

Artemia sites in Iran

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Field surveys were conducted in order to collect information on the occurrence of wild *Artemia* populations in hypersaline environments such as salt lakes, lagoons and salty rivers. The mating behaviour of *Artemia* populations and the presence or absence of males were carefully recorded. Sampling involved the use of plankton nets. Collected cysts were characterized on the basis of their diameter and chorion thickness, while nauplii (instar-I) were characterized on the basis of their total length. *Artemia* populations were found at 17 different geographical locations scattered over 12 Iranian provinces. All Iranian *Artemia* populations are parthenogenetic with the exception of *Artemia urmiana* from Urmia Lake. During the last five years severe salinity increase has caused a dramatic reduction of population sizes in several hypersaline settings in Iran. The study of cyst and naupliar biometry revealed substantial differences between populations and can be used, to some extent, for their discrimination. Cyst diameter mean values range from 243.2 to 285.4 μm . For some Iranian parthenogens, cyst diameters were among the smallest recorded so far for parthenogenetic *Artemia*. The total length of newly hatched nauplii ranges from 455.5 to 529.8 μm .

INTRODUCTION

Artemia was first described in Lymington (England) by Schlosser in 1755 (Kuenen & Bass-Becking, 1938). Since then, many *Artemia* sites have been recorded. The first trial to list all known *Artemia* sites dates back to 1922 when Artom reported 18 of them. Later, Stella (1933) and Barigozzi (1946) reported the occurrence of *Artemia* populations in 28 and 29 sites respectively, spread over the five continents. However, the first systematic effort to make an inventory of the various known *Artemia* populations was carried out by Persoone & Sorgeloos (1980), who listed 244 sites/populations. The same authors also pointed out that many *Artemia* sites have been abandoned or destroyed (e.g. *Artemia* has disappeared from Germany and Great Britain).

Vanhaecke et al. (1987), in their updated review, reported the presence of *Artemia* in 360 geographically distinct areas. The most recent investigations on *Artemia* biogeography have been published by Triantaphyllidis et al. (1998) and Van Stappen (2002), and report 505 and 598 *Artemia* sites, respectively. The increasing number of *Artemia* sites indicates that their real number must be higher, since vast areas in sub-Saharan Africa and continental Asia remain largely unexplored (Van Stappen, 2002).

Genetic studies performed a century ago revealed two distinct modes of reproduction in *Artemia*: parthenogenesis and zygogenesis (Artom, 1907). Artom's cytogenetic analyses unveiled the existence of several ploidy levels (2n to 5n) in *Artemia* parthenogenetic forms, as opposed to the diploidy (2n=42) of bisexual *Artemia* (Artom, 1907, 1922). Initially, the binomen *Artemia salina* was used for all *Artemia* populations. It took some time before the effect of

salinity on the morphology of *Artemia* was recognized as a non-heritable factor (Gilchrist, 1960). Recently, genetic markers were adopted as more effective tools for characterizing *Artemia* species (Abatzopoulos et al., 2002b; Gajardo et al., 2004; Baxevanis et al., 2005; Mura et al., 2005).

Although the presence of *Artemia* in Urmia Lake was first reported over a century ago (Günther, 1899), a long period of time elapsed before this population was characterized. Clark & Bowen (1976) assigned the binomen *Artemia urmiana*. However, very little information has been available until recently and its mode of reproduction remains baffling. Based on cytogenetics, and analysis of repetitive DNA and heterochromatin, Barigozzi et al. (1987) and Badaracco et al. (1987) found that the Urmia population was exclusively parthenogenetic. As a result, Barigozzi & Baratelli (1989) proposed to abandon the binomen *A. urmiana*. Subsequent studies showed that both sexual and asexual populations exist in Urmia (Browne & Bowen, 1991). Therefore, a more detailed investigation was needed in order to confirm the reproductive status of *Artemia* in Urmia Lake.

Ahmadi (1987) reported the presence of a possible parthenogenetic *Artemia* population in Shurabil Lake at Ardabil (north-west of Iran). Agh & Noori (1997) and Agh et al. (2001) also reported the presence of a morphologically distinctive parthenogenetic population in the small lagoons in the vicinity of Urmia Lake. Makhdomi (1992) announced the presence of *Artemia* in the Incheh and Shor lakes while Piri & Tehrani (1997) found *Artemia* in Varmal Lake. As no other *Artemia* sites have been reported in the literature so far, this investigation aims to present an updated, systematic inventory of *Artemia* sites in Iran, providing additional information on the reproductive mode of these populations.

MATERIALS AND METHODS

Iranian inland saline lakes and lagoons were surveyed and their geographical coordinates were recorded (Table 1). Field trips were organized in order to collect information and samples from these hypersaline environments, i.e. salt lakes, lagoons and salty rivers. Information on biotic and abiotic parameters focusing mainly on water surface/volume, salinity and *Artemia* population density at the studied sites were also gathered.

