



Quality evaluation of *Artemia urmiana* Günther (Urmia Lake, Iran) with special emphasis on its particular cyst characteristics (International Study on *Artemia* LXIX)

Theodore J. Abatzopoulos^{a,*}, Athanasios D. Baxevanis^a, George V. Triantaphyllidis^b, Godelieve Criel^c, Erwin L. Pador^d, Gilbert Van Stappen^d, Patrick Sorgeloos^d

^a Department of Genetics, Development and Molecular Biology, School of Biology, Faculty of Sciences, Aristotle University of Thessaloniki, 541 24, Thessaloniki, Greece

^b Lamans Management Services S.A., 3rd Septemvriou str 56, 104 33 Athens, Greece

^c Department of Human Anatomy and Embryology, Ghent University, Godshuizenlaan 4, B-9000, Gent, Belgium

^d Laboratory of Aquaculture and Artemia Reference Center, Ghent University, B-9000, Gent, Belgium

Received 25 August 2005; received in revised form 31 October 2005; accepted 2 November 2005

Abstract

Artemia urmiana cysts were collected from seven sites in Urmia Lake, Iran. Biometrical analysis revealed that the mean values for the untreated cysts ranged from 262.7 to 286.6 μm , decapsulated cysts from 258.6 to 273.9 μm , and the chorion thickness ranged from 1.2 to 9.3 μm . The cyst samples were tested for their buoyancy at salinities of 35, 50, 100, 150 and 200 g/l. Two cyst batches from Great Salt Lake (*Artemia franciscana*) were also tested as reference material. It was found that the majority of Urmia cysts (over 60%) sank after 72 h even at the salinity of 200 g/l, while, GSL cysts reached a much lower figure (less than 10%) after the same time period. Transmission electron microscopy studies of the Urmia cyst chorion revealed a thinner alveolar layer and a thicker fibrous layer in comparison with the respective layers of *A. franciscana* cysts. *A. urmiana* instar-I nauplii biometry was also performed (total naupliar length: 466.3–505 μm). Six reproductive and four life span characteristics were investigated at salinities of 35, 50, 100, 140 and 180 g/l in order to evaluate *A. urmiana* performance at different salinities. *A. urmiana* individuals showed a preference for high salinity, since high mortality was recorded at 35 and 50 g/l. No significant differences were found between the three highest salinities (100, 140 and 180 g/l) tested ($P > 0.05$), with the exception of offspring per brood, reproductive period, and total life span. The analysis of highly unsaturated fatty acid (HUFA) profile of instar-I nauplii hatched from collected cyst batches resulted in low levels of eicosapentaenoic acid (20:5n-3) and high levels of linolenic acid (18:3n-3) ranging from 1.8 to 7.2 and from 32.7 to 54.7 mg/g DW, respectively. Only traces of docosahexaenoic acid (22:6n-3) were found.

© 2005 Elsevier B.V. All rights reserved.

Keywords: *Artemia urmiana*; Cyst buoyancy; HUFA; Chorion structure; Urmia Lake

1. Introduction

Populations of the brine shrimp *Artemia* (Crustacea, Anostraca) are found in many inland salt lakes and coastal salterns distributed all over the world (Triantaphyllidis et

* Corresponding author. Tel.: +30 2310 998301, fax: +30 2310 998256.

E-mail address: abatzip@bio.auth.gr (T.J. Abatzopoulos).

al., 1998; Van Stappen, 2002). Up to date, the following bisexual species have been described: *Artemia franciscana* and *Artemia persimilis* in the New World, and *Artemia salina*, *Artemia urmiana*, *Artemia tibetiana* and *Artemia sinica* in the Old World (Abatzopoulos et al., 2002a,b). Apart from bisexual *Artemia* species, there are numerous parthenogenetic populations found in the Old World, with ploidy levels varying from di- to tri-, tetra- and pentaploid (Abatzopoulos et al., 1986; Triantaphyllidis et al., 1998).

Artemia cyst demand in aquaculture has increased to over 2000 metric tons annually (Sorgeloos et al., 2001; Dhont and Sorgeloos, 2002). In order to sustain the fast growing aquaculture industry (Lavens and Sorgeloos, 2000), natural resources other than Great Salt Lake in Utah (USA) should be explored as alternative commercial sources (Triantaphyllidis et al., 1994; Lavens and Sorgeloos, 2000). In 1993, the Iranian government, through the Iran Fisheries Company, decided to initiate a detailed hydrobiological study of Urmia Lake in order to formulate recommendations for a sustainable exploitation of the endemic *A. urmiana* population as a cyst and biomass resource. The first results of these efforts are described in Van Stappen et al. (2001).

Urmia Lake is one of the biggest natural *Artemia* habitats in the world. It is a landlocked thalassohaline, sodium chloride lake (Löffler, 1961) located in the northwestern region of Iran (45°10'N and 30°20'E) at 1250 m above sea level (Cole and Brown, 1967; Azari Takami, 1993). Its surface area ranges from 4750 to 6100 km² and the average and greatest depths account for 6 and 16 m, respectively (Azari Takami, 1993; Van Stappen et al., 2001). For extensive information on the hydrology of Urmia Lake and its *Artemia* population dynamics, see Van Stappen et al. (2001) and references therein.

Günther (1899) reported for the first time the presence of *Artemia* in Urmia Lake. The species was designated as *A. urmiana*. Clark and Bowen (1976) demonstrated that there is reproductive isolation between *A. urmiana*, *A. salina* (formerly *Artemia tunisiana*, see Triantaphyllidis et al., 1997a) and *A. franciscana* under laboratory conditions. Barigozzi et al. (1987) reported that a sample from Urmia Lake was found to be exclusively parthenogenetic, consisting of diploid, tetraploid and pentaploid individuals. Since no bisexual *Artemia* was present in those samples from Urmia Lake, Barigozzi (1988) proposed to drop the species designation *A. urmiana*. Later, Ahmadi et al. (1990), studying *Artemia* cysts collected from the western shoreline of the lake in August 1987, showed that the *Artemia* population of Urmia Lake was a

mixture of bisexual and asexual brine shrimps. Abatzopoulos et al. (2002b) utilized a multidisciplinary approach based on allozyme analysis, RAPDs and cross-breeding experiments for investigating speciation in the genus *Artemia*. Baxevanis et al. (2005) applying morphometry confirmed previous findings, although mtDNA-RFLP analysis (16S rRNA) showed that *A. urmiana* was indistinguishable from *A. tibetiana*.

The scope of this paper is to further characterize *A. urmiana* focusing on its potential use in aquaculture. Therefore, it reports on the biometry of cysts and instar-I nauplii, and on the reproductive performance and life span characteristics of *A. urmiana* in cultures at different salinities. The poor floating capacity of the cysts collected from several sites in Urmia Lake is examined by applying simple buoyancy tests and by studying cyst chorion structure through transmission electron microscopy. Fatty acid profiles of instar-I nauplii derived from five cyst batches are also evaluated.

2. Material and methods

Artemia cysts were collected from seven sampling stations in Urmia Lake (a detailed description of Urmia Lake and results of field studies on *A. urmiana* can be found in Van Stappen et al., 2001). Fig. 1 depicts the locations of the various sampling sites in the lake while Table 1 shows the cyst sample code numbers used throughout this study, the *Artemia* Reference Center cyst

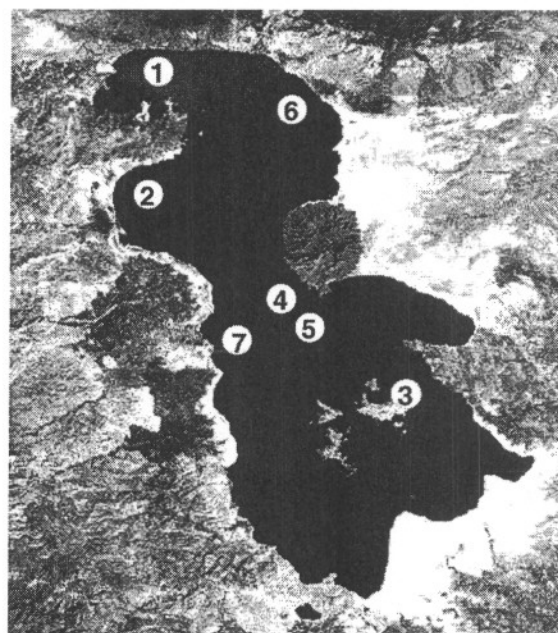


Fig. 1. Map of Urmia Lake (Iran) and location of the sampling stations.

