

## Original Article

# Comparative study on effects of different salinities on the reproduction rates of two parthenogenetic species of *Artemia* (Gaav Khooni wetlands of Isfahan and ponds around the Urmia Lake) from Iran

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**Abstract:** This paper investigated effects of different salinities on reproductive of two parthenogenetic species of *Artemia* (from Gaav Khooni wetlands of Isfahan and ponds around Lake Urmia) in Iran. Nauplii from two Iranian brine shrimp parthenogenetic populations, were grown up at three salinities (80, 120 and 150 ppt). The initial stocking density was 200 nauplii/litre at all the salinities. The Shrimps were fed according to a standard feeding table. The results show that Gaav Khooni's *Artemia* has better reproductive than the brine shrimp from Urmia ponds (except in reproductive characteristics such as number of offspring at each day of reproductive period, the number and the percent of offspring encysted). Moreover, *Artemia* from ponds around the Urmia Lake had a reduction in many characters with increasing salinity, but *Artemia* from Gaav Khooni showed best results at optimal level's of salinity (120 ppt). Therefore the present study indicated that *Artemia* from Gaav Khooni, unlike most species the decrease in salinity, (optimal level) is also vulnerable.

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## Introduction

The brine shrimp *Artemia* is a genus with a wide distribution on the five continents, inhabiting inland salt lakes, coastal lagoons and solar saltworks (Vanhaecke et al., 1987). It is among the unique organisms that can adapt to very diverse living conditions that involve salinities as low as 10 g/L (Abatzopoulos et al., 2006) to as high as 340 g/L (Post and Youssef, 1977). It comprises a complex of sibling species and superspecies defined by a criterion of reproductive isolation (Browne and Bowen, 1991).

The Gaav Khooni Lake is situated 140 km south-east of the Isfahan province. It is one of the rare wetlands of Central Iran and, in this sense, plays a critical role for migratory and native birds. It is an internationally protected natural reserve. The Gaav Khooni Lake is

located on the center of the Gaav Khooni region, which extends over an area of 2800 km<sup>2</sup>. The soil is salty throughout the region and a permanent salt crust covers a large area around the lake. It is almost nearly permanent saline lake and its major water source is the River Zayandeh Roud. During the rainy season, many smaller lagoons and lakes with fluctuating salinity appear around the central lake. The average annual precipitation is 83 mm. The dry season lasts from late March until mid-October. The temperature in the region ranges from 6.6 to 37.48°C (Asri et al., 2002). Water salinity is usually above 120 g/L. Agh et al. (2007) reported the presence of a possible parthenogenetic *Artemia* population in the Gaav Khooni Lake at Isfahan (Central of Iran). Agh and Noori (1997) and Agh et al. (2001) also reported the presence of a morphologically

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distinctive parthenogenetic population in small lagoons in the vicinity of the Urmia Lake. The lagoons are scattered at the periphery of the lake both in West and East Azerbaijan. The size of the lagoons varies from a few square meters to maximum 10000 m<sup>2</sup> surface area and their depth is always less than 0.7 m. Therefore these lagoons are temporary small water catchments that are dried during the early summer and filled up again during the winter rainfall. Water salinity in the lagoons ranges from 10-20 g/L in the early spring and gradually rises to saturation level within 10 weeks. Parthenogenetic females were observed at high densities with rare males seen only at the ratio of one male to 100 females in these lagoons (Agh and Noori, 1997; Abatzopoulos et al., 2006).

There is a lot of information on survival and growth of many parthenogenetic *Artemia* populations (Vanhaecke et al., 1984; Wear and Haslett, 1986; Browne et al., 1984, 1991; Browne and Wanigasekera, 2000; Triantaphyllidis et al., 1995, 1997; Baxevanis et al., 2004; El-Bermawi et al., 2004; Abatzopoulos et al., 2004, 2006; Agh et al., 2007). But, the effect of salinity on *Artemia* population in the Gaav Khooni Lake at Isfahan (Central of Iran) have been poorly examined or has not been studied at all therefore, the present study aimed to survey effects of salinity on reproduction rate of two parthenogenetic species of *Artemia* (Gaav Khooni wetlands of Isfahan, ponds around lake Urmia) from Iran.