The mating behaviour, the sex ratio and the presence or absence of males were carefully recorded. Samples were collected using plankton nets and adult animals were transferred to the laboratory for further examination. Thirty adult females from each population were collected and placed individually in 50 ml Falcon tubes containing brine (salinity was 80 g/l). Females were checked daily for offspring production and part of the culture medium was renewed every 48 h. The animals were fed with the unicellular algae (*Dunaliella tertiolecta*) according to the feeding schedule described by Coutteau et al. (1992).

Cyst samples (if available) were collected from each lake, lagoon or salty river. The cysts were characterized

on the basis of their diameter and chorion thickness (Sorgeloos, 1997). For this purpose, 1 g of cysts from each sample was first hydrated and then fixed in 1% Lugol overnight. The fixation time had no apparent impact on the biometry of encapsulated cysts. The diameter of 400 cysts was measured using a light microscope equipped with an eyepiece containing a graticule. The graticule was calibrated against a standard and the measurements had an accuracy of 1 μm (Sorgeloos, 1997). One gram of cysts from each sample was decapsulated (according to the methodology described in Sorgeloos et al., 1986), fixed with Lugol for 3–5 min and the diameter of 400 of these cysts was promptly measured. In order to measure the length of newly hatched nauplii, cysts from each population were hatched in cyllindroconical flasks following the standard protocol described by Sorgeloos et al. (1986). Four hundred instar-I nauplii were fixed in 1% Lugol solution at 35 g/l D&K medium and measured under a microscope to the nearest μm . Also, 400 free-swimming nauplii were transferred into 1-l cones in four replicates for each population and reared to adulthood in 80 g/l salinity culture medium (prepared by diluting brine from Urmia Lake). After reaching maturity, the sex ratio, the

Table 1. *Distribution of Artemia populations in Iran.*

	Name of the biotope/nearby city and province	Reproductive mode	Geographical coordinates
1	Urmia Lake Urmia, West Azerbaijan province	Bisexual Parthenogenetic	37°20'E–45°40'N
2	Lagoons around Urmia Lake Urmia and Fesendooz, West Azerbaijan province	Parthenogenetic	37°20'E–45°40'N 37°15'E–45°85'N
3	Lagoons around Urmia Lake Dasht-E-Tabriz, East Azerbaijan province	Parthenogenetic	37°50'E–46°40'N
4	Maharlu Lake Shiraz, Fars province	Parthenogenetic	29°57'E–52°14'N
5	Bakhtegan Lake Shiraz, Fars province	Parthenogenetic	29°40'E–53°50'N
6	Tashk Lake Shiraz, Fars province	Parthenogenetic	29°60'E–53°50'N
7	Incheh Lake Gonbad, Golestan province	Parthenogenetic	37°24'E–54°36'N
8	Shor Lake Gonbad, Golestan province	Parthenogenetic	37°25'E–54°41'N
9	Varmal catchment Zabul, Sistan and Baluchestan province	Parthenogenetic	30°80'E–61°50'N
10	Mighan Lake Arak, Central province	Parthenogenetic	34°20'E–49°80'N
11	Qom Salt Lake Qom, Qom province	Parthenogenetic	34°40'E–51°80'N
12	Houze Sultan Lake Qom, Qom province	Parthenogenetic	34°50'E–51°20'N
13	Gaav Khooni Lake Hasan Abad, Isfahan province	Parthenogenetic	32°20'E–52°58'N
14	Kale Shoor, Gonabad, Khorasan province	Parthenogenetic	35°10'E–57°50'N
15	Kale Shoor, Khorram Abad, Lorestan province	Parthenogenetic	32°40'E–48°54'N
16	Nough catchment Nough, Kerman province	Bisexual (<i>A. franciscana</i>) Parthenogenetic	30°60'E–56°50'N
17	Shurabil Lake (extinct) Ardabil, Ardabil province	Parthenogenetic	38°25'E–48°55'N
18	Kale Shoor Hashtgerd Karaj, Tehran province	Parthenogenetic	35°90'E–50°78'N

composition of populations (mixture of different strains or not) and the reproductive mode were recorded. During the culture period, *Artemia* was fed on a mixed diet consisting of the algae *D. tertiolecta* and treated yeast (Lansy PZ, INVE, Belgium), following the feeding schedule of Coutteau et al. (1992).

Data were statistically treated by analysis of variance (ANOVA), using the Statistical Package for the Social Sciences software (version 9). Averages were compared using Duncan's test.

OBSERVATIONS AND RESULTS

Description of Artemia sites in Iran

Eighteen salt lakes, lagoons or salty rivers were investigated for the occurrence of brine shrimp. *Artemia* populations were found in 17 of these sites (Figure 1 and Table 1).