Table 1
Cyst sample codes (see Fig. 1), their *Artemia* Reference Center (ARC) cyst bank number, hatching percentage for each sample after 48-h incubation under standard conditions and localities

Sample	ARC cyst bank no.	Hatching percentage (%)	Locality
Urmia 1	1293	33.4±3.4	Khantakhti
Urmia 2	1292	97.8±3.4	South of Khantakhti
Urmia 3	1294	20.2±3.0	Kaboudan
Urmia 4	1295	17.3±2.7	Causeway East
Urmia 5	1296	13.7±1.4	South of Aq Gonbed
Urmia 6	1302	72.9±2.3	Sharaf Khaneh
Urmia 7	1303	22.5±3.1	Golman Khaneh

bank code numbers and the localities. Sampling was performed by dragging a 100- μ m mesh size net (60 cm opening, 20 cm cod end and 2.5 m length) over a length of 400 m in each of the designated transect stations. The collected cysts were packed in polyethylene plastic bags for transportation. Cysts were cleaned according to Sorgeloos et al. (1986). *A. franciscana* cysts from Great Salt Lake (ARC No: GSL-1 1287, GSL-2 1286) were used as reference material when needed.

2.1. Biometry of cysts and nauplii

Decapsulation of cysts was performed according to Sorgeloos et al. (1986). Prior to measurement, decapsulated and non-decapsulated cysts were hydrated in a 10 g/l artificial Dietrich and Kalle (D&K) medium (Kalle, 1971) which was prepared following the modifications of Vanhaecke et al. (1984) and filtered through a 0.45- μ m cartridge filter (Sartobran®-PH capsule from Sartorius). The hydrated cysts were measured under a microscope equipped with an eyepiece micrometer Leitz device containing a graticule. The graticule was calibrated against a standard microscale and the measurements had an accuracy of 1 μ m. Cysts were hatched in filtered D&K hatching medium (i.e. salinity was 35 g/l, temperature 27±1 °C, pH 8.75, illumination ~2000 lux). Instar-I nauplii were harvested and fixed in 1% lugol solution at 35 g/l D&K medium and measured under the microscope to the nearest μ m. The hatching percentages of all samples were also calculated according to Sorgeloos et al. (1986) (Table 1).

Data were analyzed using Statistica (version 6) and STATGRAPHICS Plus 5. The diameter of untreated and decapsulated cysts, as well as the length of instar-I nauplii were analyzed by a standard single factor ANOVA (Sokal and Rohlf, 1981). Bartlett's, Hartley's, Cochran's and Levene's tests were applied to determine the homogeneity

of variances. For post-hoc comparisons, Least Significant Difference tests (LSD) were employed.

2.2. Survival, growth, reproductive and life span characteristics

Cysts of Urmia 2 sample were incubated in 0.45- μ m filtered 35 g/l D&K medium under standard conditions (Sorgeloos et al., 1986). This sample was chosen because it showed the highest hatching percentage (Table 1) and the quantity of cysts was sufficient for conducting experiments on survival, growth and life history traits. The hatched nauplii were transferred to 1-l cylindroconical glass jars containing 0.45- μ m filtered D&K medium of 35, 50, 100, 140 and 180 g/l salinity, at an initial animal density of 2 nauplii/ml. The density was reduced after day 8 to one metanauplius per 4 ml. The temperature was kept at 25±1 °C and mild aeration was supplied from the bottom of the culture jars, which were covered with perforated Petri dishes to minimize evaporation. The salinity of the culture medium was daily monitored and adjusted at the desired level by adding distilled water when needed. Three replicates were set up for each salinity value. The animals were fed on a mixed diet of the alga *Dunaliella tertiolecta* Butcher and the yeast-based formulated feed LANSY PZ (INVE Aquaculture SA, Belgium), following the feeding schedule of Triantaphyllidis et al. (1995).

Survival was monitored at each water renewal, i.e. on day 8, 11, and every 3 or 4 days thereafter until the end of the experiment. Survival data were log-transformed and analyzed through regression and ANCOVA (for details, see El-Bermawi et al., 2004).

For each growth experiment, one hundred nauplii were transferred immediately after hatching in separate 50-ml Falcon tubes at 100, 140 and 180 g/l salinities (culture experiments at salinities of 35 and 50 g/l resulted in very low survival, see Results). Total length of individuals was recorded at each water renewal. Animals were anaesthetized in chloroform-saturated seawater and measured under a dissection microscope equipped with a camera Lucida and a digitizer (measurement precision was 1 μ m). Growth analysis was performed separately for males and females identified at sexual maturity. Data on total lengths measured at a specific time for each salinity allowed the calculation of the Von Bertalanffy equation parameters. Details about this method can be found in El-Bermawi et al. (2004).

The reproductive performance of individuals from Urmia 2 sample was based on six reproductive and four life span characteristics which were recorded following the methodology described by Browne et al. (1984, 1988),

Table 2
Cyst diameter, decapsulated cyst diameter and length of instar-I nauplii (mean±S.D.) and the chorion thickness for *A. urmiana* samples

Sample	Cysts (µm)	Decapsulated cysts (µm)	Chorion thickness (µm)	Length (µm) of instar-I nauplii
Urmia 1	262.7±15.2 ^a	258.6±17.4 ^a	2.1	466.3±48.1 ^a
Urmia 2	276.7±18.3 ^b	274.4±18.4 ^e	1.2	497.2±39.0 ^e
Urmia 3	274.5±14.9 ^b	270.3±14.8 ^{cd}	2.2	505.0±29.7 ^e
Urmia 4	273.4±16.6 ^b	266.9±15.1 ^{bc}	3.2	497.1±30.2 ^e
Urmia 5	280.8±19.5 ^c	264.3±14.1 ^b	8.3	488.4±26.3 ^b
Urmia 6	286.5±12.3 ^d	267.8±15.7 ^{bc}	9.3	471.7±28.7 ^b
Urmia 7	286.6±14.3 ^d	273.9±16.6 ^{de}	6.4	473.9±29.6 ^a
F-value	27.7	16.1	n.a.	22.6
df	6693	6693		6693

n.a. = non applicable.

F-value and degrees of freedom (df) for ANOVA are given for each variable. Values for each treatment sharing the same letter(s) are not significantly different (LSD tests, $P>0.05$).

i.e. offspring per brood, broods per female, offspring per female per day during the reproductive period, days between broods, percent of encysted embryos, total offspring per female, pre-reproductive period, reproductive period, post-reproductive period and total life span (the last four characteristics were measured in days). As soon as males and females were sexually mature, couples were isolated and transferred to 50-ml Falcon tubes with 45 ml of 0.45-µm filtered D&K medium of desired salinity. Prior to transfer, acclimatization of the animals was performed (Baxevanis et al., 2004). For each salinity experiment, twenty couples were observed daily for reproduction and survival. Males were replaced at death.

Tests for homogeneity of variances (Levene's test, Bartlett's test and Hartley's F_{\max} test) showed significant deviations from equal variances for most of the

studied characteristics. Since log or square root transformation did not remove heteroscedasticity, an approximate test for equality of means was performed when variances were assumed to be unequal and sample sizes varied (Games and Howell, 1976).

