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Biology of *Artemia persimilis* Piccinelli and Prosdocimi, 1968 (Crustacea: Anostraca) at the highest salinities reported for the species under natural conditions

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Abstract

Artemia is one of the few organisms that inhabit hypersaline lakes. In Argentina the genus is represented by two species: the introduced *A. franciscana* and the autochthonous *A. persimilis.* Since there are few data about the ecology of the latter in natural conditions, the aim of this paper is to present information on *A. persimilis* population density and structure in relation to environmental parameters in an Argentinean shallow hypersaline lake. Bimonthly samples were collected between October 2011 and August 2012, in Guatraché, a shallow lake located at the Southeast of La Pampa. Mean salinity reached 345.4 (±48.2) $g.L^{-1}$, ranging between 279 $g.L^{-1}$ and 418.5 $g.L^{-1}$. Mean phytoplankton chlorophyll-*a* concentration was 8.39 (±6.97) $mg.m^{-3}$. *A. persimilis* was recorded in all samples but not in April. Its mean density was 11.5 (±17.2 $ind.L^{-1}$, and no correlations were found between the densities and environmental variables. Nauplii predominated in most of the sampling occasions. Since the species was found at salinities greater than 400 $g.L^{-1}$, when the population was reproducing (males and females with eggs were observed), this finding constitutes a record that extends the tolerance range known for this species in a natural water body.

Key Words: *Artemia persimilis*, hypersaline lakes, salinity tolerance, Argentina

Introduction

Hypersaline lakes (Hammer, 1986) can only be inhabited by relatively few organisms with physiological mechanisms to withstand osmotic stress (Herbst, 2001; Gajardo and Beardmore, 2012). Among them is *Artemia*. This genus, widely spread throughout the world (Medina *et al*., 2007; Clegg and Gajardo 2009), has several species economically important as they are a common food source for aquaculture (Sorgeloos, 1986).

In Argentina this genus is represented by two bisexual species: *A. franciscana* Kellogg, 1906 and *A. persimilis* Piccinelli and Prosdocimi, 1968 (Ruiz *et al*., 2008). The first one extends up to 36°S and it was introduced from North America due to its higher productivity. The second one is autochthonous and it was recorded southward up to 37°S (Ruiz *et al*., 2008) in Argentina and Chile (Castro *et al*., 2006, De los Ríos, 2008, De los Ríos-Escalante, 2010).

In Argentina, some biological aspects of *A. persimilis* have been studied under laboratory conditions (Pastorino *et al*., 2002) to understand productivity related aspects (Sato *et al*., 2004; Mechaly *et al*., 2004, 2013; Medina *et al*., 2007). Unfortunately there are few data about its ecology in natural conditions (Browne and Wanigasekera, 2000) as this species is at risk of displacement by its cogeneric *A. franciscana*. It has been suggested that this second species has a great phenotypic plasticity (Browne and Wanigasekera, 2000; Medina *et al*., 2007; Clegg and Gajardo, 2009; Vikas *et al*., 2012), and could be considered an invasive species that eliminates native ones due to its higher adaptive capacity (Medina *et al*., 2007; Ruebhart *et al*., 2008).

Considering this, the aim of this paper is to present information on population density and population structure of *A. persimilis* in relation to environmental variables in a hypersaline lake located in central semiarid Argentina. We also report the presence of this species at the highest salt concentration recorded.

Materials and Methods

Study area

Guatraché (37º 45'S, 63º 33'W) is a large hypersaline lake located in a Provincial Reserve at the Southeast of the province of La Pampa (Fig. 1). It belongs to the phytogeographical province of the "Espinal" (Cabrera, 1976), a region characterized by the presence of the caldén (*Prosopis caldenia* Burkart) forest. The mean annual regional precipitation is 600 mm (Casagrande *et al*., 2006); with peaks in November (late spring) and March (late summer). Potential evapotranspiration is approximately 800 $mm.year^{-1}$ (Roberto *et al*., 1994). The lake is in a deep depression with the North side covered by natural vegetation but most of the South side is deforested and used for agriculture and extensive cattle grazing.

