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NASER AGH<sup>\*,1,2</sup>, THEODORE J. ABATZOPOULOS<sup>3</sup>, ILIAS KAPPAS<sup>3</sup>,  
GILBERT VAN STAPPEN<sup>1</sup>, SEYED M. RAZAVI ROUHANI<sup>4</sup>  
and PATRICK SORGELOOS<sup>1</sup>

<sup>1</sup>Laboratory of Aquaculture & *Artemia* Reference Center, Ghent University,  
Rozier 44, B-9000, Ghent, Belgium

<sup>2</sup>*Artemia* & Aquatic Animals Research Institute, Urmia University, Urmia 57153, Iran;  
e-mail: n.agh@mail.urmia.ac.ir

<sup>3</sup>Department of Genetics, Development & Molecular Biology, School of Biology,  
Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

<sup>4</sup>Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

## Coexistence of Sexual and Parthenogenetic *Artemia* Populations in Lake Urmia and Neighbouring Lagoons

key words: *Artemia urmiana*, brine shrimp, salinity, asexual, co-occurrence

### Abstract

We studied the *Artemia* populations existing in Lake Urmia (north-western Iran), one of the largest habitats of *Artemia* in the world, in order to settle the long-standing controversy over the sexual status of the endemic *Artemia* populations. Experiments were carried out in the laboratory and in the field. Cysts, collected from different sites of the lake and peripheral lagoons, were hatched and cultured to adults in the laboratory. Adult sexual and parthenogenetic animals were isolated and newly hatched nauplii from them were cultured to maturity in different salinities, ranging from 15–80 ppt. Survival levels and percentage of animals attaining adulthood were measured over a period of 30 days. In the field experiment, cysts taken from Lake Urmia were hatched and the resulting nauplii were inoculated into six earthen ponds (80–140 ppt) constructed in the vicinity of the lake. Population composition in each pond was determined over a period of two years. Results indicated that both sexual and parthenogenetic *Artemia* coexist in Lake Urmia. While the lake itself is dominated by sexual *Artemia*, the asexual populations were found to be restricted to particular areas in or near the lake. *Artemia* appearing seasonally in the lagoons adjacent to the lake were exclusively parthenogenetic. Parthenogens could grow, mature and reproduce at very low salinities (15–33 ppt), whereas higher salinities (above 50 ppt) were required for *A. urmiana* to attain sexual maturity. We consider salinity to be a major abiotic factor determining the distribution of these sexually different populations within and outside the lake.

### 1. Introduction

Lake Urmia is located between the provinces of West and East Azerbaijan (37°42' N, 45°18'60" E), 21 km east of the city of Urmia, Iran. It is one of the largest hypersaline permanent water bodies in western Asia. Lake Urmia is thalassohaline with oligotrophic characteristics, situated at an altitude of 1250 m above sea level. The total surface area is between 4750 and 6100 km<sup>2</sup> (AZARI TAKAMI, 1987). Maximum length and width of the lake are about 130 and 50 km, respectively. The average and maximum depths are reported to be about 6 and 16 m, respectively. The lake is subdivided into North and South arms, separated by a causeway, which connects the city of Urmia (West Azerbaijan) with Tabriz (East Azerbaijan). The causeway bears a 1 km-gap, which allows a limited exchange of water

\* Corresponding author

between the two parts of the lake. A number of permanent rivers (10–12), flowing through agricultural areas, discharge into the South arm of the lake, while the number of rivers flowing into the North arm is certainly smaller. Due to severe drought (during 1999–2002) and the construction of a number of dams converting permanent inflowing rivers into temporary ones, salinity at both arms of the lake is similar, at least during six months of the year. During those years, the lake experienced grave conditions as a result of a dramatic increase in salinity reaching a maximum of 220 ppt in 1999 and over 300 ppt during 2001–2002.

From early 2003 onward, due to favorable climatic changes, water salinity dropped to about 225 ppt at the surface and 265 ppt at the bottom in the South arm of the lake. In contrast, salinity remained high (265–270 ppt) at both surface and bottom in the North arm. Thus, it seems that the return to a dense *Artemia* population, as previously reported by SORGELOOS (1997) and VAN STAPPEN *et al.* (2001), is still far away and may require many years of sufficient rainfall for a further decrease of the salinity in the lake. Moreover, it seems that the single opening (nearly 1 km) across the lake causeway does not permit enough water exchange between the two arms of the lake. Therefore, more breaches may be necessary to ensure that circulation and water exchange are adequate to restore a suitable environment for *Artemia* in both arms of the lake.

