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Characterization of *Artemia* Populations from Iran

Thesis submitted in fulfillment of the requirements for the degree of Doctor (Ph. D.) in Applied Biological Sciences (Aquaculture)

Dutch translation of the title: “Karakterisatie van *Artemia* populaties uit Iran”

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Last but not least, I have to extend my most sincere thanks to my mother, my wife and my sons. I thank my mother for her daily prayers for my health, success and happiness. I had to work hard all these years and could not spare much time for my wife and my children. I know they have suffered a lot due to my absence from home. But future is ahead and we should plan and look forward to build happier life to compensate the bitter days of past. I will do my best to create livelier environment for my family in future.

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CHAPTER 1

GENERAL INTRODUCTION and THESIS OUTINE

General Introduction and Thesis Outline

Artemia is a unique cosmopolitan anostracan living in hypersaline and saline lakes, ponds, lagoons and man-made salterns. It is characterized by communities with low species diversity and simple trophic structures compared to fresh and marine water environments (Por, 1980; Lenz, 1987). *Artemia* is very well adapted to the severe physiological demands imposed by these ecosystems. Covered by a nearly impenetrable exoskeleton and able to pump out excess of salt, brine shrimp can tolerate the widest salinity range of any other multicellular organism (Browne, 1993). Due to its unique osmoregulatory capacity, *Artemia* is found in a wide range of saline environments with salinity levels ranging from 10 to 340 g/l, which are characterized by diverse chemical compositions (Post and Yossef, 1977; Persoone and Sorgeloos, 1980; Agh, 2002).

The genus *Artemia* is comprised of a number of sexual species and numerous parthenogenetic populations. In the New World, only two sexual species of *Artemia*, *Artemia persimilis* (Pincinelli & Prosdocimi), and *Artemia franciscana* (Kellogg, 1906) have been reported. But in the Old World, five sexual species and many parthenogenetic populations are reported. The sexual species include *Artemia salina* (Schlosser, 1755) from Spain, North Africa, Italy, and Cyprus (Triantaphyllidis *et al*., 1997abc), *Artemia urmiana* from Lake Urmia (Iran) (Gűnther, 1890), *Artemia sinica* from P.R. China (Cai, 1989), *Artemia* sp. from Kazakhstan (Pilla and Beardmore, 1994; Lavens and Sorgeloos, 2000) and *Artemia tibetiana* from Tibet (P.R. China) (Abatzopoulos *et al*., 1998, 2002ab). Parthenogenetic populations are spread all over Asia, Europe, Africa and Australia.

Artemia was first described by Schlosser in 1755 (Kuenen and Baas-Becking, 1938) From early 1900 it attracted the attention of the scientists and since then thousands of research activities were carried out on different issues, ranging from ecology, biology, molecular biology, physiology, toxicology, and radiobiology, and especially on its use in aquaculture. Its use in larviculture of fish and shellfish on one hand brought about a substantial development in the aquaculture industry, and on the other hand it stimulated more and more scientists to look for advanced techniques aiming to upgrading of *Artemia* applications: e.g. promote higher quality

larvae with improved survival rates. Its use in aquaculture, therefore, increased year by year and attained a record annual consumption of over 2000 metric tonnes of *Artemia* cysts and tens of thousands of *Artemia* biomass (Van Stappen and Sorgeloos, 1993; Lavens and Sorgeloos, 2000).

The introduction of *Artemia* nauplii as food source for 'newborn' fish larvae by Seale (1933) and Rollefsen (1939), paved the way for accelerated development of hatchery activities of fish and shrimp (Sorgeloos, 1980). Aquaculture activities, especially aquaculture of economically important marine fish and shellfish species has shown a constantly increasing trend over the last few decades. The decline of *Artemia* cyst harvests from the Great Salt Lake in Utah, USA since 1977 (Lavens and Sorgeloos, 2000) has intensified the search for alternative resources, especially in inland lakes that are sufficiently large and productive to justify commercial exploitation.

Urmia Lake in Iran, with more than 5000 km^2 surface area, has always been considered as a potentially appreciable natural resource of *Artemia*. It is indeed comparable to the Great Salt Lake of Utah and could have undoubtedly a crucial role in contributing to the world demand for *Artemia* cysts and biomass. However, Iranian *Artemia* resources are not only limited to Urmia Lake: due to the numerous salt water bodies, the presence of *Artemia* in different parts of the country seemed very likely. Moreover, Iran with its extensive coastal areas in the south of the country and with climatic conditions favourable for *Artemia* culturing has large potentials for *Artemia* pond production. There are tens of thousands of hectares of salt pans at the vicinity of the Persian Gulf and the Oman Sea, which provide excellent opportunities for *Artemia* production in the southern and south-eastern Iran.

Aquaculture activity has started in Iran in 1960 with trout culture and has developed to a rapidly growing industry during the last decade. According to data published by FAO, Iran ranked first among the top ten aquaculture producers in terms of annual growth rate in the period from 2000 till 2002 (Table 1.1) (FAO, 2004).

Table 1.1. Top ten aquaculture producers in terms of growth (FAO, 2004)

Country	2000	2002	APR
	(1000 tonnes)		(%)
Iran	40.6	76.8	37.6
Faeroer Islands	32.6	50.9	25.0
Laos	42.1	59.7	19.1
Brazil	176.5	246.2	18.1
Chile	391.6	545.7	18.0
Russia	74.1	101.3	16.9
Mexico	53.9	73.7	16.9
Taiwan	243.9	330.2	16.4
Canada	127.6	172.3	16.2
Myanmar	98.9	121.3	10.7

APR refers to Average Annual Percentage growth rate

Expansion of aquaculture activities in Iran mainly covers freshwater fish (carp, trout, etc), sturgeon and marine fish and shrimp culture. Freshwater fishes are cultured all over Iran and account for almost 90% of overall cultured fish. The consumption per capita of fish has been growing in Iran since the last decade and therefore, aquaculture has grown into a profitable industry. The Iran Fisheries Organization has plans to expand sturgeon fish and shrimp culture extensively in the north and south Iran. Caspian sturgeon is used to supply 90% of the world caviar until 2002. But sturgeon catch and export of caviar has been banned since 2002 due to the extreme reduction of their resources in the Caspian Sea that happened as a result of over-exploitation, water contamination and invasion by a Cnidarian jellyfish that predates on zooplankton and has caused a dramatic change of the ecosystem affecting negatively the sturgeon food cycle. Therefore, pond culture of sturgeon is being advocated by both international agencies for the preservation of endangered species, and by the local authorities. This program, which has started some 7-8 years ago, has gained in importance, and more land in the vicinity of the Caspian Sea is being allocated for this purpose. Shrimp culture started in the early 1990's and according to preliminary studies, there is a potential of more than 100,000 hectares of suitable

land along the Persian Gulf and Oman Sea for this activity, of which some 40,000 hectares of land is allocated to investors so far. Thirdly, research and pilot plants for the culture of local marine fish species, hamoor (grouper) (*Epinephelus coioides*); shanak (yellow fin sea bream) (*Acanthopagrus latus*) and sobeity (silver bream) (*Sparidentex hasta*) has begun in Khuzestan, a southern province of Iran, since 2003. This program will also include many other valuable local species in the future.

Successful management and guaranteed achievements in all these programs are highly dependent on proper use and modern applications of *Artemia* as a valuable source of food, and also as a carrier of essential nutrients for the fish and shrimp larvae at early stages of growth. Therefore, a special interest is paid to the identification of natural resources of *Artemia* in Iran and to the possibilities of exploitation of existing resources as an alternative to the high cost of imported cysts. Moreover pond culture of *Artemia*, particularly in south Iran, also has received special attention and preliminary pilot plants are being set up.

Research objectives

The general objectives of this thesis were:

- To present an overview of *Artemia* biotopes in Iran.

- To identify new biotopes and to characterize them on the basis of their ecological and biological characteristics.

- To study the responses of some populations from larger biotopes situated at varying geographic locations at different salinities, in order to clearly identify these populations for better use in Iranian aquaculture projects.

- To clarify the long lasting confusion about the reproductive status of *Artemia urmiana* in Lake Urmia and the possible coexistence of sexual and asexual populations in this lake.

- To find out the role of salinity in distinguishing the bisexual from the parthenogenetic populations.

- To study Reproductive, morphometric and genetic characteristics aiming to find out the relationship between the *Artemia* populations existing in Iran.

- To support the hypothesis proposed by Browne et al. (1991) and Browne (1992), stating that the ***parthenogenetic lineage*** might have been derived from *A. urmiana*.

- To provide some valuable information for the selection of more suitable strains of *Artemia* in pond culture projects.

The specific objectives can be summarized as follows:

- Identification of new biotopes of *Artemia* in Iran and preliminary characterization of the populations based on their reproductive status and biometry of cysts and nauplii.

- Coexistence of bisexual *Artemia urmiana* and parthenogenetic populations in Lake Urmia and in the lagoons in the vicinity of the lake.

- Reproductive, morphometric and genetic characterization of some sexual and asexual *Artemia* populations found in Iran.

- Performance of the Iranian *Artemia* populations, subjected to different salinities, with regard to their growth, survival, and reproductive and life span characteristics.

- Enrichment of *Artemia urmiana* cysts aiming at improving its nutritional quality.

This thesis includes the following chapters:

Chapter I (*general introduction*) reviews the life history, taxonomy and morphology of *Artemia* and its use in aquaculture.

Chapter II (*Artemia sites in Iran*) reports on new *Artemia* populations from different geographic locations in Iran and defines their reproductive status in nature and in the laboratory and their biometry of cysts and nauplii, comparing them with other, previously described populations from other parts of the world.

Chapter III (*Co-existence of bisexual and parthenogenetic Artemia populations in Lake Urmia and neighboring lagoons*) aims at clarifying the long standing confusion on the sexual status of *Artemia* in Lake Urmia, the presence of a mixed population in the lake, and at finding out the determining factor for localization of asexual populations in small lagoons in the periphery of the lake.

Chapter IV *(Effects of salinity on survival, growth, reproductive and life span characteristics of Artemia populations from Lake Urmia region)* presents important aspects of the effects of salinity on growth, survival, maturity, reproductive and life span characteristics under standard laboratory conditions. The aim of this study is to have information about the adaptation capacity of different *Artemia* populations of Iran to varying salinities, aiming at a better application in aquaculture and pond culture activities.

Chapter V *(Life cycle characteristics of six Artemia populations from Iran)* is comparing the maturation, reproductive and life span characteristics of six Iranian populations of *Artemia* in order to distinguish and select the best qualified strain for use in pond culture programs in Iran.

Chapter VI (*Studies on the enrichment of Artemia urmiana cysts for improving fish food value)* aims at improving nutritional quality of the newly hatched nauplii. Special emphasis is put on elevation of EPA and DHA levels in cyst and nauplii.

Chapter VII *(Morphometric and genetic characteristics of Artemia populations from Iran)* is examining the adult morphometry and genetic characteristics based on RFLP finger printing techniques to find out morphometric and genetic similarities and dissimilarities among the *Artemia* populations from Iran.

Chapter VIII (*General discussion and conclusion***)** discusses the overall results of this thesis in the framework of the research objectives.

References contain all the bibliography cited in the general introduction and all chapters included in this thesis.

CHAPTER 2

Artemia sites in Iran

T.J. Abatzopoulos, N. Agh, G. Van Stappen, S.M. Razavi Rouhani and
P. Sorgeloos (2006)

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Abstract

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 absences 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 (Gunther, 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 2.1). 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 cylindroconical 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 form Urmia Lake). After reaching maturity, the sex ratio, the 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).

Table 2.1. 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 (*A. urmiana*) Parthenogenetic	37°20'E-45°40N
2	Lagoons around Urmia lake Urmia & 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 Bisexual (*A. franciscana*)	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 e Kavoos, Golestan Province	Parthenogenetic	37°25'E-54°41'N
8	Shor Lake Gonbad e Kavoos, Golestan Province	Parthenogenetic	37°24'E-54°36'N
9	Varmal Catchment Zabol, Sistan & 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 Bisexual (*A. franciscana*)	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	Parthenogenetic Bisexual (*A. franciscana*)	30°60’E-56°50’N
17	Shurabil Lake Ardabil, Ardabil Province	Parthenogenetic (extinct)	38°25’E-48°55’N
18	Kale Shoor Hashtgerd Karaj, Tehran province	Parthenogenetic	35°90’E-50°78’N
19	Solar Salt Ponds Mahshahr, Khuzestan province	Bisexual (*A. franciscana*)	30°33’N-49°11’E

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.

Results

Description of Artemia sites in Iran

Nineteen salt lakes, lagoons, solar salt ponds or salty rivers were investigated for the occurrence of brine shrimp. *Artemia* populations were found in 18 of these sites (Figure 2.1 and Table 2.1).

Urmia Lake (West Azerbaijan province)

Urmia Lake is located 21km 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 1250m above sea level. The total surface area ranges between 4750 km^2 and 6100 km^2 (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 6.0m 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 1km, 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 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’.

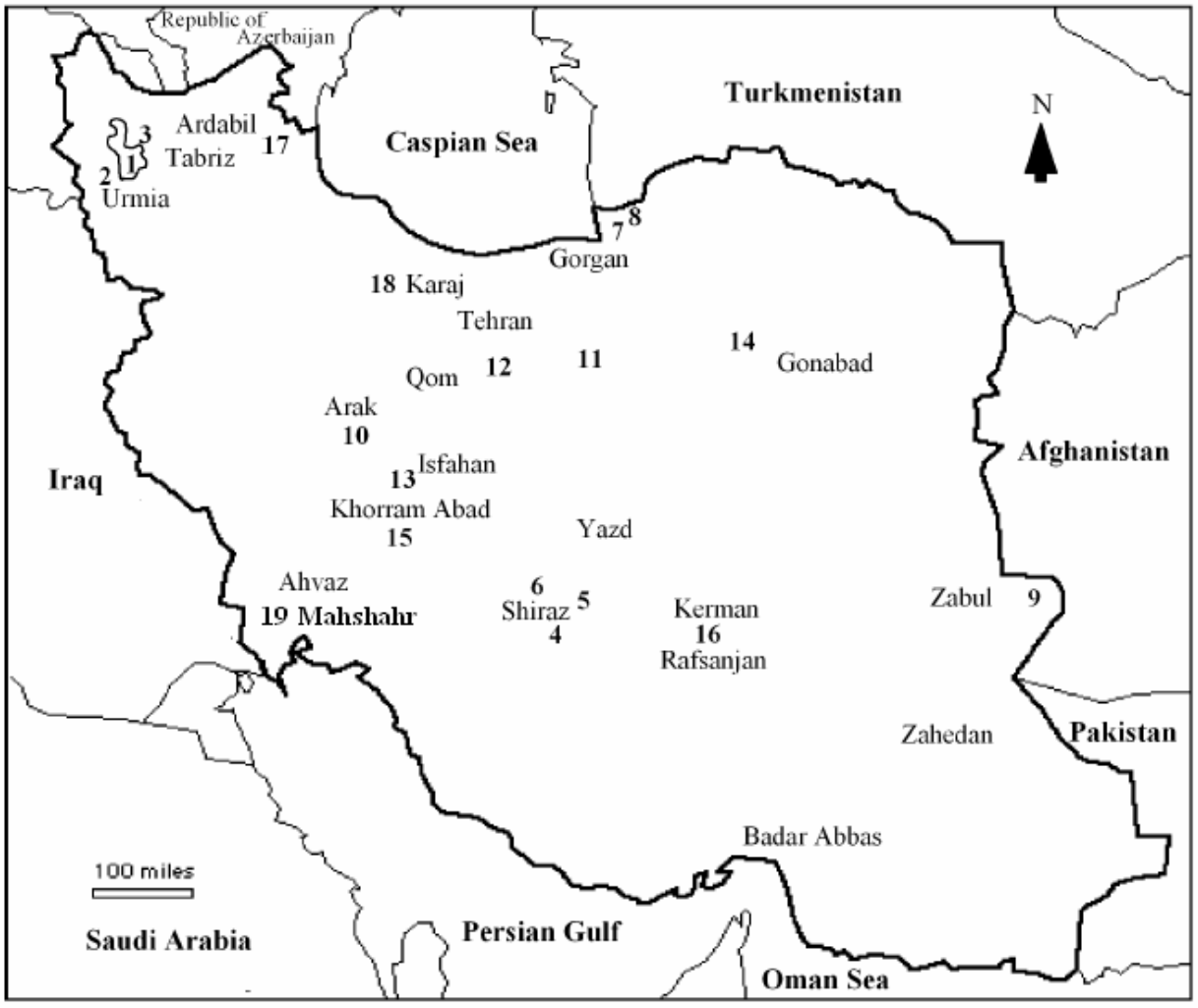

Figure 2.1. Distribution of *Artemia* sites in Iran. The names on the map refer to main cities while the numbers indicate the studied *Artemia* sites. 1, Urmia Lake; 2, Lagoons-West Azerbaijan; 3, Lagoons-East Azerbaijan; 4; Maharlu Lake; 5, Bakhtegan Lake; 6, Tashk Lake; 7, Incheh Lake; 8, Shor Lake; 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 Hashtgerd; 19, Solar Salt Ponds, Mahshahr. Geographical coordinates are given in Table 2.1.

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 10,000m^2 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 km^2. Maharlu Lake is situated at an altitude of 1454m above sea level and its surface varies from 175 to 250 km^2. The average depth of the lake is 0.55m (reaching 1m during the rainy season). The deepest part of the lake is 2.5m and is located at its north-east region. The total water volume of the lake is estimated to be 130 million m^3. 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 km^2. 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 2.0m and the average is 0.4m. 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 km^2. *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 km^2. 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 cysts and cultured in the laboratory developed into parthenogenetic females only.

Table 2.2. *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
	- Coastal areas in Lake Urmia	URM-Par
Bisexual *Artemia urmiana*		
	- Ashk island harvesting site	ASH
	- Islamic island harvesting site	ISL
	- Golmankhaneh harvesting site	GOL

Varmal catchments (Sistan and Baluchestan provinces)

The Varmal Lake is located west of the city of Zabol, in eastern Iran close to the borders with Pakistan. Its surface area has been reported to range between 1.3 and 60 km^2. The water salinity varies from 18 to 45 g/l. Average depth is about 1.5m and maximum depth is 5 m. The lake's total water volume is 90 million m^3 (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 1670m above sea level. Its surface area is about 112 km^2. Mighan Lake is a seasonal lake fed by three main rivers (Tabrene, Ashtian and Kareh) 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 2.0m, 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 km^2. Its length and width are 80 and 30 km respectively, and it is situated 800m 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 km^2. It dries up in summer, resembling a salty desert. It refills during winter and spring. Houze Sultan Lake is located 790m above sea level. Water salinity varies from 150 to 250g/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 37.48°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 (~ 0.8m) but their depth may reach 2m 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.

Nough catchments (Kerman province)

A catchment of about 0.4 km^2 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.

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.

Shurabil Lake (Ardabil province)

The Shurabil Lake is an alkaline lake located south of Ardabil city at an altitude of 1260m. Its surface area is about 64 hectares and it has an average depth of 2.0m (maximum 3.5m 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.

Solar Salt Ponds, Mahshahr (Khuzestan province)

These Solar Salt Ponds are situated close to the Mahshahr Petrochemicals, near Mahshahr city. The total surface area of the ponds is about 12 Km^2. Water enters the ponds as a result of tidal increase in water level from the Persian Gulf. *A. franciscana* was introduced into these ponds by a private company engaged in *Artemia* cyst business.

Biometrical analysis

Hydrated and decapsulated cyst diameters, chorion thickness and length of nauplii were measured. The results are summarized in Table 2.3 (for abbreviations see Table 2.2).

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.

Table 2.3. Cyst diameter, length of instar-I nauplii and cyst chorion thickness from different *Artemia* population from Iran

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

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

Discussion

Artemia populations

In 1899, Gunther 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 *A. urmiana*. The present survey shows that many parthenogenetic populations inhabit different Iranian saline lakes, lagoons and salty rivers, and many more wait 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 lowas10 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 percent, 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 2.3). 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 2.3); 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 *A. 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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CHAPTER 3

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

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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 g/l. 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 g/l) 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 g/l), whereas higher salinities (above 50 g/l) 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, 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^2 (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 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 on major rivers discharging into the lake, both arms of the lake look alike, at least during six months of the year. Since the construction of dams all permanent rivers have been effectively converted into temporary ones. As a result, no significant differences in salinity have been observed at both arms of the lake. During those years, the lake experienced grave conditions as a result of a dramatic increase in salinity reaching a maximum of 220 g/l in 1999 and over 300 g/l during 2001–2002.

From early 2003 onward, due to favorable climatic changes, water salinity dropped to about 225 g/l at the surface and 265 g/l at the bottom in the South arm of the lake. In contrast, salinity remained high (265–270 g/l) 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 VanStappen *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 (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, proper, 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; Triantaphyllidis *et al*., 1997; Abatzopoulos *et al*., 2002; Baxevanis *et al*., 2005).

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 at the perimeter of Lake Urmia and from 6 different sampling sites 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) (Figure 3.1). 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 g/l) 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 g/l) and kept in a freezer at -20ºC for a period of 2 months to induce diapause deactivation. Then the cysts were stored at 4ºC and used for hatching after one week.

Hatching was carried out in cylindro-conical flasks according to Sorgeloos *et al*. (1986) using 15 g/l diluted Lake Urmia water for 24 h at 28°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 that 75%. The hatched nauplii were transferred to cones containing diluted lake water with different salinities varying from 15 to 80 g/l, each in 6 replicates. Initial salinities of 15, 20 and 25 g/l in the first 3 treatments were raised to 33 g/l within 6, 4 and 2 days, respectively. In the next 3 treatments 33, 50 and 80 g/l were used from the beginning and throughout the 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 g/l. 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 Coutteau *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. Adult virgin females were transferred to individual 50 ml Falcon tubes and were observed for their reproductive mode in order to determine 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. *Artemia urmiana* presents 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.

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

Figure 3.1. 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.

2.2. Field Experiments

Eight ponds of 2890 m^2 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 g/l in six ponds (for *Artemia* culture) and 60 g/l 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 g/l throughout the experiment, which is significantly lower compared with the current salinity range of Lake Urmia (200–300 g/l).

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 g/l. 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 × 30 × d cm^3; 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 μm-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 μm-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 season. However, salinity never dropped below 190 g/l even at these areas, whereas salinity in off coast sites was always higher than 220 g/l. The salinity in the lagoons ranged from 10 g/l to saturation by the end of the season. The results obtained from the laboratory experiments are summarized in Tables 3.1 and 3.2 and in Figure 3.2 (a–g).

Artemia hatched from cysts collected in the lake did not perform well at the very low start-up salinities of 15–25 g/l in the culture experiments. Mortality as high as 100% was observed in almost all lake samples when the culture was started at 15 g/l, and very low survival was observed at 20 and 25 g/l. 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 g/l, significantly higher ($p < 0.05$) than those hatched from lake samples in these salinities (Figure 3.1).

We found that nauplii hatched from coastal samples, when grown in salinities lower than 33 g/l, gave rise to parthenogenetic females (maturity was attained between days 14 and 17 of culture), although survival was very low (0.00–4.21%). 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 (Figure 3.1). Furthermore, at higher salinities most or even all adult animals were sexual. Significant differences ($p < 0.05$) were observed in survival and reproductive mode of *Artemia* with regard to salinity (Figure 3.1). *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 g/l), as they do in their natural habitat, but the parthenogenetic populations sampled from Lake Urmia preferred a slightly higher salinity (33–50 g/l) to begin with (Figure 3.1). Highest survival (48–63%) was observed at 80 g/l for *Artemia* hatched from Lake Urmia cysts, resulting in sexual animals, whereas survival was highest (67.37%) at 33 g/l in parthenogenetic *Artemia* grown from

lagoon cysts. The lowest survival was recorded at 80 g/l for this parthenogenetic population (Figure 3.1). The survival of *Artemia* reared at 80 g/l was significantly higher in all samples originating from Lake Urmia compared with other salinities (Figure 3.2a–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.

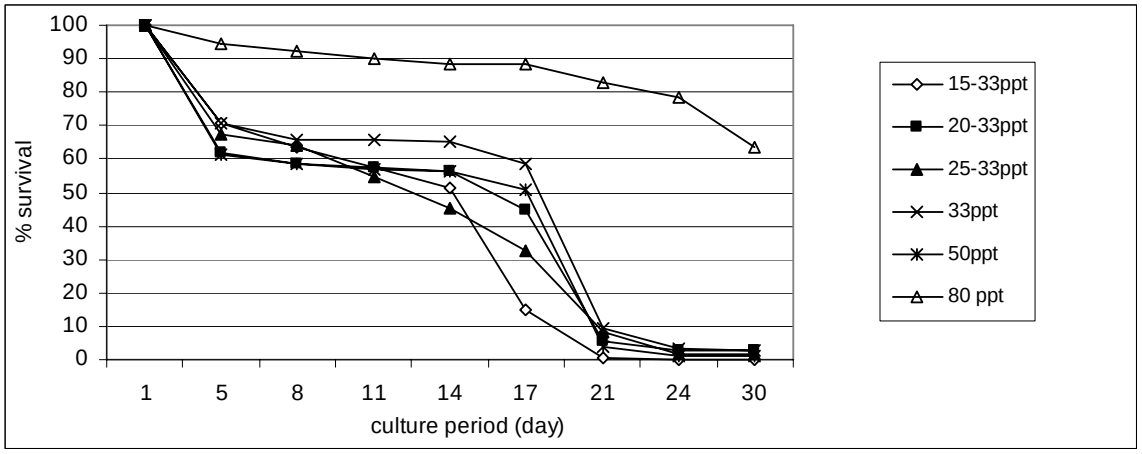
Figure 3.1. Survival values of *Artemia* hatched from cyst samples harvested from different sites of Lake Urmia and cultured at different salinities. Salinity groups (per population) sharing the same superscript are not significantly different (p level 0.05).

