

Effects of salinity tolerances on survival and life history of 2 cladocerans

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Received: 12.04.2013

Accepted: 23.12.2013

Published Online: 21.03.2014

Printed: 18.04.2014

Abstract: Salinity alterations in freshwater ecosystems greatly affect the survival and life history of zooplankton and, therefore, have an effect on higher trophic levels. Salinity is an essential and critical factor in determining the presence, dominance, and succession of organisms. After being collected in the field, *Scapholeberis mucronata* and *Simocephalus vetulus* were brought to the laboratory in water. Under laboratory conditions (24 ± 1 °C; 16:8 h photoperiod), we evaluated the effect of different salt concentrations on the neonates. The LC_{50} values were determined at different halotolerance levels for *S. mucronata* (0.375 g L^{-1}) and *S. vetulus* (0.250 g L^{-1}). Results of experiments demonstrated that *S. vetulus* was more sensitive than *S. mucronata*, which has hyponeustonic behavior. Morphometrics of the body in salinity stress were measured for *S. mucronata*. In the measurements of body length, body width, and spine lengths, there were significant differences determined by post hoc analysis between the control, 0.250, and 0.375 g L^{-1} dose groups and the 0.500 and 0.625 g L^{-1} dose groups. However, no clear relation between ovum size and salinity concentration was found. This study demonstrated how an increase in salinity significantly affects survival and life history properties, which can cause changes in the zooplankton community structure.

Key words: *Scapholeberis mucronata*, *Simocephalus vetulus*, salinity gradient, population growth, survival parameters, morphometry

1. Introduction

Saline and freshwater environments require completely different adaptations for the animals inhabiting them to have suitable osmotic pressure and cell homeostasis (Smolders et al., 2005; Grzesiuk and Mikulski, 2006; Ak et al., 2012; Gonçalves et al., 2012). Nevertheless, as salinity varies substantially in many habitats, such as estuaries or coastal lakes, local populations may be characterized by the presence of microevolutionary changes, the expansion of tolerance ranges, and greater phenotypic plasticity (Hall and Burns, 2002). Information on how increasing salinity affects the various life stages of the biota is limited (De Decker, 1983).

Salinization can lead to variations in the physical environment, which directly or indirectly affect ecosystem processes and zooplankton population structures. As a result of increasing salinity, toxic effects cause physiological changes, resulting in loss of species. Indirect changes can occur, as well; for instance, increasing salinity modifies community structures by removing taxa that obtain food or modify predation stress (Lignot et al., 2000; Nielsen, 2003). Cladocerans, one of the most abundant primary consumer groups, are a key component in lentic ecosystems. They display different physiological tolerances to several ionic compounds (Martínez-Jerónimo and

Martínez-Jerónimo, 2007; El-Deeb Ghazy et al., 2009; Loureiro, 2012). The use of cladocerans in toxicity studies provides many advantages: easy handling, asexual reproduction by parthenogenesis, a short lifespan, and great sensitivity to toxic compounds (Martínez-Jerónimo and Martínez-Jerónimo, 2007).

A species sensitivity distribution of the examined cladoceran species is descriptive for ecological risk assessment, even though the information is limited to only a particular functional group (Dumont and Negrea, 2002; Gliwicz, 2003). The purpose of this study is to present the current state of knowledge regarding the effects of salinity on the life parameters and body size structures of the freshwater crustaceans *Scapholeberis mucronata* (Schoedler 1858) and *Simocephalus vetulus* (Muller 1873). In this study, the salinity tolerance capacities of these 2 species collected from a lake ecosystem reflect possible changes occurring in the ecosystem.