The existence of *Artemia* in Lake Urmia was initially reported by GÜNTHER (1899). CLARK and BOWEN (1976) first demonstrated reproductive isolation of this species using laboratory crosses with *A. franciscana* and *A. salina* (formerly *A. tunisiana*) and characterized it as a separate sexual species which was named *A. urmiana*. In contrast, eleven years later BADARACCO *et al.* (1987) and BARIGOZZI *et al.* (1987) referred to *A. urmiana* as an exclusively parthenogenetic population, showing various ploidy levels (di-, tetra- and pentaploid). As a result of these findings, BARIGOZZI and BARATELLI (1989) proposed to cancel the binomen *A. urmiana*. At about the same time, AZARI TAKAMI (1989) reported the coexistence of sexual and parthenogenetic populations in Lake Urmia. Based on his observations he claimed that the parthenogenetic strain dominates the sexual population during spring and summer. The coexistence of sexual and parthenogenetic *Artemia* populations in the lake was also proposed by AHMADI *et al.* (1990) and BROWNE *et al.* (1991). VAN STAPPEN *et al.* (2001), based on a population monitoring campaign, although not excluding a possible coexistence, confirmed that the *Artemia* population in Lake Urmia is, at least, predominantly sexual.

In recent years, a number of studies have again demonstrated the presence of sexual *Artemia* in Lake Urmia. Regular observations of the populations in the lake for over 10 years (1992–2003, AGH, unpublished data) and the experiments performed by PADOR (1995), SORGELOOS (1997), VAN STAPPEN *et al.* (2001), AGH (2002), AGH *et al.* (2002), NOORI and AGH (2002) showed that *Artemia* from Lake Urmia, actually, reproduce sexually.

AGH and NOORI (1997) reported the occurrence of an exclusively parthenogenetic population, with distinct morphological differences from *A. urmiana*, in many small lagoons in the vicinity of the lake. This finding further supported the possible coexistence of sexual and asexual *Artemia* in the lake itself, as these lagoons are situated close to the periphery of the lake. During the last ten years, a number of molecular markers (SDS-PAGE of embryonic proteins, AFLPs, RAPDs, RFLPs, allozymes) have also been utilized for the delineation of *Artemia* species and *A. urmiana* in particular (ABATZOPOULOS *et al.*, 1997; TRIANTAPHYLIDIS *et al.*, 1997a; ABATZOPOULOS *et al.*, 2002; BAXEVANIS *et al.*, 2005; MURA *et al.*, 2006).

The occurrence and/or coexistence of different *Artemia* strains in other sites in Iran, such as lagoons and salty rivers (ABATZOPOULOS *et al.*, 2006a), seems to be partially explained by substantial fluctuations in salinity. Moreover, there is a tendency for parthenogens, in general, to establish themselves in such astatic environments, presumably as a result of their considerable advantages for effective colonization under conditions of severe “population dilution”. This is valid not only for *Artemia* but also for other continental aquatic invertebrates. Ecological preferences in relation to wide fluctuations in salinity seem to be responsible for shaping patterns of sympatry in *Brachionus* rotifers (GÓMEZ *et al.*, 1995; SERRA *et al.*, 1998).

Considering the wealth of reports and often contradictory information on *A. urmiana*, detailed research and specific experiments are necessary in order to establish the actual biological status of *Artemia* from Lake Urmia. The present work was undertaken to resolve this long-standing uncertainty, and to evaluate the possible coexistence of sexual and parthenogenetic populations through experiments on life-history traits.

## 2. Methods

### 2.1. Laboratory Experiments

*Artemia* cysts were collected from lagoons (May 2005) at the perimeter of Lake Urmia and from 6 different sampling sites (September 2005) in the lake itself: 3 coastal sites (Golmankhaneh, Tappeh Shahi and Rashakan) and 3 off-coast areas of the lake (Kaboudan, Ashk and North arm sites) (Fig. 1). Water salinity was recorded in the sampling areas. Two of the coastal sites, Golmankhaneh and Rashakan, were selected due to their vicinity to the discharge points of two temporary rivers. The cysts were brought to the laboratory and after proper cleaning they were transferred into 4-liter jars containing saturated brine (~280 ppt) and incubated for a period of 15 days at room temperature. The cyst suspension was stirred thoroughly 3 times daily during this period. The cysts were then washed with lake water (150 ppt) and kept in a freezer at  $-20\text{ }^{\circ}\text{C}$  for a period of 2 months to induce diapause deactivation. Then the cysts were stored at  $4\text{ }^{\circ}\text{C}$  and used for hatching after one week.

Hatching was carried out in cylindro-conical flasks according to SORGELOOS *et al.* (1986) using 15 ppt diluted Lake Urmia water for 24 h at  $28\text{ }^{\circ}\text{C}$  with vigorous aeration and continuous illumination (~2000 lux). The hatching medium was adjusted to pH 8.0. The cyst samples used in this experiment showed hatching percentage higher than 75%. The hatched nauplii were transferred to cones containing diluted lake water with different salinities varying from 15 to 80 ppt, each in 6 replicates. Initial salinities of 15, 20 and 25 ppt in the first 3 treatments were raised to 33 ppt within 6, 4 and 2 days, respectively. In the next 3 treatments 33, 50 and 80 ppt were used from the beginning and throughout the

