Combined effect of temperature and salinity on hatching characteristics of three fairy shrimp species (Crustacea: Anostraca)

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ABSTRACT
The combined effects of temperature and salinity on hatching performance of three anostracan species, Phallocryptus spinosa, Branchinecta orientalis and Streptocephalus torvicornis from East and West Azerbaijan, Iran, were studied. The cysts were kept for 10 days at seven different temperatures (12, 15, 18, 21, 24, 27 and 30°C) and four salinity conditions (0, 5, 10, and 15 gL⁻¹), and the effects of the resulting 28 experimental conditions on hatching patterns (duration of pre-hatching period, hatching percentage at first day of hatching, cumulative hatching success) were examined. Results were tested by ANOVA and multiple regression was applied to generate contour models by polynomial equation. The hatching performance in all species was significantly affected by temperature and salinity. Based on contour plot analysis, maximum hatching for P. spinosa, B. orientalis and S. torvicornis cysts was registered at temperatures 19-25°C, 18-23°C and 16-20°C, respectively, within the same salinity range of 0-1 gL⁻¹. The highest cumulative hatching success among the species was observed in P. spinosa at the combination of 24°C and 0gL⁻¹ (88.98%). No hatching was observed for eggs of S. torvicornis and B. orientalis incubated at lower (<15°C) and higher (>27°C) temperature, respectively. The pre-hatching period was prolonged with increase in salinity and decrease in temperature and was highest in P. spinosa (7.7 days at 12°C and 15 gL⁻¹ salinity). High hatching success was observed over wide ranges of temperature and salinity in P. spinosa eggs which demonstrates one of the possible mechanisms responsible for the wide distribution of this species.

Key words: Anostraca, Phallocryptus spinosa, Branchinecta orientalis, Streptocephalus torvicornis, cumulative hatch.

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INTRODUCTION
Fairy shrimp (Branchiopoda: Anostraca) are often the most conspicuous invertebrates associated with the temporary aquatic habitats that are typically characterized by variation in timing, frequency and duration of inundations; a number of variables that together shape the hydrological regime (i.e., hydroregime) of the habitat (Hulsmans et al., 2008). Like many other diapausing crustaceans, anostracans produce encysted embryos that can remain viable in the sediments for years (Marcus, 1996), providing a significant source of recruitment to the water column (Viitasalo, 1992) and of dispersal in time (Hastinon and Cáceres, 1996). For most of the year, anostracan cysts are far more accessible than the corresponding adult or juvenile forms due to the much longer dry season when compared to the length of the inundated phase (Simovich and Hathaway, 1997).

The distribution of large branchiopods is affected by their drought-resistant cysts, which are efficient agents of passive dispersal, so that populations occur on remote islands, and are apparently found wherever there are suitable habitats (Longhurst, 1955). The diapausing cysts may be dispersed by wind, water or birds, which regularly visit seasonal water bodies (Proctor, 1964; Proctor et al., 1967; Figuerola et al., 2003; Green and Figuerola, 2005; Green et al., 2005). Furthermore the extremely sticky eggs could also disperse presumably by adhering to land animals (Longhurst, 1955; Frank, 1988; Gottwald and Eder, 1999; Bohonak and Roderick, 2001; Coulson et al., 2002). Dormancy ends when the appropriate environmental cues (e.g., light, temperature) occur with hydration (Brendonck, 1996; Brendonck et al., 1996, 1998; Hathaway and Simovich, 1996). A certain fraction of the eggs resumes metabolism when favourable environmental conditions are restored, while others remain paused until one or more seasonal cycles have passed. This observed delay in cyst hatching is supposed to be an adaptation to overcome unpredictable seasonal changes that could be fatal for the adult life-phase. In this sense, the existence of a marked inter- and intraspecific variation observed in the hatching pattern of fairy shrimps (Merta, 2003; Zarattini, 2004; Zarattini and Mura, 2007), has provided evidence that a different cyst reactivity exists, and this is related to the degree of environmental unpredictability (Belk and Cole, 1975).