Site	Salinity (g/l)	% Survival (± s.d.)								Reproductive status
		Day 1	Day 5	Day 8	Day 11	Day 14	Day 17	Day 21	Day 24	Day 30	
Golmankhaneh (coastal)	15–33	100	70.88 (1.291)	63.38 (2.565)	57.63 (1.780)	52.40 (2.758)	15.10 (3.362)	0.40 (0.254)	0.00	0.00 ^{c}	Parthenogenetic
	20–33	100	61.63 (2.811)	58.75 (1.556)	57.25 (1.856)	56.20 (0.975)	44.80 (1.247)	5.50 (1.112)	2.88 (0.559)	2.54 ^{b} (0.481)	Parthenogenetic
	25–33	100	67.25 (1.905)	64.13 (2.271)	54.88 (1.790)	45.60 (2.764)	32.40 (3.160)	8.30 (2.113)	1.62 (0.461)	1.45 ^{b} (0.306)	Parthenogenetic
	33	100	70.72 (1.768)	65.92 (2.601)	65.92 (3.156)	65.30 (1.232)	58.60 (1.742)	9.70 (1.450)	3.11 (1.270)	2.68 ^{b} (1.267)	Parthenogenetic
	50	100	61.13 (2.350)	58.38 (2.136)	57.00 (2.356)	56.40 (2.345)	50.90 (2.517)	3.80 (1.176)	1.25 (0.449)	1.00 ^{b} (0.249)	Mixed
	80	100	94.73 (0.958)	92.09 (1.676)	89.95 (2.025)	89.70 (1.479)	88.40 (1.061)	83.10 (1.550)	78.33 (1.658)	63.48 ^{a} (2.001)	Bisexual
Tappeh Shahi (coastal)	15–33	100	72.25 (1.713)	64.86 (1.874)	59.88 (1.698)	53.10 (1.574)	18.30 (2.746)	2.00 (0.097)	0.50 (0.125)	0.25 ^{c} (0.062)	Parthenogenetic
	20–33	100	60.26 (1.806)	56.66 (1.772)	55.97 (1.047)	53.32 (1.346)	41.70 (3.022)	4.50 (1.470)	2.45 (0.572)	1.65 ^{c} (0.539)	Parthenogenetic
	25–33	100	62.48 (1.553)	60.85 (0.494)	51.62 (2.467)	42.55 (3.198)	29.50 (3.206)	6.50 (1.547)	2.22 (0.597)	1.45 ^{c} (0.413)	Parthenogenetic
	33	100	73.85 (1.562)	68.21 (1.906)	62.54 (1.596)	60.21 (1.474)	55.70 (2.520)	12.30 (3.217)	4.54 (1.454)	4.21 ^{c} (1.165)	Parthenogenetic

	50	**100**	**65.97 (1.778)**	**61.25 (1.715)**	**58.20 (1.797)**	**56.36 (1.604)**	**51.90 (2.405)**	**16.90 (2.318)**	**11.26 (3.242)**	**8.98 [b] (2.302)**	**Mixed**
	80	**100**	**92.55 (0.743)**	**90.21 (0.457)**	**87.96 (0.646)**	**86.22 (0.727)**	**84.50 (1.663)**	**81.50 (2.670)**	**77.25 (2.462)**	**60.30 [a] (4.811)**	**Bisexual**
Rashakan (coastal)	**15–33**	**100**	**68.64 (1.515)**	**60.96 (3.007)**	**55.69 (2.357)**	**49.56 (1.521)**	**17.20 (4.235)**	**1.60 (1.097)**	**0.33 (0.328)**	**0.00 [c]**	**Parthenogenetic**
	20–33	**100**	**57.25 (2.615)**	**53.26 (1.784)**	**52.05 (1.089)**	**49.73 (1.528)**	**39.20 (4.181)**	**3.60 (2.150)**	**1.96 (1.588)**	**1.00 [b] (0.808)**	**Parthenogenetic**
	25–33	**100**	**59.35 (2.543)**	**57.20 (1.957)**	**54.54 (1.694)**	**51.29 (1.834)**	**46.50 (2.527)**	**5.20 (3.226)**	**1.77 (1.490)**	**0.80 [b] (0.736)**	**Parthenogenetic**
	33	**100**	**70.16 (1.695)**	**64.12 (1.797)**	**58.16 (2.692)**	**55.17 (2.356)**	**42.60 (3.219)**	**9.90 (2.923)**	**3.63 (0.566)**	**3.24 [b] (0.653)**	**Parthenogenetic**
	50	**100**	**62.67 (2.274)**	**57.58 (2.523)**	**54.13 (2.692)**	**51.38 (2.539)**	**48.70 (2.414)**	**10.50 (3.021)**	**8.33 (2.576)**	**6.68 [b] (1.878)**	**Mixed**
	80	**100**	**87.92 (2.195)**	**84.80 (2.530)**	**81.80 (2.276)**	**79.44 (2.597)**	**76.10 (2.806)**	**65.20 (2.630)**	**61.80 (2.470)**	**55.32 [a] (2.154)**	**Bisexual**
Kaboudan (interior)	**15–33**	**100**	**65.91 (0.846)**	**58.31 (1.845)**	**51.86 (1.524)**	**41.20 (2.572)**	**10.59 (2.213)**	**0.26 (0.433)**	**0.00**	**0.00 [c]**	**No animals survived**
	20–33	**100**	**57.31 (2.433)**	**54.05 (2.373)**	**51.53 (2.417)**	**45.10 (2.685)**	**31.33 (2.597)**	**3.85 (1.515)**	**1.73 (0.677)**	**0.50 [c] (0.312)**	**No animals survived**
	25–33	**100**	**62.54 (1.825)**	**59.00 (1.717)**	**49.39 (2.540)**	**36.20 (3.443)**	**22.67 (4.777)**	**5.78 (3.091)**	**0.97 (0.164)**	**0.00 [c]**	**No animals survived**
	33	**100**	**65.77 (1.731)**	**60.65 (1.809)**	**59.33 (1.772)**	**51.98 (2.396)**	**36.85 (4.119)**	**6.76 (2.967)**	**1.87 (0.833)**	**1.00 [c] (0.327)**	**No animals attained adulthood**
	50	**100**	**62.55 (2.625)**	**58.98 (2.063)**	**56.59 (2.503)**	**52.47 (2.515)**	**42.62 (2.613)**	**29.36 (4.120)**	**17.42 (4.254)**	**6.77 [b] (2.523)**	**Bisexual**

	80	**100**	**88.10 (1.731)**	**84.72 (1.788)**	**80.96 (2.197)**	**76.54 (2.217)**	**72.89 (2.352)**	**69.55 (1.707)**	**62.35 (2.584)**	**55.39 [a] (2.556)**	**Bisexual**
Ashk (interior)	**15–33**	**100**	**59.32 (2.511)**	**49.56 (1.878)**	**40.45 (2.609)**	**26.78 (3.201)**	**6.35 (2.613)**	**0.13 (0.167)**	**0.00**	**0.00 [c]**	**No animals survived**
	20–33	**100**	**51.58 (2.238)**	**45.94 (2.413)**	**40.19 (3.401)**	**29.32 (3.184)**	**14.35 (3.455)**	**1.93 (1.580)**	**0.00**	**0.00 [c]**	**No animals survived**
	25–33	**100**	**56.29 (2.572)**	**50.15 (2.556)**	**38.53 (3.360)**	**23.53 (4.234)**	**13.60 (3.397)**	**2.89 (1.886)**	**0.00**	**0.00 [c]**	**No animals survived**
	33	**100**	**59.20 (1.157)**	**51.55 (1.817)**	**46.28 (3.405)**	**33.78 (3.274)**	**20.48 (2.720)**	**3.38 (1.739)**	**3.11 (0.968)**	**0.00 [c]**	**No animals survived**
	50	**100**	**56.29 (3.361)**	**50.13 (3.380)**	**44.14 (2.681)**	**36.54 (2.556)**	**28.64 (3.352)**	**22.87 (2.581)**	**12.37 (2.560)**	**4.65 [b] (1.273)**	**Bisexual**
	80	**100**	**79.29 (2.617)**	**72.02 (2.425)**	**63.14 (2.610)**	**61.13 (2.572)**	**58.31 (1.585)**	**55.36 (1.780)**	**52.46 (2.556)**	**50.33 [a] (0.962)**	**Bisexual**
Central region of North arm (interior)	**15–33**	**100**	**55.65 (2.315)**	**44.59 (2.556)**	**38.12 (3.405)**	**22.87 (2.515)**	**9.48 (2.629)**	**1.00 (0.492)**	**0.00**	**0.00 [c]**	**No animals survived**
	20–33	**100**	**52.57 (2.617)**	**47.54 (2.552)**	**40.44 (2.527)**	**25.67 (3.018)**	**12.35 (1.596)**	**2.34 (0.767)**	**0.00**	**0.00 [c]**	**No animals survived**
	25–33	**100**	**59.84 (1.478)**	**54.39 (0.923)**	**42.55 (0.094)**	**31.45 (1.727)**	**19.62 (0.976)**	**6.21 (0.531)**	**1.45 (0.340)**	**0.00 [c]**	**No animals survived**
	33	**100**	**61.05 (4.116)**	**56.75 (1.957)**	**52.34 (1.123)**	**44.22 (1.837)**	**34.55 (0.890)**	**31.66 (0.386)**	**26.58 (1.392)**	**20.69 [b] (0.284)**	**No animals attained adulthood**
	50	**100**	**66.46 (2.484)**	**58.25 (2.729)**	**54.38 (1.490)**	**46.14 (2.487)**	**36.31 (1.887)**	**33.46 (1.666)**	**29.44 (2.768)**	**24.55 [b] (0.902)**	**Bisexual**
	80	**100**	**80.21 (3.442)**	**75.51 (2.523)**	**71.24 (1.711)**	**68.90 (1.483)**	**65.92 (2.418)**	**64.59 (1.637)**	**55.48 (2.539)**	**48.22 [a] (1.642)**	**Bisexual**

Lagoons	**15–33**	**100**	**87.36 (0.898)**	**82.87 (1.450)**	**78.27 (2.274)**	**74.16 (1.801)**	**69.48 (2.294)**	**64.72 (2.645)**	**62.55 (2.787)**	**60.44 [a] (1.796)**	**Parthenogenetic**
	20–33	**100**	**88.92 (1.792)**	**84.64 (1.829)**	**81.39 (2.201)**	**78.48 (1.825)**	**73.79 (2.499)**	**67.12 (1.449)**	**65.28 (1.053)**	**61.64 [a] (1.143)**	**Parthenogenetic**
	25–33	**100**	**89.55 (2.157)**	**85.38 (2.229)**	**82.88 (1.837)**	**80.35 (2.017)**	**76.92 (2.613)**	**74.46 (1.336)**	**71.61 (1.441)**	**66.52 [a] (2.356)**	**Parthenogenetic**
	33	**100**	**90.68 (0.935)**	**87.49 (2.258)**	**84.18 (2.752)**	**79.73 (1.797)**	**77.56 (0.940)**	**73.55 (1.666)**	**70.30 (3.712)**	**67.37 [a] (1.829)**	**Parthenogenetic**
	50	**100**	**89.98 (1.008)**	**84.61 (1.788)**	**80.44 (1.474)**	**73.38 (2.278)**	**67.81 (1.615)**	**61.47 (2.465)**	**59.33 (1.280)**	**51.59 [b] (2.807)**	**Parthenogenetic**
	80	**100**	**89.63 (1.862)**	**80.25 (3.059)**	**71.75 (3.121)**	**66.63 (2.601)**	**55.38 (3.233)**	**43.88 (3.251)**	**41.65 (1.097)**	**39.29 [b] (0.798)**	**Parthenogenetic**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culture period (day)
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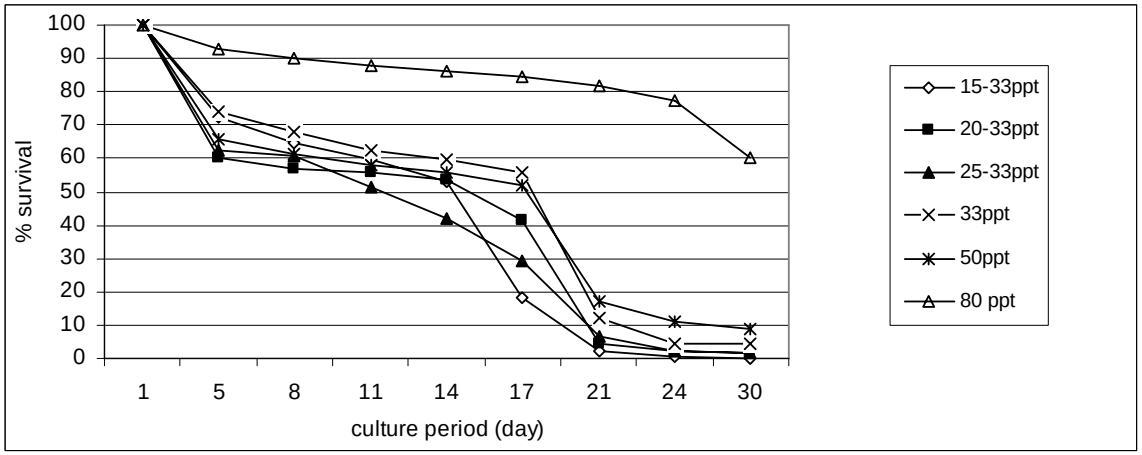
(a)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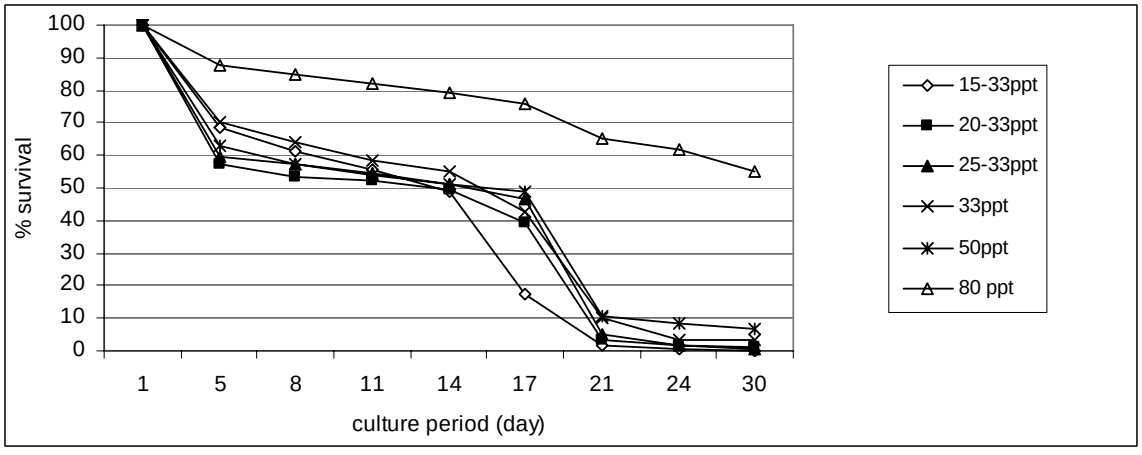
80 ppt

(b)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culture period (day)
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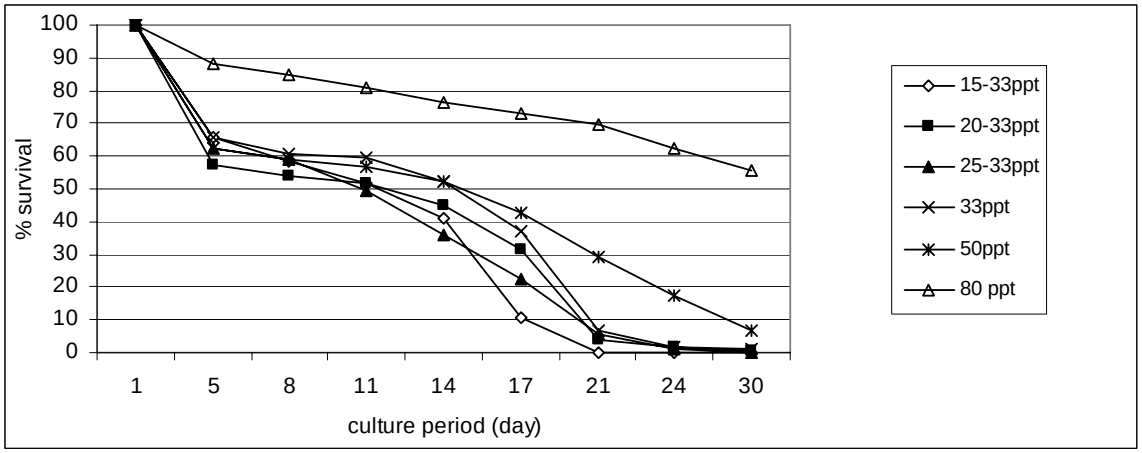
25-33ppt
33ppt
50ppt
80 ppt

(c)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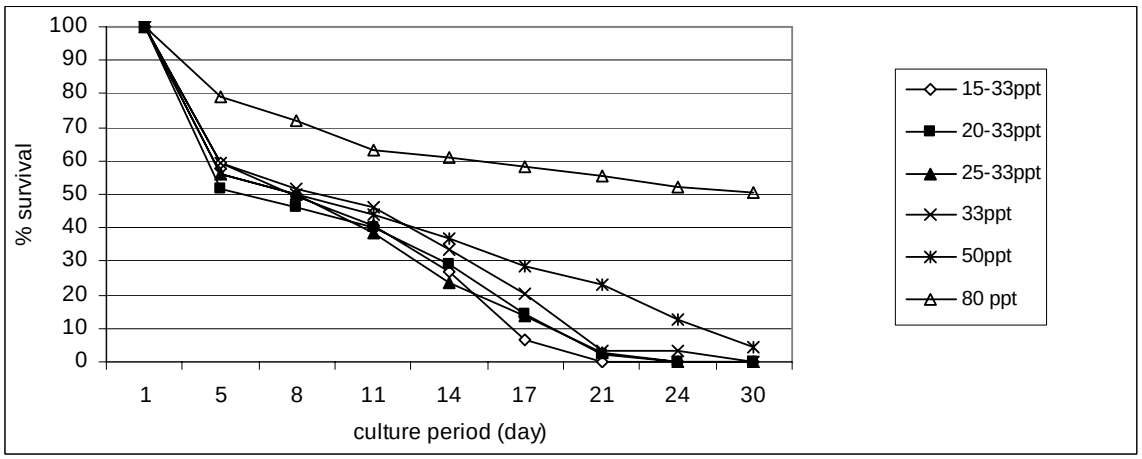
(d)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culture period (day)
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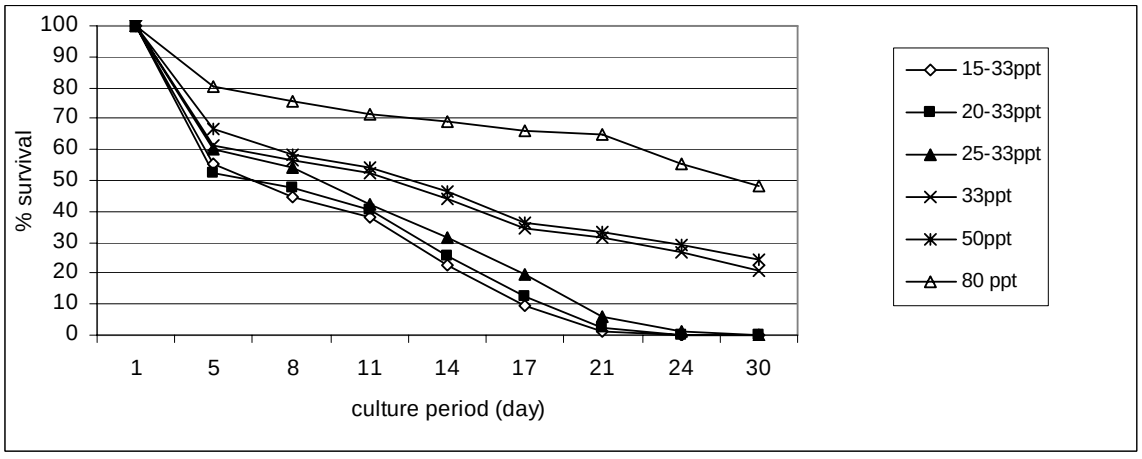
20-33ppt
25-33ppt
33ppt
50ppt
80 ppt

(e)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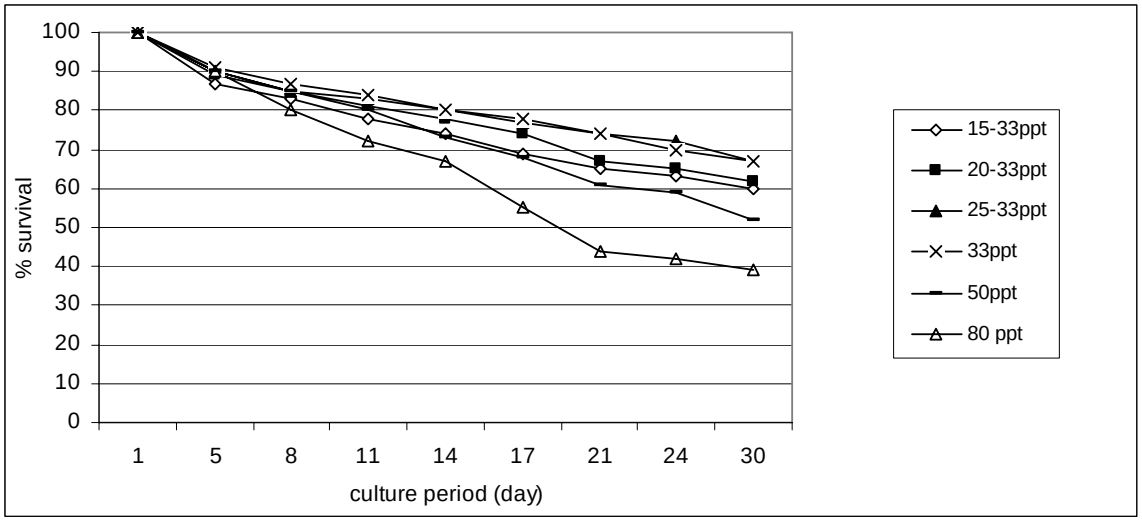
11
14
17
21
24
30
culture period (day)
15-33ppt
20-33ppt
25-33ppt
33ppt
50ppt
80 ppt

(f)



(g)

Figure 3.2. 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.

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 g/l), 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 3.2). 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.

Table 3.2. 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

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 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 g/l 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 g/l 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 g/l 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 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) have demonstrated 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 (Triantaphyllidis *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 (Triantaphyllidis *et al*., 1997) 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 radial 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 g/l, 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 g/l. 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 appropriate sampling 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. Due to an idiosyncratic cyst buoyancy behavior, a significant fraction (> 60%) of *A. urmiana* cysts sinks after 72 h even at salinities of 200 g/l (Abatzopoulos *et al*., 2006b). 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. These findings 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.

5. Acknowledgements

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CHAPTER 4

Effects of Salinity on Survival, Growth, Reproductive and life span characteristics of *Artemia* populations from Urmia Lake and neighbouring lagoons

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P. Sorgeloos (2007)

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Abstract

This paper deals with effects of different salinities on the survival, growth, reproductive and lifespan characteristics of three *Artemia* populations from Urmia Lake and small lagoons at the vicinity of the lake under laboratory conditions. Experimental salinities ranged from 75 to 175 g/l. Salinity was proved to have significant impact on the majority of the characters studied in this survey. Growth and survival in bisexual *A.urmiana* and parthenogenetic *Artemia* from Lake Urmia were significantly higher with respect to the parthenogenetic *Artemia* from lagoons at most of the salinities tested. Reproductive characteristics such as total number of broods, total offspring, number of offspring in each brood and number of offspring at each day of reproductive period reduced with increasing salinity. Moreover higher salinity prolonged the pre-reproductive period but shortened the total reproductive period. Higher salinities also affected the percentage of encystment and post-reproductive period, showing significantly higher values in parthenogenetic populations in comparison to bisexual *A. urmiana*.

1. Introduction

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

Lake Urmia with about 5000 km^2 is one of the largest permanent water catchments in West Asia. Lake Urmia is a thalassohaline, sodium chloride lake (Löffler, 1961) with oligotrophic characteristics, located at an altitude of 1250 m above sea level (Cole and Brown, 1967; Azari Takami, 1993). Its surface area was reported to range from 4750 to 6100 km2 and the average and greatest depths were between 6 and 16 m, respectively (Azari Takami, 1993; Van Stappen et al. 2001). However, according to recent studies by Agh (2006) the surface area of the lake has reduced to less than 4000 km2 and the average and greatest depths are between 3 and 6 m, respectively, due to the prolonged drought (since 1999) and the construction of a number of

reservoirs/dams on major inflowing rivers. Lake Urmia water salinity that used to fluctuate from 140 to 220 g/l before 1999, has never dropped below 250 g/l since then, and is in saturation state almost throughout the year (Agh, 2006).

A. urmiana was first reported in Lake Urmia by Günther in 1890. Many other researchers confirmed presence of this bisexual species of *Artemia* in Lake Urmia (Clark & Bowen, 1976; Barigozzi, 1989; Pador, 1995; Sorgeloos, 1997; Van Stappen et al. 2001; Agh, 2002; Noori & Agh, 2002). Most recently Agh et al. (in press) confirmed that a parthenogenetic population of *Artemia* coexists with the bisexual *A. urmiana* in Lake Urmia.

A parthenogenetic population of *Artemia* was reported from small lagoons at the vicinity of the Lake Urmia by Agh & Noori, 1997. These lagoons are scattered at the periphery of the lake in both West and East Azerbaijan. The size of the lagoons varies from a few square meters to maximum 10000 m^2 surface area and their depth is always less than 0.7 m. Therefore these lagoons are considered as temporary small water catchments that are dried during early summer and filled up again during winter rains. Water salinity in the lagoons ranges from 10-20 g/l in early spring and gradually rises to saturation level within about 10 weeks. Parthenogenetic females were observed at high densities with rare males seen only at the ratio of one male to 100 females in these lagoons (Agh & Noori, 1997, Abatzopoulos et al. 2006). Parthenogenetic *Artemi*a in these lagoons grow to maturity at very low salinities (as low as 10 g/l) and start reproduction at salinity of 15-20 g/l. The lagoons get saturated with brine within this period, but still a very high density of dark red coloured *Artemia* could be observed in these lagoons before they finally dry up.

There is considerable literature information on survival, growth, morphometry, reproductive and life span characteristics of many bisexual and parthenogenetic *Artemia* populations (Vanhaecke et al., 1984; Wear & Haslett, 1986; Browne et al., 1984, 1991; Browne & Wanigasekera, 2000; Triantaphyllidis et al., 1995, 1997a,b; Baxevanis et al., 2004; El-Bermawi et al., 2004; Abatzopoulos et al., 2004, 2006). Most of these studies have contributed to the evaluation of genetic and environmental components of variance in sexual and or clonal *Artemia*. They have also enabled the comparison of life history characteristics and strategies between different

populations (Browne et al., 2002; Abatzopoulos et al., 2003; Baxevanis and Abatzopoulos, 2004; Kappas et al., 2004). However, the effects of salinity on *A. urmiana* and parthenogenetic populations from Iran have been poorly examined or not studied at all.

In this study we will survey the effect of salinity on growth, survival, reproductive and life span characteristics of three *Artemia* populations occurring in Urmia Lake region in order to understand their adaptation patterns to various salinities, thereby providing additional data on the characterization of *Artemia* populations from Iran.

2. Materials and methods

2.1. Culture procedure

Cysts of *Artemia* from the Lake Urmia and from the lagoons around the lake were hatched under standard conditions (Sorgeloos et al. 1986). The larvae hatched from the cyst samples of each biotope were siphoned into separate beakers and then transferred into separate 30-liter aquaria, where they were cultured until adulthood. One hundred sexually matured bisexual couples (*A. urmiana*; URM) and equal number of adult parthenogenetic female *Artemia* (P-URM) were separated from the Urmia Lake sample on the basis of their different morphology and similarly 100 parthenogenetic females from the lagoon sample (LAG) (Table 4.1) were all transferred into separate 1.5-liter cylindro-conical containers. The adult bisexual and parthenogenetic *Artemia* were allowed to reproduce inside the cones and the newly produced cysts and live nauplii were eliminated every alternate day until 2 weeks. This ensures that each population reproduces in its respective pattern of reproduction, sexually or asexually. This step was then followed by transferring six replicates of 200 actively moving nauplii from each population into 500 ml conical containers filled with 400 ml of filtered water with different salinities (50, 75, 100, 125, 150 and 175 g/l). The animals were cultured at 27±1˚C under constant aeration. The salinity in each cone was checked twice a day in order to maintain salinities according to the experimental set up. *Artemia* were fed unicellular algae *Dunaliella tertiolecta* and chemically treated yeast Lansy PZ® (INVE Technologies, Baasrode, Belgium) adopted from Coutteau et al. (1992).
Density of *Artemia* was adjusted to one animal/ml at the beginning of the experiment, but the density was gradually decreased to one animal per 3 ml on day 8 and per 4 ml on day 14 of growth.

Table 4.1. List of the populations studied and abbreviations used

Site	Strain	Abbreviation
Urmia Lake	Bisexual	URM
Urmia Lake	Parthenogenetic	P-URM
Lagoons	Parthenogenetic	LAG

2.2. Determination of survival and growth

Survival and total length were determined on days 8, 11, 14, 17, 20 and 23, according to Triantaphyllidis et al. (1995). For determining the growth 10 animals from each replicate were measured from the most anterior part of the head up to the last abdominal segment (telson) using a light microscope equipped with a phototube and micrometer. Drawings were later digitized using a digitizer connected to a computer.

2.3. Reproductive and life span characteristics

As animals attained maturity, 30 pairs of coupling bisexual *A. urmiana* and 30 adult parthenogenetic females from each population (from all salinities) were transferred into separate 50 ml falcon tubes in order to study their life cycle characteristics. Separate culture of individuals or couples continued as long as the female *Artemia* were alive. In the Falcon tubes containing bisexual *A. urmiana,* dead males were immediately replaced with actively swimming males during the experiment (Browne et al. 1988). The Falcon tubes were checked every day for the production of cysts or nauplii, which were counted and recorded separately. Finally the reproductive characteristics (number of broods, total amount of offspring, brood size, offspring/day during the reproductive period, brood intervals and percentage of encystment) and the life span characteristics (pre-reproductive period, reproductive period, post-reproductive period and life span) were determined for each population according to Browne et al. (1984; 1988). The results were statistically analyzed using SPSS (version 13) analysis of variance (ANOVA) (Sokal & Rohlf, 1981; Triantaphyllidis et al., 1995) and the averages were compared using Duncan's test.

3. Results

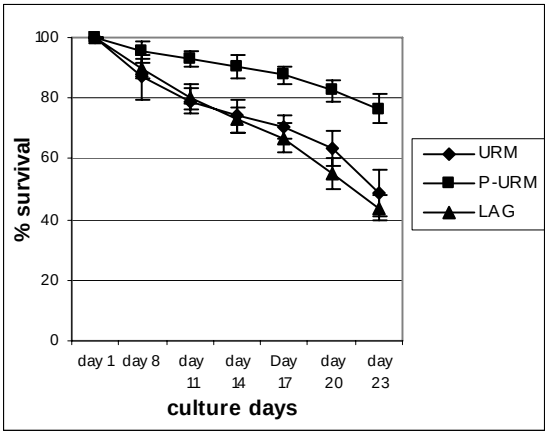
3.1. Survival

Records of the survival after 23 days of experiment indicate that in the majority of the cases survival declines in all populations when salinity increases. P-URM had significantly higher survival at salinities 75, 100 and 125 g/l compared to URM and LAG (Table 4.2, Figure 4.1, ANOVA, $p < 0.05$). The bisexual URM had significantly higher survival only at 150 g/l compared to the asexual populations. No significant differences were observed in the survival rate of the three populations at 175 g/l. Maximum survival of URM was observed at 100 g/l, significantly higher compared to the values obtained at other salinities at intrapopulation level (Table 4.2, ANOVA, $p < 0.05$), while minimum survival for this population was observed at 175 g/l. No significant differences were observed in survival of URM at 75, 125 and 150 g/l salinities. The two asexual populations demonstrated significant differences in survival rate at all salinities.