2.3. Buoyancy tests

A simple buoyancy test was applied to observe the floating capacity of hydrated cysts at different salinities. Seven cyst samples were collected from Urmia Lake (Iran) and two from Great Salt Lake (Utah, USA) with known chorion thickness and were tested. Prior to buoyancy experiments, cysts were dehydrated: five grams of each sample were placed into small aluminum foil trays and oven dried at 37 °C for 24 h. The dehydrated samples were placed into 50-ml glass test tubes containing D&K medium of 35, 50, 100, 150 and 200 g/l salinity (in triplicates). The tubes were lightly shaken and the proportion of floating cysts was determined (by weighing) after 1, 2 and 3 h for all tested salinities, and again after 72 h for the salinities of 100, 150 and 200 g/l, since hatching was prominent at 35 and 50 g/l. The experimental procedure described above was repeated with the addition of an extra step. After dehydration, cysts were re-hydrated in 10 g/l for an hour to ensure that all start from the same hydration level. However, no differences were observed (data not shown).

2.4. Transmission electron microscopy (TEM) study

For the TEM study, cysts were fixed overnight in a glutaraldehyde–paraformaldehyde mixture (Karnovsky, 1965) diluted 3:1 with cacodylate buffer 0.2 M, pH 7.4,

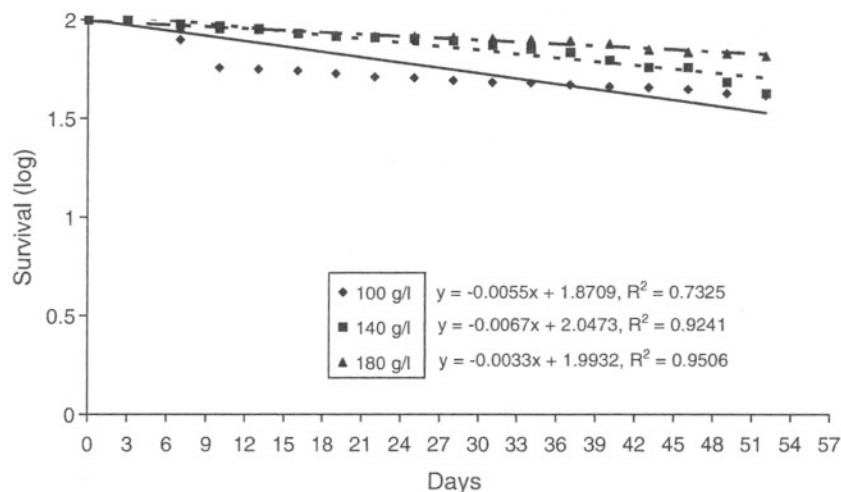


Fig. 2. Survival of *A. urmiana* individuals cultured at three different salinities (100, 140 and 180 g/l).

rinsed in cacodylate buffer and postfixed in 2% osmium tetroxide in cacodylate buffer. After additional buffer rinses, the tissue was dehydrated in an ascending series of ethanol concentrations to absolute ethanol and embedded in Epon 812 or LX resin. Ultrathin sections were stained with uranyl acetate followed by lead citrate and examined with a JEOL 100 (Criel, 1992).

2.5. Lipid analysis

Fatty acid methyl esters (FAME) were prepared through direct acid-catalyzed transesterification following the procedure described in Triantaphyllidis et al. (1996). An internal standard, 20:2(n-6), was added before the reaction. Hexane was used for the extraction of FAME and after the evaporation of the solvent, FAME were prepared for injection (by redissolving them in *iso*-octane). Quantitative determination was performed on a Chrom-pack CP9001 gas chromatograph equipped with auto-

sampler. A polar 50-m capillary column BPX70 (0.32 mm diameter and a layer thickness of 0.25 μm , connected to a 2.5-m methyl-deactivated precolumn) was used for 0.2- μl injections. The carrier gas was hydrogen at a pressure of 100 kPa and the detection mode FID. The temperature profile at the oven was programmed as follow: initial temperature at 85 $^{\circ}\text{C}$, increase at 150 $^{\circ}\text{C}$ (at a rate of 20 $^{\circ}\text{C}/\text{min}$), increase to 152 $^{\circ}\text{C}$ at 0.1 $^{\circ}\text{C}/\text{min}$, from 152 $^{\circ}\text{C}$ to 174 $^{\circ}\text{C}$ at 0.7 $^{\circ}\text{C}/\text{min}$ and from 174 $^{\circ}\text{C}$ to 180 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$. The last temperature was kept for 2 min. Identification was based on standard reference mixtures (Nu-Chek-Prep., Inc., USA).

3. Results

3.1. Hatching percentage

The hatching percentages of the seven samples studied were not satisfactory (less than 35% for most

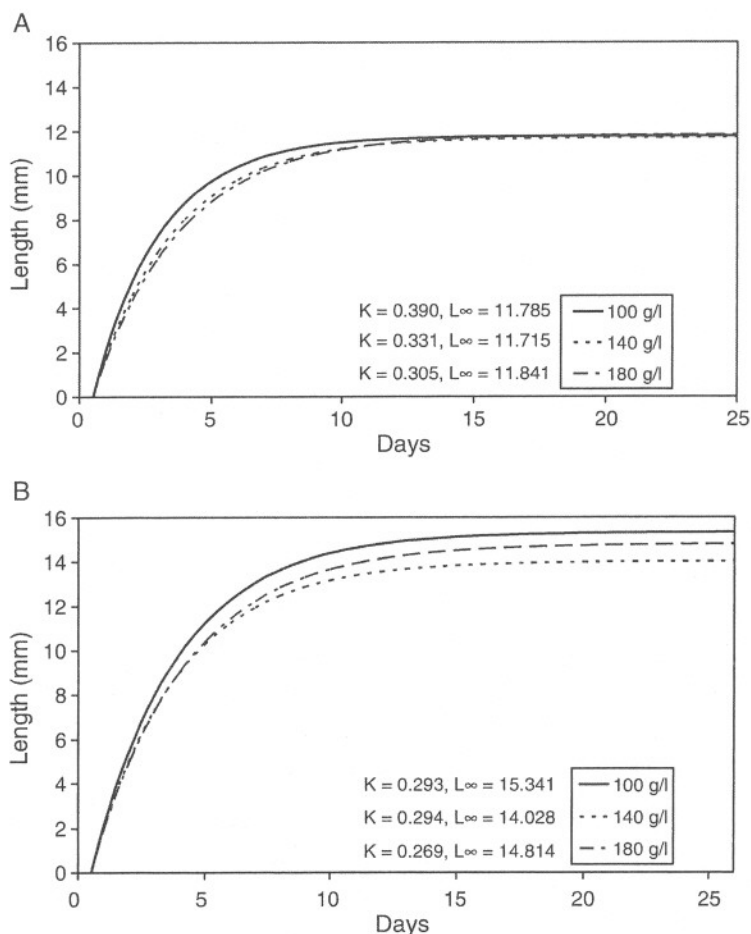


Fig. 3. Growth curves of *A. urmiana* males (A) and females (B) reared at three different salinities (100, 140 and 180 g/l).

of them after 48 h of incubation, see Table 1), with the exception of the Urmia 2 and Urmia 6 samples (97.8% and 72.9%, respectively). Accordingly, and because of the limited number of Urmia 6 cysts, the Urmia 2 sample was the only one used for studying survival, growth, reproductive and life span characteristics of *A. urmiana* (see below).