Little is known regarding the environmental conditions...
Temperature and salinity combined effects on shrimp present in temporary ponds that may stimulate hatching of resting eggs. Some variables, such as temperature (Brendonck et al., 1998) and salinity level (Nielsen et al., 2003) have been suggested as hatching cues for temporary pool invertebrates. The influence of temperature and salinity levels on hatching of resting eggs has been studied in zooplankton such as Artemia (Sorgeloos et al., 1980; Abatzopoulos et al., 2003), copepods (Grice and Marcus, 1981; Hansen et al., 2010) and rotifers (Lubzens et al., 1980; Pouriot and Snell, 1983; Garcia-Roger et al., 2006). However, high variability in hatching behavior and lack of ecological background information of the test species make it difficult to generalize or to correlate hatching characteristics with environmental conditions (Bond, 1934).

Three fairy shrimp species, Phallocryptus spinosa Milne Edwards, 1840, Branchinecta orientalis G. O. Sars 1901 and Streptocephalus torvicornis Waga, 1842 (Mura and Azari Takami, 2000; Mehdizadeh Fanid et al., 2007; Atashbar et al., 2014) live in temporary wetlands whose hydroregimes patterns are extremely unpredictable and which often fill in the early spring and dry in the summer time. P. spinosa occurs in fresh, brackish to saline waters, while B. orientalis and S. torvicornis are found in fresh to brackish water pools. Field studies and personal observation demonstrate that in P. spinosa and S. torvicornis cysts hatch through mid-spring and adult fairy shrimps may persist into the summer but are usually hard to find after July. In contrast, hatching of B. orientalis occurs through the late winter from March to May and the adults usually start disappearing from the ponds in early May.

There are no literature data available on the reproductive characteristics and hatching performances of Iranian P. spinosa, B. orientalis, and S. torvicornis. Hence, our experiment aimed to test the impact of critical environmental factors such as salinity and water temperature, on the hatching characteristics of these species of fairy shrimps.

**METHODS**

**Study sites**

Diapausing eggs of the three anostracan species, Streptocephalus torvicornis, Phallocryptus spinosa and Branchinecta orientalis were collected from the sediments of three water bodies located in the northwestern region of Iran (East and West Azerbaijan) (Fig. 1). All these habitats had been previously identified through live animal samplings performed during the wet period. Each locality was visited 4-5 times during April-May. Length and width of each pool were measured to estimate surface area as the surface area of an ellipse. Temperature was measured using sensors (Crisron MM40, Alella, Barcelona, Spain). A hand refractometer (Atago, Tokyo, Japan) was used to measure salinity. During the desiccation process, water salinity increased up to levels as high as 40-60 gL⁻¹ in different biotopes. Altitude and coordinates were measured using a portable GPS (Garmin, Olathe, KS, USA) (Tab. 1).

**Hatching experiments**

The soil containing cysts of anostracans was collected in August after complete drying out of the habitat. The soil samples were transferred into a 300-L tank filled with tap water and subsequently washed over 500 and 150 µm sieves to collect the eggs. The separated dormant eggs were dried at 37°C in an incubator (Munuswamy et al., 2009) and kept in a refrigerator at 4°C for one month (Atashbar et al., 2012).

Hatching fractions were compared in a multifactorial design at seven temperatures (12, 15, 18, 21, 24, 27 and 30°C) and four salinity conditions (0, 5, 10, and 15 gL⁻¹; prepared by dissolving Urmia Lake salt in dechlorinated tap water under constant fluorescent light (Atashbar et al., 2012). The choice of these salinity levels was inspired by the salinity registered after inundation of the habitats. The selected temperatures were based on field records collected when the first stages of the fairy shrimps were observed in the pools. Hatching under experimental temperatures was performed in six replicates of 30 dormant eggs each, incubated in multi-well (10 mL) plastic trays for a period of 10 days (Hulsmans et al., 2006; Atashbar et al., 2012). The numbers of hatched nauplii were counted, expressed as percentage, and they were removed from the hatching trays daily. Dormant eggs that remained unhatched at the end of the experiment were tested for their viability by checking for the presence of a first nauplius.
of a yolky embryo according to the method described by Van Stappen (1996). Hatching performance was corrected based on the number of empty eggs found in each sample (Van Stappen, 1996).