Table 4.2. Mean values (standard deviations in parenthesis) of survival percentage of three *Artemia* populations studied. Abbreviations in Table 4.1

		day 1	day 8	day 11	day 14	day 17	day 20	day 23
			87.0[a]	79.0[a]	74.2[a]	70.5[a]	63.5[a]	48.8[a]
URM	75 g/l	100	(7.26)	(4.16)	(3.68)	(3.87)	(6.03)	(7.72)
			95.3[a]	92.8[b]	90.3[b]	87.8[b]	82.5[b]	76.5[b]
P-URM	75 g/l	100	(3.59)	(2.50)	(3.68)	(2.87)	(3.70)	(4.80)
			89.8[a]	80.3[a]	72.8[a]	66.8[a]	55.3[a]	43.8[a]
LAG	75g/l	100	(2.99)	(4.27)	(4.36)	(4.79)	(5.32)	(4.27)
			89.5[a]	87.3[b]	81.4[b]	77.3[b]	74.0[b]	64.0[b]
URM	100 g/l	100	(2.08)	(2.75)	(3.87)	(4.65)	(4.55)	(6.06)
			96.8[b]	93.8[c]	91.6[c]	90.3[c]	85.0[c]	75.3[c]
P-URM	100 g/l	100	(1.26)	(2.22)	(2.98)	(3.10)	(2.58)	(3.50)
			90.8[a]	74.8[a]	67.2[a]	56.0[a]	49.8[a]	38.0[a]
LAG	100g/l	100	(2.50)	(3.77)	(4.14)	(4.40)	(4.79)	(5.35)
			87.0[b]	78.8[b]	73.5[b]	69.0[b]	66.8[b]	49.5[b]
URM	125 g/l	100	(3.56)	(2.63)	(4.25)	(7.07)	(7.18)	(5.69)
			93.8[c]	91.0[c]	89.3[c]	87.0[c]	80.3[c]	68.3[c]
P-URM	125 g/l	100	(1.71)	(1.83)	(2.15)	(5.58)	(2.99)	(4.79)
			77.8[a]	62.0[a]	54.6[a]	48.0[a]	41.5[a]	37.5[a]
LAG	125g/l	100	(2.50)	(7.48)	(7.28)	(7.53)	(6.61)	(5.45)
			85.8[b]	71.5[b]	65.3[b]	59.5[b]	56.3[b]	47.5[b]
URM	150 g/l	100	(3.77)	(5.80)	(6.73)	(7.14)	(6.80)	(5.97)
			76.3[b]	66.3[b]	58.6[b]	44.8[a]	32.5[a]	24.5[a]
P-URM	150 g/l	100	(3.50)	(4.57)	(5.76)	(6.40)	(5.20)	(3.87)
			55.0[a]	45.8[a]	39.8[a]	35.0[a]	30.8[a]	26.8[a]
LAG	150g/l	100	(7.53)	(5.85)	(5.48)	(5.60)	(5.50)	(6.08)
			77.3[b]	39.8[a]	28.5[a]	19.8[a]	17.8[a]	13.5[a]
URM	175 g/l	100	(2.75)	(5.85)	(4.82)	(4.03)	(4.03)	(4.20)
			72.5[b]	54.8[b]	38.6[a]	24.8[b]	19.3[a]	14.8[a]
P-URM	175 g/l	100	(4.43)	(4.57)	(4.69)	(5.62)	(3.77)	(3.50)
			50.3[a]	34.8[a]	32.2[a]	30.0[bc]	23.3[a]	21.8[a]
LAG	175g/l	100	(6.08)	(5.12)	(4.75)	(4.40)	(4.79)	(4.79)

Populations sharing similar letters in each column are not significantly different at respective salinities ($p<0.05$).



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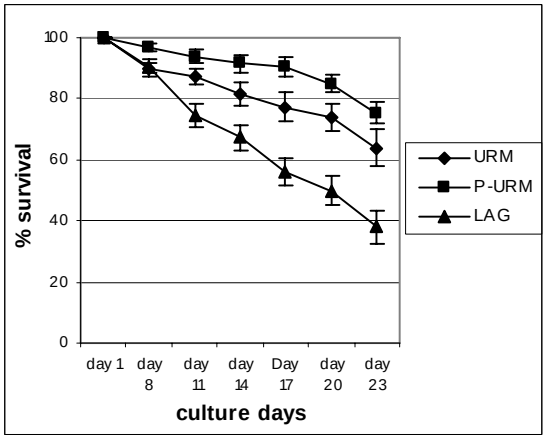

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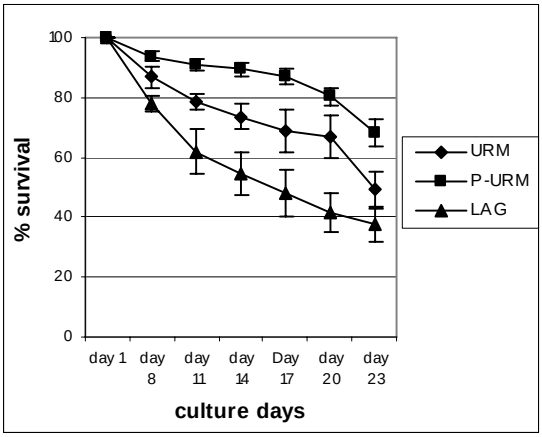
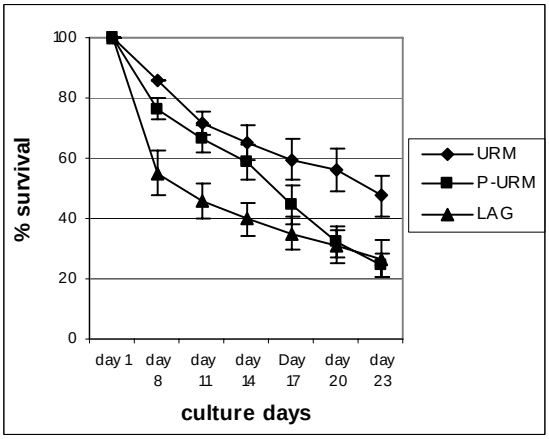
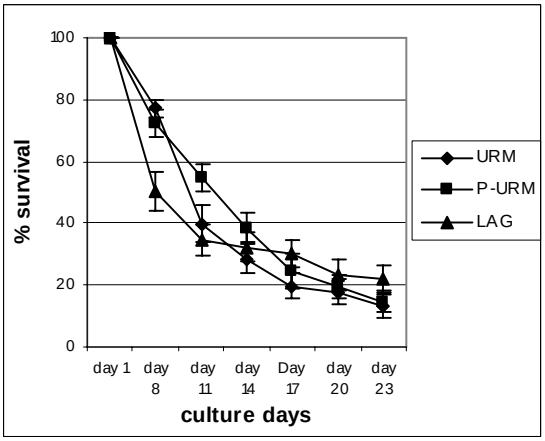


125 g/l



150 g/l



175 g/l

Figure 4.1. Survival percentage of three *Artemia* strains from Urmia Lake and neighbouring lagoons as a function of culture salinity. (Error bars indicate standard deviations)

3.2. Growth

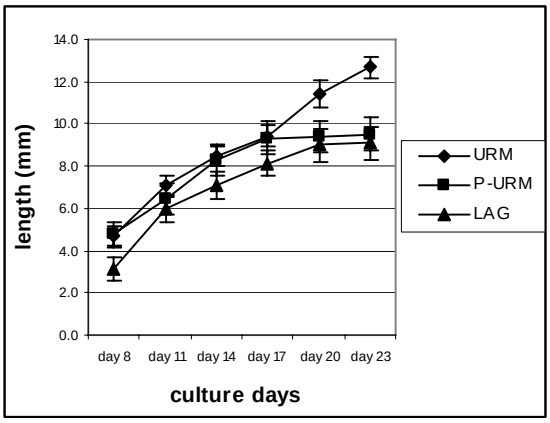
The bisexual URM had the highest growth values in all salinities compared to the asexual populations (Table 4.3, ANOVA, $p < 0.05$). The maximum total length of URM was significantly larger than this of the parthenogenetic strains (URM: 12.7 ± 0.5 mm while for the asexual strains it was less than 9.7 mm). Growth rates of URM were almost similar at 75 and 100 g/l (Table 4.3 and Figure 4.2) while this was significantly smaller at the three higher salinities (ANOVA, $p < 0.05$).

No considerable differences were found in the total length of the parthenogenetic strains (P-URM and LAG) at 75 and 100 g/l (Table 4.3, Figure 4.2), but the same were significantly different at 125, 150 and 175 g/l. (ANOVA, $p < 0.05$). Maximum total length for P-URM (9.7 mm) was observed at 125 g/l while maximum growth for LAG (9.1 mm) was seen at 75 and 100 g/l.

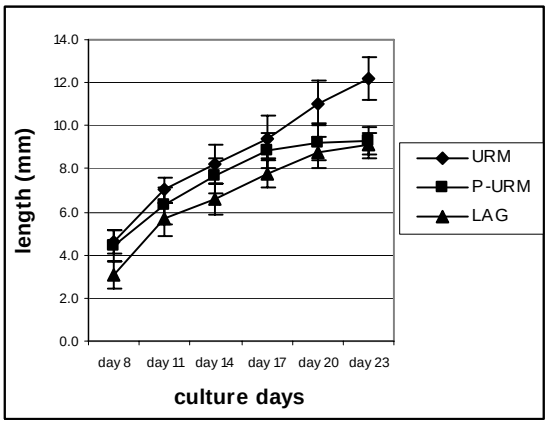
Table 4.3. Mean values (standard deviations in parenthesis) of growth rate (in mm) of three *Artemia* populations studied. Abbreviations in Table 4.1

		day 8	day 11	day 14	day 17	day 20	day 23
		4.7[b]	7.1[c]	8.5[b]	9.4[b]	11.4[b]	12.7[b]
URM	75 g/l	(0.47)	0.49)	(0.48)	(0.49)	(0.67)	(0.53)
		4.8[b]	6.4[b]	8.3[b]	9.3[b]	9.4[a]	9.5[a]
P-URM	75 g/l	(0.60)	(0.72)	(0.74)	(0.77)	(0.75)	(0.77)
		3.1[a]	6.0[a]	7.1[a]	8.2[a]	9.0[a]	9.1[a]
LAG	75g/l	(0.54)	(0.60)	(0.62)	(0.64)	(0.81)	(0.80)
		4.6[b]	7.0[c]	8.2[c]	9.4[c]	11.1[b]	12.2[b]
URM	100 g/l	(0.56)	(0.59)	(0.88)	(1.04)	(1.04)	(0.99)
		4.4[b]	6.3[b]	7.7[b]	8.8[b]	9.3[a]	9.3[a]
P-URM	100 g/l	(0.75)	(0.86)	(0.81)	(0.80)	(0.82)	(0.64)
		3.0[a]	5.7[a]	6.6[a]	7.8[a]	8.8[a]	9.1[a]
LAG	100g/l	(0.64)	(0.78)	(0.71)	(0.66)	(0.75)	(0.58)
		4.2[b]	6.3[c]	8.1[b]	9.2[b]	10.1[c]	11.2[c]
URM	125 g/l	(0.59)	(0.85)	(0.94)	(1.04)	(0.85)	(1.22)
		3.9[b]	5.8[b]	7.8[b]	9.1[b]	9.4[b]	9.7[b]
P-URM	125 g/l	(0.74)	(0.77)	(0.73)	(0.79)	(0.69)	(0.68)
		2.2[a]	4.0[a]	5.3[a]	6.8[a]	7.6[a]	7.8[a]
LAG	125g/l	(0.60)	(0.63)	(0.75)	(0.88)	(0.89)	(0.78)
		2.1[a]	4.4[b]	6.2[b]	7.6[b]	8.8[b]	9.3[b]
URM	150 g/l	(0.40)	(0.56)	(0.72)	(0.81)	(1.07)	(0.95)
		2.6[b]	4.5[b]	6.5[b]	8.0[b]	8.6[b]	8.7[b]
P-URM	150 g/l	(0.51)	(0.65)	(0.78)	(0.90)	(0.89)	(0.97)
		1.9[a]	3.4[a]	4.7[a]	5.9[a]	6.7[a]	6.8[a]
LAG	150g/l	(0.49)	(0.47)	(0.68)	(0.77)	(0.67)	(0.88)
		2.2[b]	2.9[b]	4.9[c]	6.3[c]	7.5[c]	7.6[c]
URM	175 g/l	(0.39)	(0.68)	(0.74)	(0.76)	(0.47)	(0.54)
		1.6[a]	2.7[b]	3.8[b]	5.0[b]	5.9[b]	6.5[b]
P-URM	175 g/l	(0.36)	(0.44)	(0.48)	(0.55)	(0.56)	(0.59)
		1.5[a]	2.3[a]	3.2[a]	4.4[a]	4.9[a]	5.3[a]
LAG	175g/l	(0.41)	(0.42)	(0.56)	(0.68)	(0.72)	(0.74)

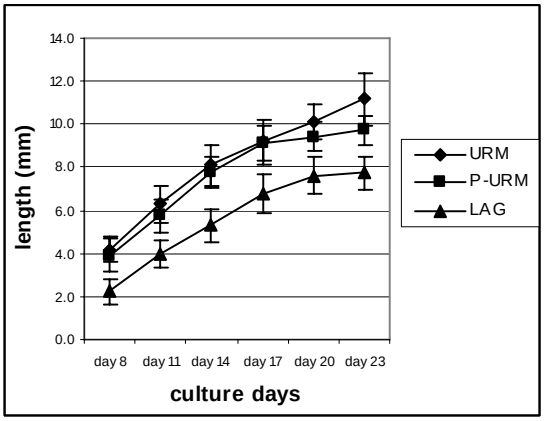
Populations sharing similar letters in each column are not significantly different at respective salinities ($p<0.05$).



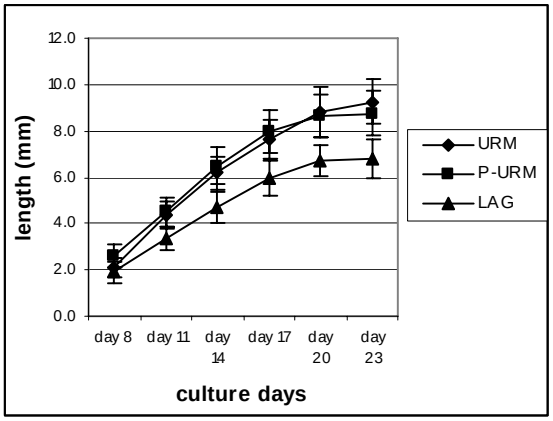
75 g/l



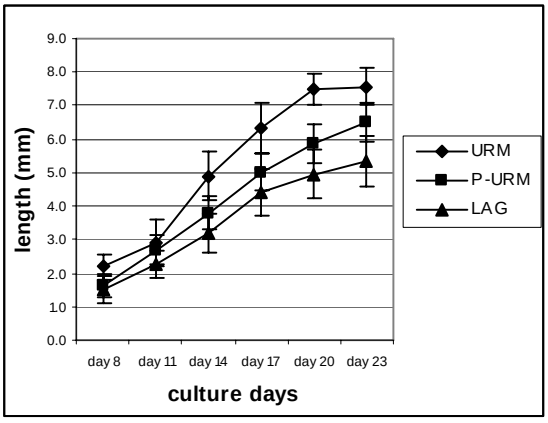
100 g/l



125 g/l



150 g/l



175 g/l

Figure 4.2. Growth rate of three *Artemia* strains from Urmia Lake and neighbouring lagoons under function of different salinities. (Error bars indicate standard deviations)

3.2. Reproductive and Life Span Characteristics

Seven reproductive and four life span characteristics from the three Iranian *Artemia* populations (URM, P-URM, LAG) cultured at five different salinities are summarized in Table 4.4. At 75 g/l, statistical analysis revealed that the bisexual strain (URM) was different from the parthenogenetic ones in six out of eleven measured characteristics. However, URM had higher values only in the total number of nauplii produced, offspring per brood and post-reproductive period compared to those of parthenogenetic strains (P-URM and LAG). URM had significantly lower values in the percentage of encysted embryos and total life span compared to the asexual populations, while P-URM demonstrated significantly higher values in five characters (i.e. number of broods per female, total number of cysts produced, total offspring, reproductive period and total life span compared to URM and LAG (ANOVA, $p < 0.05$, Table 4.4). At 100 g/l, URM strain was statistically different from the parthenogenetic strains in four out of eleven reproductive and life span characteristics (i.e. total number of cysts, total number of nauplii, percentage of encysted embryos and post-reproductive period), being significantly higher only in total number of nauplii produced, while P-URM had significantly higher values in number of broods per female, total number of cysts produced, total offspring and total life span compared to URM and LAG strains (ANOVA, $p < 0.05$, Table 4.4).

At 125 g/l URM had significantly higher values only in offspring per brood and total number of nauplii, while P-URM showed significantly higher values in the same 5 characters as in 75 g/l (ANOVA, $p < 0.05$, Table 4.4). At 150 and 125 g/l, the reproductive and life span pattern were similar. But at 175 g/l the asexual P-URM had higher values in almost all studied characters compared to sexual URM, while LAG strain did not reproduce any offspring in this salinity. Comparisons of reproductive and life span variables in the five salinities revealed that most of the characters studied, were statistically different at these salinities. Two variables, percentage of encysted embryos and pre-reproductive period, were significantly affected by salinity, exhibiting a parallel increase with salinity increase up to 150 g/l in most cases. Intervals between broods and total life span are hardly affected by salinity but there was a decrease in the rest of the characters as salinity became higher. Highest values for total offspring and offspring per brood in all populations was observed at 75 and 100 g/l (Table 4.4) indicating that the best reproductive performance of these populations was observed at 75 and 100 g/l salinity. Longest life span was

observed at 100 g/l for all three strains. P-URM lived significantly longer than the other two populations in all salinities.

4. Discussion

4.1. Survival

Although thriving *Artemia* populations are found in salinities as high as 340 g/l (Post and Youssef, 1977), its culture and maintenance in laboratory at salinities higher than 200 g/l has always been difficult (Wear and Haslett, 1986, Wear et al. 1986). Browne and Hoopes (1990) reported only 9 % survival at 190 g/l and no survival at all at 230 g/l in a parthenogenetic *Artemia* from Salin de Giraud (France). Dana and Lenz (1986) studying the bisexual *Artemia* from Mono Lake, California, USA, found low survival in 159 and 179 g/l under laboratory conditions. Triantaphyllidis et al. (1995) reported over 80 % mortality of both parthenogenetic *Artemia* from Tanggu area (China) and *A. franciscana* at 180 g/l at 25˚C over a 23 days culture period. On the contrary, they reported above 75 % survival for *A. franciscana* and higher than 50 % survival for parthenogenetic *Artemia* at salinities lower than 100 g/l.

In the experiments performed by El-Bermawi et al. (2004) on *Artemia* populations from Egypt, 100 % mortality was observed in bisexual *A. salina* from Wadi El-Natrun in 150 and 200 g/l within 17 days, but the high salinity had little effect on the ability of parthenogenetic populations to survive. Total mortality did not occur in our experiments with sexual and asexual strains *Artemia* from the Urmia Lake and the neighbouring lagoons within the range of 75-175 g/l salinities. Browne and Wanigasekera (2000) reported an increase in survival of parthenogenetic *Artemia* from Margherita di Savoia (Italy) and *A. salina* when salinity of the culture medium was increased from 60 to 120 g/l at 15˚C, but this percentage sharply decreased in three other bisexual species (*A. sinica, A. franciscana and A. persimilis*). Inversely, at 24˚C they got completely different results, obtaining significantly higher survival at higher salinity. El-Bermawi et al. (2004) observed similar results with parthenogenetic *Artemia* populations from Egypt. Contrary to these two findings, Triantaphyllidis et al. (1995) reported a steady decrease in survival in both parthenogenetic from Tanggu (China) and bisexual *A. franciscana* cultured in

Table 4.4. Average reproductive and life span characteristics of 3 *Artemia* populations from Urmia Lake region under the function of salinity. Populations sharing similar letters are not significantly different for the respective reproductive variable at different salinities, as determined by ANOVA ($p<0.05$). (Figures in brackets indicate ± S.E)

Reproductive characteristics	URM 75	P-URM 75	LAG 75	URM 100	P-URM 100	LAG 100	URM 125	P-URM 125	LAG 125	URM 150	P-URM 150	LAG 150	URM 175	P-URM 175	LAG 175
Number of broods per female	3.7^{a} (2.05)	5.5^{b} (2)	3.6^{a} (1.56)	3.6^{a} (2.43)	5.5^{b} (2.02)	4.4^{a} (0.98)	2.5^{a} (1.92)	4.3^{b} (1.97)	2.2^{a} (0.84)	2.1^{a} (1.56)	2.8^{a} (1.51)	0.5^{b} (0.62)	2.0^{a} (1.51)	3.3^{b} (1.52)	0 0
Intervals between broods	4.7^{a} (2.49)	5.0^{a} (1.66)	4.9^{a} (2.32)	6.0^{a} (3.49)	5.1^{a} (1.24)	5.1^{a} (1.34)	4.3^{a} (2.86)	4.9^{a} (2.67)	4.2^{a} (2.63)	3.4^{a} (2.45)	6.4^{b} (5.08)	2.4^{c} (2.37)	3.5^{a} (2.13)	6.7^{b} (2.43)	- -
Offsprings per brood	47.5^{a} (18.73)	43.7^{a} (10.33)	31.7^{b} (12.34)	49.2^{a} (21.23)	48.3^{a} (12.37)	35.2^{b} (10.27)	37.6^{a} (16.05)	31.9^{b} (7.51)	22.4^{c} (6.48)	23.8^{a} (14.23)	31.0^{b} (17.3)	15.1^{c} (5.62)	29.1^{a} (10.72)	32.5^{b} (9.18)	0 0
Total No. of cysts produced	97.9^{a} (18.2)	180.9^{b} (18.59)	84.4^{c} (9.01)	86.4^{a} (13.13)	234.9^{b} (21.17)	137.6^{c} (9.55)	33.3^{a} (6.97)	104.5^{b} (11.54)	49.2^{c} (3.80)	40.8^{a} (8.51)	62.8^{b} (6.94)	7.56^{c} (1.61)	41.2^{a} (9.29)	63.9^{b} (7.71)	0 0
Total No. of nauplii produced	96.3^{a} (12.83)	61.8^{b} (14.55)	27.2^{c} (3.83)	82.5^{a} (13.95)	37.0^{b} (9.04)	18.5^{c} (3.38)	63.0^{a} (11.23)	37.8^{b} (7.54)	0.9^{c} (0.73)	30.5^{a} (7.96)	22.6^{b} (6.40)	0 0	20.4^{a} (4.00)	41.9^{b} (8.04)	0 0
Total Offsprings	194.3^{a} (23.58)	242.7^{b} (18.37)	111.6^{c} (10.42)	168.9^{a} (22.36)	271.9^{b} (21.42)	156.0^{a} (10.22)	96.2^{a} (15.24)	142.3^{b} (13.82)	50.1^{c} (3.88)	71.3^{a} (12.43)	85.4^{b} (10.70)	7.6^{c} (1.61)	61.6^{a} (10.61)	105.8^{b} (9.98)	0 0
Percentage of encysted embryos	43.6^{a} (32.81)	77.5^{b} (26.54)	72.4^{b} (23.58)	50.9^{a} (31.29)	87.6^{b} (16.03)	83.6^{b} (12.06)	40.3^{a} (32.60)	77.0^{b} (22.19)	98.7^{c} (5.66)	54.8^{a} (35.42)	81.1^{b} (22.80)	100^{c} 0	67.1^{a} (32.27)	64.3^{a} (30.52)	- -
Pre-reproductive period	21.5^{a} (1.83)	28.4^{a} (2.30)	24.4^{a} (2.55)	24.9^{a} (11.97)	29.8^{a} (3.53)	24.9^{a} (1.90)	29.6^{a} (2.78)	28.3^{a} (2.99)	31.5^{a} (2.91)	30.0^{a} (3.20)	29.5^{a} (4.06)	41.8^{b} (5.01)	34.9^{a} (2.97)	39.4^{a} (3.71)	- -
Post-reproductive period	1.2^{a} (2.08)	11.4^{b} (13.47)	10.1^{b} (12.2)	8.8^{a} (8.70)	13.2^{b} (14.63)	12. 8^{b} (10.35)	3.9^{a} (6.98)	13.7^{b} (15.93)	11.0^{b} (8.17)	3.8^{a} (5.54)	6.9^{b} (8.28)	6.8^{b} (4.93)	1.1^{a} (2.96)	7.6^{b} (8.33)	- -
Reproductive period	20.8^{a} (16.42)	28.4^{b} (12.17)	18.0^{a} (9.32)	25.9^{a} (18.53)	28.7^{ab} (11.71)	22.1^{c} (5.71)	14.7^{a} (14.6)	23.2^{b} (14.32)	9.8^{c} (6.34)	9.4^{a} (11.59)	19.0^{b} (16.18)	2.9^{c} (3.11)	8.7^{a} (9.22)	22.4^{b} (11.13)	0 0
Lifespan	43.3^{a} (16.57)	68.2^{b} (22.58)	52.8^{c} (17.14)	57.6^{a} (28.83)	71.8^{b} (21.67)	59.4^{a} (12.73)	46.7^{a} (16.06)	63.9^{b} (23.74)	51.7^{a} (9.76)	42.2^{a} (12.98)	55.8^{b} (22.60)	51.6^{b} (7.15)	44.9^{a} (8.39)	69.7^{b} (13.56)	48.3^{a} (4.04)

the range of 60 to 180 g/l. Triantaphyllidis et al. (1995) reported 70-80 % survival for *A. franciscana* at 60 g/l, but Browne and Wanigasekera (2000) observed only 16 % survival for this species at the same salinity. Vanhaecke et al. (1984) reported high survivorship for *A. sinica* and *A. salina* at 60 g/l, whereas survival was zero for these two species at the same salinity in the experiments performed by Browne and Wanigasekera (2000).

Browne and Wanigasekera (2000) claimed that differences in the culture conditions and intra-species and population-dependent characteristics could be among the reasons for the different results obtained by different researchers. Our experiments are in agreement with the findings of Triantaphyllidis et al. (1995), showing a constant decline in survival when salinity increases from 100 to 170 g/l. Different findings with different *Artemia* strains could be an indication for strain-specific adaptation patterns of various *Artemia* populations to diverse physical, chemical and biotic characteristics of their own biotopes.

Abatzopoulos et al. (2006) reported very low survival for *A. urmiana* in salinities of 35 and 50 g/l. But they found high survival at 100, 140 and 180 g/l. However, they did not find significant differences in the survival of bisexual *A. urmiana* in the later elevated salinities.

Our findings are not in agreement with findings of Abatzopoulos et al. (2006). In present study we found survival percentages of both sexual and asexual populations that decrease with increasing salinity. According to the results obtained in our study, sexual URM can tolerate higher salinities compared to the two asexual strains. However, high mortality was observed in URM at 175 g/l, whereas parthenogenetic populations begin experiencing sharp mortality at 150 g/l. Unlike the Egyptian parthenogenetic populations as reported by El-Bermawi et al. (2004), the Iranian asexual strains had very low survival at high salinities.

4.2. Growth

According to Gilchrist (1960), Triantaphyllidis et al. (1995) and El-Bermawi et al. (2004) growth is inversely proportional to salinity. Triantaphyllidis et al. (1995) reported significant differences

in the growth of *Artemia* especially in the parthenogenetic population from Tanggu (China) cultured at different salinities. According to their experiments maximum growth in *A. franciscana* was observed at 35 g/l, whereas growth in parthenogenetic *Artemia* showed no differences in 35, 60 and 100 g/l. But parthenogenetic *Artemia* at 180 g/l attained only 50 % of the length of those at 35, 60 and 100 g/l. *A. franciscana* at 180 g/l achieved 60 % of the length in comparison to animals grown at 35 g/l. El-Bermawi et al. (2004) did not observe big differences in growth of parthenogenetic and bisexual populations of *Artemia* from Egypt grown in the laboratory at salinities ranging from 35 to 200 g/l. Abatzopoulos et al. (2006) found that growth rate of *A. urmiana* was not affected by the increase of salinity.