3.2. Biometry of cysts and nauplii

The diameter of hydrated non-decapsulated and decapsulated cysts, the chorion thickness and the instar-I naupliar length are presented in Table 2. The mean value of the diameter of non-decapsulated cysts ranged from 262.7 to 286.6 μm , while for the decapsulated cysts it varied from 258.6 to 274.4 μm . Samples of both untreated (non-decapsulated) and treated (decapsulated) cysts showed statistical differences (ANOVA, $*P < 0.05$) (see Table 2). The LSD test showed that there are four distinctive sample classes regarding untreated cysts: i) Urmia 1 (lowest value), ii) Urmia 2, 3 and 4, iii) Urmia 5 and iv) Urmia 6 and 7 (highest values) (see Table 2). The LSD test on treated cysts did not show any particular grouping; Urmia 1 decapsulated cysts were the smallest while those of Urmia 5 sample were the largest (Table 2). Chorion thickness mean values ranged from 1.2 to 9.3 μm (Table 2). Total naupliar length was measured from 466.3 to 505.0 μm showing significant differences (ANOVA, $*P < 0.05$) (Table 2). The LSD test for instar-I naupliar length revealed that cysts from Urmia 1, 6 and 7 produced the smallest nauplii, while cysts from Urmia 2, 3 and 4 produced the largest ones (Urmia 5 naupliar length was intermediate, see Table 2).

3.3. Survival, growth, reproductive and life span characteristics

Since it had the highest hatching percentage and the biggest available amount of cysts, the Urmia 2 cyst sample was also chosen for culturing experiments and for recording the reproductive and life span characteristics. Furthermore, the Urmia 2 cyst sample is representative of the existing *Artemia* population in that region of the lake.

Culture experiments at salinities of 35 and 50 g/l resulted in very low survival (less than 10% after day 7 and 5% after day 10 of the culture period, data not shown). Therefore, reproductive performance in these salinities was not recorded. Survival of *A. urmiana* cultured for ~50 days at 100, 140 and 180 g/l is shown in Fig. 2. Log-transformed survival values regressed on

time showed that survival was high at all salinities. No significant differences were found between salinities (ANCOVA, $P > 0.05$). However, the regression analysis showed a tendency for *A. urmiana* to perform better at 180 g/l (Fig. 2).

Growth rates for *A. urmiana* females and males reared at 100, 140 and 180 g/l are given in Fig. 3 (A and B). Female individuals (Fig. 3A) showed the same growth rate (K) at all salinities (Kruskal–Wallis, $P > 0.05$), while the growth rate of males at 100 g/l was higher compared with that at 140 and 180 g/l (Kruskal–Wallis, $*P < 0.05$). The recorded maximum total length of females and males was not affected by the increase of salinity. It should be noted that females displayed higher values for maximum total length compared with males, while the latter showed higher values in growth rate (K) at all salinities (see Fig. 3A, B).

Table 3 summarizes the results of the tests on the six reproductive and four life span characteristics of *A. urmiana* (Urmia 2 sample) cultured at 100, 140 and 180 g/l. Statistical analysis did not reveal significant differences in five out of six reproductive traits, i.e. individuals reared at 180 g/l had a significantly lower number of offspring per brood than those reared at 100 and 140 g/l ($*P < 0.05$). Two out of four life span characteristics were affected by salinity increase, i.e. individuals cultured at 140 and 180 g/l showed a significantly longer reproductive period and total life span than those at 100 g/l ($*P < 0.05$) (Table 3).

Table 3
Reproductive and life span characteristics (mean \pm S.D.) of *A. urmiana* individuals reared at three different salinities

Characteristic	Salinity		
	100 g/l	140 g/l	180 g/l
Offspring per brood	43.2 \pm 8.3 ^b	48.3 \pm 10.3 ^b	34.18 \pm 6.3 ^a
Broods per female	7.2 \pm 5.9 ^a	11.4 \pm 3.9 ^a	10.5 \pm 6.1 ^a
Offspring per female per day	13.8 \pm 14.6 ^a	8.54 \pm 2.6 ^a	6.91 \pm 1.4 ^a
Days between brood	4.3 \pm 2.3 ^a	5.69 \pm 1.0 ^a	5.53 \pm 1.6 ^a
Percent of encysted embryos	58.2 \pm 38.5 ^a	75.9 \pm 28.5 ^a	80.7 \pm 22.7 ^a
Total offspring per female	341.1 \pm 274.2 ^a	536.6 \pm 223.7 ^a	416.65 \pm 262.6 ^a
Pre-reproductive period	31.2 \pm 3.9 ^a	32.0 \pm 2.79 ^a	31.0 \pm 3.76 ^a
Reproductive period	38.4 \pm 26.3 ^a	65.5 \pm 27.8 ^b	65.9 \pm 23.8 ^b
Post-reproductive period	0 ^a	0.2 \pm 1.1 ^a	3.2 \pm 2.4 ^a
Total life span	69.7 \pm 31.9 ^a	97.7 \pm 28.9 ^b	99.8 \pm 27.0 ^b

Values for each characteristic that share the same letter(s) are not significantly different ($P > 0.05$). Twenty pairs were analyzed for each salinity.

3.4. Buoyancy tests and TEM study of cyst chorion structure

During processing, Urmia cysts were sinking faster compared with cysts from Great Salt Lake. Therefore, we performed simple buoyancy tests in order to investigate the floating ability of Urmia cyst batches when hydrated in the salinities of 35, 50, 100, 150 and 200 g/l. The results were compared with those of the two GSL strains, which were used as controls (Fig. 4). The samples 2, 3 and 4 showed very low percentages of buoyant cysts (from ~10% to ~40%) at all salinities, even after 72 h (Fig. 4). However, the cyst batches 5, 6 and 7 did not show similar floating ability: the percentage of sinking cysts decreased with the increase of salinity (up to 150 g/l) in all hydration

periods. At 200 g/l, more than 60% of the cysts sank and this also applied to all collected batches (Fig. 4). The only exception was the Urmia 1 sample (Fig. 4), which showed a floating capability very similar to the control material (GSL 1 and 2) in all salinities and hydration periods (see Fig. 4). This triggered the investigation of cyst chorion structure. Hence, cysts were examined by means of TEM to obtain information on both the chorion and on the structure of the various layers surrounding the embryo. The results are shown in Fig. 5. Although the total shell thickness (consisting of the chorion and four additional layers or membranes) was approximately the same in both *A. franciscana* (GSL) and *A. urmiana* (varying from 10 to 13 μm), electron microscopy revealed striking differences between the layers of these two species. GSL cyst shell

