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-Short Communication-

Combined effects of salinity and temperature on survival of two autochthonous parthenogenetic populations of *Artemia* (Crustacea: Anostraca) in Portugal (Rio Maior and Aveiro saltworks)

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Abstract

The Iberian Peninsula comprises two native species, *Artemia salina* with bisexual reproduction and parthenogenetic population of *Artemia* with asexual reproduction; moreover, some populations were found with the presence of *Artemia franciscana*, native from the American continent. In Portugal there are only two places referenced with the presence of parthenogenetic *Artemia* species. The aim of this study was to estimate the combined effect of two temperatures (24ºC and 28ºC) and two salinities (70 ppt and 120 ppt) on the survival of these two parthenogenetic *Artemia* populations. In this study we found a different physiological response in the two populations caused by the stress of the combined effect of temperature and salinity. Aveiro population appears to be less sensitive when compared to Rio Maior population regarding to the increasing water temperature, but it has better survival rates when exposed to higher salinities. These results show that even within the same species, these two populations respond differently to different environmental conditions.

Key Words: *Artemia*, Parthenogenetic Population, Temperature, Salinity, Mortality, Portugal

Introduction

Artemia (Branchiopoda, Anostraca) is a crustacean that is distributed over all continents except Antarctica (Triantaphyllidis *et al*., 1998). There are nine reproductively isolated species described, with sexual and parthenogenetic reproduction (Browne & Bowen, 1991). The Iberian Peninsula comprises two native species, *Artemia salina* with bisexual reproduction and parthenogenetic population of *Artemia* with asexual reproduction; moreover, some populations were found with *Artemia franciscana* presence, native from the American continent (Amat *et al*., 1995). In the Iberian Peninsula almost all types of biotopes described and colonized by the *Artemia* genus are present, including coastal and inland saltworks, which differ in ionic composition (Lenz & Browne, 1991). One of the problems that the *Artemia* genus faces relates to the loss of biodiversity that has occurred in the Iberian saltworks due to introduction of exotic species of *Artemia* and loss of habitat (Amat *et al*., 2007). In Portugal there are only two places referenced with the presence of parthenogenetic populations of *Artemia*: the Rio Maior saltworks and the Aveiro saltworks (Amat *et al*., 2007), which are quite distinct in terms of the ionic composition of their water. The Rio Maior saline is supplied by a long and deep streak of rock salt while the Aveiro saline is supplied by sea water. The Rio Maior saline is a particular case in Portugal, and even in Europe. The occurrence of salt water about 30 km from the sea is a major characteristic of this saline. According to chemical analysis, the salinity of this salt water is much higher than seawater, around 150 ppt (Personal communication) at the well output that supplies the saltwork. In addition, the chemical composition is very different, namely the concentration of magnesium, which is much lower than in seawater (Calado & Brandão, 2009).

There are numerous studies on the effect of salinity and/or temperature on most of the populations of *Artemia* to understand/assess the greater or less capacity to physiologically respond to these factors (Vanhaecke *et al*., 1984; Abatzopoulos *et al*., 2003; Medina *et al*., 2007; Agh *et al*., 2008), thus giving bases to support the already known theory, that the parthenogenetic strains have a wide variation in response to physiological stress caused by the combined effect of temperature and salinity (Browne & Wanigasekera, 2000).

The aim of this study was to estimate the combined effect of different temperatures and salinities on the survival of these two strains, in order to understand the impact of different environmental stressors in the permanence of native Portuguese populations.

Materials and Methods

Artemia Sample

Cysts were collected from biotope Rio Maior and Aveiro saltworks.

Culture conditions

Solutions of different salinities (70 ppt and 120 ppt) were prepared using natural sea water and Tropic Marin Sea Salt. The two experimental temperatures (24ºC and 28ºC) were maintained using water baths and the temperatures were being confirmed by thermometer and maintained at ±1°C.

Survival experiments

Artemia were mass hatched from cysts in sea water (35 ppt) and 30 individuals were transferred into jars containing 100 ml of brine at 70 ppt and 120 ppt with ± 300000 cel.ml^{-1} of *Tetraselmis suecica.* Jars were placed at appropriate temperatures under 12h fluorescent lighting /12h darkness. For each combination (population, salinity, temperature) ten jars were established, the medium was renewed every two days and the mortality rate was evaluated at each change of medium.