The present study confirms that growth rate in *Artemia* populations from Urmia Lake region (Iran) is inversely proportional to salinity, supporting the findings of Gilchrist (1960), Triantaphyllidis et al. (1995) and El-Bermawi et al. (2004). In our experiment URM and LAG at 175 g/l could attain almost 60 % of the total length of those grown at 75 and 100 g/l., while P-URM at 175 g/l achieved almost 75 % of the length of 75 g/l grown animals. Our result are not in agreement with the findings of Abatzopoulos et al. (2006), and prove that *A. urmiana* grows best at 75 -100 g/l and that its growth rate is significantly affected by elevated salinity. It seems that performance of *A. urmiana* and probably the asexual strains are affected by different sources of brine water used for culture experiments. In present study we used diluted Lake Urmia water adjusted to 75, 100, 125, 150 and 175 g/l throughout the experiment, whereas Abatzopoulos et al. (2006) used artificially prepared D&K medium of 35, 50, 100, 140 and 180 g/l salinity in their experiments. Our results in combination with literature data suggest that adaptation to different salinities and growth rate are species-specific and in addition dependent on the culture conditions.

4.3. Reproductive and Life Span Characteristics

A number of investigations have reported on effects of salinity on the reproductive and life span characteristics of *Artemia*. Gilchrist, (1960), Dana and Lenz, (1986) and Triantaphyllidis et al. (1995) who worked on *A. salina, A. franciscana* from Mono lake and Tanggu parthenogenetic *Artemia* and *A. franciscana* from San Francisco Bay respectively, reported that maturation is

achieved fastest at salinities lower than 100 g/l, and much slower above 140 g/l. Abatzopoulos et al. (2003) reported faster maturity at 50 and 80 g/l in comparison to 120 g/l for a parthenogenetic *Artemia* from Megalon Embolon (Greece). Similarly Baxevanis et al. (2004) reported early maturation at 35 g/l in three parthenogenetic populations and at 80 g/l in the bisexual *A. salina* from Lake of Wadi El-Natrun, all from Egypt. It was found that this bisexual *Artemia* died before attaining maturity at 150 and 200 g/l. But Browne and Wanigasekera (2000) who performed the experiments at various combinations of temperature and salinity with five *Artemia* populations (one parthenogenetic and four bisexual) reported parthenogenetic *Artemia* from Margherita di Savoia (Italy) as a niche specialist attaining maturity and reproducing only at salinities higher than 120 g/l at 24˚C. This *Artemia* was not able to reproduce at 60 g/l at 15˚C or 30˚C. According to their findings maturation time in all four bisexual populations was more temperature-dependent than salinity-dependent. Within different temperature treatments at lower salinity, maturity was achieved earlier than at higher salinities, except in *A. persimilis* which had the shortest maturation time (9.7 days) at 30˚C at 180 g/l (Browne and Wanigasekera, 2000).

Triantaphyllidis et al. (1995) did not find significant differences in the reproductive characteristics (offspring per brood, broods per female, offspring per female per day, days between broods, total offspring per female and percentage of encystment) in Tanggu parthenogenetic *Artemia* and *A. franciscana* from San Francisco Bay at interpopulation level, but there were significant differences at intrapopulation level at salinities below 100 g/l. Abatzopoulos et al. (2003) studying an *Artemia* clone from Megalon Embolon observed significant differences in all reproductive characteristics except for number of broods in all three salinities (50, 80, 120 g/l). Baxevanis et al. (2004) reported significant differences in most of the reproductive parameters between the bisexual and parthenogenetic populations he studied at 35, 80 and 120 g/l. Moreover they observed statistically significant differences between the inland and coastal parthenogenetic populations of *Artemia* from Egypt, but no differences were evident between the two coastal strains. Browne and Wanigasekera (2000) reported that all five populations in their study had highest reproduction period and peak production at 24˚C at either 120 or 180 g/l. According to their findings Old World species (*A. sinica* and *A. salina*) and the parthenogenetic population are more limited by temperature and salinity for reproduction, whereas New World species (*A. franciscana and A. persimilis*) are euryhaline and eurythermal

being able to reproduce more successfully at more diverse salinity-temperature combinations. But our results indicate that *A. urmiana* and the two asexual populations from Iran are able to reproduce very well at all salinities below 150 g/l and peak production was observed at 75 and 100 g/l.

In this study, which was carried out at five different salinities ranging from 75 to 175 g/l at 27±1˚C, all three populations did produce offspring in all salinities, except for the parthenogenetic *Artemia* from lagoons, which was not reproductive at 175 g/l. Our results are in agreement with findings of Gilchrist, (1960), Dana and Lenz (1986), Triantaphyllidis et al. (1995), Abatzopoulos et al. (2003) and Baxevanis et al. (2004) proving that reproductive and life span characteristics are inversely affected by salinity. Our results also proved that early maturation is achieved by sexual URM and the two asexual populations from Lake Urmia and neighbouring lagoons in low salinities. Unlike findings of Triantaphyllidis et al. (1995) with Tanggu (China) parthenogenetic *Artemia* and *A. franciscana* from San Francisco Bay, we found significant differences in most of the reproductive and life span characteristics of URM and two asexual populations from Iran both at inter- and intrapopulation levels in all salinities. In accordance with findings of Baxevanis et al. (2004) on Egyptian sexual and asexual population, we also found significant differences in reproductive and life span characteristics of Iranian sexual and parthenogenetic strains under function of different salinities.

Abatzopoulos et al. (2006) did not find significant differences in five out of six reproductive traits and two out of four life span characteristics of *A. urmiana* in different salinities. According to them *A. urmiana* cultured at 140 and 180 g/l showed a significantly longer reproductive period and total life span than those at 100 g/l. Same study also revealed that *A. urmiana* has higher tendency for encystment with salinity increase. Baxevanis et al. (2004) observed that the salinity increase was positively correlated with the number of produced encysted embryos in Egyptian sexual and asexual *Artemia* populations. Unlike findings of Abatzopoulos et al. (2006), we observed significant differences in 3 major reproductive characteristics (i.e. total offspring, number of cysts and number of nauplii produced) in *A. urmiana* cultured at different salinities, higher offspring production was observed at lower salinities (75 & 100 g/l). But our results

support findings of Abatzopoulos et al. (2006) and Baxevanis et al. (2004) on percentage of encystment by *Artemia* populations at elevated salinities, although in our study total cyst production decreased significantly at 150 and 180 g/l compared to 75 and 100 g/l.

The present study supports findings by many studies on the negative impact of salinity values above 120–140 g/l on reproductive and life span characteristics in many other *Artemia* species or strains (Vanhaecke et al., 1984; Wear & Haslett, 1986; Triantaphyllidis et al., 1995; Browne and Wanigasekera, 2000; Baxevanis and Abatzopoulos, 2004; Baxevanis et al., 2004). In accordance with previous laboratory investigations on several *Artemia* species (Browne et al., 1991; Triantaphyllidis et al., 1995; Baxevanis et al., 2004), it was found that the optimal range for growth, survival and reproduction of Iranian sexual and asexual strains of *Artemia* from Lake Urmia region lies between 75 and 125 g/l.

Although salinity was usually very high, ranging from 250-300 g/l during the last ten years in most parts of the Lake Urmia (Agh, 2006); considerably high *Artemia* biomass concentrations could be seen at limited areas in the lake during spring and summer. It should be remembered that numerous quantities of *Artemia* cysts hatch in Lake Urmia during spring and summer in areas with low salinities. Therefore it could be assumed that the newly hatched nauplii go through an adaptation period before migrating to deeper parts of the lake where salinity never dropped below 250 g/l since 1998. However there are no accurate data on mortality rates of *Artemia* in Lake Urmia. Apparently the total number of adults in the lake is much less than the total number of nauplii hatching from cysts in spring and summer (Agh, unpublished data). This would mean that even in a natural biotope only limited number of animals that can withstand high salinity levels can survive and the rest die off. This could be a logic explanation for thriving *Artemia* populations at very high salinities in nature. This phenomenon could also help us to understand why culture and maintenance of *Artemia* in laboratory at salinities higher than 200 g/l has always been difficult and why very high mortality occurs under function of high salinities in laboratory culture of *Artemia*. Thus it could be concluded that, despite extraordinary tolerance levels of *Artemia* to high salinities, it seems that salinity is the crucial limiting factor in controlling the population density of *Artemia* both in nature and in the laboratory.

5. Acknowledgements

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CHAPTER 5

Life cycle characteristics of six *Artemia* populations from Iran

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Abstract

In this paper we present the life cycle characteristics of six *Artemia* populations, one bisexual and five parthenogenetic, from Iran. The cysts of asexual strains were collected from Maharlu, Incheh, Varmal and Qom salt lakes and also from Lagoons at the periphery of Lake Urmia. Cysts of the bisexual *Artemia urmiana* were collected from the Lake Urmia. Cysts were hatched according to the standard procedures (Sorgeloos 1986) and the nauplii from all populations were reared at 80 g/l at laboratory conditions. Survival and total length of the *Artemia* were measured on days 8, 11, 14, 17, 20 and 23 of culture. Randomly selected adult animals were studied for eight reproductive and four life span characteristics according to Browne et al. (1984; 1988).

Results showed that parthenogenetic *Artemia* from Maharlu (73.4%), Incheh (62.8%) lakes and from Lagoons at the vicinity of the Lake Urmia have significantly higher ($P<0.05$) survival when compared to bisexual *A. urmiana* (49.6%) and parthenogenetic strains from Qom (29.2%) and Varmal (23.2%) lakes. Statistically non-significant differences were observed in the growth level of these populations when cultured under similar laboratory conditions. *Artemia* populations from Maharlu and Qom lakes and also from Lagoons in many occasions had significantly higher ($P<0.05$) reproductive values in comparison to the other three populations including the bisexual *A. urmiana*. Due to high standard variations in reproductive and life span characteristics observed in parthenogenetic populations from Iran, it was concluded that there could be high heterogeneity and intrapopulation variations among them.

Introduction

Artemia can be found in a great variety of habitats in terms of anionic water composition (Browne et al. 1985; Lenz, 1987; Browne et al. 1988), altitude (Abatzopoulos et al. 1998; Traintaphyllidis et al. 1998; Van Stappen, 2002) and climatic conditions, from humid-sub humid to arid (Vanhaecke et al. 1987).

Many studies have been carried out on growth, survival, reproductive and life span characteristics of *Artemia* populations from different parts of the world cultured under standardized laboratory conditions (Browne et al., 1984; Wear & Haslett, 1986; Gajardo et al,

1998; Abatzopoulos et al., 2003; Traintaphyllidis et al., 1995; Browne & Wanigasekera, 2000; El-Bermawi, 2004; Baxevanis et al., 2004). Most of these studies confirm that different ecological conditions such as salinity and temperature have major influence on reproductive characteristics of *Artemia*.

Historical overview of *Artemia* populations from Iran is discussed in detail by Abatzopoulos et al. (2006). Until recently only 3-4 populations of *Artemia* were reported from Iran (Artom, 1922; Stella, 1933; Barigozzi, 1946; Persoone and Sorgeloos, 1980; Vanhaecke et al. 1987 and Triantaphyllidis et al. 1998). It was only as a result of recent surveys by Agh et al. (2001-2002a, b) Abatzopoulos et al. (2006) that occurrence of *Artemia* from many new geographic locations from Iran were reported. All these populations except *Artemia urmiana* from Urmia Lake are parthenogenetic populations. Considerable differences were observed in their habitats in terms of ionic composition, temperature, altitude and climatic conditions (Abatzopoulos et al., 2006).
The life cycle of these different *Artemia* populations from Iran has not been documented so far. This paper reports on a study on growth, survival, reproductive and life span characteristics of five inland parthenogenetic and one inland bisexual *Artemia* population from Iran. The main aim of this study is to present data on further characterization of *Artemia* populations from Iran, based on their growth and survival patterns, and also on their reproductive and life span characteristics.

Material and methods

Strains studied

The *Artemia* populations studied were from 6 different geographic regions of Iran including the bisexual *A. urmiana* from Lake Urmia (North West) and five inland parthenogenetic populations: the lagoons at the vicinity of Lake Urmia, Incheh Lake (North East), Qom Salt Lake (central region), Maharlu lake (South) and Varmal lake (South East).

Culture experiments

Cysts of *Artemia* were collected from each lake and lagoon and were hatched under standard conditions (Sorgeloos, et al. 1986). From each population 400 newly hatched larvae were transferred into separate 1-liter cones containing 800 ml of 80 g/l brine water in four replicates.

They were cultured under controlled standard conditions using *Dunaliella tertiolecta* and Lansy PZ as food. Growth and survival rates were determined at each water renewal on days 8, 11, 14, 17, 20 and 23. As soon as males started to clasp females (in case of bisexual *A.urmiana*) or parthenogenetic females showed signs of ovarian development, 30 randomly collected couples or parthenogenetic females were removed from each population's mass culture and placed in 50 ml cylindroconical falcon tubes to begin the isolated culture. Females were considered mature when migration of the oocytes into the uterus was observed (Triantaphyllidis et al., 1995).

Clonal cultures continued as long as the female *Artemia* were alive. In the falcon tubes containing bisexual *A. urmiana*, dead males were immediately replaced with actively swimming males during the experiment (Browne et al. 1988). The falcon tubes were checked every day for the production of cysts or nauplii, which were counted and recorded separately. Finally the reproductive characteristics (brood size, total number of offspring, number of nauplii, number of cysts, offspring in each reproduction, offspring/day during the reproductive period, brood intervals and percentage of encystment) and the life span characteristics (pre-reproductive period, reproductive period, post-reproductive period and life span) were determined for each population according to Browne et al. (1984; 1988). The results were statistically analyzed using SPSS (version 14) analysis of variance (ANOVA) (Sokal & Rohlf, 1981; Triantaphyllidis et al., 1995), and the averages were compared using Tukey test. The results of reproductive and life span characteristics were also processed with discriminant function analysis (Kachigan, 1986; Hontoria & Amat, 1992a, b; Triantaphyllidis et al., 1995; Abatzopoulos et al., 2003).

Results

Survival and growth

Survival results after 23 days of experiment demonstrate significant differences ($P<0.05$) when different populations are cultured under similar laboratory conditions (Figure 5.1). The asexual *Artemia* (MAH) had significantly higher survival (73 %) compared to the sexual URM and other asexual populations. Two other asexual populations (INC and LAG) also had significantly higher survival in comparison to URM (ANOVA, $p < 0.05$). Results showed that parthenogenetic strains when cultured under standardized conditions performed differently. MAH, INC (62.8 %)

and LAG (62.8 %) populations had significantly higher survival in comparison to QOM (23.2 %) and VAR (29.2 %) after 23 days (Figure 5.1, Table 5.1, ANOVA, $p < 0.05$).

Table 5.1. Survival percentage of *Artemia* populations from Iran

	day 1	day 8	day 11	day 14	day 17	day 20	day 23
Inche (INC)	100	95.7	73.5	71.7	69.5	65.0	62.8
Qom (QOM)	100	65.8	41.9	41.9	31.8	29.3	23.1
Lagoon (LAG)	100	93.4	83.8	83.8	76.1	71.9	62.8
Maharlu (MAH)	100	95.0	92.9	91.6	87.6	82.5	73.4
Varmal (VAR)	100	70.2	58.3	48.1	39.4	35.0	29.2
A.urmiana (URM)	100	87.0	79.0	75.3	70.5	63.4	49.6

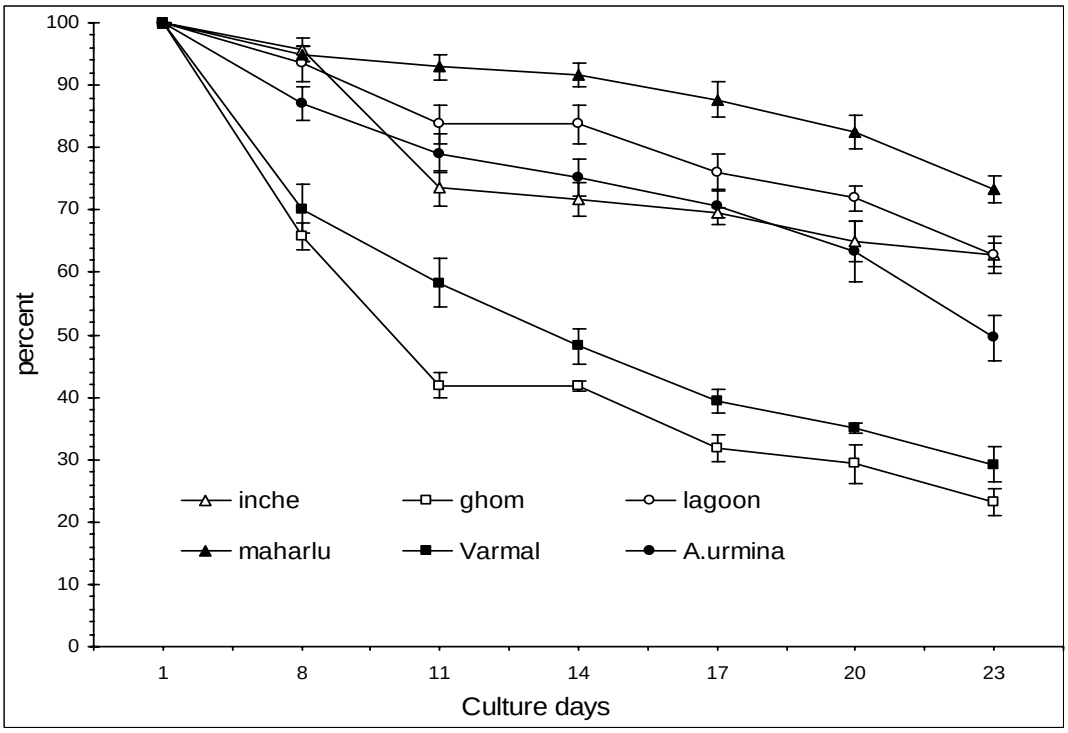

Figure 5.1. Survival of *Artemia* populations from Iran.

The sexual URM had significantly higher growth rate in comparison to the parthenogenetic strains ($p<0.05$) throughout the growth period. The recorded maximum total length of URM was significantly bigger than this of the parthenogenetic strains (URM: 11.67 mm while for the

parthenogenetic strains it was less than 10 mm). Asexual populations exhibited significant differences in the growth rate among them ($p<0.05$). Maximum and minimum total length for these strains was 9.98 and 9.32 mm respectively. Among the parthenogenetic populations LAG had significantly higher growth followed by MAH. VAR had the lowest growth rate, significantly lower than all populations studied (Figure 5.2, Table 5.2, ANOVA, $p<0.05$).

Table 5.2. Growth rate of 1 sexual and 5 asexual populations of *Artemia* from Iran.
The abbreviation of populations can be found in Table 5.1

Culture period (days)	INC	QOM	LAG	MAH	VAR	URM
1	0.49	0.49	0.45	0.50	0.50	0.52
8	4.06	3.57	4.26	4.76	4.76	4.61
11	5.98	5.91	6.09	6.44	6.49	6.88
14	7.62	7.40	7.49	7.86	7.90	8.05
17	8.11	7.76	8.37	8.99	8.30	9.43
20	9.42	8.10	9.51	9.25	9.04	10.44
23	9.95	9.64	9.98	9.46	9.33	11.67

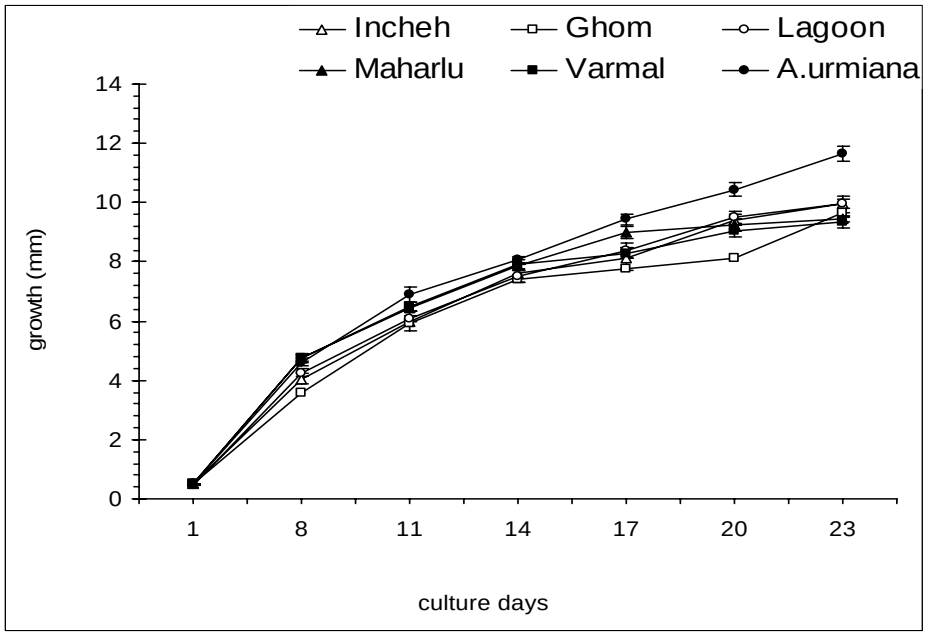

Figure 5.2. Growth rate of the *Artemia* populations cultured at laboratory condition.

Reproductive and Life Span Characteristics

Eight reproductive and four life span characteristics from the six *Artemia* populations from Iran (URM, INC, QOM, LAG, MAH, VAR) cultured under standardized laboratory conditions are summarized in Table 5.3. Statistical analysis using ANOVA indicated that significant differences exist among the different populations in most of the characters studied. It was revealed that the sexual URM had lowest values in total number of offspring and number of broods in comparison to all asexual strains (Figures 5.3 and 5.4). QOM and LAG strains had significantly higher values in the total offspring and nauplii production than other asexual strains and the sexual URM (Figures 5, ANOVA, $P<0.05$). QOM strain had the highest number of cyst production, offspring per day and offspring per brood (Figures 5.4 and 5.5, Table 5.3). URM had significantly longer intervals between broods (7.7 days) than the asexual strains, showing its slower reproducing capacity in comparison to parthenogenetic populations (Figure 5.7). Minimum intervals between two consecutive broods were observed in LAG strain (4.8 days). MAH strain produced significantly higher percentage of encysted embryos (79.07 %) in comparison to all strains studied, followed by QOM strain (58.39 %) (Figure 5.6, ANOVA, $p<0.05$).

MAH showed significantly longer pre-reproduction and life span periods ($P<0.05$) in comparison to all other populations. INC and VAR demonstrated shortest pre-reproduction period significantly less than other populations except *A. urmiana*. Maximum and minimum reproduction period was observed in QOM and INC respectively. But only QOM and LAG strains were significantly different from INC and VAR. MAH lived for an average of 11.9 days after its last production showing significantly longer post reproduction period in comparison to other strains (ANOVA, $P<0.05$). MAH strain had longest life span (67.9 days), significantly longer than any other population, followed by QOM and LAG strains (ANOVA, $P<0.05$). Sexual URM lived for 43.3 days, significantly shorter than MAH and QOM strains (Figure 5.7).

Table 5.3. Mean of various reproductive and life span characteristics for six *Artemia* populations from Iran (standard deviations in parenthesis). Significant differences were determined by ANOVA test ($P < 0.05$). Values in each row that share the same superscript are not significantly different. For abbreviation of populations see Table 5.1

	INC	QOM	LAG	MAH	VAR	URM
Number of offspring	256.1^{b} (279.5)	576.1^{a} (567.5)	429.0^{a} (382.9)	214.1^{b} (104.6)	213.1^{b} (117.7)	194.3^{b} (130.6)
Number of nauplii	197.8^{ab} (198.4)	290.5^{a} (359.6)	263.6^{a} (237.7)	55.8^{c} (72.9)	133.1^{bc} (87.4)	96.3^{bc} (70.3)
Number of cysts	58.2^{d} (100.4)	285.6^{a} (317.2)	165.4^{bc} (182)	188.3^{b} (103.7)	80.6^{cd} (49.9)	97.9^{cd} (99.7)
Number of offspring per brood	50.1^{b} (18.5)	75.2^{a} (41.4)	53.6^{b} (22.9)	44.0^{b} (10.0)	47.0^{b} (17.2)	47.5^{b} (18.7)
Number of offspring per day	12.6^{bc} (5.2)	16.0^{a} (5.9)	14.0^{ab} (4.8)	9.6^{e} (4.5)	10.3^{dc} (3.1)	10.7^{dc} (4.9)
Brood	4.5^{bc} (2.7)	6.2^{ab} (4.5)	7.3^{a} (4.5)	5.5^{bc} (2.1)	4.1^{d} (1.4)	3.7^{d} (2.0)
Days between Broods	5.4^{c} (1.2)	6.7^{ab} (3.2)	4.8^{c} (1.3)	6.5^{b} (2.0)	5.1^{c} (0.5)	7.7^{a} (2.8)
Percent offspring encysted	23.3^{e} (19.9)	58.4^{b} (34.7)	37.0^{cd} (27.6)	79.1^{a} (23.9)	37.0^{cd} (27.6)	43.6^{c} (32.8)
Pre Reproductive period	20.0^{d} (2.1)	21.6^{bc} (2.9)	22.0^{b} (1.2)	28.4^{a} (2.5)	20.5^{cd} (2.1)	21.5^{bc} (1.8)
Reproductive period	19.2^{b} (11.0)	35.2^{a} (26.1)	$31. 9^{a}$ (21.6)	28.6^{ab} (10.8)	19.7^{b} (7.6)	26.6^{ab} (14.1)
Post Reproductive period	1.2^{b} (1.7)	1.4^{b} (3.2)	0.8^{b} (1.6)	11.9^{a} (13.8)	2.1^{b} (0.6)	1.2^{b} (2.0)
Lifespan	39.8^{d} (11.8)	54.5^{b} (27.1)	52.9^{bc} (21.6)	67.9^{a} (23.2)	42.3^{d} (8.0)	43.3^{cd} (16.6)

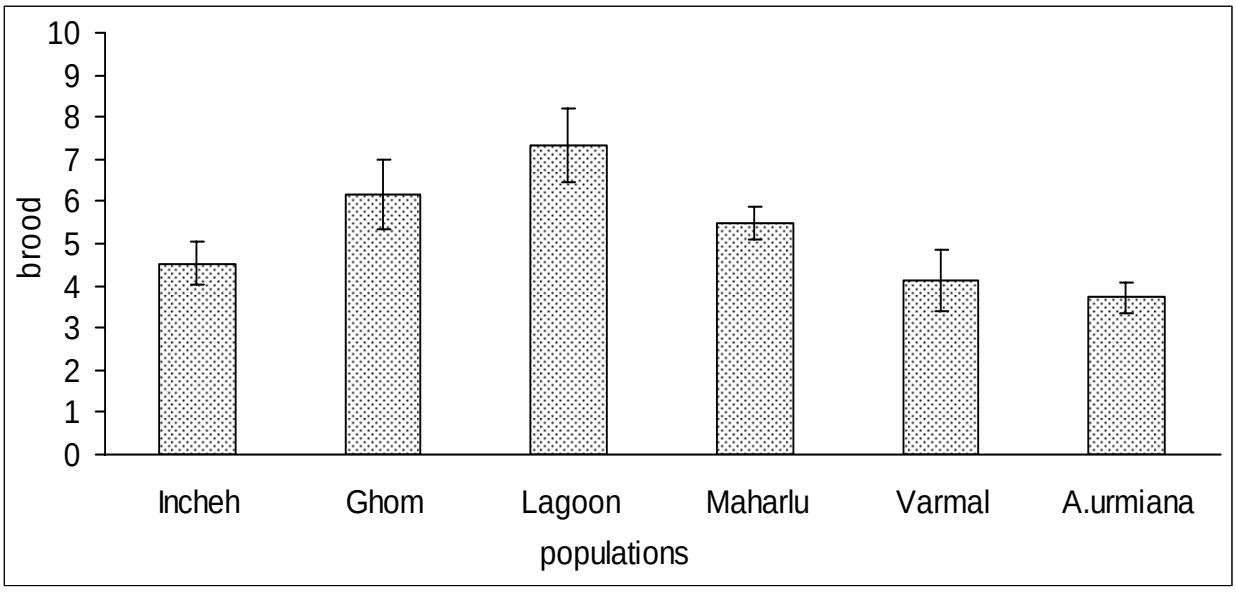

Figure 5.3. Number of broods in the Iranian populations of *Artemia.*

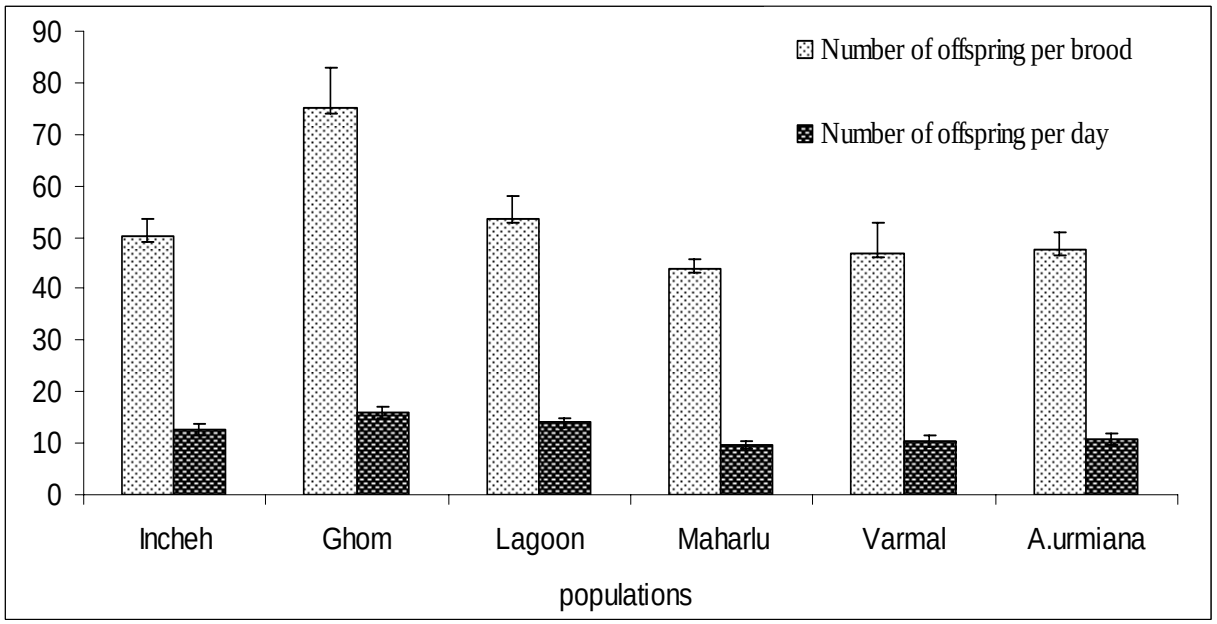

Figure 5.4. Number of offspring per brood and number of offspring per day in the Iranian populations of *Artemia.*

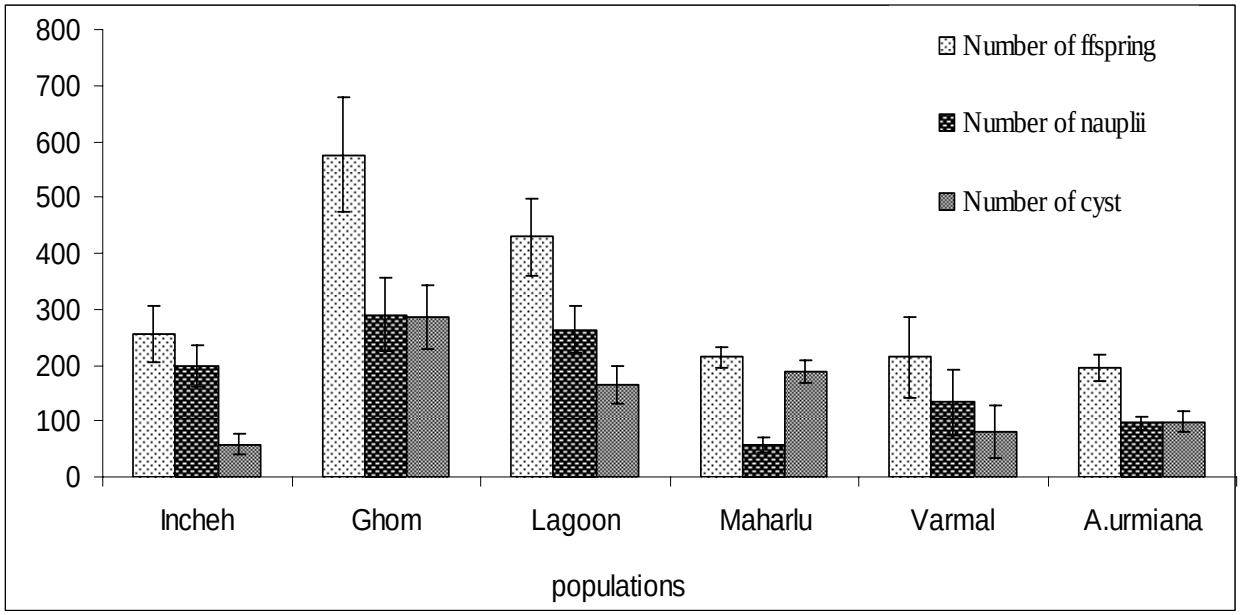

Figure 5.5. Total number of offspring, number of nauplii and number of cysts produced by the Iranian populations of *Artemia*.