Urmia Lake (West Azerbaijan province)

Urmia Lake is located 21 km east of Urmia city. It is one of the largest permanent hypersaline water catchments in West Asia. Urmia Lake is an oligotrophic lake of thalassohaline origin. It is located at an altitude of 1250 m above sea level. The total surface area ranges between 4750 km² and 6100 km² (Azari Takami, 1987). The maximum length and width of the lake are 128–140 km and 50 km, respectively. The average and maximum depths are reported to be about 6.0 m and 16.0 m, respectively. The lake is divided into a north and south arm separated by a causeway connecting the city of Urmia in West Azerbaijan with Tabriz in East Azerbaijan. The causeway has a gap of about 1 km, which allows for a limited exchange of water between the two parts of the lake. The construction of this causeway and the possible effects of this partitioning have been a matter of concern for many years, as a number of rivers (10–12) running through agricultural areas flow into the southern part of the lake. On the contrary, the number of rivers flowing into the northern part of the lake is

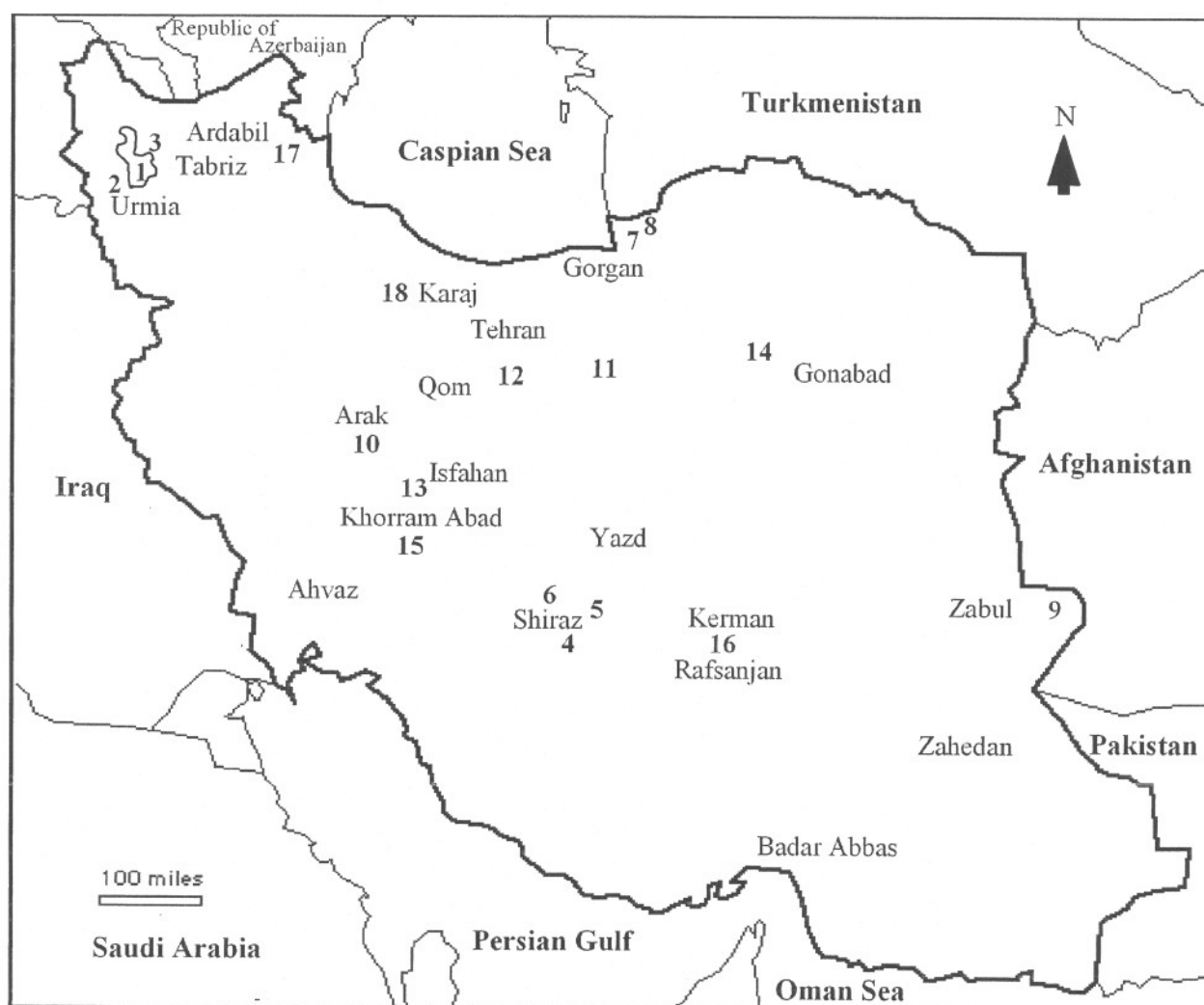


Figure 1. Distribution of *Artemia* sites in Iran. The names on the map refer to main cities while the numbers indicate the studied *Artemia* sites. 1, Urmia Lake; 2, Lagoons—West Azerbaijan; 3, Lagoons—East Azerbaijan; 4, Maharlu Lake; 5, Bakhtegan Lake; 6, Tashk Lake; 7, Incheh Lake; 8, Shor Lake; 9, Varmal Catchments; 10, Mighan Lake; 11, Qom Salt Lake; 12, Houze Sultan Lake; 13, Gaav Khooni Lake; 14, Kale Shoor—Gonabad; 15, Kale Shoor—Khorram Abad; 16, Nough catchments; 17, Shurabil Lake; 18, Kale Shoor Hastgerd. Geographical coordinates are given in Table 1.

Table 2. *Artemia strains studied for biometric analysis.*

Source of cysts	Site	Abbreviation used
Parthenogenetic strains:	Incheh Lake	INC
	Shoor Lake	SHO
	Qom Salt Lake	QOM
	Houze Sultan Lake	SUL
	Maharlu Lake	MAH
	Varmal Lake	VAR
	Lagoons around Lake Urmia	LAG
Bisexual <i>Artemia urmiana</i>	Coastal areas of Lake Urmia	URM-Par
	Sites in the Lake Urmia:	
	Ashk Island	ASH
	Islamic Island	ISL
	Golmankhaneh	GOL

smaller. Due to the prolonged drought (from 1999 to 2002) and the construction of a number of reservoirs/dams on major inflowing rivers, water salinity in the two arms of the lake remains relatively invariable. During the years of drought, the water salinity increased from 220 g/l in 1999 to more than 300 g/l. This increase in salinity made the coastline resemble huge 'crystallizers'.

Early in 2003, however, improved climatic conditions made water salinity drop to 250 g/l in the southern arm of the lake, whereas the salinity remained as high as 280 g/l in the northern arm. Due to high salinity, *Artemia* has not been able to recover fully in the northern arm, resulting in different population densities in the two arms.