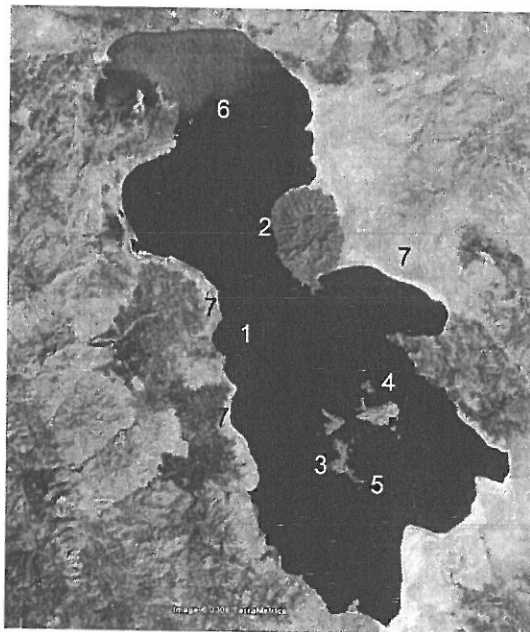


Figure 1. Sampling sites in Lake Urmia region. 1: Golmankhaneh, 2: Tappeh Shahi, 3: Rashakan, 4: Kaboudan, 5: Ashk, 6: Central region of North arm, 7: Lagoons.



experiment. The reason for adopting two different experimental setups in the laboratory for rearing *Artemia* was the observation that cysts of parthenogenetic *Artemia* from the lagoons hatch, and the larvae start growing, in water of salinity as low as 10–15 ppt. Thus, an effort was made to simulate environmental conditions as far as salinity is concerned.

All treatments contained 800 ml culture medium and 400 newly hatched nauplii. The animals were fed *Dunaliella tertiolecta* according to COUTEAU *et al.* (1992). Survival levels and percentage of animals attaining adulthood in each salinity test were recorded every three days after day 5 (until day 24) and on day 30. 30 adult virgin females and adult pairs (in case of *A. urmiana*) were transferred to individual 50 ml Falcon tubes and were observed for their reproductive mode and the ratio of sexual to parthenogenetic females in each treatment. The adult females were distinguished as sexual or asexual based on their reproductive mode and morphological differences. Sexual *Artemia* from Lake Urmia present a longer abdominal region and a small furca with only 2–3 setae on each branch as opposed to a shorter abdomen and a longer furca with many setae of parthenogenetic females (AGH and NOORI, 1997; TRIANTAPHYLIDIS *et al.*, 1997b).

Results were analyzed in SPSS (version 9.0) using one-way analysis of variance (ANOVA) and Duncan's *post hoc* multiple comparison test.

## 2.2. Field Experiments

Eight ponds of 2890 m<sup>2</sup> each were constructed in the vicinity of Lake Urmia (Ghobadlu region), an area with no local parthenogenetic population. The ponds were filled with a mixture of underground fresh water and highly saline water from Lake Urmia, adjusting the salinity to 80 ppt in six ponds (for *Artemia* culture) and 60 ppt in the remaining two (for phytoplankton culture). All ponds were fertilized with chicken manure and chemical fertilizers (urea and di-ammonium phosphate) to promote the growth of unicellular algae. *Artemia* cysts from Lake Urmia were hatched in bulk and the newly hatched nauplii were introduced into the *Artemia* ponds. The salinity in the *Artemia* ponds was maintained between 80 and 140 ppt throughout the experiment, which is significantly lower compared with the current salinity range of Lake Urmia (200–300 ppt).

The population composition of the *Artemia* growing in the ponds was studied in each pond throughout the experimental period from April until September. The ponds were drained by the end of the season (mid autumn), leaving cysts that had settled to the bottom. The ponds were allowed to fill with rain water during winter and early spring, dissolving the bottom salts and attaining salinities of 25 to 40 ppt. The cysts in these ponds hatched during April and the growing *Artemia* population was observed for its reproductive mode. The population composition was determined each year by monthly collections of 20 samples from 20 different sampling sites in each pond. For this purpose, we used a plankton net that allowed the entire water column ( $30 \times 30 \times d$  cm<sup>3</sup>;  $d$  = depth) to be filtered at each sampling site. Total biomass was collected in a 500 ml container attached to the bottom of the plankton net. Samples collected from each site were separately washed through 850 and 500  $\mu$ m-size sieves in order to separate the different age classes. Each filtrate was then transferred to a cylindro-conical flask containing 1 l of sea water and aerated thoroughly. Six sub-samples of 10 ml each were taken from the cones with the suspensions from the 850 and 500  $\mu$ m-size sieves. *Artemia* in each sub-sample were sacrificed by adding a few drops of lugol solution and were then classified as adults, pre-adults, juveniles, metanauplii and nauplii. The above procedure was carried out in order to select those samples with the highest percentage of mature individuals. These samples were used for the determination of the percentage of parthenogenetic *Artemia* in the experimental ponds based on key morphological characters described previously.

## 3. Results

### 3.1. Laboratory Experiments

Salinity records at different sampling areas from Lake Urmia indicated no considerable differences at the time of sampling. But due to the vicinity of the coastal sites to the regions where some temporary rivers discharge, a drop in salinity was recorded during the rainy sea-

son. However, salinity never dropped below 190 ppt even at these areas, whereas salinity in off coast sites was always higher than 220 ppt. The salinity in the lagoons ranged from 10 ppt to saturation by the end of the season. The results obtained from the laboratory experiments are summarized in Table 1 and in Fig. 2(a-g).