Statistical analyses

Normality and homogeneity of variances of group distributions were first assessed using the Shapiro-Wilks and the Levene's tests, respectively. The arcsine transformation was applied to the data to fulfil the normality assumption. Data were then analysed by means of a three-way analysis of variance with interaction. Population, temperature, and salinity were taken as sources of variation. Because heterocedasticity was observed, when significant

differences were found the Games-Howell test was used to identify their origin. Statistically significant differences were accepted when P<0.05. All statistical analyses were carried out on IBM SPSS Statistics (V. 20).

Results and Discussion

A significant interaction between population and temperature was found (Tab. 1). The mortality was significantly higher at 28ºC, particularly in the population from Rio Maior for which the increase in mortality from 24ºC to 28ºC was greater than that recorded for the Aveiro population (Fig. 1). The Rio Maior saltwork is composed by tanks with much greater depth than those composing the Aveiro saltwork. This probably increases the average water temperature per year in the Aveiro saltwork as opposed to Rio Maior saltwork and consequently showed higher mortality rate in Rio Maior saltwork due to lack of adaptation to higher temperature.

Tab. 1: Results of a factorial ANOVA performed for 25-day mortality values of the populations from Rio Maior and Aveiro saltwork exposed to 4 temperature-salinity combinations.

Source of variation	*df*	*F*	*p*
Population	1.69	1.17	ns
Temperature	1.69	83.96	<0.001
Salinity	1.69	1.55	ns
Population x Temperature	1.69	6.08	0.016
Population x Salinity	1.69	83.96	<0.001
Temperature x Salinity	1.69	33.58	<0.001
Population x Temperature x Salinity	1.69	16.71	<0.001

An opposite effect of salinity on mortality of the two populations was found indicating a different sensitivity of the populations from the two saltworks to salinity (Tab. 1). Significantly higher mortality was recorded at 70 ppt salinity in the Aveiro saltwork population, whereas in the population from Rio Maior saltwork mortality was significantly higher at 120 ppt salinity (Fig. 1). This result is in agreement with what was expected because during in situ observations *Artemia* individuals were located predominantly in evaporation ponds where the average salinities are going to be of intermediate level as postulated by Vieira & Bio (2011). This salinity is not too far from the maximum salinity tested by the herein work and consequently led to suitable adaptation of Aveiro strain to salinities around 120 ppt.

In the case of Rio Maior saltwork, once the water that supplies the saltwork displays at the output of the well salinity around 150 ppt, the *Artemia* from this biotope should be more resistant to high salinity and recorded low mortality at 120 ppt. The Rio Maior saltwork is composed mostly by crystallization ponds with high salinities where the *Artemia* presence will be scarce. A large amount of *Artemia* individuals will be in old ponds that in the past served to supply the crystallization ponds. Probably due to mixing with rain waters, the salinity of these pounds is lower than 100 ppt. *Artemia* individuals are mostly present in the previous tanks where the salinity is considerably lower and in turn may explain the high affinity of this strain to salinities lower than 120 ppt.

The significant interaction between population, temperature and salinity (Tab. 1) indicates different sensitivity of these two populations to the combined effect of temperature and salinity. In the population from Aveiro saltwork, significant higher mortality was recorded for the combination of 28ºC and 70 ppt salinity. In the opposite, the population from Rio Maior saltwork cultured at the combination of 28ºC and 120 ppt salinity showed 100% mortality (Fig. 1). The combined effect of temperature and salinity in the mortality rate for Rio Maior strain are going to be in concordance as previously evaluated for a single factor.