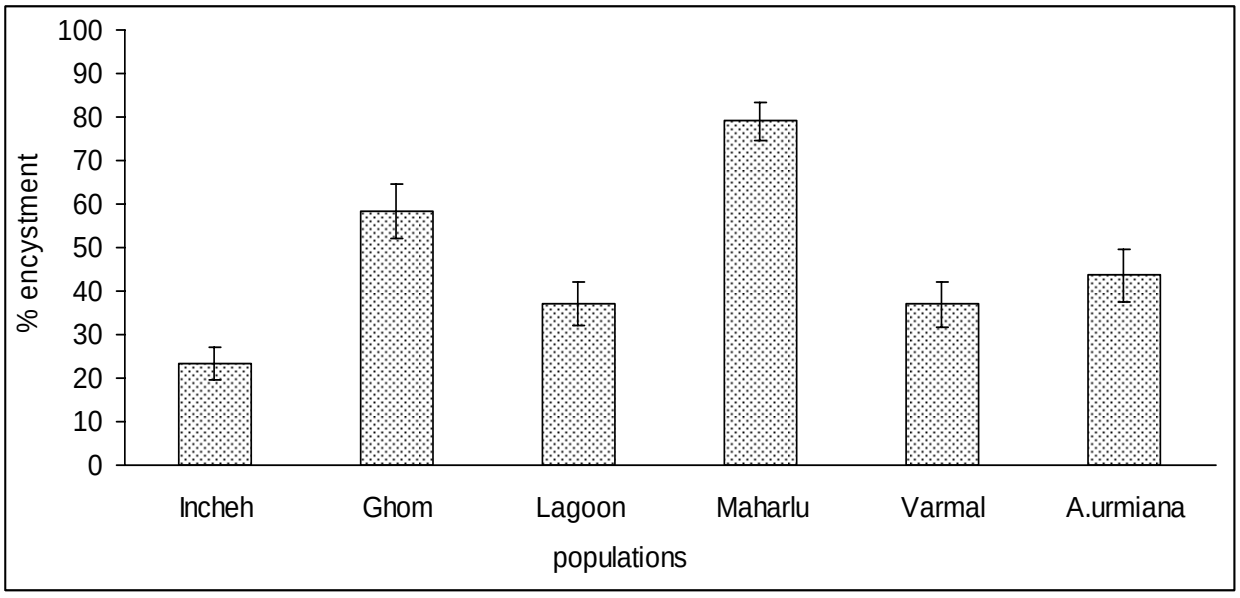

Figure 5.6. Percentage of encystment in Iranian populations of *Artemia*.

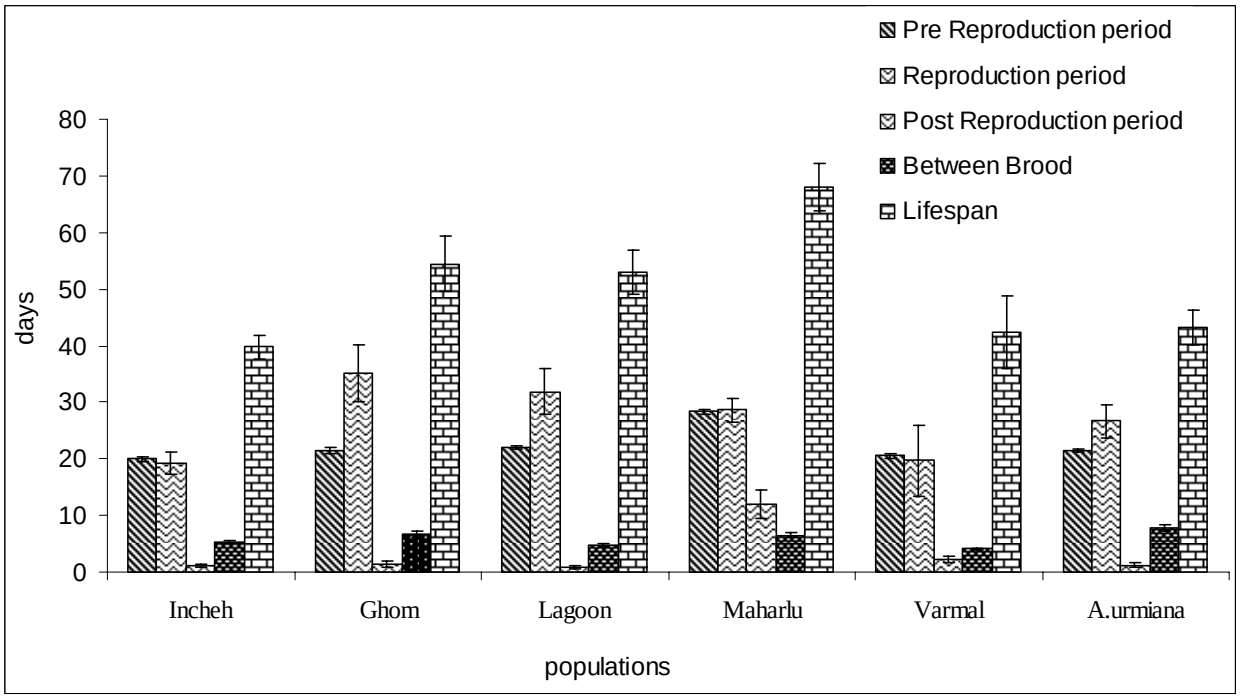

Figure 5.7. Life span characteristics of the Iranian populations of *Artemia*

Discriminant analysis based on the origin of each population as a separation criterion resulted in 70.90 % separation of original groups. However this analysis could separate the sexual *A. urmiana* only by 66.7 % predictability from the parthenogenetic populations. The predictability value for MAH strain was 96.7 %, but the respective values for the other four parthenogenetic populations ranged from 46.7 to 86.7 % (Tables 5.4, 5.5, 5.6). Figure 5.8 depicts the plot of the discriminant analysis based on the 2 out of 5 roots that were produced. The MAH population is clustered separately from other populations suggesting its significantly different reproductive and life span characteristics. Sexual URM was clustered together with VAR, LAG and QOM populations, while INC strain was placed in a separate group.

Table 5.4. Discriminant analysis of the reproductive and life span parameters of female *Artemia* populations. Classification functions produced by discriminant analysis for each population. For abbreviations, see Table 5.1

Variables	Classification Function Coefficients for each population					
	INC	QOM	LAG	MAH	VAR	URM
Number of offspring per brood	0.154	0.074	0.137	0.151	0.190	0.118
Number of offspring per day	2.649	2.970	2.826	2.538	2.151	2.992
Number of broods per female	9.785	8.670	10.878	9.828	8.390	9.098
Time interval in-between broods (in days)	7.348	8.013	7.777	7.453	6.183	7.987
Encystment rate (%)	-0.068	-0.028	-0.052	-0.014	-0.023	-0.054
Total number of offspring per female	-0.103	-0.095	-0.110	-0.116	-0.096	-0.113
Number of cysts	0.028	0.033	0.031	0.041	0.024	0.033
Pre-reproductive period	4.959	5.236	5.402	7.141	5.111	5.451
Reproductive period	-0.524	-0.378	-0.540	-0.370	-0.351	-0.240
Post-reproductive period	-0.424	-0.399	-0.443	-0.100	-0.326	-0.457
Total lifespan	0.167	0.151	0.163	0.142	0.132	0.164
(Constant)	-100.486	-113.029	-117.771	-157.851	-92.085	-118.617

Table 5.5. Standardized coefficients produced by discriminant analysis for canonical variables. Eigenvalues and Cumulative percentages are presented.

	Standardized coefficients for canonical variables				
Variables	Root 1	Root 2	Root 3	Root 4	Root 5
Number of offspring per brood	.028	-.907	.128	-.065	-.084
Number of offspring per day	-.086	1.468	.503	-.659	.206
Number of broods per female	.302	.304	3.311	.505	.347
Time interval in-between broods (in days)	.075	1.298	.552	-.440	.443
Encystment rate (%)	.178	-.072	-.420	.373	-.608
Total number of offspring per female	-.947	-.196	-2.095	2.298	.426
Number of cysts	.418	.483	.157	-.140	.512
Pre-reproductive period	.893	.037	.120	-.028	.090
Reproductive period	.234	.166	-1.600	-1.827	-1.880
Post-reproductive period	.389	-.263	-.235	.316	.349
Total lifespan	-.050	.133	.207	-.159	.282
Eigenvalues	3.295	0.796	0.523	0.262	0.069
Cumulative %	66.6	82.7	93.3	98.6	100.0

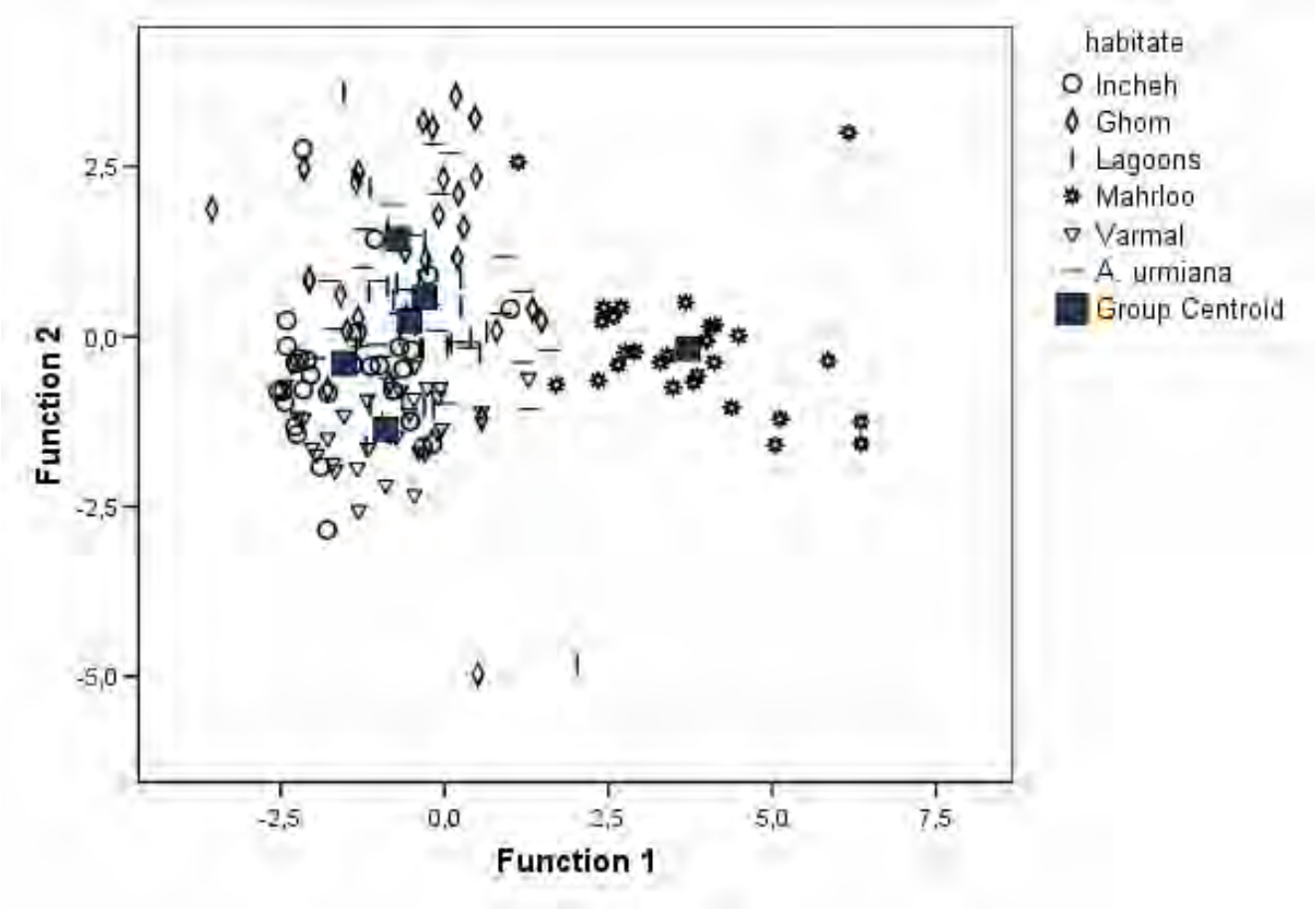

Figure 5.8. Scatterplot resulting from the discriminant analysis (canonical scores) based on reproductive and life span characteristics and using the origin of each population as a separation criterion

Table 5.6. Classification results of discriminant analysis showing the percentages of populations classified in each group. The percent of "grouped" cases correctly classified is 70.60 %. For abbreviations see Table 5.1

		habitat	Predicted Group Membership						Total
			INC	QOM	LAG	MAH	VAR	URM	
Original	Count	INC	21	1	2	0	5	1	30
		QOM	7	17	2	0	2	2	30
		LAG	5	2	14	0	4	5	30
		MAH	0	0	0	29	0	1	30
		Varmal	4	0	0	0	26	0	30
		URM	6	1	0	0	3	20	30
	%	INC	70.0	3,3	6.7	0.0	16.7	3.3	100.0
		QOM	23.3	56.7	6.7	0.0	6.7	6.7	100.0
		LAG	16.7	6.7	46.7	0.0	13.3	16.7	100.0
		MAH	0.0	0.0	0.0	96.7	0.0	3.3	100.0
		Varmal	13.3	0.0	0.0	0.0	86.7	0.0	100.0
		URM	20.0	3.3	0.0	0.,0	10.0	66.7	100.0
Total predictability			70.6 %						

Discussion

Survival and Growth

There are a number of studies on laboratory culture of *Artemia* at 80±20 g/l showing that most *Artemia* grow and reproduce well in this range of salinity (Pador, 1995; Triantaphyllidis et al. 1995; Browne and Wanigasekara, 2000; Abatzopoulos et al. 2003; El-Bermawi, et al. 2004; Baxevanis et al. 2004). These studies proved that most *Artemia* populations demonstrated different responses with regard to survival, growth and reproductive characteristics at the same salinity. According to Vanhaecke and Sorgeloos (1989) who compared survival and growth of *Artemia* larvae in 25 geographical strains, bisexual strains had significantly higher survival and growth compared to asexual populations. However Gilchrist (1960) reported that a

parthenogenetic *Artemia* from La Palme (France) grew faster than a bisexual strain from San Diego (California, USA). El-Bermawi et al. (2004) reported that the bisexual population from Wadi El-Natrun of Egypt had higher survival in compare to the parthenogenetic strains when cultured at 80 g/l. But they did not find any significant differences in their growth rate.

According to Triantaphyllidis et al. (1995) the survival rate of parthenogenetic *Artemia* from Tanggu China and bisexual *A. franciscana* did not show any significant differences when grown at 60 and 100 g/l. But they observed significantly higher growth in parthenogenetic population compared to *A. franciscana.*

Browne and Wanigasekara (2000) found significant differences in the survivorship of bisexual *Artemia* (*A. franciscana, A. salina, A. sinica and A. persimilis*) and *A. parthenogenetica* from Italy cultured at different combinations of salinity and temperature. Their findings confirmed that in terms of survival, there is no one optimal temperature – salinity combination, with each species having a different optimum.

In our experiments the survival rate of the Iranian bisexual population, *A. urmiana*, exhibited considerable differences with the parthenogenetic populations. *A. urmiana* had significantly higher survival compared to VAR and QOM, but at the same time significantly lower than LAG, MAH and INC populations (ANOVA, $p<0.05$). But in terms of growth, the sexual URM grew significantly faster than the asexual strains. Significant differences were recorded in both survival and growth among the parthenogenetic strains too.

There is no literature data available on the survival and growth rate of *Artemia* populations from Iran, but in comparison with results obtained by other researchers on *Artemia* populations from other geographic locations, our findings are in agreement with a number of literature observations: strain differences in survival and growth rate can not be ascribed to the mode of reproduction. It seems that each population has its own optimal environmental conditions for growth and better survival. The culture condition that is considered standard for rearing *Artemia*

may be optimal for some strains, but not for others. Moreover our study provides further evidence that growth and survival rates of *Artemia* are strain-dependent.

Reproductive and Life Span Characteristics

Many researchers have studied reproductive and life span characteristics of parthenogenetic and bisexual *Artemia* populations from different geographic locations (Gilchrist, 1960; Dana and Lenz, 1986; Triantaphyllidis, et al. 1995; Browne and Wanigasekara, 2000; Abatzopoulos et al. 2003; El-Bermawi, et al. 2004; Baxevanis et al. 2004). Triantaphyllidis et al. (1995) who compared the reproductive and lifespan characteristics of Tanggu parthenogenetic *Artemia* and *A. franciscana* from San Francisco Bay, reported no significant differences in pre-reproductive period between them. Baxevanis et al. (2004) also did not find any statistical difference in this parameter between the parthenogenetic and bisexual strains from Egypt.

A. franciscana from San Francisco Bay had significantly longer reproductive period than Tanggu parthenogenetic *Artemia* (Triantaphyllidis et al., 1995) but the Egyptian bisexual *Artemia* had a significantly shorter reproductive period (12.3 days) in comparison to the parthenogenetic *Artemia* (23.2 – 27.6 days). Abatzopoulos et al. (2003) found still a shorter reproductive period (21.8 days) in the parthenogenetic population of Megalon Embolon saltworks from Greece when cultured in similar conditions.

Triantaphyllidis et al. (1995) did not find any significant differences in the total life span of *A. franciscana* and parthenogenetic *Artemia* from Tanggu. Similarly the Egyptian bisexual population also did not exhibit any significant differences in total life span with 2 out of 3 asexual strains (El-Bermawi, 2003; Baxevanis et al. 2004).

Total number of offspring, offspring per brood and number of brood per female were significantly higher in *A. franciscana* when compared with Tanggu parthenogenetic *Artemia* (Triantaphyllidis et al., 1995). But Egyptian bisexual *Artemia* produced significantly less offspring, and had lesser number of offspring per female per brood and fewer broods per female

in comparison to the parthenogenetic strains (Baxevanis et al. 2004). Percentage of offspring encysted was significantly high both in *A. franciscana* and in Egyptian bisexual *Artemia* compared to the asexual *Artemia*.

Triantaphyllidis et al. (1995) claimed that high standard deviations observed for reproduction and lifespan characteristics of the bisexual *A. franciscana* presumably reflects the presence of high heterogeneity and intrapopulation variance, whereas much lower variance levels for the Tanggu parthenogenetic population are expressed due to their lower genetic diversity compared to the bisexual species. But Baxevanis et al. (2004) found higher standard deviations among Egyptian parthenogenetic populations in comparison to the bisexual species.

In our study INC and VAR had shortest maturation time significantly different from other populations including the bisexual *A. urmiana* (ANOVA, $P < 0.05$). But however no significant differences were observed in pre-reproductive period between *A. urmiana* and parthenogenetic populations from QOM, LAG and VAR. Parthenogenetic *Artemia* from MAH had a significantly longer maturation time in comparison to all populations studied (ANOVA, $P < 0.05$), proving that pre-reproductive period is an individual characteristic for each strain, regardless of their reproductive mode. We found no significant differences in reproductive period of the bisexual *A. urmiana* and the parthenogenetic strains. But significant differences were found among the parthenogens themselves with regard to this characteristic. QOM and LAG strains had significantly longer reproductive period compared to INC and VAR populations (ANOVA, $P < 0.05$). Moreover the bisexual *A. urmiana* demonstrated a significantly shorter life span compared to MAH and QOM populations, but no significant differences were observed with the same parameter in INC, LAG and VAR strains. Therefore it could be assumed that the life span characteristics are strain-specific for the Iranian populations of *Artemia*.

In our experiment the bisexual *A. urmiana* had least number of offspring compared to parthenogenetic strains from Iran, significantly less than that in QOM and LAG populations (ANOVA, $P < 0.05$). It was also found that the number of broods in URM was significantly lower in comparison to the asexual strains except from that in VAR strain. No significant differences were found in the number of offspring per brood among most Iranian strains except for MAH

population that was highly significant from others. Moreover the percentage of *A. urmiana* producing encysted embryos was significantly lower than QOM and MAH populations, and only significantly higher than the INC strain. Comparison of the parthenogenetic populations revealed no significant differences in the majority of the characters assayed between INC and VAR on one hand, and QOM and LAG on the other.

In this study we found high standard deviations in Iranian parthenogenetic strains compared to the bisexual *A. urmiana* in most of the reproductive characters, indicating that some other parameters like culture conditions and ionic differences of the culture medium might be important in determining this high standard deviation rather than the genetic diversity. This is in agreement with findings of Baxevanis et al. (2004) with Egyptian parthenogenetic strains but does not support findings of Triantaphyllidis et al., (1995) and Browne and Hoopes (1990).

We also tried to define possible grouping of six *Artemia* populations based on reproductive characteristics. Based on the reproductive parameters studied, discrimination models resulted in total predictability of 70.60 %. The sexual URM showed significantly higher values not even in a single reproductive and life span characteristics when compared to the asexual populations. On the contrary, the asexual MAH strain showed significant differences from URM and other parthenogenetic populations in 5 out of 12 reproductive and life span characteristics (ANOVA, $P<0.05$). The reproductive parameters that contributed to the discrimination of the MAH from URM and other asexual strains were the pre-reproductive period; post-reproductive period; total life span; number of offspring per day and percent offspring encysted. The first four variables were significantly higher and the fifth variable was significantly lower compared to all populations studied (ANOVA, $P < 0.05$). Our results are different from findings of El-Bermawi (2003) and Baxevanis et al. (2004) who could statistically discriminate an Egyptian sexual strain from asexual strains of Egypt based on reproductive and life span characteristics. According to our findings sexual URM was grouped close to asexual strains from QOM, LAG and VAR habitats. This reveals that based on reproductive and life span characteristics, sexual URM is not much different from the Iranian parthenogenetic populations of *Artemia*.

These findings show that bisexuality is not always a determining factor for better reproductive and life span characteristics for a population of *Artemia*, but we should look into more favourable culture conditions in order to have a higher reproductive result with asexual strains of *Artemia*. The studied populations of *Artemia* live in biotopes far away from each other with considerably different environmental conditions. Our results are therefore in agreement with Browne et al. (2002) who reported that reproductive characteristics of *Artemia* are highly influenced by the environmental components.

Acknowledgments

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CHAPTER 6

Studies on the enrichment of *Artemia urmiana* cysts for improving fish food value

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Abstract

Since no artificial feed formulation is yet available to substitute completely for *Artemia*, feeding *Artemia* cysts to young fish larvae still remains essential in commercial hatchery operations. The nutritional quality of commercially available *Artemia* strains is relatively poor in eicosapentaenoic acid (EPA, 20:5*n*−3) and docosahexaenoic acid (DHA, 22:6*n*−3), two major determinants of fish food value and also the price of *Artemia* cysts. It is a common practice to enrich these *Artemia* cysts with emulsions of marine oils. In the study reported, fatty acids of *Artemia urmiana* cysts were studied by GC, both before and after their enrichment with fish oil. Decapsulated cysts of *A. urmiana* were dried for 24 h at 60 ◦C, fatty acids were extracted with diethyl ether, converted to the methyl esters of fatty acid (FAME) by methanolic KOH (2N), and the esters were extracted with *n*-heptane. FAMEs were injected to GC for the determination of the composition and quantity of fatty acids. In the next stage, *A. urmiana* decapsulated cysts were enriched with fish oil to increase its EPA and DHA fatty acid levels and analysed by GC. The result demonstrated that this enrichment method brought about an increase in the levels of EPA from 13 to 24.6 mg/g and DHA from 0.5 to 10.6 mg/g.

1. Introduction

Urmia Lake with over 5500 km2 area, situated in northwest of Iran, is one of the largest biotopes of *Artemia* in the world (Van Stappen et al., 2001). *Artemia urmiana* is one of the eight bisexual *Artemia* species known to date. *Artemia*, a rich source of protein (about 600 g/kg in cyst) and fatty acids (about 240 g/kg in enriched *Artemia* cysts), has been consumed extensively as a nutritious food for humans, and used as a food for domesticated animals and birds. *Artemia* is also used in aquaculture as a nutritious food for fish. Since the development of commercial marine fish culture in the late 1970s, the demand for *Artemia* cysts has increased dramatically from a few metric tons to approximately 800 metric tons per annum (Lavens and Sorgeloos, 2000). Moreover, *Artemia* could be enriched and used as a carrier for various other essential nutrients and medicinal compounds for fishes and shrimps, such as highly unsaturated fatty acids, vaccines, antibiotics, and vitamins. Dietary lipid is incorporated into feeds to provide with metabolisable energy, promote the synthesis of cell membranes, and as a precursor for eicosanoid fatty acids. Although lipid is quantitatively a major dietary component for fish

growth, lipid requirements, particularly in larval stages of marine species, remain poorly understood (Coutteau and Mourente, 1997).

The dietary requirements of *n*−3 poly-unsaturated fatty acids (PUFA), particularly docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) have been documented for various species of marine fish (Izquierdo et al., 1992; Sargent et al., 1999; Watanabe, 1993). DHA, as well as its ratio to EPA, appears to be critical during the early larval stages as it affects growth and survival of marine fish (Furuita et al., 1996; Reitan et al., 1994; Sargent et al., 1997; Watanabe, 1993). Because of its role as a precursor of the eicosanoids, arachidonic acid was later added to the list of dietary essential fatty acids for marine fish (Castell et al., 1994a,b; Estevez et al., 1999; Sargent et al., 1999). The discrepancy between the essential fatty acid requirements of marine fish larvae and the fatty acid composition of the live preys has resulted in the development on enrichment protocols improving the PUFA content of rotifers and *Artemia* species (Han et al., 2001; Rainuzzo et al., 1994; Takuchi et al., 1992; Watanabe, 1993). Various enrichment emulsions have been formulated differing in the fatty acid composition of their triglycerides. To reduce the risks for oxidation of these fatty acids, higher concentrations of Vitamin E are incorporated into the emulsions. Also, Vitamin C has been incorporated in booster formulations that increase the level of ascorbic acid in *Artemia* to 2000-ppm (Sorgeloos et al., 2001). All these changes in the formulation of the enrichment diets offer more possibilities to satisfy the needs of different species and help to reduce problems related to diseases, stress resistance, malformation, and pigmentation in numerous fish species (Sorgeloos et al., 2001). The current programme reports the analysis of fatty acids composition in *A. urmiana* cysts by GC, before and after the implementation of the enrichment method with fish oil.

2. Materials and methods

2.1. Equipments and chemicals

GC-14A (Shimadzu, Japan) equipped with a flame-ionization detector was used. All chemicals were purchased from Merck Chemical Company and standards were purchased from PolySclence Corporation.

2.2. Decapsulation of the cysts

Cysts were washed by seawater maintained at a salinity of 100 g/l at 25 °C and filtered through filter paper (125 _m). All the filtered cysts were transferred to a conical flask containing 11–13% of NaOCl solution and the pH was adjusted above 10 by adding 2N NaOH. The hydrated cysts were kept in the above solution for a period of 5–15 min. Complete decapsulation of the cysts was frequently monitored by a stereomicroscope. Appearance of greyish colour among cysts indicated the completion of decapsulation. At this stage, the cysts were filtered through 125 µm filter paper and washed to remove chlorine from the solution. To neutralise the cysts containing solution, 0.1N HCl was added and kept in this condition for 1 min.

2.3. Extraction of lipids from the decapsulated cysts

Decapsulated cysts were dried in the oven at 60 °C for 24 h, powdered, and double extracted by diethylether. The ether was let to evaporate and the remaining residue lipids were collected.

2.4. Preparation of FAMEs from the above lipid residue

The lipid residue was converted to the fatty acid methyl ester (FAME) derivatives by adding *n*-heptane (1 ml) and 2N NaOH (0.05 ml). It was shaken continuously for a period of 15 min.

2.5. The analysis of FAME by GC

The GC analysis was carried out under the following running conditions: injector temperature 200 ◦C; nitrogen carrier gas flow rate 1 ml/min; injection volume 1_l; splitting ratio 1:25; CBP20 Shimpak column; column temperature increased from 100 to 180 °C with a period of 30 min by linear gradient. After 40 min, 19 peaks were detected. These peaks were compared with related FAMEs standard and were identified.

2.6. Enrichment of decapsulated cyst

The decapsulated cysts (20 g) were mixed with fish oil (0.3 ml). *n*-Heptane (0.05–0.1 ml) was added for assisting with the penetration of fish oil into the cysts for a period of 1, 3, and 24 h. After enrichment, the fatty acids were analysed by GC using the above methods.

2.7. Statistical analysis

One-way analysis of variance was applied to determine differences between four groups (enrichment after 0, 1, 3, and 24 h). The level of significance was chosen at $p < 0.001$. The linear and non-linear correlations of the results were compared.

3. Results and discussion

This is the first report on the analysis of fatty acids composition of *A. urmiana* cysts by GC, before and after the implementation of the enrichment method with fish oil. However, other species of *Artemia* were previously analyzed for their fatty acids composition and enrichment of fatty acids was accomplished with them.

In the study reported here, the fatty acids profile of the decapsulated *A. urmiana* cysts before enrichment included predominantly palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1*n*−7), linoleic acid (18:3*n*−3) and EPA (20:5*n*−3),. After enrichment while the profile changed to palmitic acid, palmitoleic acid, oleic acid (18:1*n*−9), EPA and DHA (22:6*n*−3), the total amounts of *n*−3 HUFA of *A. urmiana* cysts increased from 26 to 42 mg/g. It was evident that this enrichment method significantly improved EPA from 13 mg/g (*Artemia* decapsulated cysts) to 24.6 mg/g (decapsulated-enriched cysts after 24 h) and DHA from 0.5 mg/g (*Artemia* decapsulated cysts) to 10.6 mg/g (decapsulated-enriched cysts after 24 h) of total fatty acids (Table 6.1). The amount of DHA/EPA ratio of *Artemia* decapsulated cysts was 0.04, which was increased to 0.43 after 24 h enrichment.

The studies on enrichment of *Artemia* cysts have so far been mainly focused on increasing the levels of *n*−3 highly unsaturated fatty acids (HUFA) and particularly DHA, EPA, and DHA/EPA ratio. The amounts of EPA and DHA have been implicated to be essential for larval growth and development in a number of fish species (Watanabe et al., 1983). They are also related to the food value as well as the price of *Artemia*. Since most *Artemia* strains naturally contain a certain level of EPA (Watanabe et al., 1978), the incorporation of DHA was always accompanied by an increase in EPA level, indicating the metabolic conversion of DHA to EPA by nauplii during the enrichment process (Han et al., 2001). In order to achieve this high DHA/EPA ratio, a special formulation and an *Artemia* species with low DHA catabolising activity were necessary. Besides

DHA, HUFA of the *n*−3 series and arachidonic acid (ARA, 20:4*n*−6) may also be significant. It has been suggested that ARA may improve larval growth and pigmentation in several marine fish species since it provides precursors for eicosanoid production (Castell et al., 1994a,b; Estevez et al., 1997). However, our results showed that enrichment with cod liver oil could not increase the content of ARA of *A. urmiana* decapsulated cysts.

Table 6.1. Type and composition (mg/g) of fatty acids in decapsulated *Artemia urmiana* cysts, and decapsulated-enriched cysts at different periods

Fatty acid type	Fatty acid (mg/g)				
	Decapsulated cysts	Decapsulated-enriched cysts			Fish oil (mg/g)
		1h	3h	24h	
14:0	2.0	1.2	1.8	4.4	0.1
14:1n-5	1.2	-	1.1	2.8	5.2
15:0, 15:1n-5	-	0.7	-	4.4	0.8
16:0	13.6	13.8	16.9	22.7	10.7
16:1n-7	10.3	8.9	11.1	17.4	12.3
17:0	-	1.6	2.8	1.6	1.4
17:1n-7	-	-	5.8	1.0	-
18:0	12.7	1.7	12.8	3.3	0.6
18:1n-9	8.2	8.1	9.8	15.9	31.1
18:2n-6	1.4	6.1	2.3	6.7	10.2
18:3n-3	8.9	10.0	-	3.1	10.9
18:4n-3	3.5	1.8	-	4.0	-
20:0	1.4	-	-	1.3	0.2
20:2n-6	-	-	-	-	0.6
20:4n-6	-	-	-	-	-
20:5n-3	13	14.3	17.3	24.6	5.8
22:0	-	-	-	1.0	-
22:6n-3	0.5	6.9	6.9	10.6	10.1
Other	5.6	14.2	7.6	1.3	1
DHA/EPA	0.04	0.46	0.4	0.43	1.74
Total fatty acids (mg/g)	82	89	96	126	-

(-) non-detectable

4. Conclusion

The method discussed in this paper for the enrichment of fatty acids in *A. urmiana* cysts in order to improve its quality and fish food value by increasing significantly the levels of EPA, DHA, and EPA/DHA ratio using fish oil can be considered as a cheap and easily adoptable method for commercial hatchery operations.

5. Acknowledgment

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CHAPTER 7

Morphometric and preliminary genetic characteristics of *Artemia* populations from Iran

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Submitted

Abstract

Six *Artemia* populations, one bisexual and five parthenogenetic, from different parts of Iran were compared using morphometric and genetic characteristics. The discriminant analysis based on 19 body measurements showed that there are significant differences between the studied populations based on their morphological characteristics, where 85.9 % of original grouped cases were correctly assigned. The bisexual *Artemia urmiana* however exhibited a 100% separation from the parthenogenetic populations. Moreover, a 1500bp mitochondrial rDNA showed similar RFLP patterns for all Iranian populations confirming earlier reports of a close genetic relationship between *A. urmiana* and parthenogenetic *Artemia*.

Introduction

The brine shrimp *Artemia* (Crustacea, Anostraca) is found abundantly in athalassohaline and thalassohaline environments at salinity levels ranging from 10 g/l Agh et al., 2007 to 340 g/l (Post & Youssef, 1977). The genus is a complex of bisexual and parthenogenetic species and probably superspecies defined by the criterion of reproductive isolation (Browne & Bowen, 1991; Pilla & Beardmore, 1994). All bisexual species are diploid while asexual populations may be diploid, polyploid or a mixture of different ploidies (Abatzopoulos et al., 2003). Bisexual species are grouped as (1) the New World species: *A. franciscana* and *A. persimilis* and (2) the Old World species: *A. salina, A. sinica, A. urmiana, A. tibetiana* and *Artemia* sp. Kazakhstan (Pilla & Beardmore, 1994, Abatzopoulos et al., 2002b). The parthenogenetic forms are found only in the Old World and are grouped, rather controversially, under the binomen *A. parthenogenetica* (Abatzopoulos et al., 2002a,b). Populations of *Artemia* are found in more than 600 habitats distributed across the world in salt lakes and natural and man-made salterns (Van Stappen, 2002). Their distribution reflects the flight paths of some migratory birds and deliberate inoculations for commercial purposes by man (Persoone & Sorgeloos, 1980). Agh et al. (2002) and Abatzopoulos et al. (2006) reported *Artemia* populations from 17 biotopes in Iran, all parthenogenetic populations except for the bisexual *Artemia urmiana*. Due to the wide distribution of saline lakes and lagoons in Iran, the presence of *Artemia* in more geographic locations seems likely. Although the presence of *Artemia* in Urmia Lake was reported more than 100 years ago (Günther, 1899), Iranian populations have remained among the least studied populations so far.

It is both due to the economic importance of *Artemia* and its critical role in larviculture of fish and shellfish that, in recent years, there has been a worldwide effort to discover new *Artemia* strains with specific responses to environmental conditions and to characterize them with regard to their potential use in aquaculture. Therefore, the characterization of *Artemia* populations and/or species has been a continuous endeavor since the second half of the previous century (Baxevanis et al., 2004).

Many scientists consider the genus *Artemia* as a complex of sibling species although many studies show that there are morphological differences among the individuals of different species (Amat, 1980; Hontoria & Amat, 1992a, b; Pilla, 1992; Triantaphyllidis et al., 1997b,c; Baxevanis et al., 2005). In a recent experiment using relatively few parthenogenetic *Artemia* and sexual *A. urmiana*, significant differences were demonstrated in morphometry of the same *Artemia* strain when reared at various salinities ranging from 20 to 240 g/l (Agh, unpublished data). Similar differences were reported in morphometry of both sexual and asexual *Artemia* populations from different parts of the world when reared under different environmental conditions (Triantaphyllidis et al., 1995; El-Bermawi et al., 2004). Amat (1980) reported that differences in morphometric characters may unveil a striking heterogeneity between Spanish sexual and asexual *Artemia* strains and, in some cases, within the same strain.

Polyploidy is a common phenomenon among parthenogenetic populations (Barigozzi, 1974; Abatzopoulos et al., 1986; Abreu-Grobois, 1987). Amat (1980) and Hontoria & Amat (1992a, b) studying parthenogenetic populations from the Western Mediterranean basin demonstrated that ploidy level affects the morphology of *Artemia*. Barigozzi et al. (1987) and Badaracco et al. (1987) described various ploidy levels (i.e. di-, tetra- and pentaploid) in parthenogenetic *Artemia* from the Urmia Lake region. Abreu-Grobois & Beardmore (1991) studied allozyme variation encoded by 22 enzyme loci in samples of *A. urmiana* and *A. monica*, compared with *A. franciscana*, *A. persimilis*, *A. salina* and diploid, triploid, tetraploid and pentaploid parthenogenetic forms. They demonstrated a close relationship between *A. urmiana* and asexual populations. Very recent studies of Baxevanis et al. (2006) based on joint analysis of ITS1 sequences and 16S rRNA RFLP markers from global isolates of *Artemia* populations indicate significant interspecific divergence as well as pronounced diversity for parthenogens, matching that of their sexual ancestors. According to the same study, on the basis of nuclear DNA sequences and cytoplasmic markers, a genetically diverse assemblage of

parthenogens is inferred in close affinity with Asian bisexuals (*A. sinica, A. urmiana,* and *A. tibetiana*). The same study demonstrated the presence of a common 16S rRNA haplotype, indicating that a number of clones may have captured mtDNA from *A. urmiana* or *A. tibetiana*. This implies that, for some genetic or ecological reasons, loss of sex is more frequent in the Old World *Artemia* (Baxevanis et al., 2006).

A variety of DNA fingerprinting techniques has been used for describing the diversity within the genus *Artemia*, namely RAPD (Random Amplified Polymorphic DNA) (Badaracco et al., 1995; Abatzopoulos et al., 1998, 2002b; Sun et al., 1999a; Camargo et al., 2002), AFLP (Amplified Fragment Length Polymorphism) (Triantaphyllidis et al., 1997a; Sun et al., 1999b), cytochrome c oxidase subunit I (COI) and cytochrome b gene sequences analysis (Perez et al., 1994), digestion of genomic DNA with *Eco*RI and *Alu*I (Badaracco et al., 1991); analysis of nuclear 5S rRNA (Cruces et al., 1989) and 16S rRNA gene fragments (Thomas, 1995), RFLP (Restriction Fragment Length Polymorphism) (Bossier et al., 2004; Kappas et al., 2004; Baxevanis et al. 2005) and recently sequencing of nDNA regions (Baxevanis et al., 2006; Qiu et al., 2006) .

There is a small number of studies based on different experimental setups and techniques suggesting a close relationship between *A. urmiana* and parthenogenetic populations from different parts of the world (Beardmore and Abreu-Grobois, 1983; Browne et al., 1991; Abatzopoulos et al., 1997; Triantaphyllidis et al., 1997a; Sun et al., 1999b; Bossier et al., 2004; Baxevanis et al., 2006).

In this paper we study morphometric characteristics of bisexual *A. urmiana* and 5 newly-sampled asexual *Artemia* populations living in geographically isolated water bodies from different parts of Iran. A preliminary genetic characterization was also performed based on an RFLP marker. The scope of this study is to present data for further characterization of these *Artemia* populations.

Material and methods

The origin of 1 bisexual and 5 asexual *Artemia* populations and the abbreviations used to present these are shown in Table 7.1 and Figure 7.1.

Table 7.1. List of studied populations and the abbreviations used

Population	Abbreviation	Location
Incheh Lake	INC	Golestan province, north east Iran
QOM salt lake	QOM	QOM province, central Iran
unnamed lagoon	LAG	West Azerbaijan Province, north west Iran
Maharlu Lake	MAH	Fars province, south Iran
Varmal Lake	VAR	Sistan & Baluchestan province, south east Iran
Urmia Lake	URM	West Azerbaijan Province, north west Iran

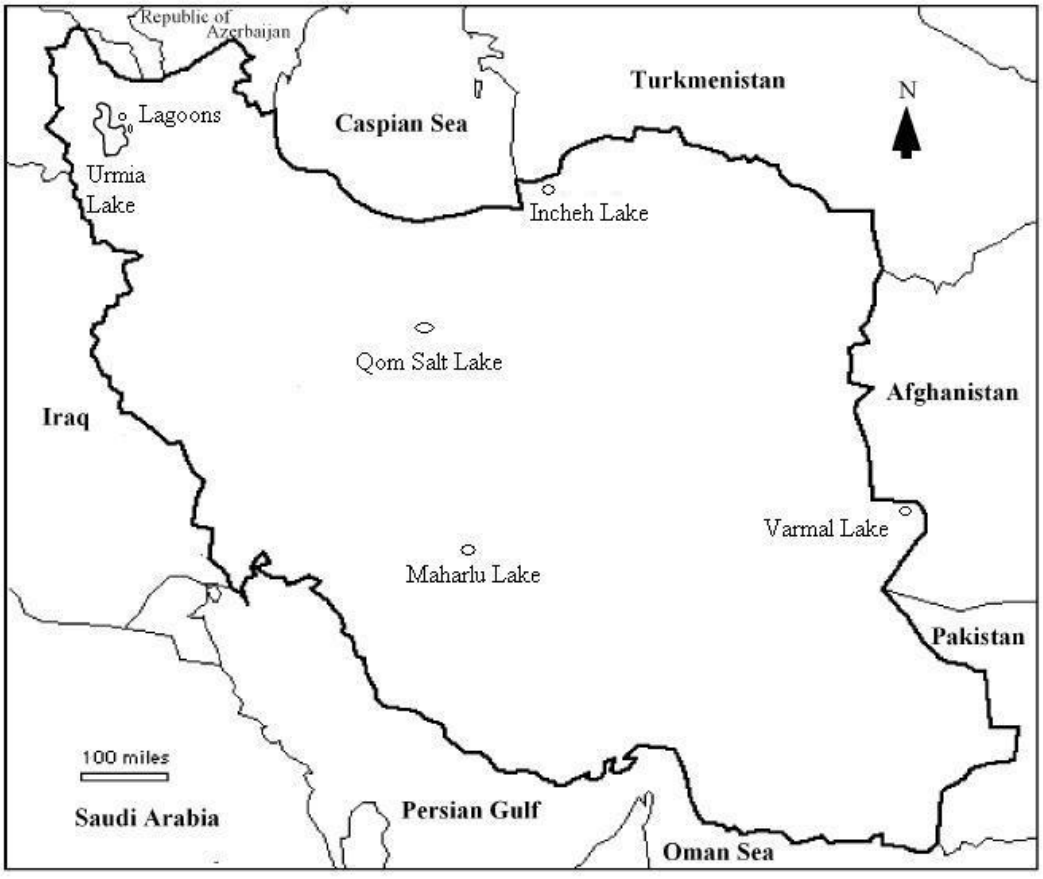

Figure 7.1. Map of Iran showing locations of studied populations

Culture conditions

Cysts from each sample were incubated in freshly prepared 0.45-µm filtered 33 g/l diluted Urmia Lake water. Hatching conditions were according to Sorgeloos et al. (1986). After 24 hours of incubation the newly-hatched nauplii were transferred to 1-liter cylindroconical glass cones containing 0.45-µm filtered 80 g/l saline water. The initial number of nauplii per tube was 400. Initial animal density was 1 nauplius per 2 ml, while from day 8 onwards it was reduced to 1 individual per 4 ml. The animals were kept under mild aeration maintaining about 2 mg/l O_2, at 27±1ºC. Each population had 3 replicates and the animals were fed on the mixed diet of unicellular algae *Dunaliella tertiolecta* and the yeast-based formulated feed LANSY PZ from INVE Aquaculture SA, Belgium, following the feeding schedule of Triantaphyllidis et al. (1995). The feeding schedule was adapted as a function of survival at each water renewal on days 8, 11, 14, 17, 20 and 23.

Biometry

Adult females were examined morphologically when at least 50 % of the animals in each cone reached the adult stage. Females were considered mature when migration of the oocytes into the uterus was observed (Triantaphyllidis et al., 1995). The various populations required different periods for maturation. Thirteen morphometric parameters were scored on 30 individuals from each population (i.e. total length, abdominal length, width of the head, length of the furca, number of setae on each branch of the furca, length of the left and right antenna, distance between the compound eyes, diameter of the left compound eye, diameter of the right compound eye and width of the ovisac. Further data were also obtained by comparing diameter of different body parts with total length. The animals were anaesthetized in chloroform-saturated 33 g/l water (Gilchrist, 1960) and measured using a stereomicroscope equipped with drawing tube, and the data were analyzed with a digitizer.

Preliminary genetic characterization

The RFLP fingerprinting technique, applied to a 1500 bp mitochondrial rDNA fragment, was used to characterize the Iranian *Artemia* populations (Bossier et al., 2004)

DNA extraction

Laboratory-reared adults from each sampling site were transferred into 2-ml stoppered vials containing 100% ethanol and preserved in the freezer until DNA extraction was carried out. DNA was extracted from the whole body using the Wizard® Genomic Purification Kit (Promega™, mouse tail protocol). The samples were washed thoroughly in distilled water, 600µl of EDTA/Nuclei Lysis solution and 17.5µl of 20mg/ml Proteinase K were added to each sample, which were incubated overnight at 55˚C with gentle shaking. Then 3µl of RNAase Soln was added and incubated for 15-30 minutes at 37˚C. The solution was then cooled down to room temperature and 200µl of Protein Precipitation solution was then added and vortexed vigorously at high speed for 20 seconds. This was centrifuged for 4 minutes at 13000-16000; the supernatant containing DNA was transferred to a clean 1.5 ml tube containing 600 ml of room temperature isopropanol, gently mixed and centrifuged for 15 minutes at 11000 rpm. The supernatant was decanted and the DNA was washed with 70% ethanol. It was then again centrifuged for 1 minute at 13000 rpm at room temperature, after which the ethanol was removed and the DNA pellet was air dried. Subsequently the DNA was rehydrated by adding 100µl of DNA Rehydration Solution and incubation at 65˚C for 1 hour. This DNA solution was directly used for PCR.

PCR

PCR was performed in a Hybrid PCR Express (Labsystem™, Belgium). The amplification reactions were carried out in a final volume of 50 µl containing a mixture of 50-100 ng of DNA, Tris-Hcl 5 mM, $MgCl_2$ 2.5 mM, PCR nucleotide Mix 1 µl, DNA polymerase mixture (Expand™ High Fidelity PCR system, Roche™ Molecular Biochemicals), 25 µl of each primer and PCR water. The primer sequences used were 12S-SP (5´-CTAGGATTAGATACCCTA-3´) and 16S-SP (5´-CCGGTCTGAACTCAGATC-3´). These sequences were taken from the published mitochondrial rDNA sequence of *A. franciscana* (Valverde et al., 1994) based on their homology to the universal primers (16Sbr-3´ and 12SA-5´) for mitochondrial rDNA (Palumbi, 1996). The thermal cycler PCR reactions were as follows: 1 cycle of 94˚C for 2 min, 34 cycles of 1 min 15 s at 94˚C, 45 s at 52˚C, 2 min at 72˚C and a final extension cycle of 72˚C for 4 min. These primers amplify a 1500 bp mitochondrial rDNA fragment.

Purification of PCR product and Restriction digestion

The DNA was purified using the same Promega kit before restriction digestion was applied. Fifty µl of PCR reaction was added to a 1.5 ml microtube containing 100µl of Direct Purification Buffer and vortexed briefly to mix; 1 ml of resin was added to the mixture and vortexed briefly for a few times over a one-minute period. The Resin/DNA mixture was transferred into a minicolumn and washed with 80% isopropanol. The minicolumn was then transferred to a 1.5 ml microcentrifuge tube and centrifuged at 10000 rpm for 2 minutes to remove any residual isopropanol. Subsequently the minicolumn was transferred to a new microcentrifuge tube, 50µl of water or TE buffer was added and centrifuged for 20 seconds at 10000 rpm to elute the DNA fragment. The purified DNA is stored in the microcentrifuge tube at 4˚C or -20˚C. In order to display the polymorphism in the mitochondrial rDNA gene, each sample was digested with the restriction enzymes *Hpa*II and *Nde*II (*Roche, Switzerland*). The digested products were separated through an agarose gel (2.5%), stained with ethidium bromide or sybr gold and photographed with a Polaroid film under an Ultra Violet transilluminator.

Statistical analysis:

Results obtained by morphometry were analyzed by SPSS (version 14) using ANOVA and discriminant analysis for each of the nineteen characters listed in Table 7.2. (Sokal & Rohlf, 1981; Games & Howell, 1976; Hontoria & Amat, 1992a,b; Triantaphyllidis et al., 1995).

Results

The mean values of morphometric characters measured on adult females are presented in Table 7.2. The ANOVA analysis demonstrated statistically significant differences for all characters ($P<0.005$). The characters which show the highest interpopulation variability and which, thus most contribute most significantly to the separation of the various populations are length of abdomen, number of setae on furca, total body length and length of furca. These characters are, therefore, of high diagnostic value.

The bisexual strain (URM) had significantly higher or lower values in 9 out of 13 main morphometric characters (12 out of 19 overall parameters) when compared to the parthenogenetic strains (INC, QOM, LAG, MAH, VAR – Table 7.2), i.e., total length, abdominal length, length of

telson, length of furca, number of setae on right furca, length of furca, number of setae on left furca, diameter of right compound eye, distance between compound eyes, abdominal length/total length; length of furca/total length and length of antenna/total length. The variables that presented the larger F ratios were distance between compound eyes (188.78), length of telson (55.04), abdominal length (44.78), number of setae on right furca (42), number of setae on left furca (36.98), total length (31.75) and length of furca (29.03), length of furca/total length (46.83). The VAR population exhibited minimum morphometric values in 10 out of 13 main characters while INC was the closest population to VAR, with no significant differences in 8 major morphometric parameters.

Discriminant analysis based on the origin of each population as a separation criterion resulted in 85.90 % separation of the original groups. The bisexual *A. urmiana* however exhibited a 100 % separation from the parthenogenetic populations (Table 7.3). Figure 7.2 depicts the plot of the discriminant analysis based on the 2 out of 5 roots that were produced. Discriminant analysis could separate the sexual URM strain with 100 % predictability but separation among the parthenogenetic populations is less good. The predictability values for the 5 asexual populations were between 73.3 and 93.3 %. Discriminant analysis resulted in 5 canonical discriminant functions. The first four are statistically highly significant ($P < 0.0001$) obtaining a cumulative separation percentage of 99.1. (Tables 7.3 and 7.4).

RFLP screening revealed no differences between the sexual and asexual populations from Iran. Figure 7.3 shows the *Hpa*II pattern of the 1500 bp fragment. The results with the *Hpa*II enzyme were similar in all samples. Only two *Hpa*II haplotypes were detected (pattern A and B), with haplotype A being predominant. Digestion by *Nde*II resulted in similar patterns (data not shown) for the bisexual and parthenogenetic *Artemia*.

Table 7.2. Mean values (standard deviations in parenthesis) of morphometric characters of different populations studied. "Number of animals analyzed per sample: 30". Abbreviations of populations in Table 7.1. A: total length; B: abdominal length; C: length of telson; D: Width of ovisac; E: length of furca; F: number of setae on right furca; G: number of setae on left furca; H: length of right antenna; I: length of left antenna; J: width of head; K: diameter of right compound eye; L: diameter of left compound eye; M: distance between compound eyes; N: abdominal length/total length; O: width of ovisac/total length; P: length of furca/total length; Q: width of head/total length; R: length of antenna/total length; S: length of telson/total length. Values with the same superscripts in the same row are not significantly different at the 5% level.

	INC	QOM	LAG	MAH	VAR	URM	F ratio	Sig.
A	12.87^{b} (1.60)	12.95^{b} (3.18)	15.24^{c} (1.20)	13.70^{b} (1.56)	11.06^{a} (.065)	16.26^{c} (.075)	31.754	< 0.0005
B	6.99^{b} (10.0)	7.15^{b} (2.18)	8.40^{c} (.094)	7.54^{bc} (.095)	5.65^{a} (.099)	10.14^{d} (.053)	44.783	< 0.0005
C	1.56^{b} (.033)	1.34^{ab} (.035)	1.81^{c} (.030)	1.48^{b} (.014)	1.15^{a} (.015)	2.32^{d} (.028)	55.045	< 0.0005
D	1.90^{ab} (.032)	2.14^{bc} (.060)	2.10^{bc} (.019)	2.40^{c} (.058)	1.67^{a} (.017)	2.17^{bc} (.006)	9.800	< 0.0005
E	$.036^{b}$ (.006)	$.048^{c}$ (.016)	$.029^{b}$ (.010)	$.033^{b}$ (.007)	$.032^{b}$ (.007)	$.018^{a}$ (.002)	29.038	< 0.0005
F	8.83^{c} (2.61)	13.13^{d} (3.64)	6.33^{b} (2.11)	6.48^{b} (3.12)	7.33^{bc} (3.09)	2.26^{a} (.042)	42.007	< 0.0005
G	8.87^{c} (3.23)	12.67^{d} (3.92)	6.03^{b} (2.59)	6.31^{b} (2.39)	7.40^{bc} (2.8)	2.30^{a} (.046)	36.980	< 0.0005
H	1.40^{bc} (.021)	1.32^{abc} (.057)	1.57^{c} (.016)	1.26^{ab} (.023)	1.13^{a} (.011)	1.54^{c} (.042)	7.009	< 0.0005
I	1.38^{bcd} (.022)	1.22^{ab} (.049)	1.59^{d} (.014)	1.26^{ab} (.023)	1.10^{a} (.015)	1.54^{cd} (.041)	9.331	< 0.0005
J	$.095^{a}$ (.009)	$.096^{a}$ (.020)	1.06^{b} (.005)	1.07^{b} (.014)	$.087^{a}$ (.007)	1.05^{b} (.002)	12.454	< 0.0005
K	$.028^{ab}$ (.003)	$.030^{bc}$ (.005)	$.033^{cd}$ (.002)	$.031^{bc}$ (.004)	$.026^{a}$ (.002)	$.034^{d}$ (.002)	17.646	< 0.0005
L	$.028^{a}$ (.003)	$.028^{a}$ (.005)	$.032^{b}$ (.002)	$.031^{b}$ (.003)	$.027^{a}$ (.002)	$.032^{b}$ (.002)	11.729	< 0.0005
M	1.72^{a} (.020)	1.77^{ab} (.040)	2.08^{c} (.010)	1.91^{bc} (.024)	1.63^{a} (.012)	3.15^{d} (.021)	188.785	< 0.0005
N	$.054^{ab}$ (.003)	$.054^{ab}$ (.005)	$.055^{b}$ (.003)	$.055^{b}$ (.004)	$.051^{a}$ (.008)	$.062^{c}$ (.004)	21.017	< 0.0005

O	.015^{ab} (.002)	.017^{cd} (.003)	.014^{ab} (.001)	.017^{d} (.003)	.015^{bc} (.001)	.013^{a} (.001)	19.234	< 0.0005
P	.003^{c} (.001)	.004^{d} (.001)	.002^{b} (.001)	.002^{bc} (.001)	.003^{c} (.001)	.001^{a} (.000)	46.828	< 0.0005
Q	.007^{bc} (.001)	.007^{bc} (.001)	.007^{ab} (.001)	.008^{c} (.000)	.008^{c} (.001)	.006^{a} (.000)	19.120	< 0.0005
R	.011^{c} (.001)	.010^{bc} (.003)	.010^{bc} (.001)	.009^{b} (.001)	.010^{bc} (.001)	.007^{a} (.002)	18.887	< 0.0005
S	.012^{b} (.001)	.010^{a} (.001)	.012^{b} (.002)	.011^{ab} (.001)	.010^{a} (.001)	.014^{c} (.002)	21.589	< 0.0005

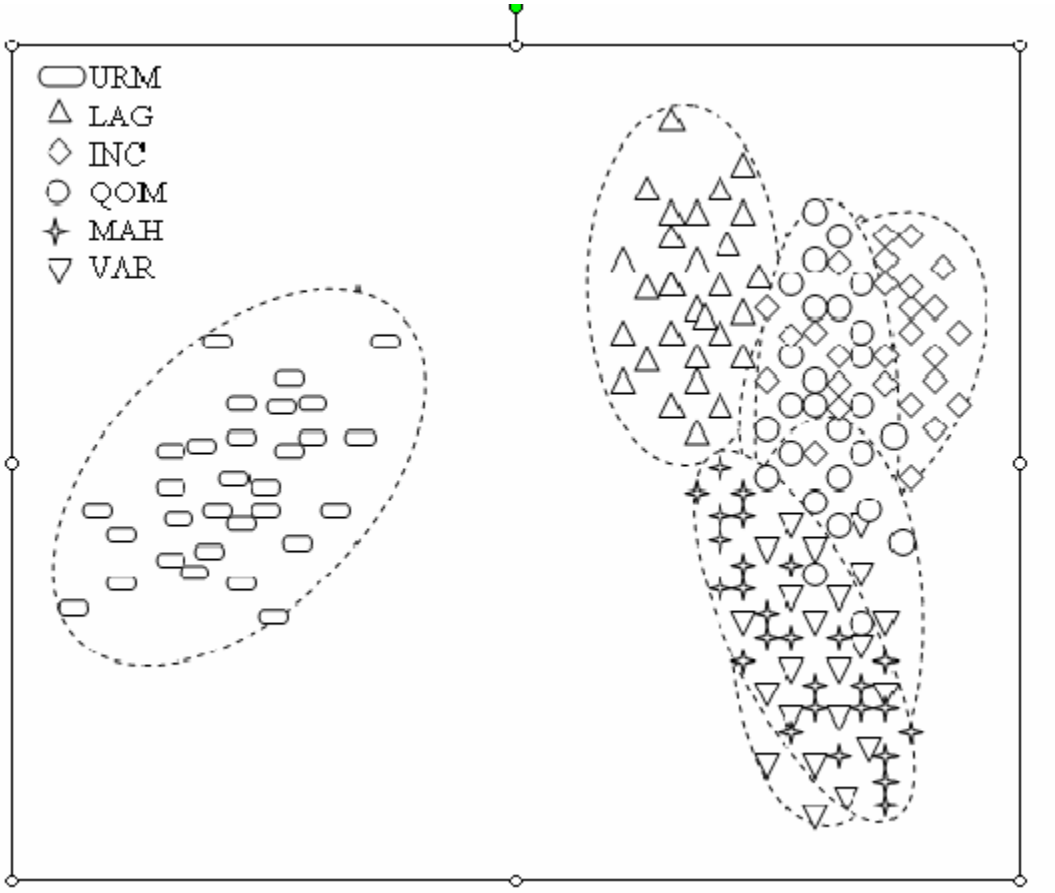

Figure 7.2. Scatterplot resulting from the discriminant analysis (canonical scores) based on morphometrics and using the origin of each population as a separation criterion. Borderlines represent 95% confidence level. Abbreviations of populations in Table 7.1.

Table 7.3. Discriminant analysis of the morphometric parameters of female *Artemia* populations. Classification functions produced by discriminant analysis for each population, Standardized coefficients for canonical variables, Eigenvalues and Cumulative percentages are presented. For the abbreviations of the habitats and variables, see Tables 7.1 and 7.2

	Classification Function Coefficients for each population					
Variables	Habitats					
	INC	QOM	LAG	MAH	VAR	URM
A	1396,955	1388,794	1400,320	1394,356	1387,955	1360,449
B	-1130,984	-1119,593	-1134,341	-1130,153	-1122,411	-1095,528
C	-1771,008	-1762,231	-1772,669	-1780,112	-1742,185	-1727,591
D	-926,433	-964,719	-980,435	-918,958	-916,070	-983,474
E	575,469	573,047	537,386	594,819	662,712	464,198
F	-2,114	-1,299	-1,994	-2,359	-2,388	-2,912
G	-4,334	-4,365	-4,429	-4,927	-4,799	-4,275
H	-238,500	-235,422	-241,246	-237,765	-237,561	-237,969
I	45,944	43,874	48,708	44,080	44,279	49,039
J	-5559,821	-5465,772	-5461,254	-5544,378	-5632,371	-5372,796
K	20,836	83,141	81,428	-4,653	-31,086	76,986
L	-348,104	-392,854	-338,426	-338,161	-310,812	-336,521
M	-8,712	-8,227	3,610	-5,342	-1,265	78,600
N	13889,838	13759,604	13932,100	13903,950	13787,762	13523,982
O	13841,587	14365,842	14456,925	13850,796	13785,327	14385,708
P	-9057,150	-8731,267	-8725,011	-9404,359	-10347,919	-7666,466
Q	75672,386	74371,763	74409,155	75597,334	76548,255	72704,851
R	5523,727	5386,532	5572,453	5443,883	5502,475	5307,118
S	24754,226	24613,881	24742,404	24804,859	24237,786	24159,022
Constant	-9287,647	-9198,832	-9361,462	-9269,331	-9164,904	-9005,364
Standardized coefficients for canonical variables						
	Root 1	Root 2	Root 3	Root 4	Root 5	
A	4,630	4,709	4,596	1,016	,728	
B	-3,171	-2,860	-4,307	-,845	-1,136	
C	-,981	-,850	-1,226	-2,586	-2,157	
D	1,556	-5,184	1,305	-1,681	6,779	
E	,910	-1,538	,111	-1,312	-1,852	
F	,188	,346	-,469	,210	-,399	
G	-,069	,314	-,246	-,221	,449	
H	-,001	-,247	-,310	,100	,067	
I	-,099	,291	,155	-,123	,014	
J	-1,502	2,594	-1,099	3,470	-1,297	
K	-,148	,745	-,344	,373	-,378	
L	-,026	-,138	,391	-,330	-,212	
M	-1,588	-,187	-,036	-,198	-,130	
N	1,254	1,097	1,984	,733	,658	
O	-,643	2,663	-,820	1,439	-4,591	
P	-,872	1,519	-,769	1,536	2,148	
Q	1,499	-2,033	1,201	-2,436	1,015	
R	,235	,445	,508	-,534	-,098	
S	,728	,945	,801	2,135	2,580	
Eigenvalues	21,076	1,952	1,402	,690	,217	
Cumulative%	83,2	90,9	96,4	99,1	100,0	

Table 7.4. Classification results of discriminant analysis showing the percentages of populations classified in each group. The percent of "grouped" cases correctly classified is 85.9 %. The abbreviations of populations can be found in Table 7.1

	Predicted Group Membership					
habitat	INC	QOM	LAG	MAH	VAR	URM
INC	73.3	3.3	3.3	6.7	13.3	0
QOM	0	80.0	6.7	6.7	6.7	0
LAG	0	6.7	93.3	0	0	0
MAH	3.4	0	0	79.3	17.2	0
VAR	13.3	0	0	0	86.7	0
URM	0	0	0	0	0	100.0

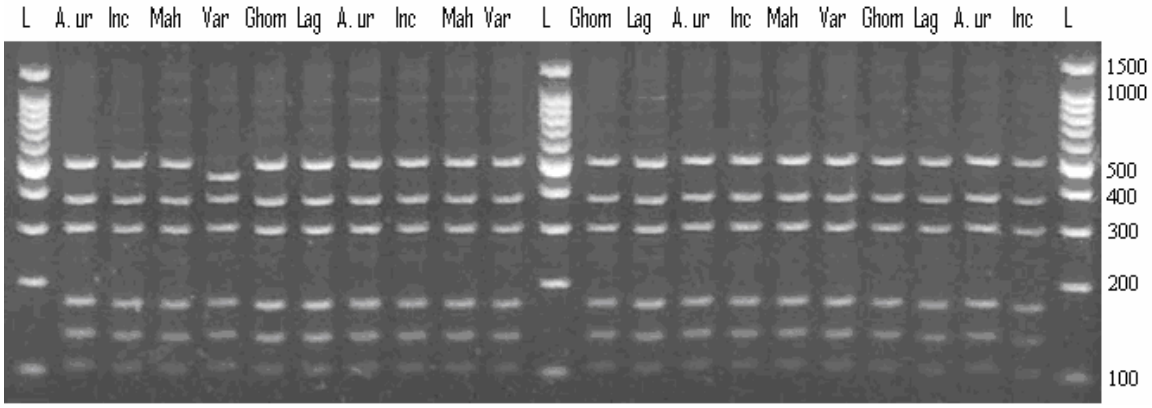

Figure 7.3. Restriction digest pattern of 1500 bp mt rDNA with enzyme *Hpa*II. L lanes are the reference ladder.

Discussion

Many evolutionary studies have used morphological characters for studying differentiation. In *Artemia*, morphological traits have been used as a basis to describe populations and species, though controversy exists on ways of selecting and using the best traits, as well as on their degree of genetic and environmental determination (Gajardo et al., 1998; Baxevanis et al. 2005). Since morphological traits can change depending on environmental variations, particularly salinity and/or other culture conditions, the simplest and perhaps most effective procedure to study the

relationships between species is to gather qualitative and quantitative data for different populations under carefully standardized laboratory conditions (Abreu-Grobois, 1987).

Discriminant analysis, proposed by Hontoria and Amat (1992a, b) and used by many investigators (Amat et al., 1995; Triantaphyllidis et al., 1995, 1997b,c; El-Bermawi et al., 2004; Mura et al. 2006), could provide significant information for the delineation of *Artemia* species or populations. Furthermore, discriminant analysis can be a useful tool for assigning an unknown individual to one of the already examined groups. This could be very essential for management or biodiversity purposes, since it is a rapid and quite accurate method for characterizing *Artemia* strains. Triantaphyllidis et al. (1995) examined a parthenogenetic strain from Tanggu (China) and *A. franciscana* and concluded that a discriminant function could predict population assignment with accuracy of almost 100%. Furthermore, Triantaphyllidis et al. (1997b) examined 15 parthenogenetic *Artemia* populations, and they produced a discriminant analysis, which had an overall prediction accuracy of 93.2%. Amat et al. (1995) used discriminant analysis that was based on morphometric variables to assign a population from Veldrif (South Africa) to Mediterranean sexual populations rather than to other known bisexual *Artemia* species. El-Bermawi et al. (2004) demonstrated the role of discriminant analysis on the correct (100% accuracy) classification of sexual and asexual *Artemia* populations from Egypt cultured at different salinities. According to the same study, discriminant analysis also proved to be a very efficient tool to classify the parthenogenetic populations from Egypt living in different environmental conditions.

Triantaphyllidis et al. (1997c) compared *A. urmiana* with four sexual populations of *Artemia* (*A. franciscana, A. salina, A. sinica* and *Artemia* sp. Kazakhstan) from 10 different geographic locations on the basis of biometrics and observed that *A. urmiana* is a more differentiated species forming a different group. Therefore it appears that *A. urmiana* has very distinct, species-specific, morphological characteristics rendering it easily distinguishable from other sexual and asexual populations (see also Baxevanis et al., 2005). Amat et al. (1995) reported that the size of the furca and the number of setae varied considerably in wild populations, due to environmental conditions. Nevertheless, the same researchers acknowledged that these morphological variables could be trustworthy as a systematic tool, provided that well-defined culture conditions are being used. They found that furcal characters were the major factors for discriminating a group of southern Spanish parthenogenetic populations from the rest of the Spanish bisexual *Artemia*. According to El-Bermawi et al. (2004) salinity affects the length and number of the furcal setae

in a relatively similar pattern in all populations, and therefore this factor could be applied as a reliable discriminating character.

In this study, we tried to define possible groupings of six Iranian *Artemia* populations by using different statistical methods based on morphometric characteristics. Based on the morphometric parameters studied, discrimination models resulted in total predictability of 85.90 %. The variables that contributed most to the discrimination were significantly different in the sexual URM compared with the asexual populations. El-Bermawi et al. (2004), found that an Egyptian sexual strain (belonging to *A. salina*) was discriminated from asexual strains of Egypt on the basis of 3 morphological characters namely, total length, abdominal length and number of setae on the furca. The bisexual URM is discriminated from asexual populations of Iran on the basis of 9 morphological parameters. This indicates that, morphologically, *A. urmiana* can be distinguished from the Iranian parthenogenetic populations. These observations provide useful evidence for species-specific morphological characteristics of *A. urmiana*, supporting the findings of Triantaphyllidis et al. (1997c).

The results of this study are also in agreement with findings of Amat et al. (1995) and El-Bermawi et al. (2004) and confirm the importance of furcal characters as important morphometric factors for discriminating sexual and parthenogenetic populations (see also Baxevanis et al., 2005). However, we also emphasize that some other morphometric characteristics, in particular the distance between the compound eyes, the abdominal length and the length of the telson, are important for the discrimination of sexual URM from asexual *Artemia* populations from Iran.

Within the asexual populations, the 2 different groups, LAG and MAH, showed no significant differences in 13 out of 19 morphometric variables. Predictability values for these two populations were 93.30 and 79.30 %, respectively. In contrast, the INC, QOM and VAR strains, that were grouped separately, showed fewer similarities. The predictability values for the last 3 strains were 73.30, 80.00 and 86.30 %, respectively. These finding once again verify the role of discriminant analysis on the characterization of strains.

Morphological data can be substantially reinforced by genetic evidence (Abreu-Grobois & Beardmore, 1991; Triantaphyllidis et al., 1997a; Sun et al., 1999a,b; Abatzopoulos et al., 2002a,b; Bossier et al., 2004; Baxevanis et al., 2005; Qiu et al., 2006). Bossier et al., (2004) working with cyst samples, used seven restriction enzymes on the same 1500 bp mitochondrial rDNA fragment and demonstrated that most bisexual populations display species-specific

patterns. It was found that the *Hpa*II digest was particularly useful and sufficient to differentiate between bisexuals and correctly allocate a cyst sample to its belonging species. However, the same study also revealed that on the basis of these seven restriction enzymes, mitochondrial rDNA of parthenogenetic *Artemia* and *A. urmiana* were hardly differentiated. Especially the *Hpa*II digest displayed an identical electrophoretic pattern, identical to the pattern shown in Figure 7.3 (haplotype A).

In the present study, a preliminary genetic screening using a RFLP marker was applied for a further characterization of the studied populations. According to the results obtained, the PCR products after digestion with the enzymes *Hpa*II and *Nde*II exhibited similar patterns for the bisexual and parthenogenetic *Artemia* from Iran. Especially with *Hpa*II the result was uniformly similar in all samples (Figure 7.3). The results of the present study are in agreement with the findings of Bossier et al., (2004), although we have used adult *Artemia* as experimental material, suggesting that these *Artemia* populations seem to be slightly differentiated at the mitochondrial DNA. In support of the mitochondrial DNA data, recent studies on the DNA sequence of a nuclear marker, namely HSP26 cDNA, revealed that parthenogenetic *Artemia* and *A. urmiana* are closely related and distinct from other species (Qiu et al., 2006). Moreover Baxevanis et al. (2006) in their studies based on ITS1 sequences and 16S RFLP markers reported a close affinity between parthenogens and Asian bisexuals (*A. sinica*, *A. urmiana*, and *A. tibetiana*) and suggested that loss of sex in these lineages leading to the emergence of parthenogens could have happened due to some genetic or ecological reasons. Hence, *A. urmiana* can be considered as a potential candidate among the sexual species that could have given rise to parthenogenetic populations.

Conclusion

According to our findings considerable morphometric differences are observed between bisexual *A. urmiana* and parthenogenetic populations and also among the asexual Iranian populations. On the contrary, RFLP results indicate a close relationship between *A. urmiana* and parthenogenetic *Artemia* which has been confirmed by many other studies using different approaches (Beardmore and Abreu-Grobois, 1983; Abreu-Grobois & Beardmore 1991; Browne et al. 1991; Abatzopoulos et al., 1997; Triantaphyllidis et al. 1997a; Sun et al. 1999b; Bossier et al., 2004; Baxevanis et al. 2006; Qiu et al. 2006).

Therefore, in view of this close genetic relationship between sexual *A. urmiana* and the Iranian parthenogens and taking into account that *Artemia* morphometrics are influenced by the environmental conditions, we suggest that within a genus even genetically similar populations may exhibit striking differences in their morphological features. On the other hand, we propose that despite these small genetic differences between these populations, detected with the DNA markers, sufficient differentiation may have happened in other genes allowing the establishment of morphological differences. Therefore, it would be interesting to carry out more specialized genetic analyses in order to reveal the basis for the morphological differentiation among sexual and asexual *Artemia* populations.

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CHAPTER 8

General Discussion and Conclusions

General Discussion and Conclusions

Iran is a large country with immense potential in aquaculture. Presently, only 1% of the water resources in Iran are used for aquacultural activities. More than 1500 km of coastline along the Persian Gulf and Oman Sea in South Iran provides excellent opportunities for aquaculture of shrimp and fish. Thousands of hectares of land at the shores of the Caspian Sea in North Iran are suitable for the culturing of sturgeon. Moreover, there are numerous rivers, lakes and springs (with fresh, brackish or saline water) which can be used for aquaculture of different fish species. Development and success in these activities is highly dependent on the availability of live food, especially of *Artemia* as starter feed.

For many years, Iran has been listed among the countries with huge natural biotope of *Artemia*, namely Urmia Lake. Many times, the total production of *Artemia* was very high in this lake and could not only fulfil the need of the local market for *Artemia* cysts and biomass, but could also contribute considerably to the world demands. Since no commercial harvest was planned, due to the lack of scientific back up, Iran has covered its requirements for *Artemia* cysts by imports from the world market. The growing aquaculture activities in Iran and the significant increase in demand for *Artemia* cysts on one hand, and the tragic decline in *Artemia* production in Lake Urmia due to the drought since the late nineties on the other hand, urged the Iranian government and the Fisheries Organization to explore, identify and characterize new resources of local *Artemia*.

The discovery of new populations can be of great importance not only for their economic value, but also for fundamental and applied research. Therefore, this study aimed to identify new resources of *Artemia* in Iran, and to characterize them on the basis of their ecological and biological features. In order to properly characterize these resources, a number of experiments had to be designed and many questions related to their biotopes, reproductive, morphological, biometric and genetic characteristics had to be answered.

This study provides insights into the:

- Biogeography of *Artemia* populations in Iran
- Reproductive status and biometrics

- Coexistence of bisexual *Artemia urmiana* and parthenogenetic populations in Lake Urmia and in the lagoons in the vicinity of the lake
- Reproductive, morphometric and genetic characterization of some major sexual and asexual *Artemia* populations
- Possible relationship between sexual and asexual strains
- Potential responses of these *Artemia* populations subjected to different salinities with regard to their growth, survival, reproductive and life span characteristics
- Possibilities for enrichment of decapsulated cysts of *Artemia*

***Artemia* sites in Iran**

Since the first report on the natural existence of *Artemia* from Lake Urmia (Günther, 1899) only few strains have been added to the list of *Artemia* populations in Iran. Through our investigation 17 *Artemia* sites were identified in Iran. Thriving *Artemia* populations were found in large and medium size saline and hypersaline lakes, small seasonal water catchments and saline rivers in different geographic regions. It is likely that further research can lead to the identification of more *Artemia* biotopes in Iran. All these populations are parthenogenetic, except for *A. urmiana* in Lake Urmia. An *Artemia* population previously reported in Shurabil Lake (Ahmadi, 1987) has become extinct now, and the local parthenogenetic population at Nogh catchment has been totally replaced by *A. franciscana*. *Artemia* production in most biotopes has suffered severely due to the prolonged drought throughout the country since 1998. This phenomenon supports the general belief that *Artemia* populations (both sexual and asexual) are greatly limited by high salinity.

Coexistence of sexual and asexual populations

The sexual status of *Artemia* in Lake Urmia has been a controversial issue since the 1980's. This study provides evidence to settle this long-standing controversy. The experiments that were carried out in the laboratory and in the field revealed that both sexual and asexual *Artemia* exist in Lake Urmia. But the lagoons in the vicinity of the Lake harbor only the parthenogenetic strain. It was found that salinity is the partitioning factor both in the lake and in the lagoons. The asexual strain is confined to specific regions of the lake with lower salinity, mostly in the vicinity of river mouths. This observation is supported by the natural occurrence of the asexual strain in lagoons in the surrounding area of the lake, where water salinity may drop to as low as 10 ppt initially when they are filled with water in early spring.

In contrary to the sexual *A. urmiana*, the parthenogenetic population can adapt rapidly to very low salinities and dominates the sexual *A. urmiana* in these conditions. This was further confirmed by the laboratory and pond culture experiments, where the asexual strain out competed the sexual population at very low salinities. Our experiments and observations by local consumers suggest that both *Artemia* populations seem to be coexisting in the lake since a very long time, one dominating the other periodically or seasonally due to salinity variations, proving the crucial role of salinity as the main partitioning factor separating sexual and asexual *Artemia* in this context.

Salinity effects on survival, growth, reproductive and life span characteristics

Investigations on the historical background of the Lake Urmia revealed the presence of cyclic changes in salinity level every 10-15 years. Based on this historical fact, culture experiments were set up at different salinities in order to evaluate the potential response of the selected *Artemia* populations from Urmia Lake and neighbouring lagoons. The results indicated that only the parthenogenetic population survives and attains adulthood at salinities below 33 g/l. This criterion thus seems to be an easy method to separate the sexual *A. urmiana* from the asexual population living in Lake Urmia and neighbouring lagoons. Although *A. urmiana* showed to be more resistant to a salinity increase, growth, survival, reproductive and life span characteristics in both sexes are negatively affected by higher salinities.

Laboratory and pond culture of *Artemia* in different salinities and regular observations of the population density of *Artemia* in Lake Urmia led us to understand why many scientists found it difficult to culture and maintain *Artemia* at high salinities in the laboratory. We found that high salinity is a limiting factor not only in the laboratory but in the natural habitats too. *Artemia* density in Lake Urmia as a natural model proved that survival is inversely proportional to salinity.

Optimization of laboratory conditions for *Artemia* culture

Artemia populations from different sources are reared under specific laboratory conditions known as “standard conditions”. But many studies have confirmed that most *Artemia* populations demonstrate different responses with regard to survival, growth and reproduction when cultured under similar conditions. The “standard conditions” may be optimum or close to optimum for some but not for all *Artemia* populations. Therefore different results with

different strains of *Artemia* originating from various sites may be expected at diverse environmental conditions. Identification of the optimal abiotic conditions, such as salinity, allows studying the exact response of the organism when these conditions are changed.

On the other hand our results, and findings by other researchers, demonstrate that the difference in survival and growth rate of *Artemia* cannot be ascribed to be related to sexual or asexual reproduction. Our study provides further evidence that growth and survival rates of *Artemia* are strain-dependent and bisexuality is not always a determining factor for better reproductive and life span characteristics.

Cyst and nauplii biometrics

It is generally accepted that the biometrical characteristics of cysts and nauplii are mainly strain-specific; however considerable intra-strain variations have been reported in literature and have been interpreted as being influenced by environmental conditions. Cyst and naupliar biometrics also have their implications on the application in aquaculture. Iranian *Artemia* populations show high variability in their cyst and nauplii biometrics. According to the results obtained in this investigation, the cyst size of the Iranian *Artemia* populations ranges from 243.2 to 285.4 µm. The naupliar size also varies from 455.5 (asexual strain) to 529.8 µm (sexual strain). However, significant differences were observed in sizes of cyst samples collected from different harvesting sites in Urmia Lake.

Iranian parthenogenetic cysts are smaller compared with many other parthenogenetic populations The cyst size of parthenogenetic *Artemia* from lagoons at the periphery of Urmia Lake (243.1 µm) and from the coastal areas of the Lake (248.5 µm) are among the smallest parthenogenetic *Artemia* cysts reported so far.

Morphometric analysis

Many evolutionary studies have used morphometric characteristics for establishing differentiation between related species. In *Artemia,* morphological traits have been used as a basis to describe the populations and species. We compared 6 *Artemia* populations from Iran based on 19 morphometric parameters. The sexual *A. urmiana* was discriminated from asexual populations from Iran on the basis of 13 morphological parameters. The results indicate that, morphologically, *A. urmiana* is very different from the Iranian parthenogenetic *Artemia* populations. These observations and earlier findings by other researchers provide firm evidence for species-specific morphological characteristics of *A. urmiana*. However, discrimination

models resulted in total predictability of 85.90 %, indicating the reliability of discriminant analysis for strain characterization. Furcal biometrics, distance between compound eyes, abdominal length and length of telson are highly significant for discrimination of sexual *A. urmiana* from asexual *Artemia* populations from Iran.

Genetic characteristics

The genetic similarity of *A. urmiana* with parthenogenetic populations and the possible descent of the asexual strains from *A. urmiana* were reported by a number of researchers. In order to verify this, a preliminary genetic screening using a RFLP marker was applied to help further characterization of the studied populations. The PCR products after *Hpa*II and *Nde*II restriction enzyme digestion exhibited similar patterns for the bisexual and parthenogenetic *Artemia* from Iran. Our findings reveal the close relationship between *A. urmiana* and the parthenogenetic populations from Iran. 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 have come to the same conclusion. Our findings once again support the idea that *A. urmiana* may be considered as a potential candidate among the sexual species that have given rise to parthenogenetic populations.

Enrichment of *A. urmiana* cysts

A. urmiana cysts and nauplii are known to be very poor with regard to their EPA and DHA content. Enrichment of newly hatched nauplii with fatty acid emulsions is routinely practiced to improve their HUFA content. But enrichment of nauplii for 24 hours results in enriched instar II metanauplii with larger size. Therefore we tried to enrich the decapsulated cysts of *A. urmiana* with EPA and DHA levels. Enrichment of decapsulated cysts during 24 h resulted in a significant increase in total fatty acid content from 82 to 126 mg/g body weight. EPA and DHA levels also increased significantly from 159 to 195 mg/g body weight and from 6 to 84 mg/g body weight respectively. The method presented for the enrichment of fatty acids in *A. urmiana* cysts in order to improve their quality as fish food by significantly increasing the levels of EPA, DHA, and DHA/EPA ratio using fish oil, can be considered as a cheap and easily adoptable method for commercial hatchery operations. This method can help us to convert any low value *Artemia* cyst into a highly nutritional and valuable product. Moreover, the hatching of these enriched cysts results in small-sized nauplii, which are the most valuable live food for marine fish and shellfish.

