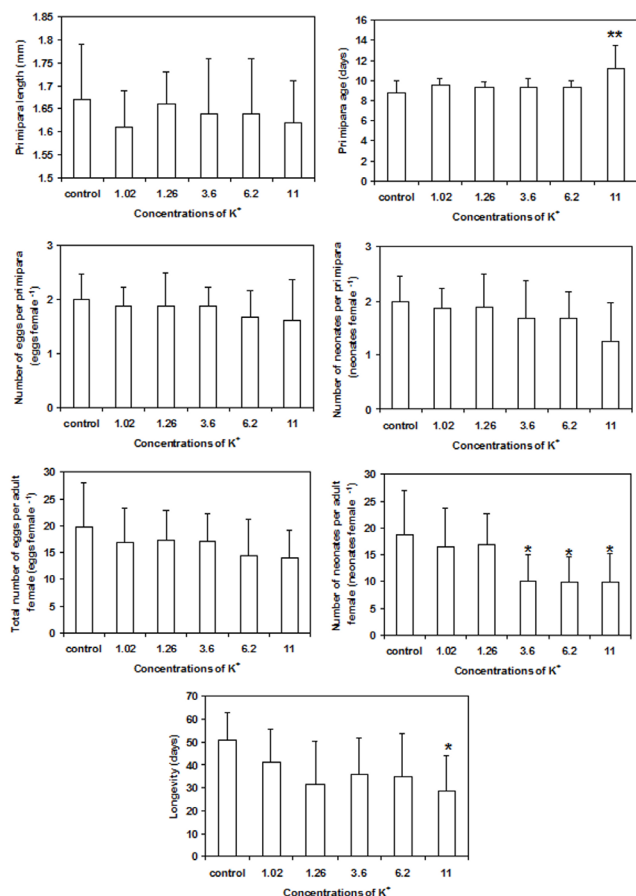


Figure 3 – Life history parameters of *P. ramosa* under influence of various concentrations of potassium (mg L^{-1}). Values are mean of 10 replicates (\pm SD). Statistically significant differences in relation to the control (Tukey’s test) are indicated by * ($p \leq 0.05$) or ** ($p \leq 0.01$).

Table 1 – NOEC and LOEC values for each life history parameter of *P. ramosa* exposed to various concentrations of sodium and potassium throughout the life cycle ($n = 10$)

Life history parameters	Na^+ (mg L^{-1})		K^+ (mg L^{-1})	
	NOEC	LOEC	NOEC	LOEC
Body length (mm)	131	249	-	-
Primipara age (days)	-	-	6.2	11
Number of neonates per primipara	< 25	25	-	-
Longevity (days)	131	249	6.2	11
Total number of neonates per female	25	41	1.26	3.6

(-) means that the value cannot be calculated.

1999) found that sodium (present as sodium bromide) caused abnormal embryonic development, an increased level of abortion and a reduced total number of neonates in *Daphnia magna*.

Potassium also negatively affected the number of neonates hatched, at concentrations above 3.6 mg L^{-1} , and therefore was about 11.4 times more toxic than sodium for the production of neonates by *P. ramosa*. Additionally, maturation (primipara age) was delayed significantly at the highest concentration (11 mg L^{-1}) of K^+ . Similarly, Teschner (1995) concluded that

clones of *D. magna* grown under brackish conditions matured later than under freshwater conditions.

Both salts (Na^+ and K^+) reduced the longevity of *P. ramosa* at the highest concentrations tested (249 and 11 mg L^{-1} , respectively). Nielsen *et al.* (2003) stated that microinvertebrates, freshwater algae and aquatic plants appear to be the biological communities least tolerant to increases in salinity.

A small increase in salinity seems to be clearly deleterious for the indigenous species *P. ramosa*. This becomes clear when we compared the values of NOEC and LOEC for this species with other freshwater cladocerans. For the daphnid *Ceriodaphnia dubia* exposed to sodium, Aragão & Pereira (2003) found a NOEC for reproduction that varied from 25 to 50 mg L^{-1} , in agreement with the value of 44 mg L^{-1} reported by Harmon *et al.* (2003). In the latter study, the LOEC for reproduction was 85 mg L^{-1} for this species, and the NOEC and LOEC for reproduction of *Daphnia ambigua* were found to be identical to those of *C. dubia*. Hence, in chronic toxicity tests with Na^+ , *P. ramosa* was as sensitive as, or more sensitive than, either *C. dubia* or *D. ambigua*, having a NOEC and LOEC for reproduction of 25 and 41 mg L^{-1} , respectively. When *P. ramosa* was exposed to potassium, the NOEC and LOEC for reproduction were found to be 1.26 and 3.6 mg L^{-1} , respectively. Utz & Bohrer (2001) tested the chronic toxicity of potassium to *Daphnia similis* and observed a LOEC for reproduction of 60 mg L^{-1} .

Note also that the concentrations of both salts that affected reproduction in *P. ramosa* were lower than those that affected the body length (for Na^+), age at first reproduction (for K^+) and longevity (for both salts) (Table 1).

Salinity is an important and crucial factor in determining the presence, dominance and succession of organisms. This study gives an insight into how an increase in the level of salts affects significantly survival and life history traits, which can cause changes in the zooplankton community structure. Other authors (e.g., Nielsen *et al.*, 2003; Bailey *et al.*, 2004) state that, if salinity rises very rapidly, organisms cannot adapt and freshwater taxa can become locally extinct, transferring dominance to salt-tolerant taxa.

The present results suggest that more attention should be paid to potassium as a toxicant to freshwater biota, seeing that it was about 11.4 times more toxic than sodium for the production of neonates by *P. ramosa*. The present study also suggest that the increasing salinity observed in many freshwater systems may have dramatic implications for species composition and biodiversity, affecting the reproduction of *P. ramosa* and eliminating this species if salinity concentrations remain above its tolerance levels. Further experiments with freshwater species under brackish and saline conditions are required in order to understand how freshwater species adapt their physiology to such stressful environments. Studies in mesocosms (more realistic and complex scenarios) may be useful for a deeper understanding of the effects of salinity and of its interactions with other factors (e.g., temperature,

nutrients), at the guild/community level, thus generating more ecologically relevant information.

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