**Statistical analysis**

Temperature and salinity effects on dependent variables (pre-hatching period, hatching percentage at the first day, and cumulative hatching percentage on day 10) were tested by 2-way factorial ANOVA (followed by Tukey test (P<0.05), in order to determine the differences between the treatments). For all dependent variables expressed as percentages the assumption of normal distribution within groups was fulfilled (Kolmogorov-Smirnov test for normality) and the Levene’s test was used to assess of the homogeneity of variances. Regression coefficients were used in the polynomial expression to generate a surface response contour:

\[ Z(H_{\text{Cum}}\%) = b_0 + b_1(T) + b_2(S) + b_3(T^2) + b_4(S^2) + b_5(T\times S) \]

where \( Z(H_{\text{Cum}}\%) \) is the surface response of the cumulative hatching percentage, \( T \) is temperature, \( S \) is salinity, \( b_0 \) is the multiple regression constant, \( b_1 \) and \( b_2 \) are the linear effects of temperature and salinity, \( b_3 \) and \( b_4 \) are the quadratic effects, and \( b_5 \) is the intersection effect. Results were analyzed using software package Statistica 10 (Stat Soft, Inc., Tulsa, Oklahoma, USA).

**RESULTS**

**Day of first hatching**

An overall significant effect of temperature and salinity on pre-hatching period was observed, as well as a significant interaction effect, for *B. orientalis*, *P. spinosa* and *S. torvicornis* (ANOVA, P<0.001) (Tab. 2).

The pre-hatching period of the three mentioned species is illustrated in Fig. 2. In *B. orientalis* hatching started first at 27°C and at salinity 0 gL\(^{-1}\) (1.56±0.20 days after inundation) and was slowest to start at the combination of 15°C and 10 gL\(^{-1}\) (5.51±1.00 days after inundation). In general, no hatching occurred at all at 30°C. No hatching was detected at the highest salinity tested (15 gL\(^{-1}\)) in combination with lower and higher temperature conditions (12, 15 and 27, 30°C). In *P. spinosa* the pre-hatching period ranged from 1 day after inundation at 30±0.1°C and 0 gL\(^{-1}\), to 7.72±1.52 days for the combination 12°C and 15 gL\(^{-1}\). In *S. torvicornis* no hatching was observed at salinity higher than 5 gL\(^{-1}\) and also at 12°C. Hatching in this species started 1 day after inundation in most of the treatments and was slowest at 15°C at salinities 0 (2.33±0.40) and 5 gL\(^{-1}\) (4.13±1.41) (Tukey, P<0.05).

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**Tab. 1.** Location and characteristics of sampling sites in the study area during April-May.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Code</th>
<th>Coordinates</th>
<th>Temperature (°C)</th>
<th>Salinity (gL(^{-1}))</th>
<th>Altitude (m.a.s.l)</th>
<th>Surface area (m(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. orientalis</em></td>
<td>Akh Gol (East Azerbaijan)</td>
<td>AKH</td>
<td>39° 33' 44° 44'</td>
<td>21-27</td>
<td>1-2</td>
<td>800</td>
<td>600,000</td>
</tr>
<tr>
<td><em>P. spinosa</em></td>
<td>Zanbil (West Azerbaijan)</td>
<td>ZAN</td>
<td>37° 44' 45° 15'</td>
<td>14-28</td>
<td>3-30</td>
<td>1300</td>
<td>25,037</td>
</tr>
<tr>
<td><em>S. torvicornis</em></td>
<td>Gom Tapa (East Azerbaijan)</td>
<td>GOT</td>
<td>38° 13' 46° 02'</td>
<td>22-26</td>
<td>0-2</td>
<td>1297</td>
<td>2100</td>
</tr>
</tbody>
</table>