The predominant species in Urmia Lake is the bisexual *Artemia urmiana*. This species is endemic to Urmia Lake and presents an interesting case of geographical isolation. However, parthenogenetic populations have been found to coexist with *A. urmiana* in coastal areas of Urmia with fluctuating salinity.

Lagoons around Urmia Lake (West and East Azerbaijan provinces)

These lagoons are located on the periphery of the lake. Their surface area varies from a few square metres to 10,000 m² and their depth is no more than 0.7 m. These lagoons are small water catchments that dry up in early summer and refill during winter. The water salinity in the lagoons is as low as 10–20 g/l in late winter, and rises gradually to saturation level. Parthenogenetic females were observed at high densities while occasionally rare males have been recorded at a maximum ratio of one male per 100 females. The study of the life cycle of this population in laboratory cultures showed that it reproduces asexually. It is worth noting that the parthenogenetic *Artemia* in these lagoons grows to maturity at very low salinities (as low as 10 g/l) and propagates at salinities of 20 g/l, which is exceptionally low compared with any other *Artemia* population investigated so far (Persoone & Sorgeloos, 1980).

Maharlu Lake (Fars province)

Maharlu basin is one of the independent basins located in the central region of Iran. It extends for 160 km from north-west to south-east. The width of the basin is about

43 km and its overall surface area is 4270 km². Maharlu Lake is situated at an altitude of 1454 m above sea level and its surface varies from 175 to 250 km². The average depth of the lake is 0.55 m (reaching 1 m during the rainy season). The deepest part of the lake is 2.5 m and is located at its north-east region. The total water volume of the lake is estimated to be 130 million m³. Water salinity ranges from 120 to 280 g/l. Few temporary rivers end in the lake, therefore, the Maharlu water surface fluctuates extensively and it is not considered as a permanent lake. A severe reduction in its water volume is observed when the precipitation is low and this was the case during this investigation. Between 1999 and 2002, the drought caused extensive evaporation. As a result, its water volume was reduced to less than one-third of its total volume. In Maharlu Lake only parthenogenetic females were observed. The asexual mode of reproduction of this population was also confirmed under laboratory conditions.

Bakhtegan Lake (Fars province)

This lake is situated 80 km east of Shiraz. Its surface area is about 3120 km². Its length is about 100 km and its width is approximately 30 km. The most important source of water feeding the lake is the River Kor. The maximum depth of Bakhtegan Lake is 2.0 m and the average is 0.4 m. Water salinity in the lake ranges from 60 to 250 g/l. It dried up in the summer of 2001 due to the excessive warm and dry conditions prevailing throughout Fars province. The local *Artemia* population is parthenogenetic.