### Materials and methods

**Culture procedure:** The origins of the *Artemia* cysts used in the experiment are as follows: (1) *Artemia parthenogenetica* from the Gaav Khooni wetlands at Isfahan, Iran; (2) *Artemia parthenogenetica* from the ponds around lake Urmia, Iran. In the laboratory, the *Artemia* cysts were separated by Floatation Separation Method and Density Separation in Freshwater (Gillbert, 1996). The cysts were maintained under conditions of continuous illumination and aeration with Gaav Khooni wetland-water collected in the sampling station and

was hatched at a constant temperature of 23–25°C and salinity of 33–35 ppt, pH was adjusted to 8.0 throughout the hatching. After hatching, the nauplii were separated from their shells and from the unhatched cysts by the method described by Amat (1985) then three replicates of 200 actively moving nauplii from each population were allocated to 500 ml conical containers filled with 400 ml of filtered water with different salinities. Solutions of different salinities (80, 120 and 150 ppt) were prepared using synthetic Gaav Khooni wetland-water and salt of Gaav Khooni wetland. The animals were cultured at 27 ± 1°C under constant aeration. The salinity in each cone was checked daily in order to maintain salinities according to the experimental set up. Twenty four h after hatching, Larvae were fed daily with yeast-based diet Lansy PZ, 1.66 gL<sup>-1</sup>, (INVE Aquaculture NV, Belgium). Density of *Artemia* was adjusted to one animal per 2 mL at the beginning of the experiment, but the density was gradually decreased to one animal per 3 mL on day 8 and per 4 mL on the day 14.

**Reproductive characteristics:** As animals attained maturity, 30 adult parthenogenetic females from each population (from all salinities) were transferred into separate 50 mL falcon tubes in order to study their life cycle characteristics. The falcon tube were checked every day for the production of cysts or nauplii, which were counted and recorded separately. Finally, the reproductive characteristics (Offspring per brood, Number of brood per female, Offspring per female per day, during the reproductive period, brood intervals, percent offspring encysted, total offspring per female, average number of cysts and larvae) were determined for each population according to Browne et al. (1984, 1988).

**Statistical analysis:** The results were statistically analyzed using SPSS (version 17). The normality of distribution of variables was examined using the Kolmogorov–Smirnov test. The homogeneity of variances was examined using the Levene's F test. The differences in the variables among the treatments were tested using one-way ANOVA at

Table 1. Comparison of two *Artemia* populations in different levels of salinity in terms of average number offspring per brood

<b>Artemia species / Salinity (ppt)</b>	<b>80</b>	<b>120</b>	<b>150</b>
Artemia Gavkhouni	<sup>a</sup> 16.785±2.04	<sup>abd</sup> 14.333±1.11	<sup>c</sup> 5.02±2.23
Artemia ponds	<sup>a</sup> 14.733±0.9	<sup>abd</sup> 14.863±1.52	<sup>ad</sup> 10.195±2.64

The rows that have no common letters are significantly different ( $P<0.05$ ).

Table 2. Comparison of two *Artemia* populations in different levels of salinity in terms of average number of brood per female

<b>Artemia species / Salinity (ppt)</b>	<b>80</b>	<b>120</b>	<b>150</b>
Artemia Gavkhouni	<sup>a</sup> 4.3±0.1	<sup>abe</sup> 5.6±0.23	<sup>c</sup> 1.3±0.11
Artemia ponds	<sup>b</sup> 3.0±0.45	<sup>ab</sup> 2.53±0.3	<sup>c</sup> 1.8±0.14

The rows that have no common letters are significantly different ( $P<0.05$ ).

Table 3. Comparison of two *Artemia* populations in different levels of salinity in terms of average number offspring per female per day, during the reproductive period

<b>Artemia species / Salinity (ppt)</b>	<b>80</b>	<b>120</b>	<b>150</b>
Artemia Gavkhouni	<sup>a</sup> 2.423±0.1	<sup>ab</sup> 1.383±0.12	<sup>a</sup> 1.527±0.15
Artemia ponds	<sup>a</sup> 2.357±0.15	<sup>abd</sup> 3.877±0.17	<sup>a</sup> 2.813±0.11

The rows that have no common letters are significantly different ( $P<0.05$ ).