**Tab. 2.** ANOVA analyses testing the effects of temperature and salinity on pre-hatching period.

<table>
<thead>
<tr>
<th>Species</th>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Mean sum of squares</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. orientalis</em></td>
<td>Temperature</td>
<td>6</td>
<td>41.80</td>
<td>53.20</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>3</td>
<td>22.50</td>
<td>17.09</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature x salinity</td>
<td>18</td>
<td>13.43</td>
<td>16.76</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td><em>P. spinosa</em></td>
<td>Temperature</td>
<td>6</td>
<td>82.62</td>
<td>147.2</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>3</td>
<td>16.85</td>
<td>30.78</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature x salinity</td>
<td>18</td>
<td>0.640</td>
<td>1.168</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td><em>S. torvicornis</em></td>
<td>Temperature</td>
<td>6</td>
<td>5.90</td>
<td>43.10</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>3</td>
<td>32.91</td>
<td>240.39</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature x salinity</td>
<td>18</td>
<td>2.76</td>
<td>20.14</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>
Hatching percentage at first day of hatching

An overall significant effect of temperature and salinity on hatching percentage at the first day of hatching was observed, as well as a significant interaction between both factors for all species (ANOVA, P<0.001, Tab. 3). In *B. orientalis* the initial hatching fraction was higher at 21°C and 0 gL⁻¹ (56.28±19.41%) compared to all others and was lowest at 27°C and 10 gL⁻¹ (2.32±3.62%). No hatching was observed at 30°C in combination with all salinity levels. No hatching at the first day of incubation was observed also at 12, 15 and 27°C in combination with 15 gL⁻¹. In *P. spinosa* it was highest at 27°C and 0 gL⁻¹ (70.31±19.08%) and lowest at 30°C and 15 gL⁻¹ (0.50±1.45%). In *S. torvicornis* the initial hatching fraction was higher at 18°C and 0 gL⁻¹ (48.70±20.43%) than in the other conditions others and lowest at 30°C and 5 gL⁻¹ (7.46±5.38%). In the latter species no hatching was observed at salinities of 10 and 15 gL⁻¹ (Fig. 3).

Cumulative hatching percentage

Overall the analysis of variance for cumulative hatching percentage produced a fully significant matrix for species, factors and their interaction (P<0.001) (Tab. 4). In *B. orientalis* cumulative hatching was highest at the combination of 21°C and 0 gL⁻¹ (79.70±12.47%) and lowest at 24°C and 15 gL⁻¹ (1.67±4.08%). No hatching occurred at 30°C at any salinity. The multiple comparisons test showed a significant difference between 18°C and all other treatment groups based on observed means. Significant differences were also seen within the salinity groups (Tukey, P<0.05). For *P. spinosa* the cumulative hatching percentage was highest at the combination of 24°C and 0 gL⁻¹ (88.98±22.50%) and lowest at 30°C and 15 gL⁻¹ (0.59±1.46%) compared to the other treatments. The mean differences were significant within the salinity levels (Tukey, P<0.05). In *S. torvicornis*, finally, it was higher at 18°C and 0 gL⁻¹ (67.07±14.18%) and lower at 30°C and 5 gL⁻¹ (7.46±5.44%) compared to the other treatments. The mean differences were significant within the salinity groups (Tukey, P<0.05). In the latter species no hatching was observed at salinities 10 and 15 gL⁻¹ (Fig. 4).

The cumulative hatching percentage after 10 days of incubation of all species under the experimental

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Fig. 2. The mean (± standard deviation) pre-hatching period at different temperatures and salinities for the three studied species (PS, *P. spinosa*; BO, *B. orientalis*; ST, *S. torvicornis*) (n=6).
conditions indicated that the linear and quadratic effects of the interaction of temperature and salinity were important factors influencing hatching percentage of these anostracan species. Contour analysis defined clear optimal combinations of temperature and salinity for the hatching of the studied species (Fig. 5). The optimal level of both temperature and salinity for hatching success of \textit{P. spinosa}, \textit{B. orientalis} and \textit{S. torvicornis} were 19-25°C; 18-23°C and 16-20°C, respectively, combined with a salinity range of 0-1 gL$^{-1}$ for the three species.