Tashk Lake (Fars province)

It is situated next to Bakhtegan Lake with which it is connected by a strait. Tashk Lake was originally a fresh water lake but due to its connection to Bakhtegan Lake, water salinity at the connecting zone has increased to levels sufficiently high to support a viable *Artemia* population. A parthenogenetic *Artemia* population, which may have entered through the connecting strait, is observed only in this particular area of the lake.

Incheh Lake (Golestan province)

It is located 40 km north of Gorgan city. The surface area of this lake is 0.6 km². *Artemia* seems to be the only zooplanktonic organism living in this lake, but a number of different phytoplankton species such as *Gyrosigma*, *Nitzschia*, *Navicula*, *Chroococcus* and *Oscillatoria* have been isolated (Makhdomi, 1992). Water salinity is at least 150 g/l. This lake also suffered severely from the drought during the period from 1999 to 2002. The presence of *Artemia* in the lake was first reported by Makhdomi (1992). Only adult females were observed in the field. Asexuality was also verified by laboratory cultures.

Shor Lake (Golestan province)

It is located 20 km from Incheh Lake, and 60 km north of Gorgan city. The surface area of this lake is 2.0 km². Due to its vicinity to Incheh Lake, similar types of zoo- and phyto-plankton are found, but in lower densities due to the high salinity. This lake dried up in 2001 due to the prolonged drought. Makhdomi (1992) first reported the presence of *Artemia* in this lake. Nauplii hatched from

Table 3. Cyst diameter, length of instar-I nauplii and cyst chorion thickness from different *Artemia* populations from Iran.

Strain	Length of nauplii (μm)	SD	Diameter of hydrated cysts (μm)	SD	Diameter of decapsulated cysts (μm)	SD	Chorion thickness (μm)
INC	491.9 ^d	41.9	268.8 ^{abcde}	17.2	255.0 ^{bc}	22.6	6.9
SHO	486.6 ^d	39.4	264.3 ^{cdef}	16.9	250.6 ^{bc}	20.9	6.8
QOM	491.3 ^d	37.3	243.7 ^g	20.8	236.8 ^c	21.4	3.4
SUL	490.4 ^d	35.6	245.9 ^g	19.2	237.5 ^c	22.4	4.2
MAH	492.4 ^d	31.6	262.4 ^{cdef}	23.8	235.2 ^c	23.1	13.6
VAR	476.0 ^c	32.4	285.4 ^a	28.1	267.0 ^a	24.9	9.1
LAG	455.5 ^a	45.2	243.1 ^g	22.8	232.6 ^d	22.5	5.2
ISL	529.7 ^b	8.2	249.8 ^g	7.3	218.4 ^c	22.2	15.6
ASH	502.1 ^d	9.4	261.8 ^{cdef}	12.5	256.3 ^{bc}	25.6	2.7
GOL	483.5 ^c	36.3	280.7 ^{ab}	23.8	259.8 ^b	26.2	10.4
URM-Par	475.4 ^c	38.8	248.5 ^g	19.3	236.4 ^c	21.2	6.0

Populations sharing the same superscript per column, are not significantly different (P -value=0.0005).

cysts and cultured in the laboratory developed into parthenogenetic females only.

Varmal catchments (Sistan and Baluchestan provinces)

The Varmal Lake is located west of the city of Zabul, in eastern Iran close to the borders with Pakistan. Its surface area has been reported to range between 1.3 and 60 km². The water salinity varies from 18 to 45 g/l. Average depth is about 1.5 m and maximum depth is 5 m. The lake's total water volume is 90 million m³ (Piri & Tehrani, 1997). The lake dried up due to severe drought during the past four years. The existence of *Artemia* in this lake was first reported by Piri & Tehrani (1997). Nauplii, hatched from cysts and reared in the laboratory, developed exclusively into females.

Mighan Lake (Markazi province)

It is located 12 km north-east of Arak city at an altitude of 1670 m above sea level. Its surface area is about 112 km². Mighan Lake is a seasonal lake fed by three main rivers (Tabrene, Ashtian and Karez) during the wet season. It is converted into a saltpan during the dry periods of the year. Average and maximum depths of the lake are 0.8 and 2.0 m, respectively. Water salinity is usually very high ranging from 150 g/l to saturation level. The density of parthenogenetic *Artemia* in the lake is very low. The few adult females brought to the laboratory reproduced asexually. No males were observed.

Qom Salt Lake and Houze Sultan Lake (Qom province)

Qom Salt Lake is a large salt lake located north-east of Kashan and south-east of Tehran, with a surface of about 2400 km². Its length and width are 80 and 30 km respectively, and it is situated 800 m above sea level. Water salinity is above 200 g/l. It is fed by four permanent and/or seasonal rivers.

Houze Sultan Lake is situated in the same basin as Qom Salt Lake, between Qom and Tehran cities. The lake is 30 km long and 15 km wide, covering a surface area of 106 km². It dries up in summer, resembling a salty desert. It refills during winter and spring. Houze Sultan Lake is

located 790 m above sea level. Water salinity varies from 150 to 250 g/l.