2. Materials and methods

2.1. Laboratory conditions

This study investigated salinity effects on *S. mucronata* and *S. vetulus* collected in Surgu Dam Lake, (Malatya, Turkey; $38^{\circ}01'511''\text{N}$, $37^{\circ}53'946''\text{E}$). Both of the cladocerans, which are the most abundant species in the zooplankton

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community in the dam lake, were used in the study as test organisms. They were brought to the laboratory in lake water. Samples of cladocerans were taken in culture in filtrated lake water (0.2‰ salinity) (Lopes et al., 2011) and fed mixed algae until the study began. Upon reproduction, neonates obtained from the third clutch were individually placed in glass containers with the same salinity value. This procedure was repeated for 3 generations and finally neonates (<24 h old) produced in the third generation in each salinity were used as test organisms in all experiments (Martínez-Jerónimo and Martínez-Jerónimo, 2007).

The experiments started when the water had approximately the ambient air temperature of the laboratory, since temperature is more important in regulating production than salinity. No other acclimation was conducted (laboratory conditions are 24 ± 1 °C and a 16:8 h photoperiod). Therefore, individuals that were not fed were used in this assay (Yu et al., 2009). Individuals were checked every day at the same hour in terms of mortality and reproductive state (presence of eggs or offspring) and transferred to freshly filtrated lake water every day (Gonçalves and Mikulski, 2006).

2.2. In situ experiments

For *S. mucronata* and *S. vetulus*, experiments were initiated with neonates (<24 h old) obtained from the same bulk culture. The experimental saline concentrations were obtained by consecutive dilutions of a stock solution of sodium chloride (NaCl) in the lake water medium; salinity concentrations ranged from 1‰ to 2.5‰ (0.250–0.625 g L⁻¹ NaCl) for *S. mucronata* and *S. vetulus*, respectively. Salinity values corresponding to the different NaCl concentrations were detected using a conductivity meter, YSI 30.

Experiments were carried out in glass beakers (5 groups per treatment) containing 250 mL of test solutions. *S. mucronata* and *S. vetulus* were exposed to different salinity concentrations for 8 days; 30 animals (randomly divided into 6 animals per 5 groups) were used per control and per NaCl concentration (0.250, 0.375, 0.500, and 0.625 g L⁻¹). Vessels were checked for immobilized individuals at 24 and 48 h to conduct posterior determination of LC₅₀ values.

The number of surviving parthenogenetic females, number of clutches produced, and age at first reproduction in each treatment group were recorded for both cladocerans. Body length (BL, the distance from anterior part of the head to the base of the caudal spine), spine length (SL, the caudal spine), body width (BW, from the dorsal to the ventral part of the valves in its widest portion), and ovum length (OL, from the anterior to end part of the ovum in clutch) were measured in *S. mucronata* for each group by using a stereomicroscope (Leica MZ 7.5 with DFC 280 camera attachment, Leica Applications

Suite software, version 2.4.0R1) throughout the study.

2.3. Data analysis

Survivorship rate (l_x), growth rate, total progeny, and net reproductive rate (R_0) were estimated for each salinity group and species. Euler's equation was used for the calculation of the population growth (De Coen and Janssen, 2003; Antunes et al., 2004). Assuming survival and fertility schemes remain constant over time, if $R_0 > 1$, the population will grow exponentially. If $R_0 < 1$, the population will shrink exponentially, and if $R_0 = 1$, the population size will not change over time.

Survivorship rate, growth rate, net reproductive rate, and total progeny of each salinity concentration group were compared between the 2 species using Student's t-test (Zar, 1996). ANOVA was performed on all results in order to determine whether significant differences appeared among treatments or not; Tukey's post hoc comparison was also performed to identify significantly different treatments among them (Zar, 1996). The LC₅₀ values and their 95% confidence intervals were determined with Probit analysis (Finney, 1971). SPSS 15.0 software program was used to conduct all statistical analyses.

3. Results and discussion

3.1. Life history parameters

In the 8-day laboratory experiments, survival rates above 90% were found in both groups (96.3% and 92.6% for the control and 0.250 g L⁻¹ salinity, respectively) for *S. mucronata*. The in situ survival rates of the other salinity concentrations decreased for 0.375 g L⁻¹, 0.500 g L⁻¹, and 0.625 g L⁻¹ (55.6%, 25.9%, and 18.5%, respectively) (Figure 1). *S. vetulus* demonstrated low survival (74.1%, 57.4%, 27.8%, 18.5%, and 16.7% for control, 0.250 g L⁻¹, 0.375 g L⁻¹, 0.500 g L⁻¹, and 0.625 g L⁻¹, respectively), indicating its unsuitability as a test organism for in situ experiments (Figure 2).