**DISCUSSION**

Our results showed that hatching was more successful in lower salinities and that an increase in salinity had a negative effect on the cumulative hatching and on the initial hatching fraction of the fairy shrimp species being studied. This finding is supported by constant observation of the hatching process in nature which mostly took place when water salinity dropped to near zero. Negative responses of freshwater invertebrates to salinity are reported for various species of Anostraca, copepods and cladocerans (Timms and Sanders, 2002; Brock \textit{et al.}, 2005; Pinder \textit{et al.}, 2005; Waterkeyn \textit{et al.}, 2010). Also the importance of temperature for anostracan egg hatching has been demonstrated by several authors (Belk and Cole, 1975; Horne, 1967; Hathaway and Simovich, 1996). In \textit{Artemia} hatching occurs within certain temperature limits (15-37°C) (Vanhaecke \textit{et al.}, 1984; Vanhaecke and Sorgeloos, 1989; Triantaphyllidis \textit{et al.}, 1994). However, the role of salinity appears to complicate the temperature effect. When \textit{Artemia} eggs are exposed to different stressors, such as salinity and temperature, for a relatively long period, the combined effect of the two stressors may be negative (Vanhaecke and Sorgeloos, 1989). Temperature tolerance in \textit{Artemia} (in the range 25-37°C) was clearly affected by salinity, though differences between Artemia species were observed.

All three species showed a significant temperature-salinity interaction effect for the duration of the pre-hatching period and for the hatching percentage at first hatching. Based on contour plot analysis maximum predicted hatching for \textit{P. spinosa}, \textit{B. orientalis} and \textit{S. torvicornis} occurred at temperature ranges 19-25°C, 18-23°C and 16-20°C respectively, at the salinity range of 0-1 gL$^{-1}$. Comprehensive information is available on the specific temperature range or regime for optimal hatching performance of various anostracan species; for some

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**Tab. 3.** ANOVA analyses testing the effects of temperature and salinity on hatching percentage at first day of hatching.

<table>
<thead>
<tr>
<th>Species</th>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Mean sum of squares</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{B. orientalis}</td>
<td>Temperature</td>
<td>6</td>
<td>1352.45</td>
<td>11.36</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>3</td>
<td>5912.68</td>
<td>49.69</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature×salinity</td>
<td>18</td>
<td>531.45</td>
<td>4.47</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>\textit{P. spinosa}</td>
<td>Temperature</td>
<td>6</td>
<td>3790.25</td>
<td>13.63</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>3</td>
<td>7667.84</td>
<td>27.58</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature×salinity</td>
<td>18</td>
<td>789.16</td>
<td>2.83</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>\textit{S. torvicornis}</td>
<td>Temperature</td>
<td>6</td>
<td>704.92</td>
<td>12.37</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>3</td>
<td>8778.84</td>
<td>154.13</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature×salinity</td>
<td>18</td>
<td>458.85</td>
<td>8.04</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

**Tab. 4.** ANOVA analyses testing the effects of temperature and salinity on cumulative hatching percentage on day 10.

<table>
<thead>
<tr>
<th>Species</th>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Mean sum of squares</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{B. orientalis}</td>
<td>Temperature</td>
<td>6</td>
<td>5473.79</td>
<td>23.06</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>3</td>
<td>17201.64</td>
<td>72.42</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature×salinity</td>
<td>18</td>
<td>860.37</td>
<td>3.62</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>\textit{P. spinosa}</td>
<td>Temperature</td>
<td>6</td>
<td>10186.02</td>
<td>39.96</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>3</td>
<td>22540.79</td>
<td>72.99</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature×salinity</td>
<td>18</td>
<td>1139.06</td>
<td>4.17</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>\textit{S. torvicornis}</td>
<td>Temperature</td>
<td>6</td>
<td>2101.76</td>
<td>38.38</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>3</td>
<td>19602.66</td>
<td>358.02</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature×salinity</td>
<td>18</td>
<td>1001.04</td>
<td>18.28</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>
species the range is as narrow as about 1°C (e.g., Branchinecta lindahlí Packard 1883, Branchinecta paludosa O.F. Müller, 1788, Streptocephalus dichotomus Baird, 1860 and Streptocephalus seali Ryder, 1879) while for others it is broader, up to 5°C (e.g., Branchinecta packardi Pearse 1912, Streptocephalus dorothae J. G. Mackin, 1942, Streptocephalus mackini W.G. Moore, 1966, Streptocephalus macrourus Daday, 1908) (Brendonck, 1996). However, we also found that P. spinosa was able to hatch at wider ranges of temperature and salinity compared to the other two species. Although the optimum temperatures and salinities usually can be estimated from the raw data, the statistical technique (surface response contour) used in this study allows to