Only adult females were observed in both lakes. Laboratory cultures confirmed the asexual status of *Artemia* populations.

Gaav Khooni Lake (Isfahan province)

Gaav Khooni Lake is situated 140 km south-east of Isfahan. It is one of the rare wetlands of Central Iran and, in this sense, it plays a critical role for migratory and native birds. It is an internationally protected natural reserve. The Gaav Khooni Lake occupies the centre of the Gaav Khooni region, which extends over an area of 2800 km². The main body of the lake runs from north to south. Its length and width are 45 and 25 km, respectively. The soil is salty throughout the region and a permanent salt crust covers large areas around the lake. It is an almost permanent saline lake and its major water source is the River Zarrineh 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 37.4°C (Asri et al., 2002). Only few adult females were observed in the lake.

Kale Shoor, Gonabad (Khorasan province)

A number of salty rivers locally known as Kale Shoor are found in Khorasan and other Iranian provinces. *Artemia* has been found in a number of them. Kale Shoor, Gonabad is one of these salty rivers. It is located 20 km away from Gonabad city in the province of Khorasan. These rivers are usually shallow (~ 0.8 m) but their depth may reach 2 m during rainy periods. They run through salty grounds, ending up in the Great Salt Desert where they gradually disappear. Water salinity in Kale Shoor, Gonabad ranges from 45 to 110 g/l, depending on the seasons.

Only adult females were observed in this salty river and no cyst samples could be collected. A number of adults were transported to the laboratory and studied for their reproductive mode. All females reproduced asexually.

Kale Shoor, Khorram Abad (Lorestan province)

In the province of Lorestan, Kale Shoor is located nearby Khorram Abad city. Water salinity fluctuates between 30 and 90 g/l. The water level rises during rainy seasons, resulting in a maximal depth of more than 1.2 m. In summer months, the water level drops due to increased evaporation. Only parthenogenetic females were observed. No cyst samples could be collected.

Nough catchments (Kerman province)

A catchment of about 0.4 km² has formed following the construction of a dam on a salty river 40 km away from the town of Nough, near the city of Rafsanjan. The maximum depth of this catchment is about 4.0 m, while its average depth is 2.0 m. Its water salinity fluctuates seasonally between 80 and 150 g/l. A parthenogenetic population of *Artemia* had colonized this environment before *A. franciscana*, originating from Great Salt Lake, Utah, USA, was introduced four years ago by a private company. The feral population is now fully dominant, since no parthenogenetic females are found anymore. Two to three metric tons of cysts (wet weight) are harvested annually from this site and are used in shrimp hatcheries of the southern provinces. There is another small lagoon of about two hectares in the vicinity of the Nough catchment, where the local parthenogenetic population is still thriving. The asexuality of this population was confirmed by laboratory culture tests.

Shurabil Lake (Ardabil province)

The Shurabil Lake is an alkaline lake located south of Ardabil city at an altitude of 1260 m. Its surface area is about 64 hectares and it has an average depth of 2.0 m (maximum 3.5 m at the centre). The climate is mesomediterranean with dry summers and cold winters. The lake is surrounded by high mountains and has no apparent outlet (Ahmadi, 1987). Until 1995, the zooplankton community in the lake was limited to *Artemia* sp., rotifers *Brachionus plicatilis* and *Hexarthra* sp., and several protozoans. Maximum densities (65 ind/l) of the *Artemia* population were observed in July and minimal densities (2 ind/l) in September. Water salinity is about 60–70 g/l. Precipitation of salt occurs in summer due to evaporation in the shallow north-western part of the lake (Ahmadi, 1987).

Kale Shoor Hashtgerd (Tehran province)

It is a salty river running a few kilometres away from Hashtgerd city. Only a few parthenogenetic females were observed in the river.

Biometrical analysis

Hydrated and decapsulated cyst diameters, chorion thickness and length of nauplii were measured. The results are summarized in Table 3 (for abbreviations see Table 2).

Significant differences ($P=0.0005$) are found both among the bisexual samples collected from different sites of Urmia Lake and among the parthenogenetic populations. There are also significant differences ($P=0.0005$) between the bisexual and the parthenogenetic samples. Cyst and naupliar sizes are usually significantly smaller in parthenogenetic populations compared with *A. urmiana*,

except for the Varmal strain, which displays the largest cyst size of all studied populations.

DISCUSSION

Artemia populations

In 1899, Günther announced the presence of *Artemia* in Lake Urmia. For nearly 90 years, there were no new reports on the occurrence of *Artemia* in other Iranian sites. Ahmadi (1987) reported the presence of an *Artemia* population in Shurabil Lake. In other reviews dealing with the biogeography of this genus, the presence of *Artemia* in Iranian sites other than Urmia Lake was rather limited (Vanhaecke et al., 1987; Triantaphyllidis et al., 1998). It is only recently that new data showed the existence of more sites than previously believed (Agh et al., 2001; Van Stappen, 2002). Nowadays, the data available enable us to suggest that the distribution of *Artemia* in Iran is definitely more extensive than expected.