In the comparison of survivorship curves, the control (0 g L⁻¹) and 0.250 g L⁻¹ treatment groups were similar in *S. mucronata*. It was always numerically more abundant than the other species. Mortality significantly increased in the 0.375 g L⁻¹ salinity group in *S. mucronata*. After ANOVA, significant differences were observed between groups (control and the other treatment groups) ($P < 0.01$, $F = 15.359$, 1-way ANOVA; Table 1). The lifespan drastically decreased at 0.250 g L⁻¹ in *S. vetulus*. According to ANOVA, there were significant differences between groups ($P = 0.001$, $F = 6.077$; Table 1). In each species, the same treatment groups were tested using Student's t-test. There were significant differences between the control and 0.250 g L⁻¹ salinity groups ($P = 0.028$, $t = 2.295$; $P = 0.012$, $t = 2.803$, respectively). There was no difference in the other treatment groups due to increasing mortality rate. The LC₅₀ values of the different salinity concentrations were defined

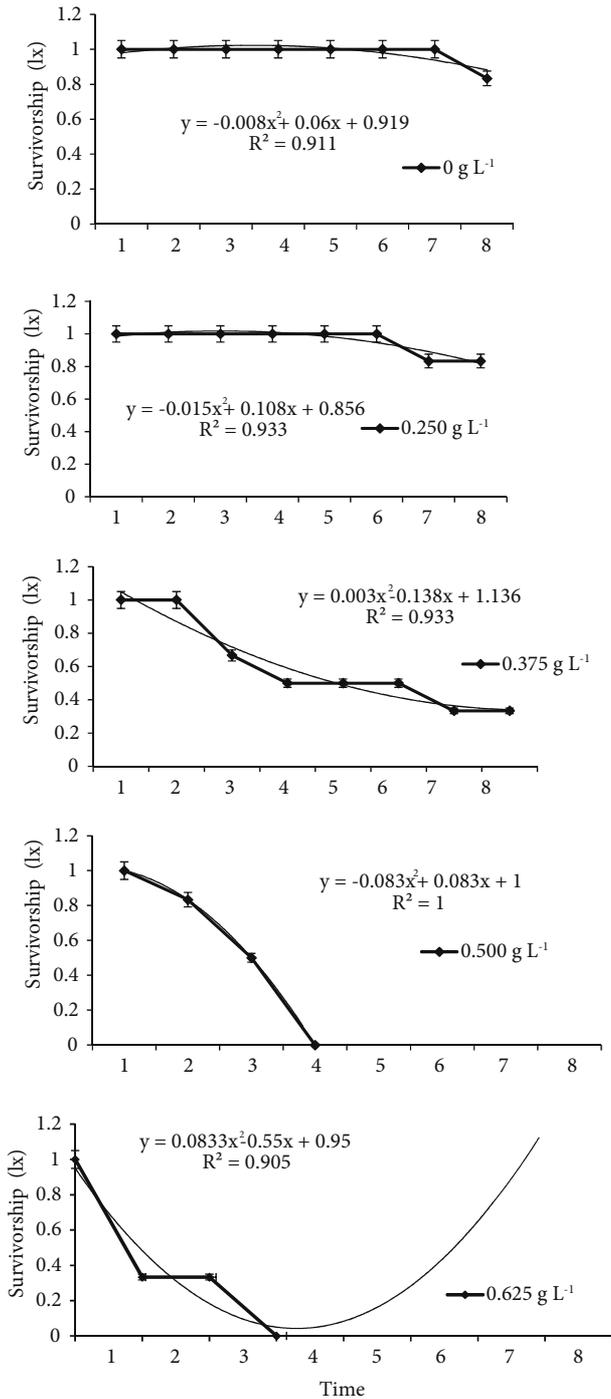


Figure 1. Survivorship curves of *S. mucronata* under different salt concentrations (with regression equations) in 8-day experiments (n = 6; ±standard error; P < 0.05).

by Probit analysis. The LC₅₀ values were 0.375 g L⁻¹ (1.5‰ salinity) for *S. mucronata* and 0.250 g L⁻¹ (1‰ salinity) for *S. vetulus*. According to LC₅₀ values, mortalities in survivorship curves increased at these concentrations (Figures 1 and 2).

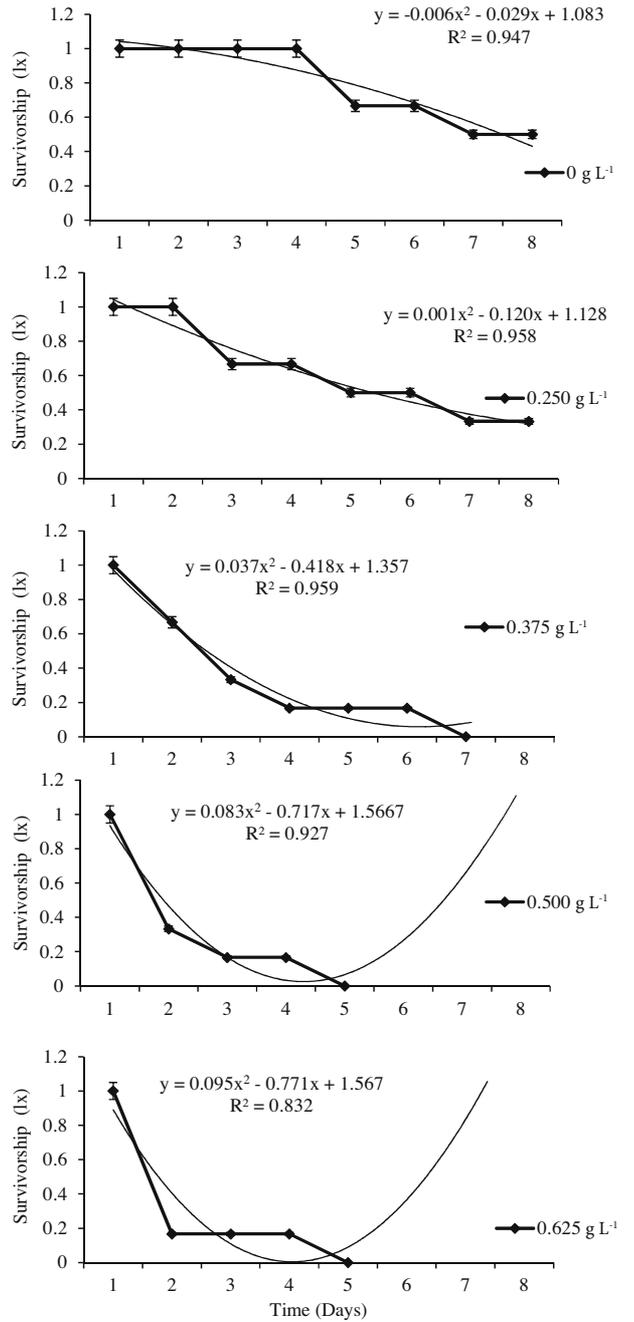


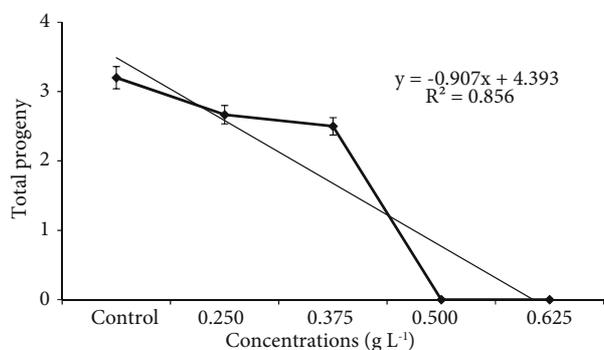
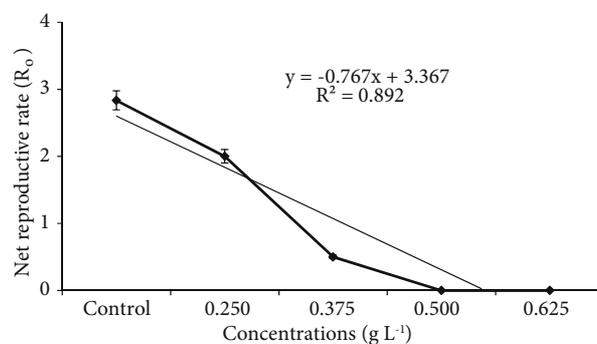
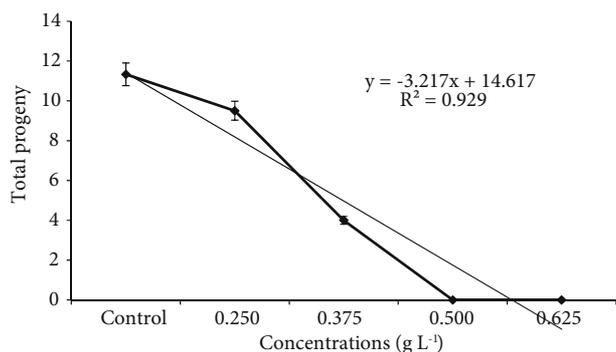
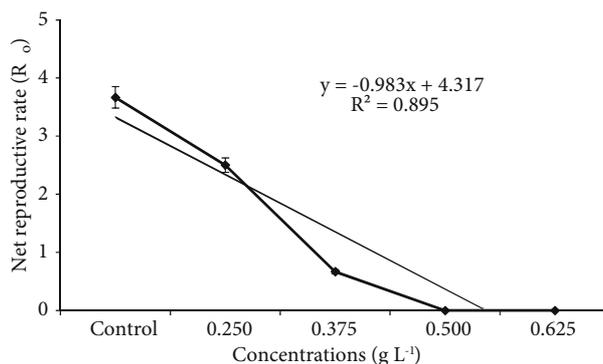
Figure 2. Survivorship curves of *S. vetulus* under different salt concentrations (with regression equations) in 8-day experiments (n = 6; ±standard error; P < 0.05).

A small increase in salinity appears to be clearly deleterious for indigenous cladocerans and it is not surprising that, under saline stress, freshwater ecosystems quickly shift to a more halotolerant assemblage (Cowgill, 1987; Nielsen, 2003).

Total progeny and net reproductive rates of the 2 species are seen in Figures 3–6. No significant differences

Table 1. Summary of 1-way ANOVAs applied to the life-history parameters of *S. mucronata* and *S. vetulus* (df, degrees of freedom; Sig, significance, $P < 0.05$).

| Parameters | <i>S. mucronata</i> | | <i>S. vetulus</i> | | | |
|-----------------------|---------------------|------|-------------------|------|------|-------|
| | F | df | Sig | F | df | Sig |
| Survivorship | 15.36 | 4.00 | 0.000 | 6.08 | 4.00 | 0.001 |
| Total progeny | 1.38 | 4.00 | 0.259 | 2.07 | 4.00 | 0.102 |
| Net reproductive rate | 2.72 | 4.00 | 0.043 | 3.90 | 4.00 | 0.009 |
| Growth rate | 3.37 | 4.00 | 0.018 | 5.91 | 4.00 | 0.001 |

**Figure 3.** Total progeny of *S. mucronata* under different salt concentrations (with regression equations) in 8-day experiments (with 95% confidence interval).**Figure 5.** Net reproductive rate (R_0) of *S. mucronata* under different salt concentrations (with regression equations) in 8-day experiments (with 95% confidence interval).**Figure 4.** Total progeny of *S. vetulus* under different salt concentrations (with regression equations) in 8-day experiments (with 95% confidence interval).**Figure 6.** Net reproductive rate (R_0) of *S. vetulus* under different salt concentrations (with regression equations) in 8-day experiments (with 95% confidence interval).

in total progeny were observed between the 2 populations of species during in situ experimentation ($P > 0.05$, Tukey's post hoc test, Table 1; Figures 3 and 4). Table 1 illustrates changes in reproductive rates based on within-group differences related to the concentration ($P < 0.05$). Reproductive rate values decreased due to the increase in salinity. According to the results of Tukey's post hoc test, multiple comparisons, especially ones among control, 0.500 g L⁻¹, and 0.625 g L⁻¹ groups, showed differences

($P < 0.05$; Figures 5 and 6). In the control and 0.250 g L⁻¹ concentration groups, the populations of *S. mucronata* and *S. vetulus* grew exponentially ($R_0 > 1$). On the other hand, in the 0.375 g L⁻¹ concentration group, populations decreased (Figures 7 and 8). An independent-samples t-test was conducted to compare each concentration between *S. mucronata* and *S. vetulus*. There were no significant differences in the score for groups of both species. No significant differences in reproduction were

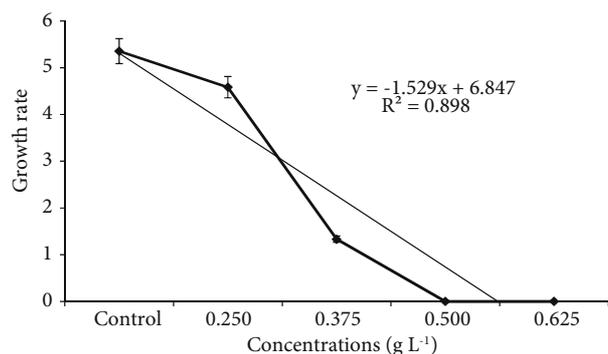


Figure 7. Growth rate of *S. mucronata* under different salt concentrations (with regression equations) in 8-day experiments (with 95% confidence interval).

monitored between the 2 populations of each species in the laboratory during the experimentation. On the other hand, *S. vetulus* had a lower tolerance. The amount of toxicant that members of Daphniidae can withstand varies depending on the size of the species. There was an inverse relation between body size of zooplankton and their numerical abundances when cultured under similar test conditions. Although morphometric parameters of *S. vetulus* were not measured, it has a smaller body size than *S. mucronata*. Smaller taxa are generally numerically more abundant than larger species (Cowgill, 1987; Sarma et al., 2005). According to Ranta (1979) and Black and Dodson (1990), *Daphnia magna*, the largest daphnid, can resist high salinity levels, as it was one of the most resistant among cladocerans to salinity increase. However, for the smaller cladocerans, LC₅₀ values for NaCl were much lower (Mount et al., 1997). These results suggest that net reproductive rate is affected by different salinity conditions, but both species demonstrated the same reproductive capacity.

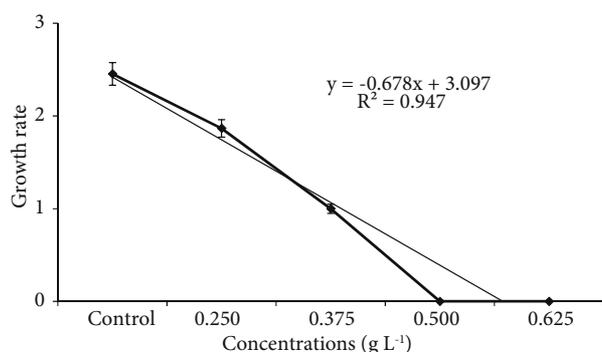


Figure 8. Growth rate of *S. vetulus* under different salt concentrations (with regression equations) in 8-day experiments (with 95% confidence interval).

Exposure to the control and salinities of 0.250 g L⁻¹ and 0.375 g L⁻¹ did not significantly affect growth rates in *S. mucronata*. Nevertheless, growth rates were decreased in the 0.500 g L⁻¹ and 0.625 g L⁻¹ treatments ($P = 0.018$, $F = 3.37$, Table 1, Figure 7). The other species was not affected by 0.250 g L⁻¹ salinity. There were significant differences between the control and 0.375 g L⁻¹, 0.500 g L⁻¹, and 0.625 g L⁻¹ treatments ($P < 0.001$, $F = 5.91$, Table 1, Figure 8). These results revealed that the growth rate of *S. vetulus* showed a strong reduction at salinity concentrations higher than 0.250 g L⁻¹ and it was more sensitive than *S. mucronata*.

Life-history parameters can also be affected by an increase in salinity, as demonstrated in this study. In this study, the most drastic reduction caused by NaCl in life history properties was observed in the number of neonates. Furthermore, the growth rates of both species were negatively affected, as well, and maturation was delayed at the highest concentrations. Salinity decreased survival, growth, and reproduction at salinity levels of 0.375 g L⁻¹ for *S. mucronata* and of 0.250 g L⁻¹ for *S. vetulus*.

3.2. Allometric changes in *S. mucronata*

The morphometric parameters of BL, BW, SL, and OL were measured in *S. mucronata*. In terms of tolerance to salinity, however, we did not find any significant relation between ovum size and salinity concentration ($P > 0.05$, Tukey's post hoc test; Table 2; Figure 9). In particular, in the measurements of BL, BW, SL, there were significant differences between the control, 0.250, and 0.375 g L⁻¹ dose groups and the 0.500 and 0.625 g L⁻¹ dose groups that were determined using post hoc analysis ($P < 0.05$, ANOVA; Table 2). In this case, concentration applications above the LC₅₀ showed differences in morphology (Figure 9). In addition, with the increase in concentrations an increase in BL and BW was observed. These results suggest that changes in the body structure of zooplankton are caused by increases in salinity (Bruçet et al., 2010).

Based on such empirical toxicity data, the community- and population-level effects of pollutants can be linked to the lethal or sublethal responses of individual species. A lower salinity of 0.250 g L⁻¹ and 0.375 g L⁻¹ (LC₅₀ values of *S.*

Table 2. Summary of 1-way ANOVAs applied to the body morphology parameters of *S. mucronata* (df, degrees of freedom; Sig, significance, $P < 0.05$).

| Parameters | F | df | Sig |
|--------------|-------|------|-------|
| Body length | 35.11 | 4.00 | 0.001 |
| Body width | 35.36 | 4.00 | 0.001 |
| Spine length | 25.92 | 4.00 | 0.001 |
| Ovum size | 0.51 | 4.00 | 0.609 |

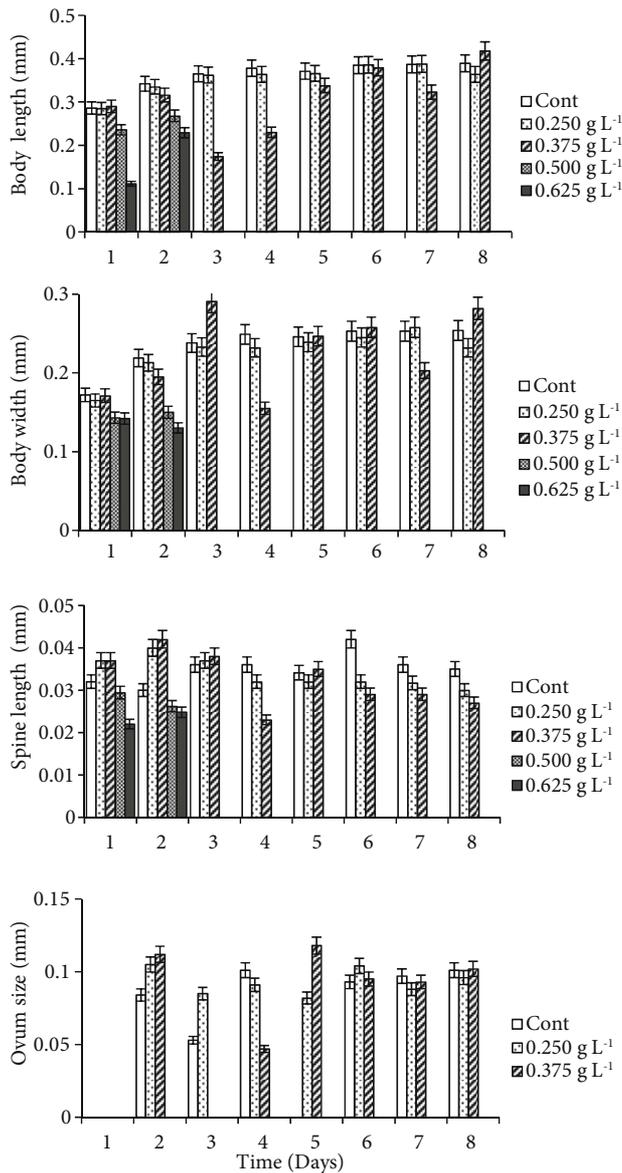


Figure 9. Body morphology parameters curves of *S. mucronata* for the various salt concentrations (\pm standard error) due to time (with 95% confidence interval).

vetulus and *S. mucronata*, respectively) had no significant effect on survival, growth, or reproduction, but above a certain threshold salinity level, physiological condition declined rapidly. Lopes et al. (2011) reported that reproduction capacity was high in *S. vetulus* and survival rates of *S. vetulus* were above 81%. Sarma et al. (2006) reported that *S. vetulus* survived and reproduced at 2 g L⁻¹.

On the other hand, in our experimental results, survival rates were lower at different concentrations. A salinity of 0.250 g L⁻¹ or lower had no significant effect on survival, growth, or reproduction, but above a certain threshold of salinity, physiological condition declined rapidly. In general, freshwater biota do not extend into slightly saline water. Therefore, this population variation pattern is shaped by locally acting selective forces (in this case, salinity). Local selective forces play an important role in genetic structure. As a result, even minor salinity increases in freshwaters may cause fine impacts in animal populations, such as the appearance of locally selected races or ecotypes (Loureiro et al., 2012). These data suggest that *S. mucronata* and *S. vetulus* have low tolerance, regardless of their habitat of origin.

Consequently, as salinity increases, the species richness and growth of freshwater biota reduce (Nielsen et al., 2003). Under stressful conditions, including salinity, the growth rates of freshwater zooplankton can be negative. Thus, there is a general acceptance that freshwater ecosystems undergo some ecological stress when subjected to salinities due to increasing climatic temperature. The native species grow under low salinity concentrations that do not support major changes that would promote their growth in freshwater. Organisms living in systems with high NaCl concentrations had to adapt themselves in order to maintain an intracellular concentration of Na lower than toxic values. Thus, an increase in salt levels in freshwater ecosystems affects the dynamics and abundance of rotifers and cladocerans (Schallenberg et al., 2003; Sarma et al., 2006). In addition, both of these groups are generally very sensitive to salinity under stressful conditions (Dodson and Frey, 2001).

In conclusion, salinity significantly influenced the growth, survival, and reproduction rates of *S. vetulus* and *S. mucronata*; 0.250 and 0.375 g L⁻¹ were the optimal salinity levels. Survival rates of both species, which lived in the same salinity during the study, differed with different salinities.

Despite the possibility that different rates of change in salinity might affect the salinity tolerance threshold of *S. vetulus* and *S. mucronata*, our results suggest that potential changes in lake water level and temperature caused by climate change will impose stress on the population and may possibly remove these species from lakes. The effects of temperature and salinity on other life stages of *S. vetulus* and *S. mucronata* could modify this process, however, and merit further study.

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