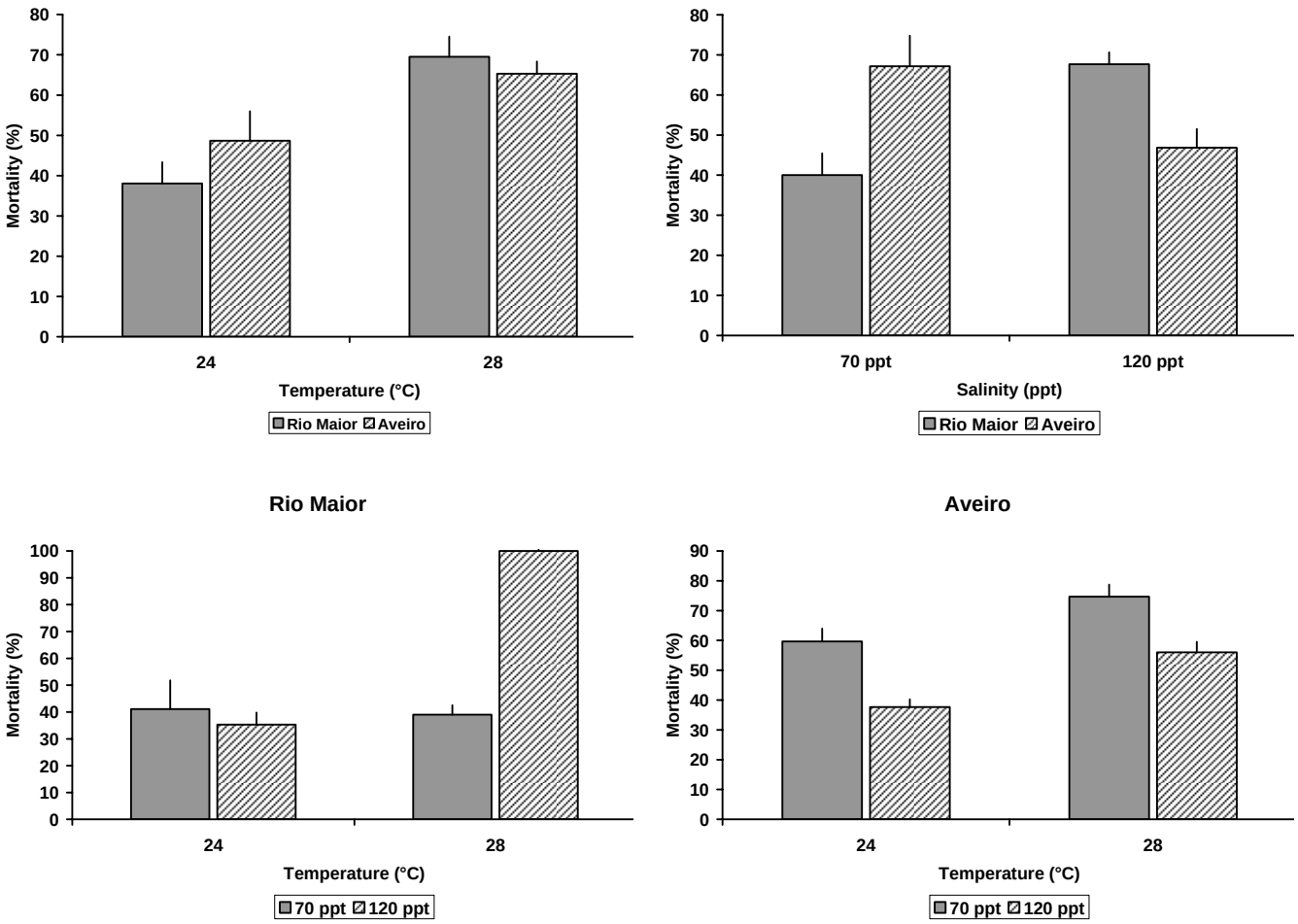

Fig. 1: Values of mortality at 25 days in parthenogenetic populations of *Artemia* from Rio Maior and Aveiro saltworks at four different combinations of salinity and temperature (Mean ± SE)

A possible adaptation by the parthenogenetic strain from Aveiro to higher salinities may also be related to the location of Aveiro saltwork in the end of Ria de Aveiro, near the Atlantic Ocean. For this reason, in the saltwork supply ponds the salinities are similar to those found in sea water (35 ppt), where *Artemia* predators are present. So Aveiro *Artemia* population exists only at salinity levels where these predators are not found (evaporation ponds), thus leading to greater adaptation by this strain to higher salinities (Vieira & Bio, 2011).

Conclusions

With this work, it is possible to conclude that the two Portuguese populations of *Artemia* showed different sensibility to the combined effect of salinity and temperature. It is necessary to increase the number of abiotic factors to attain a broader view of the physiology responses of these two parthenogenetic populations.

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