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SAMMARY

SAMENVATTING

SUMMARY

In this PhD study, we investigated the saline water resources in Iran for natural occurrence of *Artemia* and used standard methods to characterize the identified *Artemia* populations.

In chapter 2, we introduced the occurrence of wild *Artemia* populations from 17 hypersaline environments such as salt lakes, lagoons and salty rivers in Iran. The reproductive behaviour of the existing *Artemia* was studied to distinguish bisexual from parthenogenetic populations. Cysts of *Artemia* were characterized on the basis of their diameter and chorion thickness, while nauplii (instar-I) were characterized on the basis of their total length. We found that all Iranian *Artemia* populations are parthenogenetic with the exception of *Artemia urmiana* from Urmia Lake. It was revealed that for some Iranian parthenogens, cyst diameters were among the smallest recorded so far for parthenogenetic *Artemia*.

In chapter 3, we studied the *Artemia* populations existing in Lake Urmia in order to settle the long-standing controversy over the sexual status of the endemic *Artemia* populations. Both laboratory and field experiments were carried out for a period of two years. We could confirm 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. Effect of salinity was studied as a major abiotic factor determining the distribution of these sexual and asexual populations within and outside the lake. It was found that Iranian asexual population from Urmia Lake could grow, mature and reproduce at salinities much lower than reported values (15–33 g/l), whereas sexual *A. urmiana* could attain sexual maturity only at salinities above 50 g/l.

In chapter 4, we studied the effects of different salinities on the survival, growth, reproductive and lifespan characteristics of three *Artemia* populations from Urmia Lake and small lagoons in the vicinity of the lake under laboratory conditions. Experimental salinities ranged from 75 to 175 g/l. Salinity was proved to have significant impact on the majority of the characters studied. Higher salinities proved to have retarding effect on growth and survival of both bisexual *A.urmiana* and parthenogenetic strains. Most of the reproductive characteristics were also negatively affected by salinity.

In chapter 5, we present the life cycle characteristics of six *Artemia* populations, one bisexual and five parthenogenetic, from Iran. The cysts of asexual strains and the bisexual *Artemia urmiana* were hatched according to standard procedures and the nauplii from all populations were reared at 80 g/l under laboratory conditions. Survival and total length of the *Artemia* were measured on days 8, 11, 14, 17, 20 and 23 of culture. Adult animals were studied for their reproductive and life span characteristics. We found significant differences in survival when different *Artemia* strains are reared under similar conditions, but no effect on growth was observed. The parthenogenetic *Artemia* populations from Maharlu and Qom lakes and also from Lagoons near Lake Urmia had significantly higher reproductive values in comparison to the other three populations including the bisexual *A. urmiana*.

In chapter 6, we developed a technique to enrich the decapsulated cyst of *Artemia* aiming at improving its nutritional quality. The nutritional quality of *Artemia urmiana* is relatively poor in eicosapentaenoic acid (EPA, 20:5*n*−3) and docosahexaenoic acid (DHA, 22:6*n*−3), two major determinants of fish food value and also of the price of *Artemia* cysts. In this study *Artemia urmiana* cysts were enriched with fish oil dissolved in *n*-Heptane. The result demonstrated that this enrichment method brought about an increase in the levels of EPA from 13 to 24.6 mg/g and DHA from 0.5 mg/g to 10.6 mg/g. The method discussed in this paper for the enrichment of fatty acids in *A. urmiana* cysts in order to improve its quality by increasing significantly the levels of EPA, DHA, and EPA/DHA ratio using fish oil can be considered as a cheap and easily adoptable method for commercial hatchery operations.

In chapter 7, we studied morphometric and genetic techniques to characterize six *Artemia* populations, one bisexual and five parthenogenetic, from different parts of Iran. The discriminant analysis was applied to characterize and separate the populations using morphometric findings based on 19 body measurements. The results showed that there are significant differences between the studied populations based on their morphological characteristics, where 85.9 % of original grouped cases were correctly assigned. The bisexual *Artemia urmiana* however exhibited a 100% separation from the parthenogenetic populations. These finding once again verify the role of discriminant analysis on the characterization of strains. The RFLP fingerprinting technique, applied to a 1500 bp mitochondrial rDNA fragment, was used to characterize the Iranian *Artemia* populations.

This resulted in similar RFLP patterns for all Iranian populations confirming earlier reports of a close genetic relationship between *A. urmiana* and parthenogenetic *Artemia*.

SAMENVATTING

In deze doctoraatsstudie onderzochten we het natuurlijk voorkomen van *Artemia* in de zoutwaterbiotopen van Iran; we gebruikten standaardmethodes om de geïdentificeerde *Artemia*-populaties te beschrijven.

In hoofdstuk 2 gaven we een inleidende beschrijving van het voorkomen van wilde *Artemia*-populaties uit 17 hypersaliene biotopen zoals zoutmeren, lagunes en zoute rivieren in Iran. Om een onderscheid te maken tussen bisexuele en parthenogenetische populaties werd het voortplantingsgedrag van de bestaande *Artemia* bestudeerd. Cysten van *Artemia* werden gekarakteriseerd op grond van hun diameter en choriondikte, terwijl de nauplii (instar I) werden gekarakteriseerd op grond van hun totale lengte. We vonden dat alle Iraanse *Artemia*-populaties parthenogenetisch zijn, uitgezonderd *Artemia urmiana* uit het Urmia-meer. We brachten aan het licht dat de diameters van sommige Iraanse parthenogenetische rassen tot de kleinste waarden behoorden ooit opgetekend voor parthenogenetische *Artemia*.

In hoofstuk 3 bestudeerden we de *Artemia*-populaties uit het Urmia-meer, met de bedoeling de oude controverse over de sexuele status van de endemische *Artemia*-populaties te beslechten. Gedurende twee jaar werden er zowel laboratorium- als veldexperimenten uitgevoerd. We konden bevestigen dat in het Urmia-meer zowel sexuele als parthenogenetische *Artemia* leeft. Terwijl het meer zelf gedomineerd wordt door sexuele *Artemia*, werden de asexuele populaties uitsluitend gevonden in bepaalde zones in of nabij het meer. *Artemia* die seizoensgebonden gevonden werd in de lagunes naast het meer was uitsluitend parthenogenetisch. Het effect van de saliniteit werd bestudeerd als zijnde een belangrijke abiotische factor die de verspreiding van deze sexuele en asexuele populaties binnen en buiten het meer bepaalt. We vonden dat de Iraanse asexuele populatie van het Urmia-meer kon groeien, geslachtsrijp worden en zich voortplanten bij veel lagere zoutgehaltes dan de gerapporteerde waarden (15-33 g/l), terwijl de sexuele *A. urmiana* alleen bij zoutgehaltes boven 50 g/l tot geslachtsrijpheid kwam.

In hoofdstuk 4 bestudeerden we de effecten in laboratoriumcondities van verschillende zoutgehaltes op de overleving, groei en kenmerken van voortplanting en levensloop van drie *Artemia*-populaties van het Urmia-meer en kleine lagunes nabij het meer. De experimentele zoutgehaltes varieerden tussen 75 en 175 g/l. We vonden dat het zoutgehalte een significante

invloed had op de meeste kenmerken die bestudeerd werden. We bewezen dat hogere zoutgehaltes een vertragend effect hadden op groei en overleving van zowel *A. urmiana* als de parthenogenetische rassen. De meeste reproductieve kenmerken werden ook negatief beïnvloed door het zoutgehalte.

In hoofdstuk 5 rapporteren we over de kenmerken van de levenscyclus van zes *Artemia*-populaties uit Iran, één bisexuele en vijf parthenogenetische. De cysten van de asexuele rassen en de bisexuele *Artemia urmiana* werden tot ontluiking gebracht volgens standaardprocedures, en de nauplii van al deze populaties werden gekweekt bij 80 g/l in laboratoriumcondities. De overleving en de totale lengte van de *Artemia* werden gemeten na 8, 11, 14, 17, 20 en 23 dagen kweek. De kenmerken van voortplanting en levensloop van de volwassen dieren werden bestudeerd. We vonden significante verschillen in overleving wanneer verschillende *Artemia*-rassen gekweekt werden in gelijkaardige omstandigheden, maar we observeerden geen effect op de groei. De parthenogenetische *Artemia*-populaties van de meren Maharlu en Qom en ook van de lagunes bij het Urmia-meer hadden significant hogere voortplantingswaarden vergeleken met de drie andere populaties, met inbegrip van de bisexuele *A. urmiana*.

In hoofdstuk 6 ontwikkelden we een een techniek om de gedecapsuleerde cysten van *Artemia* aan te rijken, met het oog op een verbetering van zijn nutritionele kwaliteit. De nutritionele kwaliteit van *Artemia urmiana* is betrekkelijk laag voor wat betreft eicosapentaeenzuur (EPA, 20:5*n*-3) en docosahexaeenzuur (DHA, 22:6*n*-3), twee belangrijke factoren die de waarde van visvoedsel, en ook de prijs van *Artemia*-cysten bepalen. In deze studie werden *Artemia urmiana*-cysten aangerijkt met visolie, opgelost in n-heptaan. De resultaten toonden aan dat deze aanrijkingsmethode leidde tot een toename in het gehalte aan EPA van 13 tot 24.6 mg/g, en aan DHA van 0.5 mg/g tot 10.6 mg/g. De methode die behandeld wordt in deze publicatie voor de aanrijking van vetzuren in *A. urmiana*-cysten voor de verbetering van hun kwaliteit door een significante verhoging van de gehaltes aan EPA, DHA en de EPA/DHA ratio middels het gebruik van visolie, kan beschouwd worden als een goedkope en gemakkelijk toepasbare methode voor commerciële broedhuizen.

In hoofdstuk 7 bestudeerden we morfometrische en genetische technieken om zes *Artemia*-populaties te karakteriseren, één bisexuele en vijf parthenogenetische, uit verschillende delen van Iran. Discriminantie-analyse werd gebruikt om de populaties te karakteriseren en te

onderscheiden met behulp van morfometrische bevindingen, gebaseerd op 19 lichaamsmetingen. De resultaten toonden aan dat er significante verschillen bestaan tussen de bestudeerde populaties op grond van hun morfologische kenmerken, waar 85,9% van de oorspronkelijke gegroepeerde gevallen correct toegekend werden. De bisexuele *Artemia urmiana* echter vertoonde 100% scheiding van de de parthenogenetische populaties. Deze bevinding toont opnieuw de rol aan van discriminantie-analyse bij de beschrijving van rassen. De RFLP fingerprinting-techniek, toegepast op een 1500 bp mitochondriaal rDNA-fragment, werd gebruikt om de Iraanse *Artemia*-populaties te karakteriseren. Dit leidde tot gelijkaardige RFLP-patronen voor alle Iraanse populaties; dit bevestigt eerdere verslagen van een nauwe genetische verwantschap tussen *A. urmiana* en parthenogenetische *Artemia*.

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STUDIES

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- Biology course at the B.Sc level: Dept. of Biology, Urmia University, Iran (Since 1991)
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- Establishing *Artemia* & Aquatic Animals Center in 1997, which later grew to Research Institute in 2005.
- Coordination of the research activities of the *Artemia* & Aquatic Animals Research Institute at the Urmia University (scientific, technical & administrative staff of 22 persons)
- Responsible scientist in INCO Project on *Artemia* Biodiversity funded by European Commission
- Coordinated/directed more than 20 National/Regional projects on *Artemia* and fish larviculture and aquaculture

PUBLICATIONS/SYMPOSIA/CONFERENCES

A. Papers published in peer-reviewed international journals:

1. Hanaee, J.; **Agh, N.**; Hanaee, M,; Delazar, A. and Sarker S.D. (2005). Studies on the enrichment of *Artemia Urmiana* cysts for improving fish food value. **Animal Feed Science and Technology**. 120, pp. 107-112

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4. Asem, A., Rastegar, N., **Agh, N.** (Accepted for publication in 2007). Biometrical Variation Of *Artemia* Urmiana (Anostraca: Artemiidae) Cysts Harvested from the Urmia Lake (West Azerbaijan, Iran). **Turkish Journal of Zoology, 31**, (2007), 171-180

5. **Agh N.**, Abatzopoulos T., Van Stappen G., Bossier P., Lotfi G. V.[2], Sorgeloos P. Effects of Salinity on Survival, Growth, Reproductive and life span characteristics of *Artemia* populations from Urmia Lake and neighboring lagoons. (**Accepted for publication in Pakistan Journal of Biological Sciences**).

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B. Papers published in peer-reviewed national journals Published in Iran:

8. Fazeli, M.S.; Kodabande S.; **Agh N**. (1999). Effects of different salinities on chorion thickness of *Artemia urmiana* cysts produced at laboratory condition. **Daneshvar**. 26, pp.59-66.

9. Lotfi, V.G.; **Agh, N**.; Sepehri H. (2003). Effects of different salinities on survival, growth and reproductive characteristics of *Artemia* parthenogenetica from Lagoons and Maharlu Lake in Iran. **Journal of Basic Sciences**, Faculty of Science, Tehran University. 29(2) pp. 305-316.

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11. Agh, N., Hosseini Ghatre, SH. (2002). Determination of protein, lipid, and fatty acid profile of *Artemia urmiana* at different growth stages. **Scientific Journal of Padjohesh and Sazandegi**. 54, pp. 85-89.

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19. Manaffar, R.; Maleki, R.; Atashbar, B. and **Agh, N.** (2006). Use of *Azadirachta indica* against contamination by ciliates in dense culture of unicellular Alge *Dunaliella tertiolect*. **Scientific Journal of Padjohesh and Sazandegi**, 71, pp 82-85.

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23. Mohammadyari, A.; Rahimian, H.; **Agh, N.** (Submitted). Biometry of Cyst and Nauplii of *Artemia* Strains from Different Geographical Locations from Iran. **Journal of Basic Sciences**, Faculty of Science, Tehran University.

24. Hajimoradloo, A. **Agh, N**., Ghorbani, R., Soltani, S., Noori, F. and Abdoljabbar Irani (Submitted). Bioencapsulation of oxolinic acid in *Artemia urmiana* as a

means of prevention of bacterial infection caused by Aeromonas hydrophila in Acipenser persicus larvae. **Journal of the World Aquaculture Society**.

25. Hosseini Najd Geramy, E. and **Agh, N.** (Submitted). Replacement of fish oil with Soya oil in feeding regime of Acipenser persicus and determination of its impact on nutritional parameters and fatty acid profile. **Journal of Basic Sciences**, University of Tehran.

C. List of papers presented in international Conferences.

1. **Agh, N**.; Sorgeloos, P.; Abatzopoulos, T.; Razavi, RSM.; Lotfi, VG. (2001). *Artemia* resources in Iran. **International Workshop on *Artemia*, Urmia University, 12-15 May 2001, Urmia, Iran.**

2. **Agh, N**. (2001). Intensive culture of *Artemia* in Tanker. **International Workshop on *Artemia*, Urmia University, 12-15 May 2001, Urmia, Iran.**

3. Sadeghi, H.; Jahanpeyma, S.; **Agh, N**. (2001). Lethal effects of Organophosphrous Compounds on *Artemia urmiana*. **International Workshop on *Artemia*, Urmia University, 12-15 May 2001, Urmia, Iran.**

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7. **Agh, N**.; Noori, F.; Asefi, A.; Sorgeloos, P. (2001). Effects of Anti-bacterial agents on the H% and Bacterial load in the hatching medium of *Artemia urmiana* cysts. **Larvi2001-Fish and Shellfish Larviculture, *Artemia* Reference Centre, University of Gent, 3-6 Sep. 2001, Gent, Belgium.**

8. **Agh, N**. (2002). Studies on *Artemia* populations from Iran. **Global Workshop on *Artemia*, *Artemia* Reference Centre, University of Gent, 5-7 Feb. 2002, Gent, Belgium.**

9. **Agh, N**.; Lotfi, VG.; Sorgeloos, P. (2002). Effects of different salinities on performance, reproduction and life span characteristics of 3 *Artemia* populations from Iran. **Aquaculture 2002, World Aquaculture Society, 22-28 April 2002, Beijing, China.**

10. **Agh, N**.; Sorgeloos, P. (2002). Introducing five parthenogenetic populations of *Artemia* from Iran. **Aquaculture 2002, World Aquaculture Society, 22-28 April 2002, Beijing, China.**
11. **Agh, N**.; Manaffar, R.; Atashbar, B. (2002). Successful inoculation of *Artemia franciscana* (VC strain) at south of Iran. **Aquaculture 2002, World Aquaculture Society, 22-28 April 2002, Beijing, China.**

12. Noori, F.; **Agh, N**. (2002). Effects of temperature, salinity, photoperiod and feeding regime on the cyst production by *Artemia urmiana*. **Aquaculture 2002, World Aquaculture Society, 22-28 April 2002, Beijing, China.**

13. Ras, MB.; **Agh, N**.;Yahyazadeh, M. (2002). Chemical composition and nutritive value of *Artemia urmiana* in broiler rations. **Aquaculture 2002, World Aquaculture Society, 22-28 April 2002, Beijing, China.**

14. **Agh, N**. (2002). Co-existence of bisexual and parthenogenetic populations of *Artemia* at Urmia lake. **China regional workshop on *Artemia*. China National Institute of Salt Research. 23-26 Sep. 2002, Beijing, China.**

15. **Agh, N**. (2003). Seasonal Variation in the food value of *Artemia* urmiana cyst and biomass. **Aquaculture 2003, Brazil May 2003, Salvador, Brazil.**

16. **Agh, N**.; Manaffar, R. and Atashbar, B. (2003). High density pond production of *Artemia* in Iran. **Aquaculture 2003, Brazil May 2003, Salvador, Brazil.**

17. Agh, N. and Jafari D. (2003). Effects of Helium-Neon Laser on growth and survival rates and reproductive and life span characteristics of *Artemia urmiana*. **Aquaculture 2003, Brazil May 2003, Salvador, Brazil.**

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19. Agh, N. and Marden, B. (2004). Laboratory analysis and calculation methods for resource assessment of *Artemia.* **Fifth International workshop on *Artemia*, 21-25 Sep. 2004, Urmia, Iran.**

20. **Agh, N**.; Manaffar, R.; Atashbar, B. and Mahini, S. (2004). Successful pond culture of *Artemia partenogenetica* and *Artemia urmiana* at the vicinity of the Lake Urmia. **Fifth International workshop on *Artemia*, 21-25 Sep. 2004, Urmia, Iran.**

21. **Agh, N**. and Noori, F. (2004). Introducing a different enrichment material for improving HUFA levels in juvenile *Artemia urmiana*. **Fifth International workshop on *Artemia*, 21-25 Sep. 2004, Urmia, Iran.**

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22. Van Stappen, G.; Negarestan, M. and **Agh, N.** (2004). Resource Assessment of *Artemia* in Lake Urmia. **Fifth International workshop on *Artemia*, 21-25 Sep. 2004, Urmia, Iran.**

23. **Agh, N.** and Noori, F. (2005). Enrichment of *Artemia urmiana* with Highly Unsaturated Fatty Acids (HUFA) Emulsions, Fish Oils, Vitamin C and Antibiotics: Applications in Larviculture. **First Regional Workshop on Enrichment of Live Food, 7-10 March, 2005, Urmia, Iran.**

24. **Agh, N.** Protocols for enrichments with HUFAs, Fish Oils, Vitamin C and Antibiotics. **First Regional Workshop on Enrichment of Live Food, 7-10 March, 2005, Urmia, Iran.**

25. Asem, A.; Rastegar, S. and **Agh, N.** (2005). Sexual Dimorphism in *Artemia urmiana* in tour different geographical stations from the Urmia Lake, West Azerbaijan, Iran. **World Aquaculture 2005, May 9-13, 2005, Bali, Indonesia.**

26. Asem, A.; Rastegar, S. and **Agh, N.** (2005). Biometrical variations of *Artemia urmiana* Cysts from Urmia Lake **(West Azerbaijan). World Aquaculture 2005, May 9-13, 2005, Bali, Indonesia.**

27. Makhdomi, N. and **Agh, N.** (2005). Inter-relationship between egg size, fatty acid profile and growth rate of *Huso Huso* larvae. **5th International Symposium on Sturgeon fishes, May 9-13, 2005, Ramsar, Iran.**

28. Ghorbani, R.; Hajimoradloo, A.; **Agh. N.**; Noori, F. (2005). A study of bioencapsulation of Oxolinic acid in *Artemia urmiana* nauplii on survilance of *Acipenser persicus* larvae. **5th International Symposium on Sturgeon fishes, May 9-13, 2005, Ramsar, Iran.**

29. **Agh, N.**, Boosier, P.; Abatzopoulos, T.J. and Sorgeloos, P. (2005). Genetic similarities of *Artemia* populations from Urmia Lake Region. **International Workshop on Genetics and Molecular Biology of Aquatic Organisms, 26-30 Sep. 2005, Urmia, Iran.**

30. **Agh., N.**; Irani, A. and Nekoei, H. (2006). Evaluation of Replacement of fish powder with *Artemia* powder in diet of Rain Bow Trout Broodstock. **World Aquaculture 2005, May 9-13, 2006, Florence, Italy.**

31. Sarli, T.; Hajimradloo, A.; Makhdomi, N. and **Agh, N.** (2006). Effects of Fatty Acid composition and egg size on growth and survival of early stages of Persian sturgeon larvae. **World Aquaculture 2005, May 9-13, 2006, Florence, Italy.**

32. **Agh, N.** and Irani, A. (2007). Optimization of early feeding of great sturgeon *Huso Huso*. International Workshop on Advanced Techniques in Sturgeon Fish Larviculture. 2007, Urmia, Iran.

33. **Agh, N.** and Irani, A. (2007). Improvements in early feeding of Persian sturgeon (Acipenser persicus). International Workshop on Advanced Techniques in Sturgeon Fish Larviculture. 2007, Urmia, Iran.

D. List of papers presented in National Conferences organized in Iran.

34. Noori, F.; **Agh, N**. (1997). Reproduction methods and morphological changes during post-larval development of *Artemia urmiana*. Sixth Iranian Biology Conference, 25-27 Aug. 1997, University of Kerman, Kerman, Iran.
35. **Agh, N**.; Noori, F. (1997). Introduction of a parthenogenetic population of *Artemia* from lagoons around Urmia lake and its morphological comparison with *Artemia urmiana.* First Iranian Congress of Zoology, University of Teacher Education (Tarbiat Moellem), 17-18 Sep. 1997,Tehran, Iran.
36. **Agh, N**. (1997). Effects of Physico-chemical conditions on hatching of *Artemia urmiana* cysts. First Iranian Congress of Zoology, University of Teacher Education (Tarbiat Moellem), 17-18 Sep. 1997,Tehran, Iran.
37. **Agh, N**. (1997). Characteristics of *Artemia urmiana* and its role in economic development of West Azerbaijan. Seminar on Development and Construction of West Azerbaijan Province. Governors of west Azerbaijan, 19-21 Nov. 1997.

38. **Agh, N**. (1997). Urmia lake, *Artemia* and economy of West Azerbaijan. Ist exhibition on scientific and technical achievements of West Azerbaijan province. West Azerbaijan University Jihad, 5-11 Feb. 1997, Urmia, Iran.
39. **Agh, N**.; Noori, F. (1997). Biological studies of *Artemia urmiana*. Ist exhibition on scientific and technical achievements of West Azerbaijan province. West Azerbaijan University Jihad, 5-11 Feb. 1997, Urmia, Iran.
40. **Agh, N**.; Yahyazadeh, MS. (1997). Role of *Artemia* in creation of employment opportunities in West Azerbaijan. Ist exhibition on scientific and technical achievements of West Azerbaijan province. West Azerbaijan University Jihad, 5-11 Feb. 1997, Urmia, Iran.
41. **Agh, N**. (1998). High density production of *Artemia urmiana* in 1000 litres Tankers with AWL culture system. 7th Iranian Biology Conference, University of Esfahan, 22-24 Sep. 1998, Esfahan, Iran.
42. Noori, F.; **Agh, N**. (1998). Effect of Salinity on hatching of Cysts, growth of larvae, and reproduction of *Artemia urmiana*. 7th Iranian Biology Conference, University of Esfahan, 22-24 Sep. 1998, Esfahan, Iran.
43. Lotfi, VG.; Sepehri, H.; **Agh, N**. (2000). Effect of different salinities on survival, biometry and reproduction of *Artemia* from Urmia and Maharlu lakes. 9th Iranian Biology Conference, University of Tehran, 15-17 Aug. 2000, Tehran, Iran.
44. Ownagh, A.; **Agh, N**. (2001). Effects of Anti-fungal agents on growth and reproduction of *Artemia urmiana*. Ist Conference on Lake Urmia, Urmia University, 5-7 Sep. 2001, Urmia, Iran.
45. Ownagh, A.; **Agh, N**. (2000). Fungal contaminations of *Artemia urmiana* cysts. Ist Iranian Congress on Fish health and Diseases. Chamran University, 14-16 Feb. 2000, Ahvaz, Iran.
46. **Agh, N.** (2003). Diversity of *Artemia* populations at Lake Urmia. Chile Regional Workshop on *Artemia* Biodiversity, Nov. 17-19, 2003, puerto Varas, Chile.
47. Agh, N. and Jafari D. (2004). Effects of Helium-Neon Laser on hatching characteristics of *A. urmiana*. 12th Biology congress of Iran. University of Razi, Hamadan, Iran.
48. Asem, A.; Rastegar, H. and Agh, N. (2004). Biometric diversity of *A. urmiana* cyst North wing of Lake Urmia. 12th Biology congress of Iran. University of Razi, Hamadan, Iran.

49. **Agh, N.** and Jafari D. (2003). Effects of Gamma and Beta irradiations on reproductive and life span characteristics of *Artemia urmiana*. 2nd International conference on Nuclear Science and Technology in Iran. Shiaz University, Shiraz, Iran.
50. Tayyebi, L.; Seifabadi, J. and **Agh, N.** (2004). Evaluation of hatching characteristics and biochemical composition of *Artemia urmiana* nauplii. 2nd congress on applied biology. Sep.2004, Mashhad University, Mashhad, Iran.
51. Mashgholozekr, A.; Esmaeli, A. and **Agh, N.** (2005). Effects of salinity of growth rate of *Artemia* from Maharlu lake. 13th Iranian conference on Biology. Rasht University, Rasht, Iran, Aug. 2005.

MSc and PhD Thesis Supervision

- 4 PhD students thesis
- 24 MSc Students thesis
- 23 DVM students thesis

International Conference/Workshop Participation (with communication)

- International Workshop on *Artemia*, Urmia University, 12-15 May 2001, Urmia.
- Larvi2001-Fish and Shellfish Larviculture, *Artemia* Reference Centre, University of Gent, 3-6 Sep. 2001, Gent, Belgium.
- Global Workshop on *Artemia*, *Artemia* Reference Centre, University of Gent, 5-7 Feb. 2002, Gent, Belgium.
- Aquaculture 2002, World Aquaculture Society, 22-28 April 2002, Beijing, China.
- China regional workshop on *Artemia*. China National Institute of Salt Research. 23-26 Sep. 2002, Beijing, China.
- Fifth International workshop on *Artemia*, 21-25 Sep. 2004, Urmia, Iran.
- First Regional Workshop on Enrichment of Live Food, 7-10 March, 2005, Urmia, Iran.
- International Workshop on recirculation System, May 8-9, 2006, Florence, Italy
- World Aquaculture 2006, May 9-13, 2006, Florence, Italy.

- International Workshop on Genetics and Molecular Biology of Aquatic Organisms, 26-30 Sep. 2005, Urmia, Iran
- 5th International Symposium on Sturgeon fishes, May 9-13, 2005, Ramsar, Iran.
- 13th Iranian conference on Biology. Rasht University, Rasht, Iran, Aug. 2005
- World Aquaculture 2005, May 9-13, 2005, Bali, Indonesia
- 2nd International conference on Nuclear Science and Technology in Iran. Shiaz University, Shiraz, Iran.
- PhD course on physical and chemical analysis of fish as food. Fish Research Institute. Technical University of Denmark. 20-24 Nov. 2006.
- International Workshop on Advanced Techniques in Sturgeon Fish Larviculture. 2007, Urmia, Iran.

MEMBERSHIPS

- Member of Urmia University Directorate Council (since 2004)
- Member of Research Council, Urmia University, Iran (since 1999)
- Member of "*Artemia* Experts Committee", Iranian Fisheries Research Institute (1998-2002)
- Member of " Live Food Experts Committee", Iranian Fisheries Research Institute (1999-2003)
- Member of 'Urmia University Sports Council' (since 2002)
- Member World Aquaculture Society (since 2002)
- Member World Sturgeon Preservation Society (since 2005)
- Member of National Committee for Propagation and Culture of Sturgeon Fish