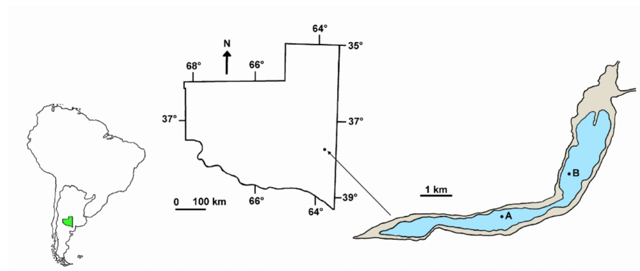

Fig. 1: Geographical location of Guatraché Lake, showing minimum (light blue) and maximum (grey) depths. A and B: Sampling sites

During the study, the lake had a maximum length and width of 8117.7 m and 1176.5 m respectively, the water surface area was 419.4 ha, and the maximum depth was 0.25 m. It is in an arheic basin, which loses water by evaporation or infiltration, and presents large level fluctuations depending on rainfall (range~0.25-2m), a temporary stream and to a lesser extent by a phreatic system.

Field and laboratory work

Samples were collected bimonthly between October 2011 and August 2012, at two stations located along the longest lake axis. Water temperature was measured with an alcohol thermometer and water transparency was measured with a 22 cm diameter Secchi disc.

Two quantitative samples of 20 L were taken at each site for zooplankton analysis. Because of the shallow depth, *Artemia* samples were taken with graduated containers that integrated the water column and filtered through a 40 micrometers pore size net. A qualitative sample was also taken. Water samples were collected and kept refrigerated until their analyses in the laboratory.

Salinity was determined applying the gravimetric method with drying at 104°C. The ionic composition was determined by standardized techniques (APHA, 1992). The pH was determined with a pH meter Corning® PS 15. Chlorophyll-*a* concentrations were measured with an Aquafluor Turner fluorometer after extractions with acetone (APHA, 1992; Arar and Collins, 1997).

Species was determined by using the descriptions of Cohen *et al.* (1998), Torrentera and Belk (2002) and Mura and Brecciaroli (2004). *Artemia persimilis* densities were determined with a Bogorov chambers under stereoscopic microscope. The stages were determined in Sedgwick Rafter chambers under optic microscope with 40-100X, using the criteria proposed for this species by Cohen *et al*. (1998).

In order to examine relationships between environmental factors and attributes of zooplankton, nonparametric correlation coefficients of Spearman (r_s) were calculated ($P \leq 0.05$).

Results

Water temperature ranged between 9.65°C (June) and 36.25°C (February) (Fig. 2). Mean salinity reached 345.4 (± 48.2) $g.L^{-1}$, ranging between279 $g.L^{-1}$ (December) and 418.5 $g.L^{-1}$ (February) (Fig. 3). The pH was slightly alkaline (7.7 ± 0.23) and remained relatively stable during the study period (Fig. 3).

Water ionic composition showed a predominance of Cl^- which represented almost 97% of the anions, followed by $SO_4^=$ that barely reached 2.7%. Among the cations, Na^+ reached 88.6%, followed by Ca^{++} whith 8.2%.

Water transparency was total as it was possible to see the bottom in all sampling occasions.

Mean phytoplankton chlorophyll-*a* concentration was 8.39 (± 6.97) $mg.m^{-3}$, with highest concentrations in autumn (April) and lowest during the coldest months (June to August) (Fig. 4). Correlation between chlorophyll and water temperature was not significant (r_s = 0.60; P = 0.2080).

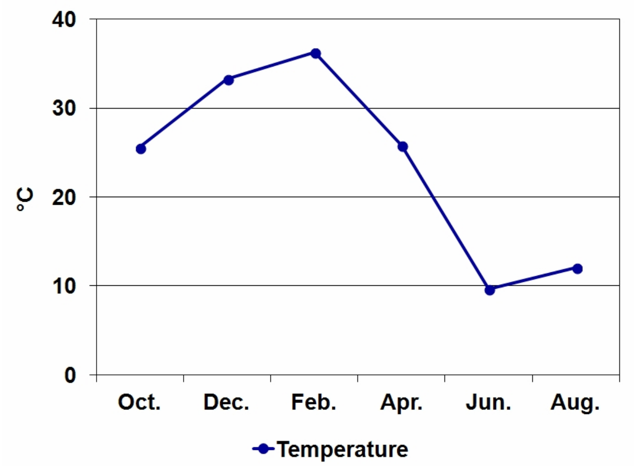

Fig. 2: Variation of water temperature in Guatraché Lake between October 2011 and August 2012.