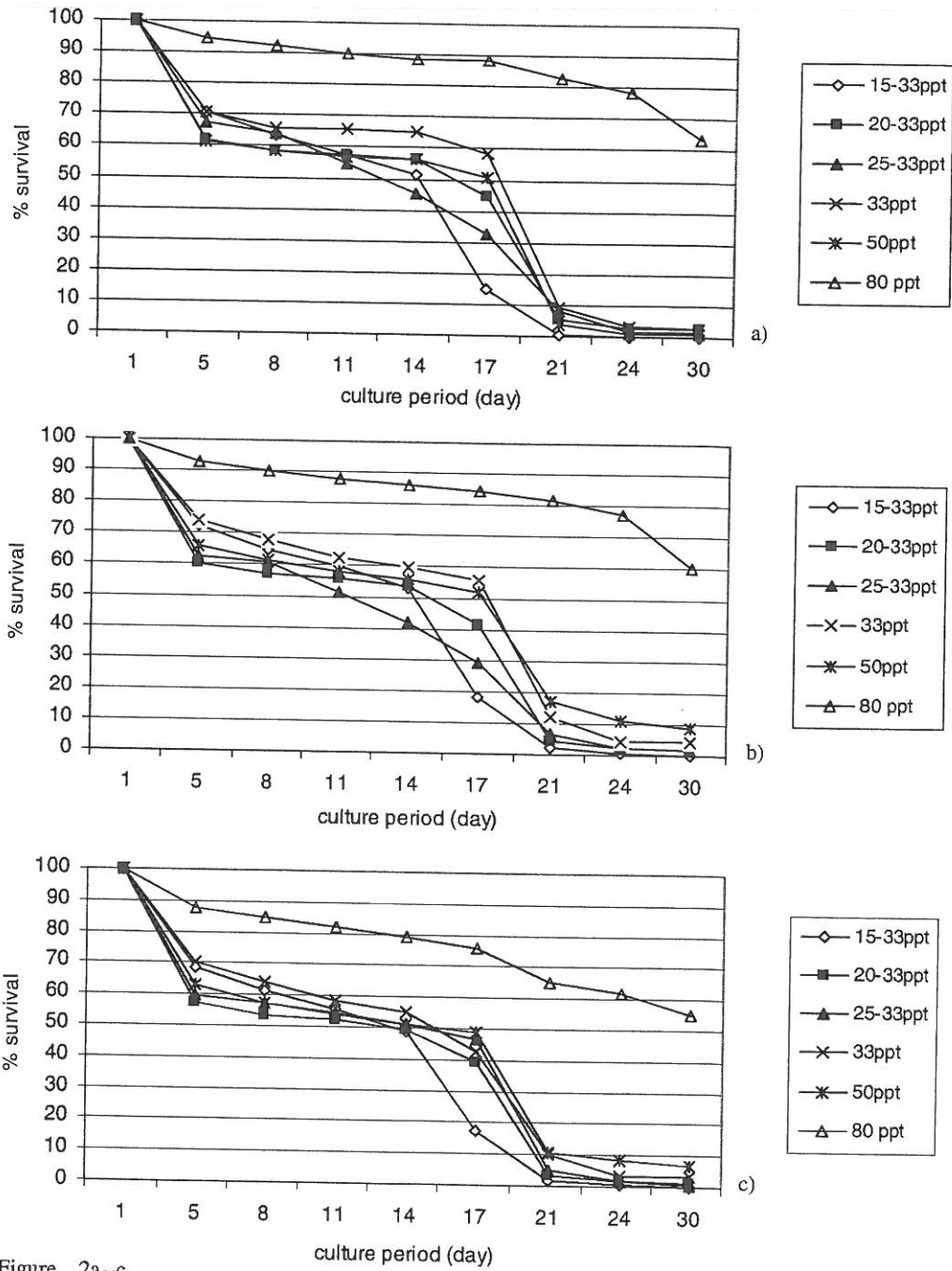


Figure 2a-c

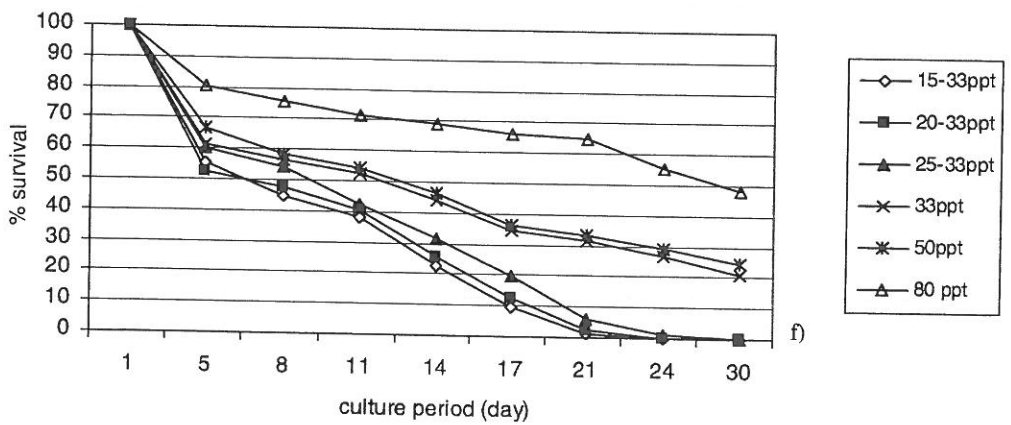
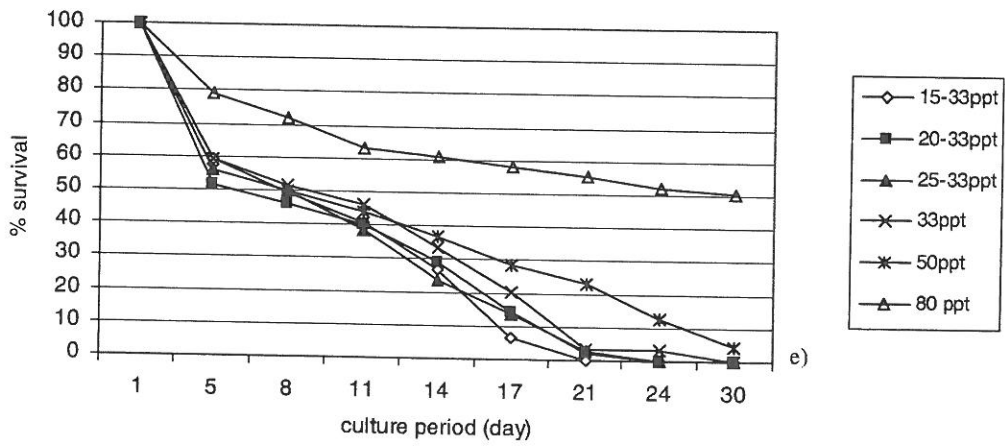
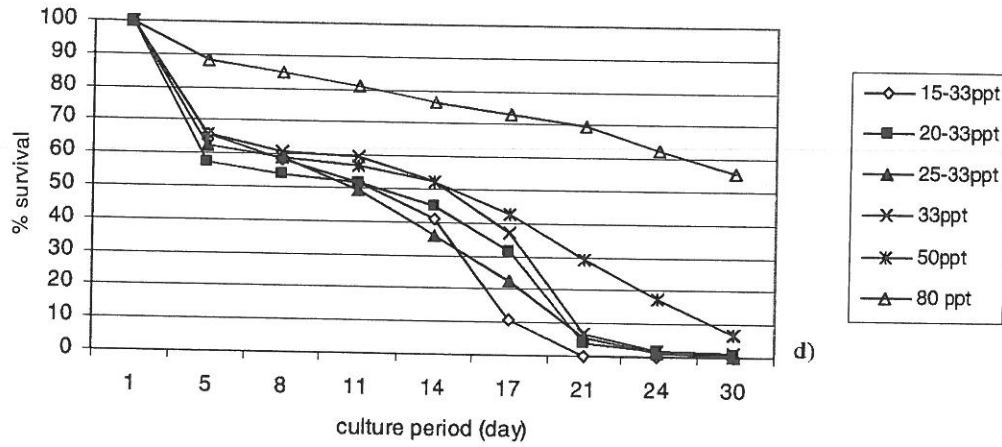


Figure 2d-f

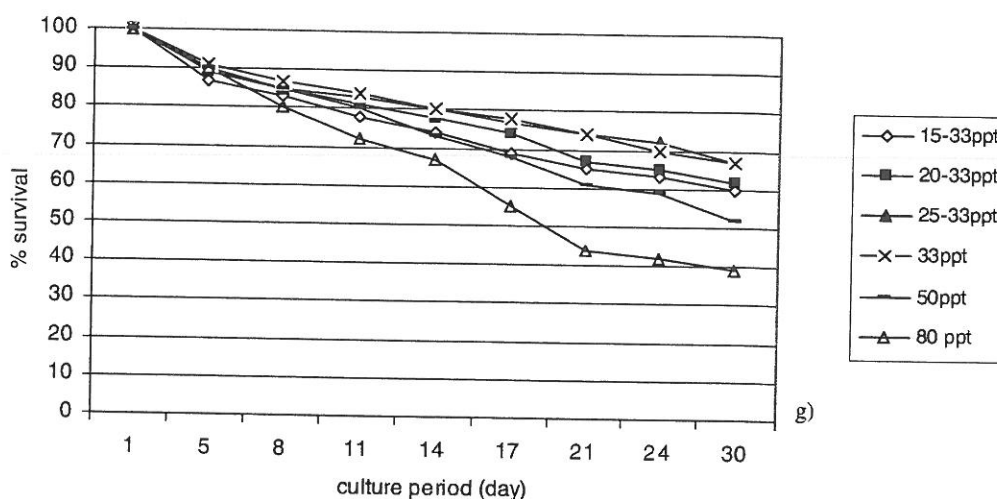


Figure 2. Percentage of survival after cultivation under laboratory conditions at different salinities. a) Golmankhaneh; b) Tappeh Shahi; c) Rashakan; d) Kaboudan; e) Ashk; f) Central region of North arm; g) Lagoons.