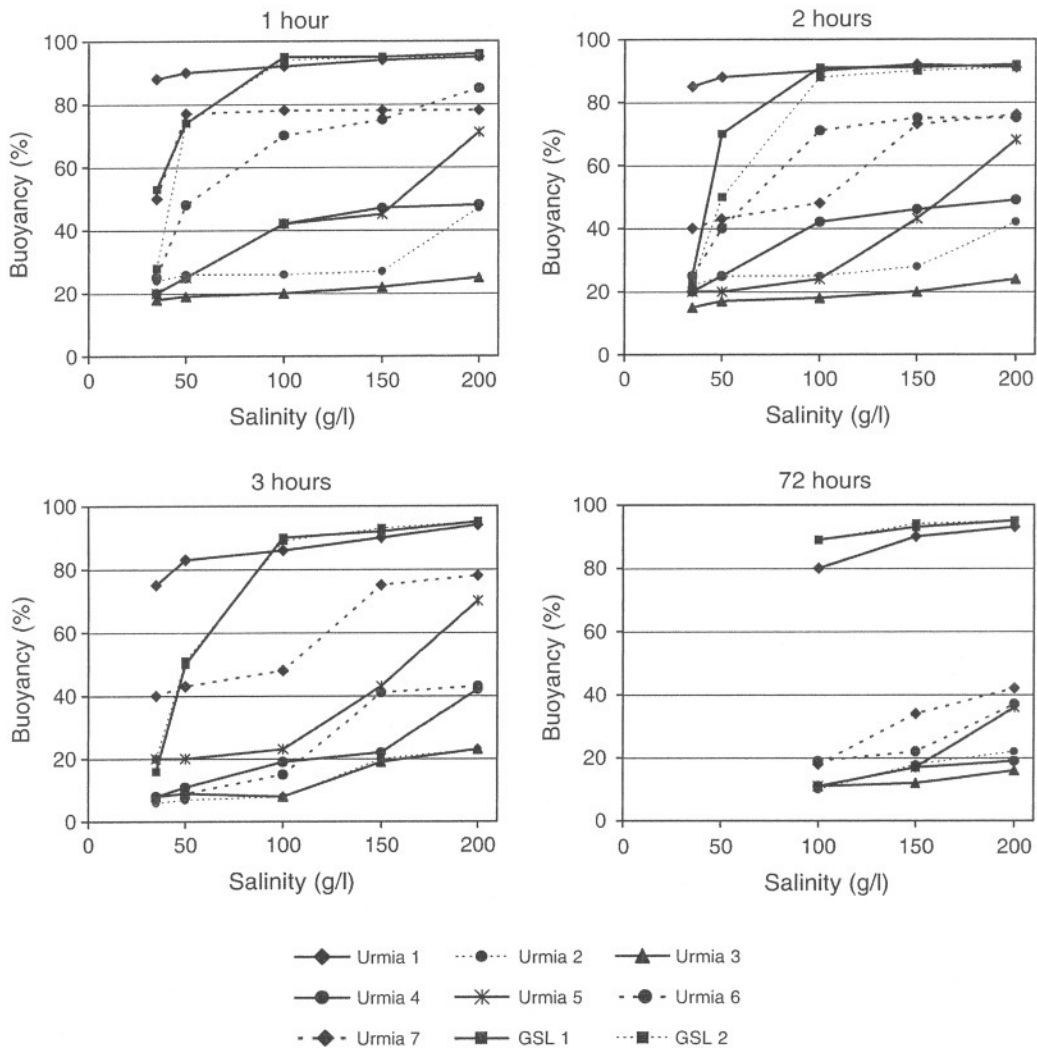


Fig. 4. Buoyancy tests of *A. urmiana* cysts at 5 different salinities. Chorion thickness of Urmia samples are given in Table 2, while for GSL1 and GSL2 cysts the respective values are 9 and 10 μm.

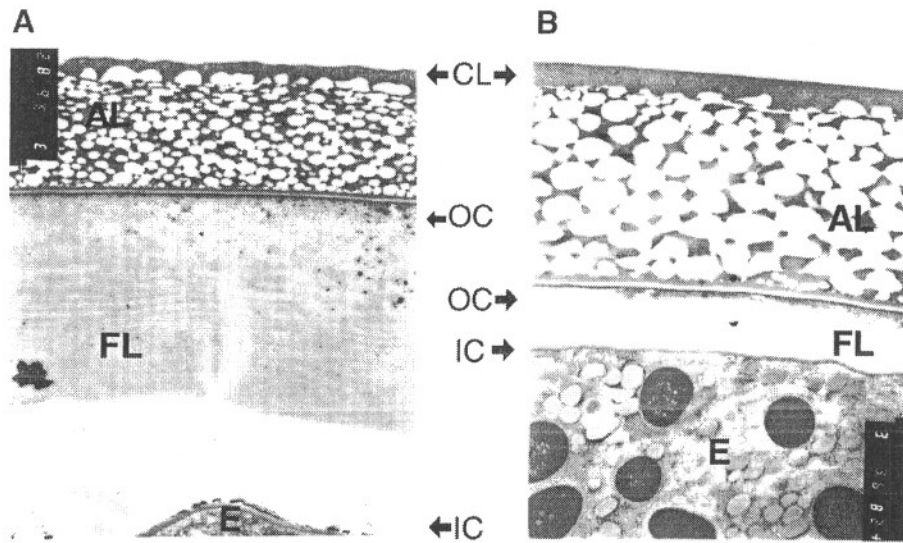


Fig. 5. TEM images for *A. urmiana* (A) and GSL (B) cysts. CL: cortical layer, AL: alveolar layer, OC: outer cuticular membrane, FL: fibrous layer, IC: inner cuticular membrane, E: embryo. Magnification $\times 14000$.

showed a relatively thicker alveolar layer (8 μm , cortical layer included) with wide alveolae and a relatively thinner fibrous layer (3 μm). In contrast, for *A. urmiana* cysts, both alveolar and fibrous layers were of equal thickness (6 μm) with narrower and more compressed alveolae (see details in Fig. 5).

3.5. FAME analysis

Table 4 shows the fatty acid composition of *A. urmiana* instar-I nauplii collected from five sampling sites. All samples exhibited high levels of linolenic acid (18:3n-3) but low levels of eicosapentaenoic acid (EPA,

Table 4
Fatty acid methyl ester analysis of *Artemia urmiana* instar-I nauplii

FAME	Urmia 1		Urmia 2		Urmia 3		Urmia 4		Urmia 5	
	Area (%)	DW (mg/g)	Area (%)	DW (mg/g)	Area (%)	DW (mg/g)	Area (%)	DW (mg/g)	Area (%)	DW (mg/g)
14:0*	1.5	2.1	1.3	1.7	1.4	1.7	1.0	1.3	0.9	1.2
14:1(n-5)	1.4	1.9	1.4	1.8	1.9	2.3	0.9	1.1	0.8	1.2
15:0*	0.3	0.5	0.3	0.4	0.3	0.4	–	–	0.2	0.2
15:1(n-5)	–	–	0.3	0.4	–	–	0.5	0.6	0.4	0.6
16:0	13.0	17.9	13.5	17.9	16.8	20.4	13.7	17.7	13.2	18.0
16:1(n-7)*	8.3	11.5	3.9	5.2	4.3	5.2	3.6	4.6	2.7	3.7
17:0	1.0	1.3	0.8	1.0	1.0	1.2	0.7	0.9	0.6	0.8
17:1(n-7)*	1.1	1.5	1.4	1.9	1.7	2.0	0.8	1.0	1.1	1.4
18:0	5.0	6.9	4.4	5.8	–	–	5.0	6.4	4.6	6.3
18:1(n-9)	14.8	20.4	14.2	18.9	6.4	7.8	14.8	19.1	14.4	19.7
18:1(n-7)*	7.4	10.3	5.2	6.9	–	–	5.7	7.4	4.4	6.0
18:2(n-6)-c	5.2	7.2	5.3	7.1	–	–	5.6	7.3	6.0	8.2
18:3(n-6)	1.0	1.4	1.2	1.6	–	–	1.2	1.5	1.3	1.7
18:3(n-3)*	25.2	34.8	32.9	43.7	45.0	54.7	32.7	42.1	37.0	50.7
18:4(n-3)*	3.5	4.8	4.2	5.6	6.2	7.6	5.4	7.0	5.5	7.5
19:0	0.4	0.5	0.3	0.3	1.7	2.1	–	–	–	–
19:1(n-9)	–	–	–	–	0.2	0.2	–	–	–	–
20:1(n-9)*	0.4	0.6	0.5	0.6	0.5	0.6	0.5	0.6	0.5	0.6
20:4(n-6)	0.8	1.0	0.6	0.8	0.6	0.7	0.2	0.3	–	–
20:3(n-3)	0.4	0.6	0.5	0.7	1.0	1.2	1.1	1.4	1.0	1.4
20:5(n-3)*	5.2	7.2	2.1	2.7	1.9	2.3	3.0	3.9	1.3	1.8
22:6(n-3)	–	–	0.3	0.4	–	–	–	–	–	–
Γ n-3: 20:3(n-3)*	8.0	–	4.3	–	4.1	–	5.8	–	3.9	–
Total FAME (mg/g)	138.3	–	133.0	–	122.0	–	129.0	–	136.9	–

20:5n-3), ranging from 34.8 to 54.7 and from 1.8 to 7.2 mg/g DW, respectively. Docosahexaenoic acid (DHA, 22:6n-3) was found only in traces in Urmia 2 sample and below detectable levels in the remaining samples. Urmia cysts also had low levels of highly unsaturated fatty acids ranging on average from 3.9 to 8.0 mg/g DW.