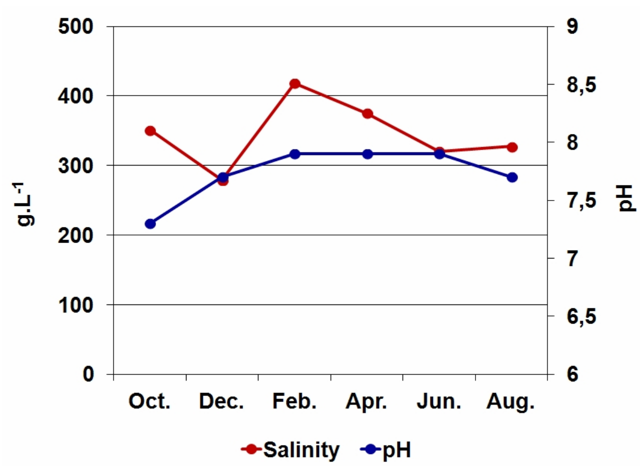

Fig. 3: Salinity and pH variation in Guatraché Lake between October 2011 and August 2012.

Artemia persimilis mean density was low throughout the study (11.5 ± 17.2 $ind.L^{-1}$), and it was not recorded in April. Its abundance declined toward the end of the study, so that in August there was a minimum of 2.72 $ind.L^{-1}$ (August). Maximum density reached 42.3 $ind.L^{-1}$ in December (Fig. 4). No correlations were found between the total population densities and any environmental variables (Table1).

Nauplii relative abundance predominated in most of the sampling occasions, with a minimum

Table 1: Results of non-parametric correlation analysis. r_s: Spearman correlation coefficient. *P*: level of significance.

	r_s	*P*
Water temperature	0,14	0,7872
pH	-0,03	0,9537
Salinity	-0,60	0,2080
Phytoplankton chlorophyll-*a*	-0,26	0,6228

density of 1 ind.L^{-1} in August, when they accounted for 37.8% of the population. Naupii were also as high as 3.7 ind.L^{-1} in June, when they accounted 67.6% of the total density (Fig. 5). The exception was verified in December, when postmetanauplii predominated with an abundance of 26.9 ind.L^{-1}, representing 63.5% of the population density. Except October; adults were recorded on all occasions. In all cases, females with eggs and males were found.

Discussion

Guatraché is a hypersaline lake (Hammer, 1986) with a dominance of sodium chloride. This characteristic is shared with most of the salt lakes of La Pampa, and especially with some extensive salt marshes of the same region under commercial exploitation, such as the Salinas Grandes of Hidalgo or Anzoategui (Cohen, 2012).

Although the lake can reach considerably deeper depths (periods when salinity is lower) during this study water levels did not exceed 0.4 m. Since water samples were taken during a dry period, salinity variations were affected by few precipitations. As salt concentration reduces heat capacity (Wetzel, 2001) it was not surprising to find large differences in water temperature between winter and summer (~27 ºC).

The fact that water transparency was total due to the low chlorophyll-*a* concentrations was not surprising either. In La Amarga Lake (66º07´W; 38º13´S), other hypersaline ecosystem of the province of La Pampa, deeper than Guatraché, Vignatti and Echaniz (unpublished data) also recorded very low phytoplankton chlorophyll-*a* concentrations and total water transparencies. However, water transparency distinguished Guatraché from other similarly deep hypersaline lakes studied in the province, as Utracán (64º36´W; 37º17´S) where water transparency reached 0.11 m, but chlorophyll-*a* concentrations were five times higher (Vignatti & Echaniz, unpublished data).