*Artemia* hatched from cysts collected in the lake did not perform well at the very low start-up salinities of 15–25 ppt in the culture experiments. Mortality as high as 100% was observed in almost all lake samples when the culture was started at 15 ppt, and very low survival was observed at 20 and 25 ppt. In contrast, nauplii hatched from cysts collected from the lagoons neighbouring Lake Urmia performed very well at low salinities and had higher than 60% survival when the start-up salinity was 15 to 25 ppt, significantly higher ( $P < 0.05$ ) than those hatched from lake samples in these salinities (Table 1).

We found that nauplii hatched from coastal samples, when grown in salinities lower than 33 ppt, gave rise to parthenogenetic females (maturity was attained between days 14 and 17 of culture), although survival was very low by day 30 of the experiment (0.00–4.21%, Figs. 2a, b, c). In contrast, all larvae hatched from cysts collected in areas away from the coast (i.e., in the lake proper) died at low salinities before reaching sexual maturity, usually on or before day 24 (Table 1). Furthermore, at higher salinities most or even all adult ani-

Table 1. Survival differences of *Artemia* hatched from cyst samples harvested from different sites of Lake Urmia and cultured at different salinities. Salinity groups (per population) sharing the same letter are not significantly different ( $p$  level 0.05). Reproductive status: par = parthenogenetic, mix = mixed, bis = bisexual; \* = no animals survived by day 24, \*\* = no animals attained adulthood.

Salinity (ppt)	Site						
	Golmankhaneh (coastal)	Tappeh Shahi (coastal)	Rashakan (coastal)	Kaboudan (interior)	Ashk (interior)	Central region of North arm (interior)	Lagoons
15–33	c/par	c/par	c/par	c/*	c/*	c/*	a/par
20–33	b/par	c/par	b/par	c/*	c/*	c/*	a/par
25–33	b/par	c/par	b/par	c/*	c/*	c/*	a/par
33	b/par	c/par	b/par	c/**	c/*	b/**	a/par
50	b/mix	b/mix	b/mix	b/bis	b/bis	b/bis	b/par
80	a/bis	a/bis	a/bis	a/bis	a/bis	a/bis	b/par



mals were sexual. Significant differences ( $P < 0.05$ ) were observed in survival and reproductive mode of *Artemia* with regard to salinity (Table 1). *Artemia* hatched from cyst samples originating from the lagoons gave rise only to parthenogenetic females, in all salinities. Parthenogenetic populations from the lagoons performed very well at very low salinities (15–33 ppt), as they do in their natural habitat, but the parthenogenetic populations sampled from Lake Urmia preferred a slightly higher salinity (33–50 ppt) to begin with. Highest survival (48–63%) was observed at 80 ppt for *Artemia* hatched from Lake Urmia cysts, resulting in sexual animals, whereas survival was highest (67.37%) at 33 ppt in parthenogenetic *Artemia* grown from lagoon cysts. The lowest survival was recorded at 80 ppt for this parthenogenetic population (Fig. 2g). The survival of *Artemia* reared at 80 ppt was significantly higher in all samples originating from Lake Urmia compared with other salinities (Figs. 2a–f). Although no considerable differences in salinity were recorded at different sampling areas at the time of sampling, results obtained from the above experiments indicate that the parthenogenetic population in Lake Urmia and adjacent lagoons is localized in areas where salinity drops markedly during specific periods of the year.

### 3.2. Field Experiments

The population composition of *Artemia* in the experimental ponds showed that, during the first year, over 98% of the population was composed of *A. urmiana* with parthenogens accounting for a negligible fraction of the total number of animals. However, during the second year of the experiment when the ponds were allowed to fill with very low salinity water (25–40 ppt), a highly different sexual ratio was observed. Low salinity medium favoured the production of parthenogenetic females, as high as 84.2–91.1% of the total number of animals in the ponds (Table 2). This indicated that cysts produced by the mixed population during the earlier season hatched and gave rise to adults capable of successful reproduction at low salinity medium, thus establishing a population of *Artemia* different from that initially inoculated in the ponds.

## 4. Discussion

The present study provides solid evidence for the status of the Lake Urmia *Artemia* population. We conclude that previous studies (CLARK and BOWEN, 1976; BADARACCO *et al.*, 1987; BARIGOZZI *et al.*, 1987; AZARI TAKAMI, 1989; AHMADI *et al.*, 1990; BROWNE *et al.*, 1991; PADOR, 1995; VAN STAPPEN *et al.*, 2001) were correct in their findings about the *Artemia* population from Lake Urmia; namely, that different batches of cysts originating from different parts of either the lake or the neighbouring lagoons could have led to contradictory conclusions concerning the sexual status of the populations. On the basis of our results, we can assume that BADARACCO *et al.* (1987) and BARIGOZZI *et al.* (1987) probably

Table 2. Percentage of adult parthenogenetic *Artemia* in the experimental ponds during two consecutive years as a function of changing salinity.