Both bisexual and parthenogenetic populations exist in the Old World (Triantaphyllidis et al., 1998). In Iran, the only existing bisexual population is *Artemia urmiana*. The present survey shows that many parthenogenetic populations inhabit different Iranian saline lakes, lagoons and salty rivers, and many more await to be found.

In Urmia Lake, parthenogenetic populations are also found at restricted coastal areas with fluctuating salinity, indicating partial co-occurrence of bisexual and asexual populations in this site. This supports the earlier findings of Agh (2002) and some unconfirmed suggestions made by Azari Takami (Sorgeloos, 1989).

The existence of these thriving parthenogenetic populations in temporary brackish–hypersaline water bodies (i.e. lagoons around Urmia Lake), with salinities as low as 10 g/l, provides evidence that *Artemia* can survive and grow at low salinity in the absence of predators. This supports the earlier observations made by Agh & Noori (1997). Moreover, it was found that these parthenogenetic strains not only survive but also propagate at salinities as low as 20 g/l. This salinity is certainly lower than optimal levels required for existence and reproduction of *Artemia* in natural environments as reported by Persoone & Sorgeloos (1980) and detrimental to *A. urmiana* that ‘prefers’ hypersaline waters (Abatzopoulos et al., 2006). This situation seems to be a result of the partitioning effect due to salinity.

During this investigation many *Artemia* sites in Iran were surveyed. Some of them were small seasonal water catchments; others were in the process of drying up or had already dried up at the time of our visit. Our survey shows that *Artemia* is present in 17 different locations in Iran. All these populations are parthenogenetic, except for *A. urmiana* in Lake Urmia. The *Artemia* population reported in Shurabil Lake (Ahmadi, 1987) has become extinct due to the drastic reduction of water salinity and the introduction of freshwater fish into the lake.

Throughout Iran, *Artemia* populations and their habitats have suffered greatly from the prolonged drought of the last years. Some of these lakes have dried up, whereas others are filled with saturated brine. Therefore, it is expected that *Artemia* populations living in other sites

than Urmia Lake may have suffered severely. A critical question is raised regarding the complete absence of parthenogens from the main body of the lake. Unlike Urmia, which is a permanent water body, the rest of the sites are characterized as astatic, temporal or ephemeral catchments. This means that they are flooded with huge volumes of water and this may cause severe 'dilution' of the populations, which reappear from cysts. As a result, organisms with asexual reproduction are expected to have a significant advantage in such conditions, and may have been selected for, compared with those reproducing sexually. The fact that parthenogenetic *Artemia* populations appear every year in the astatic lagoons situated perimetrically to Urmia Lake and, even more interesting, in the salty rivers where finding mates has become nearly impossible for *Artemia* further supports this inference.

From the existing bibliography it is easily deduced that *A. urmiana* is phylogenetically very close to parthenogenetic *Artemia* which mainly originated in the eastern Mediterranean basin. Several studies using different molecular markers (i.e. allozymes, amplified fragment length polymorphisms, randomly amplified polymorphic DNAs and/or mtDNA restriction fragment length polymorphism analyses) have reached the very same conclusion, i.e. that parthenogens are likely to be closely related to the line that led to *A. urmiana* (Beardmore & Abreu-Grobois, 1983; Triantaphyllidis et al., 1997; Abatzopoulos et al., 2002b). In this work, the co-occurrence of *A. urmiana* with asexual *Artemia* partitioned by salinity or the fact that parthenogenetic populations are found in so many sites in Iran support the close relatedness of *A. urmiana* with asexual *Artemia*. Another interesting point is the appearance of 'rare' males. The case of 'rare' males has been documented by several researchers: they do appear unexpectedly in obligate parthenogenetic *Artemia* populations bearing an undefined role (Browne & Bowen, 1991 and references therein). Although they are scarce and their frequency fluctuates between 0.15–1.2%, rare males may be also an indication for the existence of a mixed population.

Certainly, further studies involving the use of molecular markers will give valuable data on the phylogenetic relationships between the bisexual *A. urmiana* and the other parthenogens in Iran as well as on the purity of asexual *Artemia* populations.

Biometrical data

Iranian *Artemia* populations show high variability in their cyst and naupliar biometrics. Efforts have been made to detect differences that could contribute to some extent to population discrimination. According to the results obtained in this investigation, the cyst size ranges from 243.2 to 285.4 μm , with the smallest cyst size belonging to the populations from lagoons around Urmia Lake, and the largest to the Varmal parthenogenetic strain. The naupliar size also varies from 455.5 (asexual strain from the lagoons around Urmia) to 529.8 μm (population from the Islamic Island in Urmia).

In the present study, significant differences among investigated strains have been demonstrated for several biometrical parameters. Our data confirm the findings of other authors (D'Agostino, 1965; Amat, 1980; Vanhaecke & Sorgeloos, 1980; Abatzopoulos et al., 1989; Triantaphyllidis

et al., 1993; Sorgeloos, 1997) about the strain-specificity of cyst biometrics. Although these criteria have been utilized in the past for strain characterization, they cannot be considered as reliable for defining the origin of unspecified cyst samples. This is due to significant differences observed in cyst samples collected from different harvesting sites in Urmia Lake as well as in the nauplii hatched from these cysts. Such differences were also reported earlier by Sorgeloos (1997) and can be attributed to seasonal fluctuations in physico-chemical parameters and food availability in the different regions of Lake Urmia. Similar findings on size variation within the cyst batches of the same population/species were also reported in another study (Vanhaecke & Sorgeloos, 1980). In order to confirm this size variation, further investigation using more detailed sampling is necessary.

The cyst and naupliar sizes of *A. urmiana* are usually larger than the values reported for other bisexual species. Focusing on cysts, the *A. franciscana* cyst diameter is 224.7–228.7 μm for the San Francisco Bay strain and 244.2–252.5 μm for the Great Salt Lake strain while for *A. persimilis* (Argentina) it is 238.2 μm (Vanhaecke & Sorgeloos, 1980) and for *A. salina* (Tunisia) it is 235.4–258.8 μm (Van Ballaer et al., 1987). However, *A. urmiana* cysts are smaller than those of *A. tibetiana* (323–336 μm ; Abatzopoulos et al., 1998).

With the exception of the Varmal strain, the Iranian parthenogenetic cysts are smaller compared with many other parthenogenetic populations tested for their cyst biometry, such as Tuticorin, India (283.8 μm ; Vanhaecke & Sorgeloos, 1980); Margherita di Savoia, Italy (284.9 μm ; Vieira & Teles, 1984) various Chinese strains (282 μm ; Zhenqiu et al., 1991); Kara Bogaz Lake, Turkmenistan (268 μm), Bolshoe Yarovoe (276 μm) and Pavlodar, (270 μm), both in Siberia, Russia (Naessens & Van Stappen, 2001). Compared with the parthenogenetic cysts harvested in Greece, i.e. in Citros (260.2 μm) and Megalon Embolon (264.7 μm) (Abatzopoulos et al., 1989), and in Kalloni (255.4 μm) and Polychnitos (269.7 μm) (Triantaphyllidis et al., 1993), the Iranian parthenogenetic cysts are either slightly smaller or similar in size. According to the above findings, it seems that the Iranian cysts from Qom Salt Lake (243.7 μm), Houze Sultan (245.9 μm), lagoons at the periphery of Urmia Lake (243.1 μm) and coastal areas of Lake Urmia (248.5 μm), together with Spanish diploid and triploid populations (240 μm ; Amat, 1980) and a Namibian diploid strain (246.7 μm ; Triantaphyllidis et al., 1996) are among the smallest parthenogenetic *Artemia* cysts reported so far.

One of the most interesting findings in this survey concerns the large differences in chorion thickness among cysts collected from different sites (see Table 3). Certainly, chorion thickness has substantial impact on cyst floating capacity, which is also affected by the level of salinity. It has been observed that cysts from Urmia Lake tend to sink even in high salinity brines; this poor floating capacity can be attributed to the cyst chorion structure, i.e. the alveolar layer, which is mainly responsible for cyst buoyancy. The use of transmission electron microscopy has revealed a thin alveolar layer and a thicker fibrous layer in Urmia Lake cysts when they were compared with *A. franciscana* cysts (Abatzopoulos et al., 2006). Cyst

membrane protein composition and abundance, based on sodium dodecyl sulphate–polyacrylamide gel electrophoresis, have corroborated the uniqueness of the *A. urmiana* cyst structure (Triantaphyllidis et al., 1994; Abatzopoulos et al., 1997). A wide range in chorion thickness has been recorded in this study for several Iranian parthenogenetic strains (Table 3); further experimentation is needed to check whether the same is true for these asexual populations. It is generally accepted that the prevailing conditions during encystment not only affect significantly chorion formation but also trigger embryo encapsulation (Clegg, 2001; Abatzopoulos et al., 2002a).

In conclusion, a disturbing event should be noted: the establishment of *Artemia franciscana* in Nough catchments after human intervention. According to a very recent bibliography, *A. franciscana* has proven a very successful colonizer, which out-competes the endemic Old World bisexuals or parthenogens (Amat et al., 2005). Special efforts must be invested in preventing the spreading of this highly invasive species to neighbouring saline lakes or areas.

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