Fig. 3. The mean (±SD standard deviation) hatching percentage at the first day of hatching at different temperatures and salinities for three species (PS, P. spinosa; BO, B. orientalis; ST, S. torvicornis) (n=6).

Fig. 4. The mean (±SD standard deviation) cumulative hatching percentage at different temperatures and salinity for three species (PS, P. spinosa; BO, B. orientalis; ST, S. torvicornis) (n=6).
define and interpret the hatching response of fairy shrimps to a matrix of environmental factors and to determine whether the responses are different for tested organisms. In the present study, the optimal temperature and salinity for maximum hatching of *P. spinosa* eggs was 24°C and 0 gL⁻¹ (up to 88 %) and the initial hatching fraction was about 70% at this salinity and 27°C. Our findings were different from results obtained by Hulsmans *et al.* (2006) on *P. spinosa* from Sua Pan in Botswana, who reported highest cumulative hatching percentage (up to 85%) at 22°C at a salinity of 5 gL⁻¹ with an initial hatching fraction of about 30% at salinity 5 gL⁻¹. However, Dararat *et al.* (2011) obtained almost similar results in *Branchinella thailandensis* Sanoamuang, Saengphan and Murugan 2002, compared to our findings. They reported the highest hatching percentage (up to 87%) after 7 days of incubation at 24-26°C and 0 gL⁻¹, 75% of which occurred in the first day. The pre-hatching period in our study ranged from 1 day at 30°C and 0 gL⁻¹, to 5.5 days at 12-15°C and 10 gL⁻¹. The hatching was slowest to start at the combination of the lowest temperature (12°C) and highest salinity (15 gL⁻¹). A similar performance has been reported for the specimens from Botswana, ranging from 1.0 day after inundation at 32°C and 0 gL⁻¹, to 6.4 days at 14°C and 10 gL⁻¹. Intraspecific variation in the hatching pattern of fairy shrimps is also reported by a number of other researchers confirming the above findings on *P. spinosa* (see review in Brendonck, 1996; Simovich and Hathaway, 1997; Van Dooren and Brendonck, 1998; Brendonck and Riddoch, 2001). However, it should be taken into account that recent data (Ketmaier *et al.*, 2008, Alonso and Ventura, 2013; Ketmaier *et al.*, 2013) suggest that *P. spinosa* is not monophyletic. Therefore further studies may be required to confirm if differences in hatching patterns are indeed due to intraspecific variations or if they are phylogenetic differences.

In the same sense, *S. torvicornis* can be compared with other species of the genus *Streptocephalus* (*Streptocephalus purcelli* G.O. Sars, 1898; *Streptocephalus sirindhornae* Sanoamuang, Murugan, Weekers and Dumont 2000; *Streptocephalus siamensis* Sanoamuang and Saengphan 2006). In our study, the highest hatching in *S. torvicornis* (up to 67%) was detected at 18°C and 0gL⁻¹. These findings are quite different from the hatching characteristics of *S. purcelli* (75% and 32%) at 13°C and 0 gL⁻¹ from a South African rock-pool and mud-pool, respectively (De Roeck *et al.*, 2010). Results obtained by Dararat *et al.* (2011) on hatching characteristics of *S. sirindhornae* and *S. siamensis* (64% and 50%, respectively, at 24-26°C and 0 gL⁻¹) show a pattern of hatching characteristics different from earlier studies. In the current study the eggs of *S. torvicornis* hatched within 1.7 days after inundation at 18°C and 0 gL⁻¹, but at higher temperatures (21-30°C) hatching started earlier