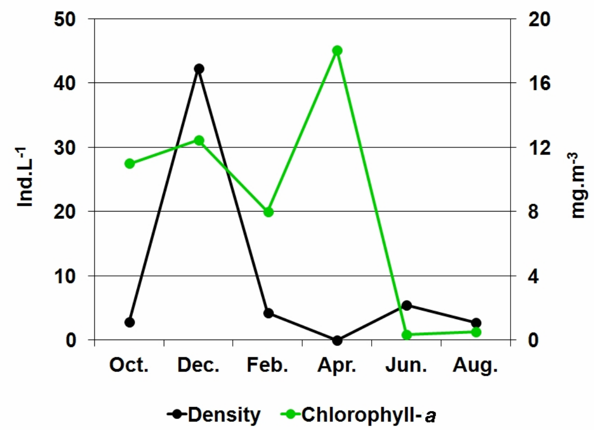

Figure 4: Chlorophyll-*a* concentration and total density of *Artemia persimilis* in Guatraché Lake between October 2011 and August 2012.

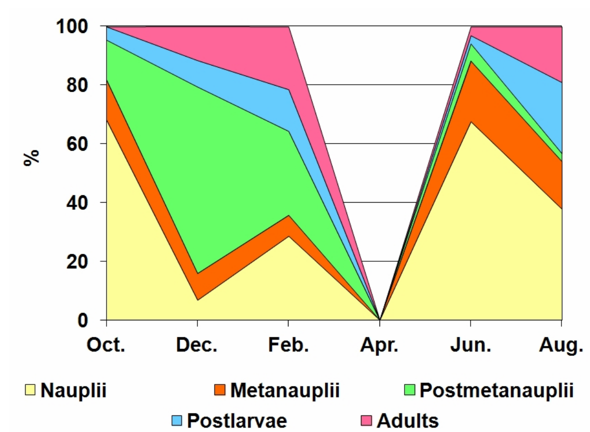

Figure 5: Percentage variation of *Artemia persimilis* population in Guatraché Lake between October 2011 and August 2012.

Mean *A. persimilis* density in Guatraché was in between La Amarga for 2007 (1.56 ind.L^{-1}) and Utracán for 2009-2010 (65.4 ind.L^{-1}) (Echaniz, unpublished data). This difference could be related to food availability as suggested by Castro Mejia *et al*. (2009). Echaniz *et al*. (unpublished data) found also that La Amarga, in spite of having a lower salinity than Guatraché, showed lower chlorophyll-*a* concentration (1.65 mg.m^{-3}), whereas Utracán had mean concentration of 44.7 mg.m^{-3}.

Many authors reported that *Artemia* can exist in several salt ponds with salinities close to the precipitation point of NaCl (340 g.L^{-1}) (Clegg and Trotman, 2002, Castro Mejía *et al*., 2013), but for reproduction to take place, salinity must be lower than that (Gajardo and Beardmore, 2012). *A. persimilis* was recorded in Guatraché Lake when dissolved solids concentration reached 418.5 g.L^{-1}. This finding constitutes a record of this species in the highest salinity for a natural water body, with the remarkable particularity that at this time, almost 29% of the population was composed of nauplii, and females with eggs and males were found; indicating that, at least until that time, the population was reproducing. However, in April, we did not record the presence of any organism (dead or alive). This may have been due to the reduction of reproduction by the increased environmental stress due to high salinity and temperature in February-March (the period before sampling; Fig. 2 and 3) (Herbst, 2001; Castro Mejía *et al*., 2009). Coincidently, in June, the population was recovering after a more benign period with less salinity and temperature (Fig. 2 and 3). The fact that the population was increasing and reproducing again is demonstrated with the high numbers of nauplii, which reached almost 68% of the total density, while adults barely reached 3% of the individuals collected.

Since in Guatraché *A. persimilis* was found with salinity greater than 400 g.L^{-1}, this study is the first contribution on the biology of this species in natural conditions that extends the tolerance range known for this species and probably also for the genus (Browne and Wanigasekera, 2000).

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