Table 4. Comparison of two *Artemia* populations in different levels of salinity in terms of average number day of brood intervals

<b>Artemia species / Salinity (ppt)</b>	<b>80</b>	<b>120</b>	<b>150</b>
Artemia Gavkhouni	<sup>a</sup> 8.68±1.11	<sup>b</sup> 11.18±0.88	<sup>c</sup> 2.35±0.32
Artemia ponds	<sup>d</sup> 5.43±0.48	<sup>edc</sup> 3.83±0.71	<sup>c</sup> 2.83±0.11

The rows that have no common letters are significantly different ( $P<0.05$ ).

regular sampling dates. Post hoc comparisons between sample means performed using by Tukey and LSD test. Data were presented as mean  $\pm$  standard error (S.E) and differences were considered statistically significant at  $P<0.05$ .

## Results

**The number offspring per brood:** The average number offspring per brood declined in the both populations when salinity increased. The brine shrimp ponds had significantly higher offspring per brood at salinities 120 ppt and 150 ppt compared to *Artemia* from Gaav Khooni. The *Artemia* from Gaav Khooni had significantly higher offspring per brood only at 80 ppt compared to the brine shrimp ponds. No significant differences were found in the offspring per brood of parthenogenetic strains at 80 ppt and 120 ppt, but significant difference was found at 80 and 150 ppt ( $P<0.05$ ). The Maximum number offspring per brood was found in *Artemia* from Gaav Khooni at 80 ppt, significantly higher than those of

the other salinities at intrapopulation level ( $P<0.05$ ). Also minimum number offspring per brood was observed in *Artemia* from Gaav Khooni at 150 ppt (Table 1).

**Number of brood per female:** The average number of brood per female declined in the both populations when salinity increased (except in *Artemia* from Gaav Khooni at salinity 80 ppt). The *Artemia* from Gaav Khooni had significantly higher number of brood per female at salinities 80 ppt and 120 ppt compared to brine shrimp ponds. The brine shrimp ponds had significantly higher number of brood per female only at 150 ppt compared to *Artemia* from Gaav Khooni. The maximum number of brood per female was found in *Artemia* from Gaav Khooni at 120 ppt, significantly higher than those of the other salinities at intrapopulation level ( $P<0.05$ ). Also the minimum number of brood per female was observed in *Artemia* from Gaav Khooni at 150 ppt (Table 2).

**Offspring per female per day, during the reproductive period:** Different results obtained in the

Table 5. Comparison of two *Artemia* populations in different levels of salinity in terms of percent offspring encysted

<b>Artemia species / Salinity (ppt)</b>	<b>80</b>	<b>120</b>	<b>150</b>
Artemia Gavkhouni	<sup>a</sup> 37.32±7.11	<sup>a</sup> 41.93±7.23	<sup>a</sup> 40.35±3.2
Artemia ponds	<sup>b</sup> 77.24±6.32	<sup>b</sup> 81.68±5.34	<sup>b</sup> 64.07±5.18

The rows that have no common letters are significantly different ( $P<0.05$ ).

Table 6. Comparison of two *Artemia* populations in different levels of salinity in terms of total offspring per female

<b>Artemia species / Salinity (ppt)</b>	<b>80</b>	<b>120</b>	<b>150</b>
Artemia Gavkhouni	<sup>a</sup> 82.87±9.23	<sup>ab</sup> 91.53±8.76	<sup>c</sup> 11.27±1.97
Artemia ponds	<sup>d</sup> 54.13±10.3	<sup>edf</sup> 45.7±4.65	<sup>f</sup> 29.67±4.44

The rows that have no common letters are significantly different ( $P<0.05$ ).

Table 7. Comparison of two *Artemia* populations in different levels of salinity in terms of average number of cysts

<b>Artemia species / Salinity (ppt)</b>	<b>80</b>	<b>120</b>	<b>150</b>
Artemia Gavkhouni	<sup>a</sup> 32.39±3.3	<sup>ab</sup> 42.05±5.93	<sup>c</sup> 6.46±0.76
Artemia ponds	<sup>d</sup> 47.56±6.67	<sup>abdf</sup> 41.92±8.41	<sup>f</sup> 28.56±2.19

The rows that have no common letters are significantly different ( $P<0.05$ ).

Table 8. Comparison of two *Artemia* populations in different levels of salinity in terms of average number of larvae

<b>Artemia species / Salinity (ppt)</b>	<b>80</b>	<b>120</b>	<b>150</b>
Artemia Gavkhouni	<sup>a</sup> 50.48±6.55	<sup>ab</sup> 49.48±5.31	<sup>c</sup> 4.81±1.2
Artemia ponds	<sup>d</sup> 6.57±0.83	<sup>abdf</sup> 3.78±0.37	<sup>f</sup> 1.1±0.68

The rows that have no common letters are significantly different ( $P<0.05$ ).

offspring per female per day, during the reproductive period for each one populations. Therefore, the maximum number offspring per female per day, during the reproductive period was observed in brine shrimp ponds at 120 ppt, significantly higher than those of the other salinities at intrapopulation level ( $P<0.05$ ), but the minimum number offspring per female per day, during the reproductive period was observed in *Artemia* from Gaav Khooni at 120 ppt (Table 3).

**Brood intervals (in terms of day):** The average number day of brood intervals declined in the both populations when salinity increased (except in *Artemia* from Gaav Khooni at salinity 120 ppt). The *Artemia* from Gaav Khooni had significantly higher number of day in the brood intervals at salinities 80 and 120 ppt compared to brine shrimp ponds. The brine shrimp ponds had significantly higher number of day in the brood intervals only at 150 ppt compared to *Artemia* from Gaav Khooni. The maximum number day of brood intervals was

observed in *Artemia* from Gaav Khooni at 120 ppt, significantly higher compared to the values obtained at other salinities at intrapopulation level ( $P<0.05$ ), also the minimum number of day in the brood intervals was observed in *Artemia* from Gaav Khooni at 150 ppt (Table 4).

**Percent offspring encysted:** No significant differences were found in the percent offspring encysted of parthenogenetic strains at all levels salinity ( $P<0.05$ ) but the maximum percent offspring encysted was observed in both parthenogenetic populations at 120 ppt (Table 5).

**Total offspring per female:** Total offspring per female declined in the both populations when salinity increased (except in *Artemia* from Gaav Khooni at salinity 120 ppt). The *Artemia* from Gaav Khooni had significantly higher total offspring per female at salinities 80 ppt and 120 ppt compared to brine shrimp ponds. The brine shrimp ponds had significantly higher total offspring per female only at 150 ppt compared to *Artemia* from Gaav Khooni.

The maximum total offspring per female was observed in *Artemia* from Gaav Khooni at 120 ppt, significantly higher than those of other salinities at intrapopulation level ( $P < 0.05$ ). Also, the minimum total offspring per female was observed in *Artemia* from Gaav Khooni at 150 ppt (Table 6).

**Average number of cysts:** The average number of cysts declined in the both populations when salinity increased (except in *Artemia* from Gaav Khooni at the salinity of 120 ppt). The brine shrimp ponds had significantly a higher average number of cysts at salinities 80 and 150 ppt compared that of the brine shrimp ponds. The *Artemia* from Gaav Khooni had significantly a higher average number of cysts only at 120 ppt compared to that of the brine shrimp ponds.

The maximum average number of cysts was observed in the brine shrimp ponds at 80 ppt, significantly higher compared to those of the other salinities at intrapopulation level ( $P < 0.05$ ), but the minimum average number of cysts was observed in *Artemia* from Gaav Khooni at 150 ppt (Table 7).

**Average number of larvae:** The average number of larvae declined in the both populations when salinity increased. The *Artemia* from Gaav Khooni had significantly higher average number of larvae at all levels salinity compared to brine shrimp ponds.

The maximum average number of larvae was observed in the *Artemia* from Gaav Khooni at 80 ppt, significantly higher compared to the those of other salinities at intrapopulation level ( $P < 0.05$ ), but the minimum average number of larvae was observed in the brine shrimp ponds at 150 ppt (Table 8).

## Discussion

**Reproductive characteristics:** We aimed to investigate the effects of different salinities on reproductive characteristics of the two parthenogenetic species of *Artemia*, one from Gaav Khooni wetlands of Isfahan and the other, from ponds around Urmia Lake.