Fig. 5. Response surface estimation of cumulative hatching percentage (contour lines) of three species of Anostraca after 10 days of inundation at different experimental temperature and salinity combinations.
(immediately after day 1) at the same salinity. In contrast to our findings, hatching in S. purcelli from a rock-pool and a mud-pool started 1 and 2 days, respectively, after inundation at 13°C and 0gL⁻¹(De Roeck et al., 2010) and started to hatch 1 day after inundation at 24-26°C and 0gL⁻¹ in both S. sirindhornae and S. siamensis (Dararat et al., 2011). Summarizing the results obtained by different authors proves that species-specific optimal factors are needed for optimal hatching in different species of fairy shrimps.

In all the species tested the duration of the pre-hatching period was directly proportional to salinity and inversely proportional to temperature. The hatching process continued during the following 10 days, especially under low salinity conditions, supporting the findings of Brendonck (1996) and Brown and Carpelan (1971) on hatching strategy. In many species, hatching extends over several days or even weeks under favourable conditions. The highest peak, however, is generally on the first or second day of hatching (Brendonck, 1996). Bet-hedging is a strategy employed by many inhabitants of temporary waters, and ensures that not all juveniles emerge before the hydropериod has become properly established (Williams, 2006). The hatching fraction is expected to be adjusted to the probability of successful completion of the life cycle before drying of the pans (Ellner, 1985; Mura, 2004; Hulsmans et al., 2006; Dararat et al., 2011). Several studies have demonstrated the spreading of the risk of a demographic catastrophe by partial delayed hatching of the anostracan egg bank (Simovich and Hathaway, 1997; Brendonck et al., 1998; Mura and Zarattini, 1999). At each inundation, only part of the egg bank hatches, while the rest serves as a buffer in case insufficient hydropériod remains to complete the life cycle. The variability in hatching response even occurs in single broods and can, therefore, be considered as a diversified bet-hedging strategy (Simovich and Hathaway, 1997; Van Dooren and Brendonck, 1998).

Combining anostracan hatching conditions with climatic data can explain the phenology of anostracans in this study area. Based on our field observations the fairy shrimps do not appear until the end of February depending on the average temperature and the amount of flooding required to fill the biotopes and to decrease salinity levels. In most cases temperature was found to be an important parameter influencing the appearance of anostracans. At the beginning of the spring when the air temperature reaches up to 12°C, only B. orientalis is found. P. spinosa gradually appear in April when the average temperature rises above 12-14°C. S. torvicornis occurs in the field when the temperature is over 15°C at the end of May (own observations). Temperature drops again to optimal ranges required for hatching in September-October, but recruitment fails in this period most likely due to high water salinity. These findings are in agreement with our laboratory results and explain the specific performance of each species with reference to the effect of salinity and temperature.

**CONCLUSIONS**

We may conclude that P. spinosa is more tolerant to high temperature and salinity levels than the other species. However there was not so much difference between the species in tolerance for low temperature and salinity. This study also showed that salinity is a more important parameter than temperature, as it affects the hatching rate of all species. It should be noted that both East and West Azerbaijan and especially the Lake Urmia region are suffering from drought since 1998, as a result of which over 80% of Lake Urmia has dried and salt storms have caused salinization of the surrounding areas including the anostracans habitats. Most of the pools inhabited by fairy shrimps are located in the vicinity of the Lake Urmia and are naturally affected by soil salinity in varying degrees. This along with incomplete filling of the pools is likely to result in high water salinity. Moreover, climate change in this region has resulted in an increase of temperature by 1.5°C compared to long-term temperature records, causing a further increase in salinity as a result of higher evaporation levels (Masoodian, 2004). Therefore, it seems that increasing salinity has been acting as an inhibiting factor on hatching performance of the anostracans living in this area.

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