	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6
1st season at high salinity	1.9	1.8	2.1	1.8	1.7	1.9
2nd season at low salinity	90.2	89.8	87.4	85.5	91.1	84.2



used cysts produced in the lagoons adjacent to the lake or harvested from the coastal areas (some of the lagoons occasionally merge with the lake). Similarly, earlier reports of sexual *Artemia* from the lake presumably referred to cyst samples produced in the main body of the lake, or to animals cultured at salinities equal to 80 ppt or higher.

Since variations in salinity are an important difference between the lake waters and those of the lagoons, this parameter appears to play a critical role in distinguishing the two populations. The lagoons are filled with rain water during winter (December to mid March), gradually dissolving the benthic salts. Initial salinities in these lagoons are about 10–15 ppt but the gradual increase in the evaporation rate from May to August results in brine saturation and eventually, desiccation. On the contrary, water salinity in Lake Urmia has been fluctuating from 200 to 300 ppt during the last 10 years. It is worth noting that salinity fluctuates substantially at the points where rivers discharge into the lake. Sudden changes in water salinity due to high precipitation at the very shallow shores of the lake are common and should be taken into account. Therefore, even in the highly saline Lake Urmia there are microenvironments where salinity differs substantially from the main body of the lake, if not throughout the year, at least during winter and spring when there is high water inflow from rain or snow. These conditions could arguably provide the environmental stimuli leading to ecological specialization of strains and, therefore, niche separation. Our data are comparable in pattern (albeit opposite in direction) to those of other field studies (AMAT, 1983) where temporal partitioning has been demonstrated due to temperature-salinity interaction. For example, in Cadiz (Spain), sexual strains dominate during winter and spring at lower salinities and temperatures while parthenogenetic strains occur during the summer and fall when temperature and salinity are higher. These findings illustrate the multiplicity of local adaptations attained by different *Artemia* strains. This is further verified by a plethora of laboratory tests (BROWNE and HALANYCH, 1989; BROWNE *et al.*, 1991; ABATZOPOULOS *et al.*, 1993; TRIANTAPHYLLIDIS *et al.*, 1995; BROWNE and WANIGASEKERA, 2000; BAXEVANIS and ABATZOPOULOS, 2004; BAXEVANIS *et al.*, 2004; EL-BERMAWI *et al.*, 2004) demonstrating divergent response profiles for various traits to salinity and temperature both between and within *Artemia* strains of different reproductive mode. A characteristic case involves the parthenogenetic population at Salin de Giraud, France where numerous clones exhibit marked distribution along a steep salinity gradient (BROWNE and HOOPES, 1990).

*Artemia urmiana* is the only sexual species found in Iran (TRIANANTAPHYLLIDIS *et al.*, 1998; VAN STAPPEN, 2002). Parthenogens are restricted to the Old World and they comprise a diverse assemblage of clones, including both narrow endemics and widespread lineages of distinctive spatial and temporal origins and genetic diversity (BAXEVANIS *et al.*, 2006). In a recent survey, ABATZOPOULOS *et al.* (2006a) recorded many parthenogenetic populations in different saline lakes, lagoons and salty rivers throughout Iran. According to BEARDMORE and ABREU-GROBOIS (1983) and ABREU-GROBOIS and BEARDMORE (1991), the phylogenetic relationship of *A. urmiana* and parthenogenetic forms is suggestive of a recent common ancestral lineage. ABATZOPOULOS *et al.* (1997) used cyst membrane protein composition to discriminate between different *Artemia* strains and found that the electrophoretic banding patterns of *A. urmiana* resembled those of parthenogenetic populations. A close relationship between *A. urmiana* and parthenogens was also confirmed by AFLP fingerprinting (TRIANANTAPHYLLIDIS *et al.*, 1997a) and RAPD analysis (ABATZOPOULOS *et al.*, 2002). Therefore, the presence of parthenogenetic *Artemia* within Lake Urmia, and in close proximity to it, illustrates its close historical affiliation to *A. urmiana*. Recently, phylogenetic analysis from global isolates has confirmed the affinity of *A. urmiana* to parthenogens and its possible role to the origin of asexuality (BAXEVANIS *et al.*, 2006). Our field tests showed the prevalence of a parthenogenetic population of *Artemia* in the subsequent season in ponds that were initially inoculated with nauplii hatched from Lake Urmia cysts. These results suggest that Lake Urmia, being situated at the core of an extensive migration route of birds between Europe and many countries in Asia and Africa, could have been an important center for radi-



al expansion of parthenogenetic *Artemia* strains to smaller lakes and lagoons far away or nearby the lake, supporting similar findings by other researchers (ABATZOPOULOS *et al.*, 2006a). The existence of parthenogens in temporary water bodies with salinities as low as 10 ppt. provides novel evidence that *Artemia* can survive and grow at such low salinities in the absence of predators. This result should be of general interest to *Artemia* biologists, notably in terms of osmoregulation.