4. Discussion

The diameter of non-decapsulated cysts of Urmia Lake samples (ranging from 262.7 to 286.6 μm) is among the largest recorded for bisexual *Artemia* species, with the only exception of the cyst size of *A. tibetiana* (from Lagkor Co in the high Tibetan plateau), the latter being the largest (ranging from 323 to 330 μm) ever recorded cyst in *Artemia* bibliography (Abatzopoulos et al., 1998). The size of untreated Urmia cysts showed considerable similarities to those of some parthenogenetic populations from China, India, Italy (Vanhaecke and Sorgeloos, 1980) and to Greek apomictic tetraploid parthenogens (Abatzopoulos et al., 1987, 1993; Triantaphyllidis et al., 1993). The important variability observed on cyst diameter of Urmia samples does not match literature data. D'Agostino (1965) reported that, within the same strain, the mean "egg size" remains constant between batches collected at different periods of the year. Vanhaecke and Sorgeloos (1980) reported that in some cases the mean cyst size within a certain strain might vary up to 10 μm from one batch to another owing to varying environmental conditions. The variation in cyst diameter of Urmia samples, which in fact were collected within a month, as well as data based on cytology (polyploid instar-I nauplii, T.J. Abatzopoulos, unpublished data) and earlier reports (Ahmadi et al., 1990), imply a probable coexistence of bisexual and parthenogenetic *Artemia* strains in Urmia Lake (Barigozzi et al., 1987) and adjacent lagoons (Naser Agh, personal communication). However, the morphometrical/morphological data of adult brine shrimps and their sex ratio in the Urmia 2 cultures (data not shown) did not ascertain the presence of any parthenogenetic individual, at least in this sample. However, application of more advanced and sensitive techniques (such as molecular markers: Gajardo et al., 2004 and Baxevanis et al., 2005) would probably shed more light to the potential coexistence between *A. urmiana* and parthenogenetic populations in Urmia Lake.

For *A. urmiana*, chorion thickness ranged from 1.2 to 9.3 μm showing great variability among the different batches studied. The observed variation in chorion thickness may be the result of several unknown factors. An intangible factor that may have provoked such a

variation could be that some cysts were obtained by squeezing gravid (egg bearing or oviparous) females collected with adult biomass; this procedure may have been producing a proportion of insufficiently developed cysts in the final cyst product collected. This may also explain the existence of a slimy, gelatinous substance in the unprocessed cyst material that could strongly affect cyst hatching ability. However, Urmia 2 sample, which had the thinnest chorion of all cysts examined in this study (i.e. 1.2 μm), showed an excellent hatching percentage, the highest among all batches collected (97.8%). Therefore, the hypothesis of having poorly developed or "immature cysts" may be safely ruled out.

There was no correlation found between diameters of untreated and decapsulated cysts (Table 2) as it was expected.

The mean length for instar-I nauplii ranged from 466.3 to 505 μm . No significant correlation between the naupliar length and the cyst diameter (decapsulated or not) was found and no grouping of naupliar length according to the geographical origin of the samples was observed. This is in accordance with the findings of Vanhaecke and Sorgeloos (1980) who found that cyst diameter had the lowest correlation value with naupliar length. Comparisons with other *Artemia* strains revealed that the average size of instar-I nauplii from Urmia Lake is larger than that of *A. franciscana* which is mainly used in aquaculture (Vanhaecke and Sorgeloos, 1980). Therefore, *A. urmiana* nauplii are suitable for use with predators where *Artemia* size is not critical (i.e. freshwater fish species), but are a less valuable diet for marine fish and shrimp larvae, where can be used only in later developmental stages (Abatzopoulos et al., 1989).

The *A. urmiana* population grown from Urmia 2 cysts showed high survival at elevated salinities (100, 140 and 180 g/l; see Fig. 2), while at low salinities (35 and 50 g/l) mortality was high (data not shown). No statistical difference in survival was observed at the three salinity regimes and the regression line slope values for *A. urmiana* at different salinities ranged from -0.003 to -0.007 (Fig. 2). The survival of *A. urmiana* individuals at elevated salinities was higher compared with that of four Egyptian strains (three parthenogenetic and *A. salina*) (El-Bermawi et al., 2004).

Growth rate analysis of *A. urmiana* females and males (see *K* values in Fig. 3) based on Von Bertalanffy's equation revealed that males grow faster at 100 g/l, while females have similar growth at all salinities tested. It should also be noted that *A. urmiana* males grow faster than females in all salinities examined (see *K* values in Fig. 3). The comparison of *K* values of Egyptian *Artemia* strains (both sexual and asexual) (El-

Bermawi et al., 2004) with those of *A. urmiana* reveals similar growth rates at salinities above 120 g/l. However, unlike *A. urmiana*, the growth rate of the Egyptian strains was strongly affected by the increased salinity (El-Bermawi et al., 2004). Recorded maximum total lengths for males were smaller to those of females at all salinities. Total length values for *A. urmiana* males and females at 100, 140 and 180 g/l were very close to those reported by Baxevanis et al. (2005) for the same species.

There is a considerable literature information on morphometry, reproductive and life span characteristics of many bisexual and parthenogenetic *Artemia* populations (Browne et al., 1984, 1991; Triantaphyllidis et al., 1997a,b; Baxevanis et al., 2004; El-Bermawi et al., 2004). Most of these studies have contributed to the evaluation of genetic and environmental components of variance in sexual and 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* populations have been poorly examined. It appears from Table 3 that the increase of salinity from 100 to 180 g/l did not produce any noticeable effect on the reproductive and life span characteristics of *A. urmiana* (Table 3). On the contrary, salinity values above 120–140 g/l were shown to induce a negative impact on many other *Artemia* species or strains (Vanhaecke et al., 1984; Triantaphyllidis et al., 1995; Browne and Wanigasekera, 2000; Baxevanis and Abatzopoulos, 2004; Baxevanis et al., 2004). Previous laboratory investigations on several *Artemia* species showed that the optimal range of salinity lies between 60 and 150 g/l (Browne et al., 1991; Triantaphyllidis et al., 1995; Baxevanis et al., 2004). However, *A. urmiana* showed better reproductive performance in culture media of high salinity (close to 180 g/l) (see Table 3). This can be explained by a prolonged reproductive period at 140 and 180 g/l as opposed to that at 100 g/l. Although there was no significant difference in the percentages of encysted embryos produced at the three salinities (Table 3), it seems that there is a tendency for encystment to increase with salinity (Table 3). Baxevanis et al. (2004) observed that the salinity increase was positively correlated with the number of produced encysted embryos. Our experiments confirm an earlier finding (Van Stappen et al., 2001) that *A. urmiana* reproduces predominantly through oviparity. It is worth noting that the *A. urmiana* population displayed high percentages of encysted offspring, which makes it attractive for future commercial exploitation.

Survival, growth, reproductive and life span characteristics of *A. urmiana* were not significantly affected by the lakewater salinity increase observed during recent years. High salinities were measured in Urmia Lake. Salinity fluctuated between 150 and 180 g/l for the period ranging from July 1994 to January 1996, exceeded 200 g/l during the winter of 1997/98 and reached 240 g/l in autumn 1999 (Van Stappen et al., 2001). Furthermore, in past years low precipitations resulted in salinities well above 280 g/l (September 2004, personal observation). It is remarkable that even at salinities as high as 300 g/l, the *A. urmiana* population was thriving.

