Research in Zoology 2014, 4(1): 8-12
DOI: 10.5923/j.zoology.20140401.02

Acute Effects of NaCl and Na_2SO_4 on *Daphnia menucoensis* Paggi, 1996 and *Moina eugeniae* Olivier, 1954 (Crustacea, Cladocera)

Cabrera Gabriela*, Vignatti Alicia, Echaniz Santiago

Facultad de Ciencias Exactas y Naturales. Universidad Nacional de La Pampa. Avda. Uruguay 151. Código Postal 6300. Santa Rosa, La Pampa

Abstract Despite the fact that halophilic cladocerans are relatively scarce, the neotropical endemic species *Daphnia menucoensis* and *Moina eugeniae* are frequent in temporary saline lakes of the semiarid centre and the north of Patagonia (Argentina). Although aspects of its biology in natural conditions are already known, there is a lack of ecophysiological information to help explain its geographical and temporary distribution. Considering that Cl^- and SO_4^{2-} are the predominant anions in La Pampa lakes, the aim of this study was to determine the effects of increasing NaCl and Na_2SO_4 concentration on neonates of both species, using acute bioassays, and to compare the results with previous information. The medium was prepared using demineralized water and different concentrations of pure salts of NaCl and Na_2SO_4 of analytical quality as well as a mixture of both salts; six concentrations for *D. menucoensis* (0.5; 1; 5; 10; 15 and 20 $g.L^{-1}$) and four for *M. eugeniae* (1; 5; 10 and 15 $g.L^{-1}$). Four replicates were performed, with five neonates per treatment. The bioassays took 48 h, without food or renewal of the medium. The survival of *D. menucoensis* was virtually zero in treatments with 15 or 20 $g.L^{-1}$, but survival was higher at lower concentrations. Survival in treatments with salt mixtures differed, but was much lower at 10 $g.L^{-1}$. The survival of *M. eugeniae* was zero in all treatments of 10 $g.L^{-1}$. In previous bioassays with salts obtained from the natural environment, the tolerance of the neonates of both species was greater. *D. menucoensis* survived up to 24 $g.L^{-1}$, while *M. eugeniae* survived up to 31 $g.L^{-1}$. The lower tolerance observed in this study might be because analytical-grade salts were used. The absence of other electrolytes that are necessary to maintain a relatively stable internal environment of the neonates might explain the limited survival at concentrations higher than 10 $g.L^{-1}$.

Keywords Bioassays, *Daphnia menucoensis*, *Moina eugeniae*, Halophyllic cladocerans, Salinity tolerance

1. Introduction

Many temporary shallow lakes of relatively high salinity exist in Argentina, especially in the semi-arid central region and in northern Patagonia. These ecosystems are characterized by associations of cladocerans and copepods, with some different elements to ecosystems in other continents, due to the presence of many endemic species with their own biogeographical patterns[1, 2, 3, 4, 5, 6, 7].

Since salinity is one of the dimensions of the ecological niche that strongly affects the distribution of organisms, the Cladocera are relatively scarce in saline lakes[8, 9]. However, the halophilous species *Daphnia menucoensis* and *Moina eugeniae* are frequent and have a wide distribution in aquatic environments of central Argentina[1, 10, 11].

Daphnia species are rare in saline ecosystems[12, 13, 14] because it was originated in freshwater where it has its major distribution[15, 16]. The frequent presence of *D. menucoensis* distinguishes between high salinity ecosystems in Argentina and those at different latitudes[17, 18].

Currently, experimental information exists concerning the negative effects of salinity on different population parameters of some zooplanktonic cladoceran species of other latitudes, such as *Daphnia magna*[9, 19, 20], *Daphnia carinata*[21], *Moina hutchinsoni*[22], *Alona rectangula*, *Ceriodaphnia dubia*, *Daphnia pulex*, *Moina macrocopa* and *Simocephalus vetulus*[23], *Daphnia exilis*[24] and *Daphniopsis australis*[25]. However, although some aspects of the biology in natural conditions are already known for the two Argentinean species[10, 11, 26], bioassays to determine salinity tolerance have only been performed recently[27]. Therefore, there is lack of information concerning other relevant ecophysiological aspects that explain their geographical and temporal distribution, such as the effects of different concentrations of Cl^- and SO_4^{2-} anions. This is because in most hypersaline and mesosaline lakes in La Pampa, where *D. menucoensis* and *M. eugeniae* occur, NaCl

* Corresponding author:
gabrielacabrera@exactas.unlpam.edu.ar (Cabrera Gabriela)
Published online at http://journal.sapub.org/zoology

and Na_2SO_4 predominate. It was also determined that the ratio between Cl^- and SO_4^{2-} is very variable[28]. Therefore, the objective of this study was to determine the effects of increasing concentrations of NaCl, Na_2SO_4 and a mixture of both salts in the same ratio, on *D. menucoensis* and *M. eugeniae* neonates using laboratory bioassays, and to compare the results with information obtained in previous studies.

2. Materials and Method

Acute bioassays were carried out, considering immobility or death of neonates as indicators of the effect of the anions.

To obtain organisms to carry out the bioassays, sediment from the dried bottom of a saline lake was collected in La Pampa province. *D. menucoensis* and *M. eugeniae* generally integrate the zooplankton of this lake during the hydrophases. The sediment was placed in a 300 L water tank outdoors. The physiochemical characteristics of the water were similar to those of the original lake when the species were recorded. Once both populations had developed and established from ephippia of the 'egg bank', parthenogenetic females were removed and were separately acclimatised in the laboratory in two 20 L aquaria for 90 days. Neonates, (younger than 24 h), which were born from the selected females, were used for the assays.

The experimental medium was prepared using demineralized water to which reagents of analytical grade were added. In bioassays with *D. menucoensis*, three series with six concentrations: 0.5; 1; 5; 10; 15 and 20 $g.L^{-1}$ of NaCl, Na_2SO_4 or mixtures of both salts in the same ratio were prepared (a total of 18 treatments). For *M. eugeniae*, three series with four concentrations: 1; 5; 10 or 15 $g.L^{-1}$ NaCl, Na_2SO_4 and a mixture of both salts were prepared (a total of 12 treatments). Four replicates per treatment were performed, comprising five neonates each. The bioassays were carried out in 20 mL glass tubes and lasted for 48 h without food or renewal of the medium. Neonates were exposed to a photoperiod of 8:16 h darkness:light. The lighting was provided by two 18 Watt fluorescent tubes and the temperature remained constant at 22 ± 1°C. For *D. menucoensis*, the observations were performed every 12 h and for *Moina eugeniae,* every 24 h.

To determine differences between the treatments, the Kruskal Wallis non-parametric test[29, 30] and the Mann-Whitney test[31] were performed.

3. Results

Daphnia menucoensis

In NaCl bioassays, neonate survival at 48 h differed significantly ($H = 21.36$; $p = 0.0079$) and was almost complete at 10 $g.L^{-1}$ but was almost zero at the two higher concentrations, since only one individual survived at 15 $g.L^{-1}$ (Figure 1A). Although the number of survivors gradually decreased at 15 $g.L^{-1}$, the decrease was abrupt in the 20 $g.L^{-1}$ treatment and no survivors were observed after 12 h.

Neonate survival in treatments with Na_2SO_4 was also significantly different ($H = 21.77$; $p = 0.0036$) (Figure 1B). As for NaCl, survival was relatively similar for treatments up to 10 $g.L^{-1}$ Na_2SO_4, although it was higher than that at the two higher concentrations. In contrast to the NaCl treatment, although survival was zero after 48 h at the highest concentration, the mortality did not decrease abruptly (Figure 1B).

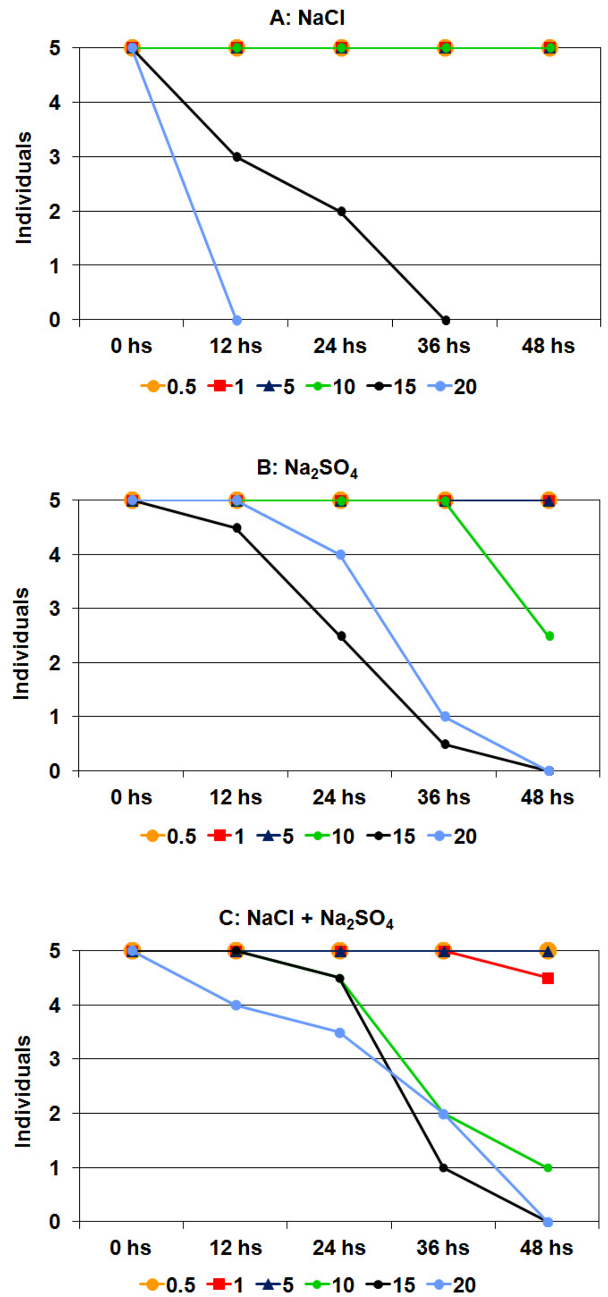

Figure 1. Results of bioassays carried out with *Daphnia menucoensis* at different concentrations of individual salts and mixtures. The me-dian of the four replicates is shown

The situation was different in bioassays with a mixture of salts (Figure 1C). The 0.5, 1 and 5 $g.L^{-1}$ treatments had a similar effect on neonate survival, but survival was significantly lower ($H = 20.9$; $p = 0.0022$) at concentrations of 10, 15 and 20 $g.L^{-1}$. In contrast to treatments with single salts, neonate survival was low in at a 10 $g.L^{-1}$ salt mixture (Figure 1C).

When the results of the different concentrations were compared (Figure 2), although the survival was slightly lower in bioassays with the mixture of salts, significant differences were only found in 10 $g.L^{-1}$ treatments ($H = 9.16$; $p = 0.0146$). In the treatments carried out with NaCl and Na_2SO_4 individually, the median of the survival was 5 and 2.5 neonates after 48 h, whereas in the mixture, it only was 1.

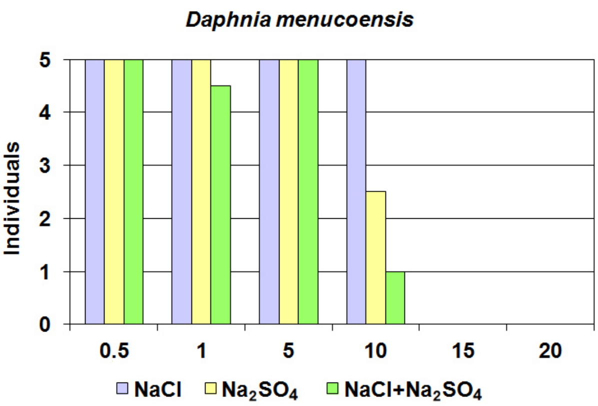

Figure 2. Comparison of bioassays results for *Daphnia menucoensis* at different salt concentrations. The bars indicate the medians

Moina eugeniae

In NaCl bioassays, the neonate survival after 48 h differed ($H = 14.12$; $p = 0.0087$). Survival was very high at concentrations of 1 and 5 $g.L^{-1}$ but was zero at 10 and 15 $g.L^{-1}$ (Figure 3A). At the two highest concentrations, complete mortality was registered at 24 h (Fig. 3A).

Similar results were obtained from bioassays carried out with Na_2SO_4. Treatments performed at concentrations of 1 and 5 $g.L^{-1}$, where survival was almost total, significantly differed ($H = 14.33$; $p = 0.0098$) from the survival at a concentration of 10 and 15 $g.L^{-1}$, where there were no survivors after 48 h (Figure 3B). However, living neonates were observed after 24 h in bioassays carried out with 10 $g.L^{-1}$ Na_2SO_4.

Bioassays with the salt mixture were not different between them. Nonetheless, the survival was much lower than with NaCl and Na_2SO_4 individually (Figure 3C).

Considering the different concentrations separately, significant differences were only found for bioassays at a concentration of 5 $g.L^{-1}$ of the salt mixture ($H = 10.67$; $p = 0.0249$), since all neonates survived with NaCl and Na_2SO_4, but only one individual survived in the mixture (Figure 4). At the two highest concentrations (10 and 15 $g.L^{-1}$), survival at 48 hours was zero.

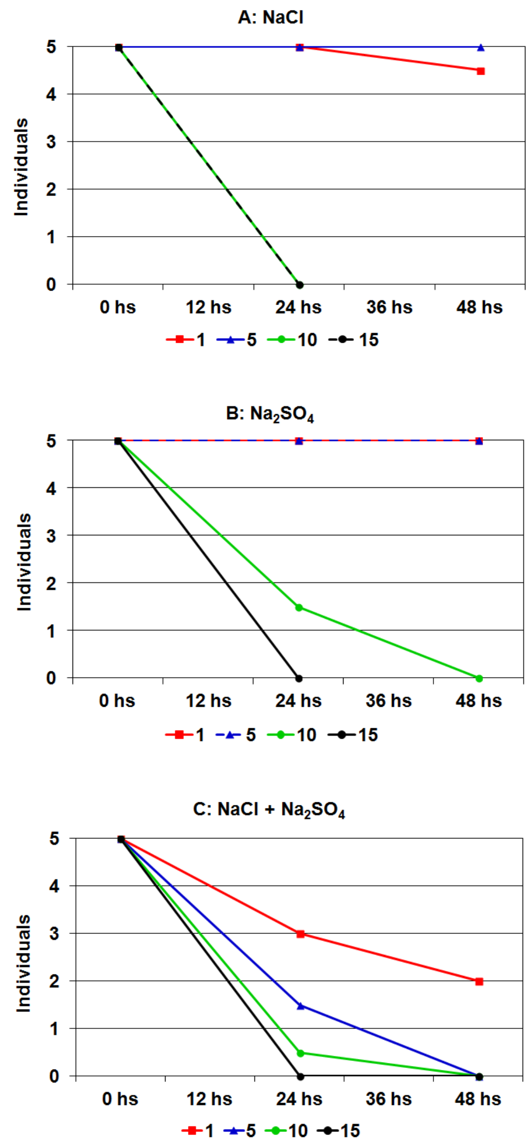

Figure 3. Results of bioassays carried out with *Moina eugeniae* and different salt concentrations. The median of four replicates is shown

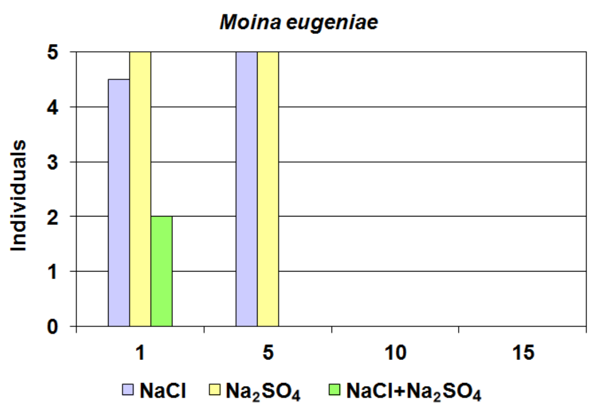

Figure 4. Comparison of bioassays carried out with *Moina eugeniae* at different salt concentrations. The bars indicate the medians

4. Discussion

The bioassays in this study confirmed that the neonate survival of *D. menucoensis* and *M. eugeniae* was very limited after 48 h. The data were different to those of Vignatti *et al*. 2012[27], in the unique laboratory study done to date to determine the salinity tolerance in neonates of both species. These authors using a similar bioassay, but using salts obtained from the natural environment. In that case, precipitated salts were collected and sterilized during the drying of a temporary lake in which the presence of both species was recorded during the previous hydrophase. In those bioassays, *D. menucoensis* survival at a concentration up to 24 $g.L^{-1}$ was observed, and the difference was even more apparent for *M. eugeniae*, whose neonates survived up to 31 $g.L^{-1}$. On the other hand, the low tolerance of both species to relatively high salt concentrations in the present study, contrasts with results obtained in field studies. In a study that included ten shallow lakes in La Pampa province, *D. menucoensis* was recorded in a salinity range between 9.64 and 29.70 $g.L^{-1}$, whereas *M. eugeniae* was found between 11.20 and 35.82 $g.L^{-1}$[28].

However, similar to results of bioassays carried out by Vignatti *et al*. 2012[27], this study showed that both species tolerate a much lower concentration of solutes than in nature. Neonates of both species survived at concentrations lower than 1 $g.L^{-1}$ (or ever lower for *D. menucoensis*), whereas in natural conditions in water bodies from La Pampa, individuals were only recorded when the salinity was above 7 $g.L^{-1}$ (*M. eugeniae*) or 5 $g.L^{-1}$ (*D. menucoensis*)[10, 11, 26, 32, 33, 34]. The absence of both cladocerans in low salinity water bodies might be due to biotic interactions that might be more important in environments with low stress than physiochemical factors in community structuring[35]. Although they are adapted to low salinity, *M. eugeniae* and *D. menucoensis* would not be successful in competition for resources with other species, such as *Moina micrura* and *Daphnia spinulata*, two frequent cladocerans in water bodies from La Pampa province, which are present at salinities lower than 5–7 $g.L^{-1}$[27, 32].

A discussion has existed concerning the convenience of developing bioassays with pure salts as a salinity source, especially NaCl, with organisms that come from water bodies with different ionic composition[36]. The limitations of the results would be higher especially when dealing with organisms whose origin is not marine[23], like the cladocerans[7, 37]. In comparison with previous bioassays, the lower tolerance range determined in this study might be because the salts used for medium preparation were of analytical grade. Therefore, the absence of other necessary electrolytes to maintain the stability of the internal medium of the neonates, might explain the limited survival at concentrations higher than 10 $g.L^{-1}$ NaCl or Na_2SO_4 or the mixture of both salts. The results also show that the information obtained from using pure salts does not necessarily depict the salinity ranges that the studied species tolerate in natural conditions.

ACKNOWLEDGEMENTS

To the Facultad de Ciencias Exactas y Naturales de la Universidad Nacional de La Pampa for the financial support.

REFERENCES

[1] Paggi, J., 1998. Cladocera (Anomopoda y Ctenopoda). Pp. 507-518. En: S. Coscarón and J. J. Morrone (eds), Biodiversidad de Artrópodos Argentinos. Ediciones Sur, La Plata.

[2] Battistoni, P. A., 1998. Capítulo 51: "Copepoda”. Pp. 519-530. En: S. Coscarón and J. J. Morrone (eds), Biodiversidad de Artrópodos Argentinos. Ediciones Sur, La Plata.

[3] Menu-Marque, S. and C. Locascio de Mitrovich, 1998. Distribución geográfica de las especies del género *Boeckella* (Copepoda, Calanoida, Centropagidae) en la República Argentina. Physis B 56: 1 - 10.

[4] Menu-Marque, S., J. Morrone and C. Locascio de Mitrovich, 2000. Distributional patterns of the south american species of *Boeckella* (Copepoda: Centropagidae): a track analysis. Journal of Crustacean Biology 20 (2): 262 - 272.

[5] Adamowicz, S., P. Hebert and M. C. Marinone, 2004. Species diversity and endemism in the *Daphnia* of Argentina: a genetic investigation. Zoological Journal of the Linnean Society (140): 171 – 205.

[6] Boxshall, G. and D. Defaye, 2008. Global diversity of copepods (Crustacea: Copepoda) in freshwater. Hydrobiologia 595:195 - 207.

[7] Forró, L., N. Korovchinsky, A. Kotov and A. Petrusek, 2008. Global diversity of cladocerans (Cladocera; Crustacea) in freshwater. Hydrobiologia 595: 177 - 184.

[8] Hobæk, A., M. Manca and T. Andersen, 2002. Factors influencing species richness in lacustrine zooplankton. Acta Oecologica 23: 155 - 163.

[9] Ghazy, M., M. Habashy, F. Kossa and E. Mohammady, 2009. Effects of Salinity on Survival, Growth and Reproduction of the Water Flea, *Daphnia magna*. Nature and Science 7(11): 28-42.

[10] Echaniz, S., A. Vignatti, S. José de Paggi, J. Paggi and A. Pilati, 2006. Zooplankton seasonal abundance of south american saline shallow lakes. International Review of Hydrobiology (91): 86 - 100.

[11] Vignatti, A., S. Echaniz and M. Martín, 2007. El zooplancton de lagos someros de diferente salinidad y estado trófico en la región semiárida pampeana (La Pampa, Argentina). Gayana 71 (1): 38 - 48.

[12] Jeppesen, E., M. Sondergaard, E. Kanstrup, B. Petersen, R. B. Eriksen, M. Hammershoj, E. Mortensen, J. P. Jensen and A. Have, 1994. Does the impact of nutrients on the biological structure and function of brackish and freshwater lakes differ? Hydrobiologia 275/276: 15-30.

[13] Scheffer, M., 1998. Ecology of shallow lakes. Chapman and Hall, London. 357 pp.

[14] Jeppesen, E., M. Søndergaard, A. Pedersen, K. Jürgens, A. Strzelczak, T. Lauridsen, and L. Johansson, 2007. Salinity Induced Regime Shift in Shallow Brackish Lagoons. Ecosystems (10): 47 - 57.

[15] Peters, R. H., 1987. Metabolism in *Daphnia*. Memorie dell´Instituto Italiano di Idrobiologia 45:193–243.

[16] Teschner M., 1995. Effects of salinity on the life history and fitness of *Daphnia magna*: variability within and between populations. Hydrobiologia 307: 33–41.

[17] Vignatti, A. M., J. C. Paggi, S. A. Echaniz and G. C. Cabrera, 2012. Zooplankton diversity and relationship with environmental changes after the filling of a temporary saline lakes in the semi-arid region of La Pampa (Argentina). Latin American Journal of Aquatic Research 40(4): 1005-1016 http://www.lajar.cl/pdf/imar/v40n4/Articulo_40_4_16.pdf (ISSN 0718-560X).

[18] Echaniz, S. A., G. C. Cabrera, P. L. Aliaga and A. M. Vignatti, 2013. Variation in zooplankton and limnological parameters in a saline lake of La Pampa, Central Argentine, during an annual cycle. International Journal of Ecosystem 3(4): 72-81. http://article.sapub.org/pdf/10.5923.j.ije.20130304.03.pdf (p-ISSN 2165-8889 e-ISSN 2165-8919).

[19] Mitchell S. E. and W. Lampert, 2000. Temperature adaptation in a geographically widespread zooplankter, *Daphnia magna*. Journal of Evolutionary Biology 13: 371-382

[20] Martínez-Jerónimo F. and L. Martínez-Jerónimo, 2007. Chronic effect of NaCl salinity on a freshwater strain of *Daphnia magna* Straus (Crustacea: Cladocera): A demographic study. Ecotoxicology and Environmental Safety 67 411–416.

[21] Hall, C. and C. Burns, 2002. Mortality and growth responses of *Daphnia carinata* to increases in temperature and salinity. Freshwater Biology 47: 451- 458.

[22] Martínez-Jerónimo, F. and F. Espinosa-Chávez, 2005. Notes on the reproduction and survival of *Moina hutchinsoni* Brehm, 1937 (Moinidae: Anomopoda) grown in media of varying salinity. Aquatic Ecology 39: 113 – 118.

[23] Sarma, S. S., E. S. Nandini, J. Morales-Ventura, I. Delgado-Martínez and L. González-Valverde, 2006. Effects of NaCl salinity on the population dynamics of freshwater zooplankton (rotifers and cladocerans). Aquatic Ecology 40:349–360.

[24] Heine-Fuster, I., C. Vega Retter, P. Sabat and R. Ramos Jiliberto, 2010. Osmoregulatory and demographic responses to salinity of the exotic cladoceran *Daphnia exilis*. Journal of Plankton Research 32 (10): 1405-1411.

[25] Ismail, H. N., J. G. Qin and L. Seuront, 2011. Regulation of life history in the brackish cladoceran, *Daphniopsis australis* (Sergeev and Williams, 1985) by temperature and salinity. Journal of Plankton Research 33 (5): 763–777.

[26] Echaniz, S., A. Vignatti, S. B. José de Paggi, J. C. Paggi, and G. Cabrera, 2010. El modelo de estados alternativos de lagos someros en La Pampa: comparación de Bajo de Giuliani y El Carancho. Libro de Trabajos del 3° Congreso Pampeano del Agua 45-53. Accesible en: http://www.lapampa.gov.ar/publicacionesrechid/24625-libro-del-3er-congreso-pampeano-del-agua-ano-2010.html. Accessed: 26-03-2013.

[27] Vignatti, A. M., J. C. Paggi, S. A. Echaniz and G. C. Cabrera, 2012. Tolerancia a la salinidad de dos cladóceros halófilos autóctonos: *Daphnia menucoensis* y *Moina eugeniae* (Artropoda, Crustacea). Biología Acuática 27: 219-231.

[28] Echaniz S., 2010. Composición y abundancia del zooplancton en lagunas de diferente composición iónica de la provincia de La Pampa. Trabajo de Tesis Doctoral. Universidad Nacional de Río Cuarto, Facultad de Ciencias Físico Químicas y Naturales.

[29] Sokal, R. and F. Rohlf, 1995. Biometría. Principios y métodos estadísticos en la investigación biológica. Ed. Blume, Barcelona.

[30] Zar, J. H., 1996. Biostatistical analysis. 3º Ed. Prentice Hall, New Jersey.

[31] Hammer, Ø., D. Harper, and P. Ryan, 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1): 1 – 9.

[32] Echaniz, S. and A. Vignatti, 1996. Cladóceros limnéticos de la provincia de La Pampa (Argentina). Revista de la Facultad de Agronomía. UNLPam 9 (1): 65 - 80.

[33] Echaniz, S. and A. Vignatti, 2002. Variación anual de la taxocenosis de cladóceros planctónicos (Crustacea: Branchiopoda) de una laguna de elevada salinidad (La Pampa, Argentina). Neotrópica 48: 11 - 17.

[34] Vignatti, A. and S. Echaniz, 1999. Presencia de *Daphnia* (*Ctenodaphnia*) *menucoensis* Paggi, 1996 en la provincia de La Pampa (Argentina). Revista de la Facultad de. Agronomía UNLPam 10 (1): 21 - 27.

[35] Herbst, D., 2001. Gradients of salinity stress, environmental stability and water chemistry as a templet for defining habitat types and physiological strategies in inland salt waters. Hydrobiologia 466: 209 - 219.

[36] Kefford B. J, C. G. Palmer, L. Pakhomova and D. Nugegoda, 2004. Comparing test systems to measure the salinity tolerance of freshwater invertebrates. Water SA 30:499–506

[37] Fritsch, M., O. Bininda-Emonds and S. Richter, 2013. Unraveling the origin of Cladocera by identifying heterochrony in the developmental sequences of Branchiopoda. Frontiers in Zoology 10:35. www.frontiersinzoology.com/content/ 10/1/35. Accessed: 11/14/2013.