Our study showed that the average number of offspring per brood declines in both populations when salinity increases. The Maximum number of

offspring per brood is found in *Artemia* from Gaav Khooni at 80 ppt, which is significantly higher than those of the other salinities at intrapopulation level. Also minimum number of offspring per brood is observed in *Artemia* from Gaav Khooni at 150 ppt. Triantaphyllidis et al. (1995) did not find significant differences in offspring per brood in Tanguu parthenogenetic *Artemia* and *A. franciscana* from San Francisco Bay at interpopulation level, but there were significant differences at intrapopulation level at salinities below 100 g/L. Abatzopoulos et al. (2003) studying an *Artemia* clone from Megalon Embolon observed significant differences in all reproductive characteristics except for number of broods in all three salinities (50, 80, 120 g/L). Browne and Wanigasekera (2000) reported that all five populations in their study had highest reproduction period and peak production at 24°C at either 120 or 180 g/L. We can justify the results that the Old World species including our two asexual populations from Iran and the parthenogenetic population are more limited by temperature and salinity for reproduction whereas New World species are euryhaline and eurythermal being able to reproduce more successfully at more diverse salinity-temperature combinations.

The average number of brood per female declines in both populations when salinity increases. The *Artemia* from Gaav Khooni has significantly higher number of brood per female at salinities of 80 ppt and 120 ppt and the maximum number of brood per female is found at 120 ppt. Also the minimum number of brood per female is observed in *Artemia* from Gaav Khooni at 150 ppt whereas the brine shrimp ponds has the highest number of brood per female at 150 ppt. The present study supports findings by many studies on the negative impact of salinity values above 120–140 g/L on reproductive and life span characteristics in many other *Artemia* species or strains (Vanhaecke et al., 1984; Wear and Haslett, 1986; Triantaphyllidis et al., 1995; Browne and Wanigasekera, 2000; Baxevanis and Abatzopoulos, 2004; Baxevanis et al., 2004). In accordance with previous laboratory investigations

on several *Artemia* species (Browne et al., 1991; Triantaphyllidis et al., 1995; Baxevanis et al., 2004), it was found that the optimal range for reproduction of Iranian asexual strains of *Artemia* from region lies between 80 and 120 ppt (especially for *Artemia* Gaav Khooni at salinity 120 ppt). Since the salinity in Gav Khooni wetland equals 120 ppt it could be anticipated that the optimum salinity for *Artemia* from Gav Khooni is 120 ppt as this is in accordance with our results.

We found that the average number day of brood intervals declines in both populations when salinity increases. Gilchrist, (1960), Dana and Lenz, (1986) and Triantaphyllidis et al. (1995) who worked on *A. salina*, *A. franciscana* from Mono lake and Tanggu parthenogenetic *Artemia* and *A. franciscana* from San Francisco Bay, respectively, reported that maturation is achieved fastest at salinities lower than 100 g/L, and much slower above 140 g/L. Abatzopoulos et al. (2003) reported faster maturity at 50 and 80 g/L in comparison to 120 g/L for a parthenogenetic *Artemia* from Megalon Embolon (Greece). Similarly Baxevanis et al. (2004) reported early maturation at 35 g/L in three parthenogenetic populations and at 80 g/L in the bisexual *A. salina* from Lake of Wadi El-Natrun, all from Egypt. It was found that this bisexual *Artemia* died before attaining maturity at 150 and 200 g/L. But Browne and Wanigasekera (2000) who performed the experiments at various combinations of temperature and salinity with five *Artemia* populations (one parthenogenetic and four bisexual) reported parthenogenetic *Artemia* from Margherita di Savoia (Italy) as a niche specialist attaining maturity and reproducing only at salinities higher than 120 g/L at 24°C.

The obtained results in the offspring per female per day were found to be different during the reproductive period for each population. Therefore, the maximum number offspring per female per day, during the reproductive period is observed in brine shrimp ponds at 120 ppt but the minimum number offspring per female per day, during the reproductive period in *Artemia* from Gaav Khooni is observed at

120 ppt. Contrary to these, Triantaphyllidis et al. (1995) in their study on Tanggu parthenogenetic *Artemia* and *A. franciscana* from San Francisco Bay found no significant differences between their study groups at interpopulation level.

We found no significant differences in the percent of offspring encysted of parthenogenetic strains at all levels salinity and the maximum percent offspring encysted can be observed in both parthenogenetic populations at 120 ppt, similar to findings observed by Triantaphyllidis et al. (1995).

In conclusion our findings show that considering the number of offspring per brood, number of brood per female, brood intervals (in terms of day), percent offspring encysted, and average number of larvae, the *Artemia* from Gav Khooni was significantly better than *Artemia* from ponds; however, *Artemia* from ponds had significantly better results considering offspring per female per day during the reproductive period, total offspring per female, average number of cysts.

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