Azari Takami (1993) describing *Artemia* cysts from Urmia Lake mentioned that during late autumn, cysts in the areas of Khantakhti (close to site 1) and Saraf Khaneh (close to site 6) (see Fig. 1) were found near the bottom or in the water column, while in Golman Khaneh (close to site 7), cysts could be collected within only a few meters from the coast by stirring the bottom sediments. Van Stappen et al. (2001) found that there was a significant amount of suspended *A. urmiana* cysts in the water column during their Urmia Lake field study. In this study, very high percentages of cysts (from 60% to 90%) had no floating capacity even after 72 h in 200 g/l medium (with the exception of the Urmia 1 sample for which microscopic analysis revealed that it contained a considerable amount of empty and cracked cysts). The fact that a high proportion of Urmia Lake cysts do not float and the need to exploit Urmia Lake, deserve further study. The following points may be addressed i) determine the salinity range allowing cysts to float, ii) evaluate the sunk proportion of cysts in Urmia Lake, iii) optimise the sampling techniques for removing suspended cysts in the water column and iv) enhance existing protocols to achieve better diapause deactivation. As to the latter point, simple dehydration protocol proved to be inappropriate or even detrimental to Urmia cysts (unpublished data). Cold storage treatment may be more effective as shown in the case of the sinking Mono Lake cysts (Drinkwater and Clegg, 1991).

It would be interesting to examine cyst samples collected from sediments of Urmia Lake and compare the results with the laboratory observations.

Observations on the floating capacity of *A. urmiana* cysts led to a more detailed study of their chorion structure through TEM. A probable explanation for the floating capacity of *A. urmiana* cysts stands in its alveolae shape and volume (Fig. 5) (alveolae contain air that allows the cysts to float). The presence of

narrower and more compressed alveolae in *A. urmiana* cysts compared with those of GSL strains (*A. franciscana*) may result in a poor capability of floating. The protein synthesis of the hatching membranes surrounding the encysted embryo has shown unexpected variation among *Artemia* species (Abatzopoulos et al., 1997). In some cases, this can be used to determine the origin of cyst samples. For instance, *A. urmiana* presented markedly different banding patterns compared with the *A. franciscana* group, although obvious resemblance existed with the parthenogenetic populations (Abatzopoulos et al., 1997).

Fatty acid content of instar-I nauplii is an important trait of *Artemia* strains used in aquaculture. Linolenic acid (18:3n-3) of *A. urmiana* instar-I nauplii showed values in the range of 32.7 to 54.7 mg/g DW (Table 4). These values were similar to or even higher than those recorded for other *Artemia* strains (28.6–40 mg/g DW for GSL, 3.6–39.3 mg/g DW for Chinese *Artemia* strains, 16.7 mg/g DW for *A. persimilis*, only traces for *A. tibetiana*) (Dhont and Sorgeloos, 2002, and references therein). Eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids in *A. urmiana* varied from 1.8 to 7.2 and from 0.3 to 0.4 mg/g DW, respectively (Table 4). The recorded values are close to those of other *Artemia* species and strains, with the exception of *A. tibetiana* which has EPA concentrations of up to 44.7 mg/g DW (Han et al., 1999).

The relationship between fatty acid composition of *Artemia* nauplii used for aquaculture purposes and the successful culture of several fish and crustacean species is well documented (for more details, see Dhont and Sorgeloos, 2002, and references therein). In particular, the levels of eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids as well as their ratio (DHA/EPA) play a crucial role in aquaculture applications for the formation of biological membranes, growth, stress resistance and pigmentation in cultured marine organisms (Mourente et al., 1993; Lavens et al., 1995). As in other natural *Artemia* populations, a high ratio of DHA/EPA is not found in *A. urmiana* (Triantaphyllidis et al., 1995). Therefore, special enrichment formulations had to be developed (Dhont and Sorgeloos, 2002).

5. Conclusions

Most of Urmia cyst samples presented poor hatching. Special attention should be paid during cyst processing since dehydration has a negative impact on cyst

hatching quality. *A. urmiana* cysts are among the largest recorded for bisexual *Artemia* species (with the only exception those of *A. tibetiana*) showing considerable similarities to the cyst size of some parthenogenetic populations from China, India, Italy and Greece. Their diameter was highly variable. Although this variability could be expected, it may, also, serve as a strong indication of co-existing bisexual and parthenogenetic *Artemia* populations in Urmia Lake. Also, considerable variability was observed in cyst chorion thickness resulting from unknown factors.

A. urmiana nauplii are suitable for use with predators where live feed size is not critical. Certainly, they can be used as live feed for later developmental stages of cultured species. Their moderate HUFA profile can be manipulated by utilising appropriate enrichment emulsions.

A. urmiana shows better reproductive performance in a high salinity and it can be considered a very efficient osmoregulator within the *Artemia* genus. It propagates by producing mainly encysted embryos, which makes it attractive for future commercial exploitation. However, its cysts are not easily harvestable since either they sink to the bottom or remain in suspension even at very high salinity brines. This could be attributed to the chorion structure specificity of *A. urmiana*.

Acknowledgements

This work has been supported by EU project ICA4-CT-2001-10020 (INCO) on *Artemia* biodiversity. The help of Nadia Fahmi with the English text is highly appreciated. The critical reading of the manuscript by Dr Ilias Kappas is also acknowledged.

References

- Abatzopoulos, T.J., Kastritsis, C.D., Triantaphyllidis, C.D., 1986. A study of karyotypes and heterochromatic associations in *Artemia*, with special reference to two N. Greek populations. *Genetica* 71, 3–10.
- Abatzopoulos, Th.J., Triantaphyllidis, C.D., Kastritsis, C.D., 1987. Preliminary studies on some *Artemia* populations from Northern Greece. In: Sorgeloos, P., Bengtson, D.A., Declair, W., Jaspers, E. (Eds.), *Artemia* Research and its Applications, vol. 1. Universa Press, Wetteren, pp. 107–114.
- Abatzopoulos, T., Karamanlidis, G., Léger, P., Sorgeloos, P., 1989. Further characterization of two *Artemia* populations from Northern Greece: biometry, hatching characteristics, caloric content and fatty acid profiles. *Hydrobiologia* 179, 211–222.
- Abatzopoulos, T.J., Triantaphyllidis, C.D., Kastritsis, C.D., 1993. Genetic polymorphism in two parthenogenetic *Artemia* populations from Northern Greece. *Hydrobiologia* 250, 73–80.
- Abatzopoulos, T.J., Triantaphyllidis, G.V., Beardmore, J.A., Sorgeloos, P., 1997. Cyst membrane protein composition as a

- discriminant character in the genus *Artemia*. (International Study on *Artemia* LV). J. Mar. Biol. Assoc. U.K. 77, 265–268.
- Abatzopoulos, T.J., Bo, Z., Sorgeloos, P., 1998. *Artemia tibetiana*: preliminary characterization of a new *Artemia* species found in Tibet (People's Republic of China). International Study on *Artemia*. LIX. Int. J. Salt Lake Res. 7, 41–44.
- Abatzopoulos, Th.J., Beardmore, J.A., Clegg, J.S., Sorgeloos, P., 2002a. *Artemia*: Basic and Applied Biology. Kluwer Academic Publishers, Dordrecht. 285 pp.
- Abatzopoulos, T.J., Kappas, I., Bossier, P., Sorgeloos, P., Beardmore, J.A., 2002b. Genetic characterization of *Artemia tibetiana* (Crustacea: Anostraca). Biol. J. Linn. Soc. 75, 333–344.
- Abatzopoulos, T.J., El-Bermawi, N., Vasdekis, C., Baxevanis, A.D., Sorgeloos, P., 2003. Effects of salinity and temperature on reproductive and life span characteristics of clonal *Artemia*. (International Study on *Artemia*, LXVI). Hydrobiologia 492, 191–199.
- Ahmadi, M.R., Leibovitz, H., Simpson, K.L., 1990. Characterization of Uromiah Lake *Artemia* (*Artemia uromiana*) by isoelectrofocusing of isozyme patterns. Comp. Biochem. Physiol. 95B, 115–118.
- Azari Takami, G., 1993. Uromiah Lake as a valuable source of *Artemia* for feeding sturgeon fry. J. Vet. Fac., Univ. Teheran. 47, 2–14.
- Barigozzi, C., 1988. The *Artemia* of Urmia Lake in Iran. Artemia Newsl. 7, 9.
- Barigozzi, C., Varotto, V., Baratelli, L., Giarrizzo, R., 1987. The *Artemia* of Urmia Lake (Iran): mode of reproduction and chromosome numbers. Atti. Accad. Lincei Rend. Fis. 81, 87–90.
- Baxevanis, A.D., Abatzopoulos, T.J., 2004. The phenotypic response of ME₂ (M. Embolon, Greece) *Artemia* clone to salinity and temperature. J. Biol. Res. 1, 107–114.
- Baxevanis, A.D., El-Bermawi, N., Abatzopoulos, T.J., Sorgeloos, P., 2004. Salinity effects on maturation, reproductive and life span characteristics of four Egyptian populations (International Study on *Artemia*, LXVIII). Hydrobiologia 513, 87–100.
- Baxevanis, A.D., Triantaphyllidis, G.V., Kappas, I., Triantaphyllidis, A., Triantaphyllidis, C.D., Abatzopoulos, T.J., 2005. Evolutionary assessment of *Artemia tibetiana* (Crustacea, Anostraca) based on morphometry and 16S rRNA RFLP analysis. J. Zool. Syst. Evol. Res. 43, 189–198.
- Browne, R.A., Wanigasekera, G., 2000. Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. J. Exp. Mar. Biol. Ecol. 244, 29–44.
- Browne, R.A., Sallee, S.E., Grosch, D.S., Segreti, W.O., Purser, S.M., 1984. Partitioning genetic and environmental components of reproduction and life-span in *Artemia*. Ecology 65, 949–960.
- Browne, R.A., Davis, L.E., Sallee, S.E., 1988. Effects of temperature and relative fitness of sexual and asexual brine shrimp *Artemia*. J. Exp. Mar. Biol. Ecol. 124, 1–20.
- Browne, R.A., Li, M., Wanigasekera, G., Simonek, S., Brownlee, D., Eiband, G., Cowan, J., 1991. Ecological, physiological and genetic divergence of sexual and asexual (diploid and polyploid) brine shrimp (*Artemia*). In: Menon, J. (Ed.), Advances in Ecology, vol. 1. Council of Research Integration, Trivandrum, India, pp. 41–52.
- Browne, R.A., Moller, V., Forbes, V.E., Depledge, M.H., 2002. Estimating genetic and environmental components of variance using sexual and clonal *Artemia*. J. Exp. Mar. Biol. Ecol. 267, 107–119.
- Clark, L.S., Bowen, S.T., 1976. The genetics of *Artemia salina*: VII. Reproductive isolation. J. Heredity 67, 385–388.
- Cole, G.A., Brown, R.J., 1967. The chemistry of *Artemia* habitats. Ecology 48, 858–861.
- Criel, G., 1992. Gametogenesis, fertilization and development in *Artemia* sp. (Crustacea: Anostraca). Thesis for the degree of “Geaggregeerde voor het Hoger Onderwijs”, University of Ghent, Belgium, 242 pp.
- D’Agostino, A.S., 1965. Comparative studies of *Artemia salina* (development and physiology). PhD thesis, New York University. 83 pp.
- Dhont, J., Sorgeloos, P., 2002. Applications of *Artemia*. In: Abatzopoulos, Th.J., Beardmore, J.A., Clegg, J.S., Sorgeloos, P. (Eds.), *Artemia*: Basic and Applied Biology. Kluwer Academic Publishers, Dordrecht, pp. 251–277.
- Drinkwater, L.E., Clegg, J.S., 1991. Experimental biology of cyst diapause. In: Browne, R.A., Sorgeloos, P., Trotman, C.N.A. (Eds.), *Artemia* Biology. CRC Press, Boca Raton, FL, USA, pp. 93–117.
- El-Bermawi, N., Baxevanis, A.D., Abatzopoulos, T.J., Van Stappen, G., Sorgeloos, P., 2004. Salinity effects on survival, growth and morphometry of four Egyptian *Artemia* populations (International Study on *Artemia*. LXVII). Hydrobiologia 523, 175–188.
- Gajardo, G., Crespo, J., Triantaphyllidis, A., Tzika, A., Baxevanis, A.D., Kappas, I., Abatzopoulos, T.J., 2004. Species identification of Chilean *Artemia* populations based on mitochondrial DNA RFLP analysis. J. Biogeogr. 31, 547–555.
- Games, P.A., Howell, J.F., 1976. Pairwise multiple comparison procedures with unequal *N*'s and/or variances: a Monte Carlo study. J. Educ. Stat. 1, 113–125.
- Günther, R.T., 1899. Contributions to the geography of Lake Urmia and its neighbourhood. Geogr. J. 14, 504–523.
- Han, K., Geurden, I., Sorgeloos, P., 1999. Enrichment of the nauplii of two *Artemia* species with docosahexaenoic acid. Crustac. Issues 12, 599–604.
- Kalle, K., 1971. Salinity. General introduction. In: Kinne, O. (Ed.), Marine Ecology. A Comprehensive, Integrated Treatise on Life in Oceans and Coastal Waters, Vol. 1: Part 2. Environmental Factors. Wiley-Interscience, New York, USA, pp. 683–688.
- Kappas, I., Abatzopoulos, T.J., Hoa, N.V., Sorgeloos, P., Beardmore, J. A., 2004. Genetic and reproductive differentiation of *Artemia franciscana* in a new environment. Mar. Biol. 146, 103–117.
- Karnovsky, M.J., 1965. A formaldehyde glutaraldehyde fixative of high molarity for use in electron microscopy. J. Cell Biol. 27, 137A.
- Lavens, P., Sorgeloos, P., 2000. The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. Aquaculture 181, 397–403.
- Lavens, P., Coutteau, P., Sorgeloos, P., 1995. Laboratory and field variation in HUFA enrichment of *Artemia* nauplii. In: Lavens, P., Jaspers, E., Roelants, I. (Eds.), Larvi'95—Book of Abstracts. Special Publication, vol. 24. European Aquaculture Society, Gent, pp. 137–140.
- Löffler, H., 1961. Beiträge zur Kenntnis der Iranischen Binnengewässer: II. Regional-limnologische Studie mit besonderer Berücksichtigung der Crustaceen-Fauna. Int. Rev. Hydrobiol. 46, 309–406.
- Mourente, G., Rodriguez, A., Sargent, J.R., 1993. Effects of dietary docosahexaenoic acid (DHA; 22:6n-3) on lipid and fatty acid composition and growth in gilthead sea bream (*Sparus aurata* L.) larvae during first feeding. Aquaculture 112, 79–98.
- Sokal, R.R., Rohlf, J.F., 1981. Biometry. W.H. Freeman and Company, San Francisco, CA, USA. 859 pp.
- Sorgeloos, P., Lavens, P., Leger, Ph., Tackaert, W., Versichele, D., 1986. Manual for the Culture and Use of Brine Shrimp *Artemia* in Aquaculture. Laboratory of Mariculture, State University of Ghent, Belgium. 319 pp.

- Sorgeloos, P., Dhert, P., Candreva, P., 2001. Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture* 200, 147–151.
- Triantaphyllidis, G.V., Abatzopoulos, T.J., Sandaltzopoulos, R.M., Stamou, G., Kastritsis, C.D., 1993. Characterization of two new *Artemia* populations from two solar saltworks of Lesbos island (Greece): biometry, hatching characteristics and fatty acid profile. *Int. J. Salt Lake Res.* 2, 59–68.
- Triantaphyllidis, G.V., Pilla, E., Thomas, K., Abatzopoulos, T.J., Beardmore, J.A., Sorgeloos, P., 1994. International study on *Artemia*: LII. Incubation of *Artemia* cyst samples at high temperature reveals mixed nature with *Artemia franciscana* cysts. *J. Exp. