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Reproduction and life span characterization of *Artemia urmiana* in Lake Urmia, Iran (Branchiopoda: Anostraca)

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Abstract

Urmia Lake is one of the largest permanent hypersaline lakes in the world. The lake can be characterized as an oligotrophic lake concerning phytoplankton production. Due to low precipitation in recent years in Urmia Lake's catchment area, the water level has declined as much as 4 m compared to 15 years ago. Irrespective of its recent evolution, Urmia Lake has always shown fluctuations in historical and geological times, which may cause some ecological partition. Regarding allopatric speciation model of *Artemia* we assume that several populations of *Artemia* are generated during last years and living in Urmia Lake. To investigate *Artemia* population diversity in Urmia Lake, *A. urmiana* Günther 1890 cysts were collected from five sites in Lake Urmia, Iran. These sites in part represent areas with different physicochemical conditions. To detect possible differences between stations, discriminant analysis (DA) was performed on data of survival, growth, and reproduction at two salinities (75 and 150 g L^{-1}) in laboratory culture tests. The performed DA implied that for the reproductive and life span characters, in both salinities, the stations of Golmankhaneh can be determined as the separated group among the studied stations which can be considered for industrial projects and evolutionary studies.

Keywords: *Artemia urmiana*, Lake Urmia, Life span, Population diversity

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Introduction

Lake Urmia is one of the largest permanent hypersaline lakes in the world (Kelts and Shahrabi, 1986; Gliwicz *et al*., 1995). It is located in northwest of Iran at an altitude of 1250 m above sea level (Van Stappen *et al*., 2001). The length is about 130 km, and the maximum width is 50 km (Loffler, 1961; Azari_Takami, 1987). The total surface area is between 4000 km² and 6100 km², depending on the balance between evaporation and water influx.

The division of the N-S elongated lake into a northern and southern part by a causeway in 1987 (Teimouri, 1998) has disrupted the natural water cycling pattern within the lake (Abatzopoulos *et al*., 2006b). Further changes have occurred following more arid conditions and a decline in river water inflow in recent years. Before 2002, the average depth exceeded 6 m and the maximum depth was 16 m (Abatzopoulos *et al*., 2006a). Due to more than 10 years of progressive drought (Alipour, 2006) and to damming of tributary rivers, the average depth decreased to 3 m and the maximum depth decreased to 5 m (unpublished data) and the surface area has been reduced to less than 4000 km² in 2006 (Agh, 2006).These changes have caused an increase in salinity, commonly reaching saturation relative to halite (NaCl) from 2003 onwards. Average salinity of the lake, which used to fluctuate between 140 and 220 g L^{-1} before 1999, has never dropped below 250 g L^{-1} since then and the lake water is halite-saturated (>300 g L^{-1} NaCl) almost throughout the year.

Field data for the brine shrimp population of Lake Urmia are rather scarce and largely limited to isolated observations and a few survey campaigns (Sorgeloos, 1997; Van Stappen *et al*., 2001, 2004; Agh, 2003, 2006; Abatzopoulos *et al*., 2006b; Agh *et al*., 2007; Asem *et al*., 2007; Eimanifar and Mohebbi, 2007).

Laboratory experiments with *Artemia urmiana* Günther, 1890 have focused on the characterization of cysts and on the study of the nutritional value of laboratory-cultured *Artemia* and its applications in aquaculture (Azari_Takami, 1993; Sorgeloos, 1997; Abatzopoulos *et al*., 2006b; Asem *et al*., 2007). Genetic characterization of *A. urmiana* has been performed by some authors (Abreu-Grobois and Beardmore, 1991; Bossier *et al*., 2004; Baxevanis *et al*., 2006), and the genetic diversity within Lake Urmia has been investigated as well (Eimanifar *et al*., 2005, 2006).

The scope of this paper is to contribute to the study of the biodiversity of *A. urmiana* in Lake Urmia by focusing on the variability of phenotypic characteristics (survival, growth, and various life history traits) when exposed to two different salinities (75 and 150 g L^{-1}).

Materials and methods

Site description and sample preparation:

Sampling was carried out at five different sites in Lake Urmia in 2004 (Fig. 1; Table 1). The sites were selected as localities for which differences in nutritional value and

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diameter of *Artemia* cysts have been reported (Asem *et al*., 2007), and also as sites reflecting the variability present in the lake's ecosystem. Bari, in the northern part of the lake, is located in the part with the greatest average water depth and with highly stable physicochemical water conditions (Alipour, 2006). All other sites are located in the central and southern part of the lake, south of the causeway. The area around Kabodan, close to a group of islands, generally shows stronger phytoplankton blooms and relatively low turbidity. Golmankhaneh is located close to a lower salinity area around the mouth of the permanent river, Shaharchay (Alipour, 2006).

The cyst samples were collected by hauling plankton net with 120 µm mesh size through the water column. Cysts were cleaned from debris and biomass according to standard methods (Lavens and Sorgeloos, 1996).

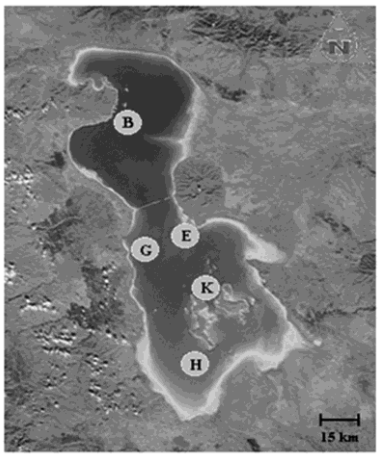


Figure 1: Lake Urmia with overview of sampling stations (H: Heydarabad, G: Golmankhaneh, B: Bari, K: Kabodan and E: Eslami).

Table 1: Coordinates and characteristics of the sampling stations.

Stations	Coordinates	pH	Salinity (g L^{-1})	Turbidity (m)	Depth (m)
Heydarabad	37º/14'/26"N-45º/30'/19"E	7.4	260	0.95	3.3
Golmankhaneh	37º/35'/03"N-45º/23'/25"E	7.3	250	2.0	3.2
Bari	37º/55'/16"N-45º/07'/44"E	7.4	267	1.8	2.2
Kabodan	37º/28'/52"N-45º/37'/53"E	7.2	278	1.5	3.4
Eslami	37º/42'/47"N-45º/33'/30"E	7.2	280	2.1	3.1

Diameter of full cysts

The diameter of the untreated cysts was measured using standard methods (Vanhaecke *et al*., 1980; Lavens and Sorgeloos, 1996). For each sample, the diameter of about 150 intact full cysts was measured using a microscope equipped with a calibrated eyepiece. Average value and standard deviation expressed in µm were calculated.

Hatching and culture experiments

The diapause of the newly harvested *Artemia* cysts was terminated by six months storage at -20ºC. Subsequently, the cysts were hatched according to standard procedures in 0.45 µm-filtered 35 g L^{-1} diluted Urmia Lake water (Lavens and Sorgeloos, 1996).

After hatching, instar-I nauplii were transferred directly to 1.5 l

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cylindroconical glass tubes with 75 or 150 g L^{-1} culture medium (diluted Urmia Lake water which was taken from middle of the lake) at 27±1ºC and at an initial density of 2 nauplii per ml^{-1}, which was lowered after 9 days, as mortality occurred, to 1 animal per 3 ml. Five replicates for each salinity were set up. The animals were reared and fed on a mixed diet of yeast-based formulated feed (LANSY PZ, INVE Aquaculture SA, Belgium) and *Dunaliella tertiolecta* (Butcher), using the feeding schedule described by Coutteau *et al*. (1992) and Triantaphyllidis *et al*. (1995).

Survival and growth of *Artemia* individuals were determined at each water renewal, on days 3, 7, 11, 15 and 20 after hatching. On those days, all individuals in each tube were gently filtered over a 150 µm sieve. The live individuals were counted and transferred to the new medium, and the length of 20 individuals (4 individuals per replicate) was measured using a stereomicroscope and digitizing equipment for biometric measurements (Triantaphyllidis *et al*., 1996). Percentage survival was calculated.

Reproductive and life span characteristics

The reproductive performance of samples from the five sites was analysed using 32 couples of each station from each salinity, 75 and 150 g L^{-1}, at 27±1ºC. As soon as males started to clasp females, the couples were removed from the mass culture and placed in 50 mL cylindroconical tubes. Males were replaced after death (Browne *et al*., 1988). The tubes were examined daily for offspring production until death of the female. Reproductive and life span characteristics were determined according to the procedures of Browne *et al*. (1984, 1988) and Triantaphyllidis *et al*. (1995). The experiment was continued until the animals' death, which typically occurred three to four months after isolating the couples.

Results

Cyst diameter

Cysts from Kabodan station (250.58±17.67 µm) were significantly larger than those harvested at Golmankhaneh station (244.62±13.77 µm) (p<0.05). All other cyst samples have a cyst diameter between these values (Table 2).

Table 2: Mean value and standard deviation (SD) of cyst diameter (µm) (n=150).

Sampling station	Heydarabad	Golmankhaneh	Bari	Kabodan	Eslami
Cyst diameter±SD	247.09±14.14^{ab}	244.62±13.77^{a}	246.59±15.21^{ab}	250.58±17.67^{b}	246.71±14.59 ^{ab}

Samples sharing similar letters are not significantly different (ANOVA, p>0.05).

Survival and total length for *A. urmiana* at both selected salinities (75 and 150 g L^{-1}) are reported in Table 3 and 4. The hatching percentage of the five samples studied was satisfactory (>70%). At 75 g L^{-1} salinity, individuals from Golmankhaneh and Heydarabad revealed significantly lower survival

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than those from Bari and Eslami at the end of the rearing period (one way-ANOVA, $p<0.05$) (Table 3). At the higher salinity significantly higher survival percentages were observed for the Golmankhaneh and Heydarabad stations and significantly lowest values for the Eslami station (one way-ANOVA, $p<0.05$) (Table 3).

Length measurements show that, at 75 g L^{-1} salinity, individuals hatched from cysts from the Heydarabad and Golmankhaneh stations have the highest total length, which was significantly higher than for all other sites (one way-ANOVA, $p<0.05$). At 150 g L^{-1} salinity, the maximum total length at day 20 was observed for Eslami station, which was significantly higher than for Heydarabad, Bari and Kabodan (Table 4).

Table 3: Survival (%) (mean value ± standard deviation, n=5) of *Artemia urmiana* cultured from cysts harvested at five sampling sites in Lake Urmia.

	Station	Day 1	Day 3	Day 7	Day 11	Day 15	Day 20
Survival 75 g L^{-1}	Heydarabad	100	75.2±3.9 ^{c}	63.5±10.2 ^{ab}	57.6±11.8 ^{ab}	34.0±10.9 ^{b}	30.7±11.8 ^{c}
	Golmankhaneh	100	81.0±5.0 ^{bc}	50.3±8.8 ^{b}	44.5±11.6 ^{b}	30.4±2.2 ^{b}	26.5±3.7 ^{c}
	Bari	100	86.5±5.1 ^{ab}	73.5±8.1 ^{a}	69.3±9.1 ^{a}	60.9±6.7 ^{a}	57.8±6.3 ^{a}
	Kabodan	100	89.4±1.6 ^{a}	71.1±4.3 ^{a}	60.1±7.2 ^{ab}	52.7±11.3 ^{a}	36.7±5.8 ^{bc}
	Eslami	100	85.9±1.6 ^{ab}	74.1±0.9 ^{a}	63.0±4.7 ^{a}	50.1±6.0 ^{a}	46.1±8.8 ^{ab}
Survival 150 g L^{-1}	Heydarabad	100	80.6±5.0 ^{a}	68.7±10.3 ^{ab}	63.3±11.3 ^{a}	58.2±11.8 ^{ab}	55.3±11.2 ^{ab}
	Golmankhaneh	100	83.7±6.7 ^{a}	75.6±5.9 ^{a}	64.2±8.9 ^{a}	57.1±8.8 ^{ab}	52.8±9.7 ^{ab}
	Bari	100	73.7±10.0 ^{a}	54.4±7.9 ^{b}	46.5±4.4 ^{b}	43.3±5.8 ^{b}	39.8±4.7 ^{b}
	Kabodan	100	85.1±6.0 ^{a}	74.2±9.5 ^{a}	66.5±10.4 ^{a}	62.4±10.2 ^{a}	59.2±9.6 ^{a}
	Eslami	100	36.7±12.7 ^{b}	28.9±11.7 ^{c}	25.2±9.3 ^{c}	22.9±8.5 ^{c}	21.1±8.8 ^{c}

Values within each column and salinity sharing the same letters are not significantly different (ANOVA; $p>0.05$).

Table 4: Total length (mm) (mean value ± standard deviation, n=20) of *Artemia urmiana* cultured from cysts harvested at five sampling sites in Lake Urmia.

	Station	Day 1	Day 3	Day 7	Day 11	Day 15	Day 20
Total length 75 g l^{-1}	**Heydarabad**	0.455±0.029 ^{b}	1.30±0.03 ^{a}	1.55±0.20 ^{c}	7.68±0.41 ^{a}	8.94±0.60 ^{a}	10.27±0.11^{a}
	Golmankhaneh	0.448±0.023 ^{c}	1.36±0.03 ^{a}	1.80±0.04 ^{ba}	6.70±0.67 ^{b}	8.97±0.52 ^{a}	10.16±0.29 ^{a}
	Bari	0.451±0.024 ^{bc}	1.40±0.04 ^{a}	1.89±0.08 ^{a}	6.83±0.24 ^{ab}	8.58±0.11^{ab}	9.17±0.57 ^{b}
	Kabodan	0.462±0.024 ^{a}	1.41±0.08 ^{a}	1.74±0.07 ^{abc}	7.11±0.31^{ab}	8.32±0.83 ^{ab}	9.30±0.37 ^{b}
	Eslami	0.455±0.026 ^{b}	1.37±0.03 ^{a}	1.68±0.08 ^{bc}	6.70±0.54 ^{b}	7.54±0.49 ^{b}	8.95±0.69 ^{b}
Total length 150 g l^{-1}	**Heydarabad**	0.455±0.029 ^{b}	1.26±0.06 ^{a}	2.44±0.20 ^{ab}	5.44±0.37 ^{a}	7.83±0.44 ^{ab}	8.90±0.29 ^{bc}
	Golmankhaneh	0.448±0.023 ^{c}	1.20±0.06 ^{ab}	2.56±0.13 ^{a}	5.40±0.33 ^{a}	7.70±0.19 ^{ab}	9.30±0.24 ^{ab}
	Bari	0.451±0.024 ^{bc}	1.16±0.05 ^{ab}	2.42±0.12 ^{ab}	5.74±0.06 ^{a}	8.20±0.40 ^{a}	9.09±0.33 ^{b}
	Kabodan	0.462±0.024 ^{a}	1.18±0.04 ^{ab}	2.30±0.24 ^{ab}	4.92±0.52 ^{a}	7.70±0.29 ^{ab}	8.70±0.06 ^{c}
	Eslami	0.455±0.026 ^{b}	1.13±0.06 ^{b}	2.20±0.07 ^{b}	5.04±0.72 ^{a}	7.40±0.51^{b}	9.70±0.05 ^{a}

Values within each column and salinity sharing the same letters are not significantly different (ANOVA; $p>0.05$).

Two-way ANOVA was used to detect significant interaction between sampling station and culture salinity (75 and 150 g L^{-1}) which revealed significant interaction ($p<0.05$) between both factors for survival data at all days of observation, whereas significant interaction for total length data was restricted to days 3, 7 and 20 ($p<0.05$).

Reproduction

Tables 5 and 6 summarize the results of the tests concerning reproductive and life span characteristics of *A. urmiana*

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from the five stations, for both selected salinities. Statistical analysis using ANOVA indicates that there were significant differences among individuals originating from the different stations in most of the studied characteristics, as discussed below.

Table 5: Reproductive and life span characteristics (mean value±standard deviation, n=32) of *Artemia urmiana* cultured at 75 g L^{-1} salinity from cysts harvested at five sampling sites in Lake Urmia.

Parameters	Heydarabad	Golmankhaneh	Bari	Kabodan	Eslami
Interval between broods (days)	3.54±3.25^{a}	2.54±3.77^{a}	4.05±5.36^{a}	4.64±3.97^{a}	4.75±4.80^{a}
Offspring per reproductive day	11.98±18.56^{a}	7.38±8.26^{ab}	3.71±2.96^{b}	7.70±8.01^{ab}	3.59±3.17^{b}
Offspring per brood	28.57±20.30^{a}	14.08±12.44^{b}	13.39±15.38^{b}	23.92±15.77^{ab}	13.63±14.57^{b}
Number of broods per female	23.48±19.28^{a}	12.42±11.93^{b}	10.81±12.91^{b}	22.10±14.15^{ac}	13.33±14.39^{bc}
Pre-reproductive period (days)	5.56±5.84^{a}	6.89±8.73^{a}	13.44±8.91^{b}	8.52±7.84^{a}	4.75±5.62^{a}
Post-reproductive period (days)	2.83±4.29^{a}	6.10±5.11^{ab}	2.88±5.40^{a}	3.21±3.8014^{ab}	4.12±5.79^{ab}
Reproductive period (days)	18.87±23.99^{a}	8.10±11.36^{b}	8.00±9.01^{b}	12.33±10.61^{ab}	9.91±9.90^{ab}
Total life span (days)	45.50±21.12^{a}	38.56±12.26^{a}	43.72±14.29^{a}	43.00±8.91^{a}	38.44±10.05^{a}

Values sharing the same letter in each row are not significantly different (ANOVA; $p>0.05$).

Table 6: Reproductive and life span characteristics (mean value ± standard deviation, n=32) of *Artemia urmiana* cultured at 150 g L^{-1} salinity from cysts harvested at five sampling sites in Lake Urmia.

Parameters	Heydarabad	Golmankhaneh	Bari	Kabodan	Eslami
Interval between broods (days)	4.31±1.44^{ab}	4.23±1.63^{ab}	4.47±1.64^{a}	3.75±1.70^{ab}	3.46±1.90^{b}
Offspring per reproductive days	7.11±2.97^{a}	6.55±1.92^{a}	6.46±2.42^{a}	6.73±4.26^{a}	6.88±3.14^{a}
Offspring per brood	37.25±13.93^{a}	32.36±15.54^{ab}	35.78±11.90^{ab}	28.75±13.51^{b}	32.54±12.53^{ab}
Number of broods per female	37.00±13.67^{a}	31.52±15.83^{ab}	34.48±13.17^{ab}	28.20±12.48^{b}	32.32±12.48^{ab}
Pre-reproductive period (days)	1.94±2.12^{a}	2.97±1.90^{a}	1.87±1.75^{a}	2.06±2.71^{a}	2.56±1.77^{a}
Post-reproductive period (days)	0.65±1.38^{a}	0.75±1.31^{a}	0.81±1.51^{a}	1.18±3.62^{a}	0.68±2.25^{a}
Reproductive period (days)	54.09±45.18^{ab}	63.06±43.59^{a}	49.84±41.08^{abc}	37.32±30.38^{bc}	31.53±22.58^{c}
Life span (days)	80.25±43.31^{a}	81.69±47.07^{a}	74.94±41.11^{ab}	62.59±31.68^{ab}	58.41±23.46^{b}

Values sharing the same letter in each row are not significantly different (ANOVA; $p>0.05$).

At the lower salinity (75 g L^{-1}), Heydarabad *Artemia* has significantly more broods and more offspring per brood than the animals from the other stations, except Kabodan ($p<0.05$). The pre-reproductive period of *Artemia* from Bari was significantly longer than other stations, except for the Kabodan station. No significant variation among stations was found for most other parameters (post-reproductive period, reproductive period, total life span, interval between broods) ($p>0.05$).

At the higher salinity, there were no differences between stations for several parameters (pre- and post-reproductive period, offspring per reproductive day) ($p<0.05$). The highest values for total number of broods per female and for offspring per brood were recorded for the Heydarabad samples and the lowest for the Kabodan samples, and sites were significantly different from each other for these parameters ($p<0.05$). A significantly shorter reproductive period and a shorter life span were

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observed for laboratory-reared *Artemia* from Eslami compared with those from Heydarabad and Golmankhaneh (p<0.05). A meaningful difference was also found in the length of the interval between broods among stations from Eslami and Bari (p<0.05).

Linear discriminant analysis (DA) was performed to define possible grouping based on the reproduction and life span traits at both salinities (Figs. 2, 3).

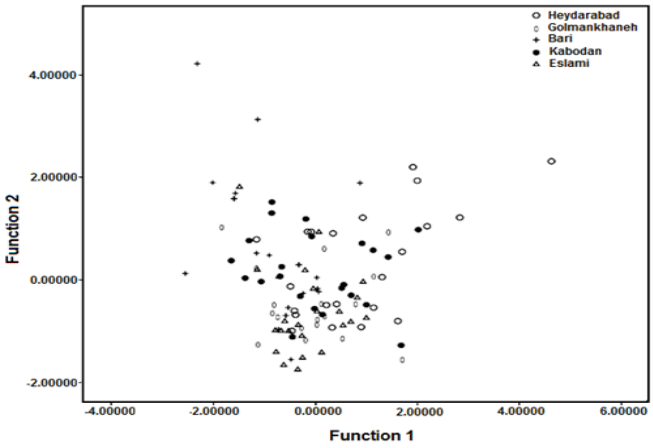


Figure 2: Scatter plot resulting from the discriminant analysis (canonical scores) based on reproductive and life span characteristics obtained in salinity 75 g L^{-1}, using the origin of each station as a separation criterion. Each symbol corresponds with one individual analyzed.

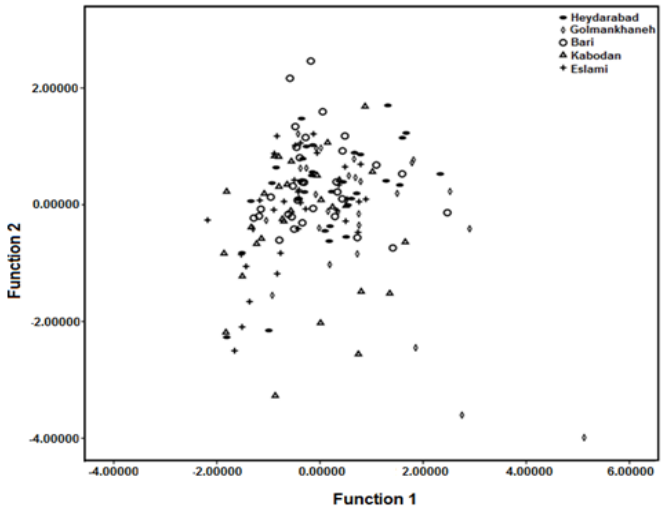


Figure 3: Scatter plot resulting from the discriminant analysis (canonical scores) based on reproductive and life span characteristics obtained in salinity 75 g L^{-1}, using the origin of each station as a separation criterion. Each symbol corresponds with one individual analysed.

At 75 g L^{-1} salinity, discriminant analysis based on the origin of each station as a separation criterion resulted in not more than 41.0% separation of the original groups. However, this analysis resulted in 50.0% separation of

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the Golmankhaneh station from all other stations. The lowest separation (26.1%) was found between Kabodan and all other stations, (Fig. 2). Intermediate values for separation were obtained for Eslami (45.8%) and Heydarabad (45.5%) from the original group. The DA functions 1 and 2 together represented 80.9% variation in the reproductive and life span variables of the characteristics set (respectively 45.6% and 35.4%). In the function 1: offspring per brood, offspring per reproductive day, number of broods per female and reproductive period are important and in the function 2: life span, pre-reproductive period, offspring per brood and offspring per reproductive day have the most important role to separation of groups, respectively.

According to the DA at 150 g L^{-1} salinity, 34.6% of the main groups were correctly classified. Golmankhaneh was the most separated station, with 41.4% predictability separation range. Heydarabad, Bari, Kabodan, and Eslami were each grouped correctly 28.1%, 37.5%, 35.5% and 31.2%, respectively (Fig. 3).

The DA axes 1 and 2 together represented 80.9% variation in the reproductive and life span variables of the characteristics set (respectively 56.7% and 24.2%). In the function 1: reproductive period, total life span, interval between broods and pre-reproductive period are important and in the function 2: offspring per brood, number of broods per female, interval between broods and lifespan have the most important role to separation of groups, respectively.

Discussion

In earlier studies of ecological variations within the Lake Urmia basin, large temporal and spatial differences in chlorophyll concentration (Van Stappen *et al*., 2001) and variations in phytoplankton population (Ryahi *et al*., 1994; Mohebbi *et al*., 2006) have been reported. In response to differences in the nutritional value of unicellular algae species, a relatively high diversity in fatty acid and protein composition was found in cysts, nauplii and adult *Artemia* sampled at different sites in Lake Urmia (Damavandi, 1997 and Porjafar, 1997). Besides qualitative and quantitative variations in primary production, available as feed for *Artemia*, significant differences in K^{+}, Ca^{2+}, Li^{+} and Mg^{2+} concentrations between the southern and northern parts of the lake have been reported, related to the greater inflow of water from rivers in the south (Alipour, 2006). Indeed, according to Bowen *et al*. (1985, 1988) and Hontoria and Amat (1992b), ionic composition of the habitat can also produce ecological isolation.

In this regard, also Abatzopoulos *et al*. (2006a) suggested that fluctuations in physico-chemical conditions and food availability in different regions of Lake Urmia can influence the population of *Artemia.* Biometrical analysis of cysts and laboratory-hatched stage-I nauplii representing seven sites in Lake Urmia showed that the diameter of the untreated cysts ranged from

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262.7 to 286.6 µm (Abatzopoulos *et al*., 2006b). Although these values were not congruent with the values found by the present study, both studies document significant inter-population variations in cyst diameter. Variations in cyst diameter and other traits (chorion thickness, diameter of decapsulated cyst) were also apparent from a study of *A. urmiana* from 26 stations in Lake Urmia (Asem *et al*., 2007).

Considering the importance of *Artemia* in aquaculture, many studies have been conducted on growth, survival and life span characteristics of different species and strains of *Artemia* grown at different salinities. These studies reveal that generally growth of *Artemia* is inversely related to salinity (Gilchrist, 1960; Triantaphyllidis *et al*., 1995; El-Bermawi *et al*., 2004), which was also confirmed for bisexual and parthenogenetic populations of *Artemia* from Iran (Agh *et al*., 2008a). Abatzopoulos *et al*. (2006b) found significant differences in survival, but not for length, of bisexual *A. urmiana* from some locations at different salinities (100, 140, 180 g L^{-1}). This is in agreement with the findings of our study, where interaction between salinity and station was significant (two-way ANOVA, $p<0.05$) for survival data throughout the period of observation, whereas this was not the case for the length data. Different response to different salinity by *A. urmiana* populations can be considered as a specific adaptation pattern to diverse physical, chemical and biotic characteristics of their biotopes (Browne and Wanigasekera, 2000).

As for survival percentages, significant differentiation was seen at 75 g L^{-1} between Bari (highest value) and Heydarabad-Golmankhaneh (lowest value) (ANOVA, $p<0.05$). In contrast, at 150 g L^{-1}, the lowest values were recorded for Bari and Eslami, whereby the value for Eslami was significantly different from those for all other stations ($p<0.05$). For total length, significant differentiation was revealed between Heydarabad-Golmankhaneh (high value) and the other stations at the lower salinity, whereas, at the higher salinity, Kabodan has the smallest total length, which was significantly different from values for Eslami ($p<0.05$).

There is also a considerable amount of literature information on the differentiation between *Artemia* species and populations based on reproduction and life span characteristics (Gilchrist, 1960; Dana and Lenz 1986; Triantaphyllidis *et al*., 1995; Browne and Wanigasekera, 2000; Browne *et al*., 2002; Abatzopoulos *et al*., 2003; El-Bermawi *et al*., 2004). Most of these studies emphasize the influence of environmental conditions on *Artemia* life span characteristics. The results of the present study imply that for most of the studied reproductive and life span traits Bari, Golmankhaneh and Heydarabad performed significantly better. These results are also congruent with the result of the discriminant analyses (DA), for both of the studied salinities, which depicted Golmankhaneh as the most separated station (albeit with a rather moderate

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separation range, which was lower than 50%).

The above results, and the findings from literature described above, indicate that *Artemia* subpopulations originating from various sites in the lake (e.g. from cysts harvested at different places) may show different performance in terms of growth, survival and reproduction when exposed to different environmental culture conditions. This implies that culture conditions considered standard for one subpopulation may not be so for others.

Due to the high salinity of the lake, *Artemia* cysts hatch in Lake Urmia during spring and summer in areas with low salinities, such as at areas of river inflow. Therefore, it could be assumed that the newly hatched nauplii undergo an adaptation (micro-evolutionary process) period before migrating to deeper parts of the lake.

It is believed that food availability under the form of primary production is the main force influencing *Artemia* densities and controlling dynamics of the *Artemia* population at the various stations of Urmia Lake. The presence of islands in the southeast of the lake as well as the incoming rivers in the southwest, in addition to the causeway, provide special niches within the lake promoting differentiation among the *Artemia* populations. It seems that increased salinity influence selectively cyst hatching which subsequently limits availability of nutrients especially unicellular algae for nauplii.

It is known that salinity both directly and indirectly effects the primary production (Nayar and Loo, 2009 and references therein). The *Artemia* population is able to influence the phytoplankton density and species composition by grazing. Therefore the interaction between abiotic conditions such as temperature and salinity, *Artemia* and phytoplankton may result in the existence of *Artemia* subpopulations in hypersaline environments. It is known that the microalgal species composition of Urmia Lake is roughly similar to the one of the Great Salt Lake, USA, which consists predominantly of *Dunaliella*, with an important fraction of diatoms such as *Nitzschi a*and *Navicula* (Gliwicz *et al*., 1995; Sorgeloos, 1997). Species composition of microalgae as the main feed source of *Artemia* has a significant effect on *Artemia* growth and reproduction rate, both in its natural habitat and in culture media (Mohebbi, 2010). In this regard Savage and Knott (1998) studied the effects of limnological factors on parthenogenetic *Artemia* populations from Lake Hayward, Western Australia. They suggested that the major mechanism controlling nauplius survival and recruitment of *Artemia* in this lake is food quality and quantity.

In Urmia Lake a substantial fraction of the cyst stock is suspended in the water column Abatzopoulos *et al*. (2006b), but the floating and sinking fractions do not show any consistent genetic differences Eimanifar *et al*. (2006). In the present study the cysts analysed originated from samples taken throughout the whole water columns up to the surface. The number of samples

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studied is relatively low in proportion to the total surface area of the lake, which should be applied more in future's studies. In a previous study Asem *et al*. (2007) used hierarchical cluster analysis to analyse samples taken from 26 different stations, but came to the conclusion that the lake could be subdivided into 6 recognizable sections. Our samples represent a number of main ecological zones of the lake, consisting of its two main arms (north and south), the western area where the main permanent rivers enter into the lake and the island area in the east of the lake. Therefore we assume that the five samples from our study are reasonably representative for the entire Urmia Lake, though this type of study would benefit from the analysis of additional samples.

Although the Bari and Golmankhaneh populations are geographically quite distant (50 km), they do represent neighbouring stations along the western lake margin. However, Eslami, located at a similar distance from Bari, and much closer to Golmankhaneh, clusters differently. Genetic drift in the presence of limited gene flow, due to the disruption in the natural water cycling which can shape up population structure, can be put forward to explain the differentiation into *A. urmiana* subpopulations in Lake Urmia.

Despite the rather weak diversification in Urmia Lake demonstrated by DA analysis, the congruence found from between the results of our phenotypic and genotypic analyses (Manaffar, 2012) implies that Golmankhaneh station can be considered as the most divergent station in this study.

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