On the other hand, laboratory experiments unambiguously confirmed that these parthenogenetic *Artemia* can grow to the adult stage and reproduce at salinities as low as 33 ppt. According to current knowledge (ABREU-GROBOIS, 1987), the radiation of asexual *Artemia* is linked to a dramatic salinity increase and habitat fragmentation in the Mediterranean basin during the Messinian salinity crisis (HSÜ *et al.*, 1997). Although this tends to provide an overall explanation for the phylogenetic history of parthenogens relative to their sexual ancestors (but see also BAXEVANIS *et al.*, 2006), it is conceivable that on a microevolutionary scale similar selective pressures may be manifested by an inverse regime, namely reductions in water salinity. In the present case, the adjacent lagoons may be considered as ephemeral populations periodically restocked from a relatively stable, mixed source population (Lake Urmia). These ephemeral sites resemble ecological models where extinction and recolonization become important forces in shaping intraspecific genetic differentiation (see AVISE, 2000 and references therein). However, they are atypical to a great extent since they involve both environmental periodicity and absence of gene flow. To this end, appropriate molecular surveys (see BAXEVANIS *et al.*, 2006) may provide valuable information on whether parthenogens have indeed achieved some degree of regional radiation or are invariably locked in a narrow zone of dynamic coexistence with *A. urmiana*.

Irrespective of their genealogical ties though, parthenogens possess additional attributes which may presumably facilitate their spread. According to BARIGOZZI *et al.* (1987), 33.3% of the parthenogenetic *Artemia* from Lake Urmia were pentaploids. AMAT *et al.* (1995) states that increased frequency of polyploids in many animal species at higher latitudes is associated with greater tolerance to cold stress and better colonizing abilities. Polyploidy in parthenogenetic *Artemia* accounts for higher heterozygosity and genetic variability (ABREU-GROBOIS, 1987; ABREU-GROBOIS and BEARDMORE, 1980, 1982). ZHANG and KING (1993) point out that polyploid parthenogenetic *Artemia* respond differently to environmental changes, showing many advantages over diploids in stressful habitats, and that they have developed a series of life-history characteristics adapting them to environments that contrast with those of diploids. As about 40% of the reported parthenogenetic *Artemia* from the Lake Urmia region are polyploids (BARIGOZZI *et al.*, 1987), there is a possibility that they could have easily adapted to new environments that were created in the vicinity or distal areas of the lake, referred to here as lagoons.

In contradiction to the conclusions of AZARI TAKAMI (1989), our study confirms the presence of a dominant sexual *A. urmiana* population in Lake Urmia throughout the year. According to present knowledge, this lake is the only natural reservoir of this species in the world and an excellent example of geographic isolation. Neither our experiments nor 10 years of constant observations support the idea of a prevailing parthenogenetic population in the lake, even at selected areas. On the other hand, our study supports the proposals of AZARI TAKAMI (1989), AHMADI *et al.* (1990) and BROWNE *et al.* (1991) for a possible coexistence of sexual and parthenogenetic *Artemia* populations in the lake. Through extensive sampling in appropriate sites (central regions, coastal sites and peripheral lagoons) we found that, besides *A. urmiana*, parthenogenetic populations also exist in restricted areas of Lake Urmia. Thus, our results provide strong evidence that Lake Urmia contains a mixed population of *Artemia*, with domination by the sexual *A. urmiana*.

The emergence of parthenogenetic populations in the experimental ponds, inoculated with nauplii hatched from cysts collected from the lake proper, proves that the parthenogenetic fraction present in the lake can generate this population in environments with much lower

salinities to which the sexual animals cannot adapt and are eliminated. This finding supports the idea that Lake Urmia could have played a crucial role in the spreading of parthenogenetic *Artemia* strains. Regarding *A. urmiana* though, salinity adaptation may not fully account for the restricted presence of this species and other factors may also be involved. Due to an idiosyncratic cyst buoyancy behavior, a significant fraction (>60%) of *A. urmiana* cysts sink after 72 h even at salinities of 200 ppt (ABATZOPOULOS *et al.*, 2006b). This undoubtedly generates a mechanism by which the number of potentially effective migrating propagules (cysts carried by birds) is greatly reduced. This may presumably explain the complete absence of *A. urmiana* from several salt lakes and lagoons throughout Iran (ABATZOPOULOS *et al.*, 2006a) despite the fact that many of these sites provide the necessary high salinity environment for *A. urmiana* growth. In addition, chances of dispersal by birds are also limited. Birds only feed at the shores of Lake Urmia (where parthenogens dominate) because deeper parts are inaccessible and the high-salinity water creates clots on their feathers, thus affecting flight. Alternatively, birds may indeed carry a mixture of parthenogenetic and *A. urmiana* cysts, but in the colonized sites parthenogens outcompete *A. urmiana* due to their two-fold reproductive advantage. All these suggest that elucidating *Artemia* biodiversity in Iran and the interaction between sexuals and parthenogens poses great challenges and may require an interesting integration of physiological, ecological and genetic assays.

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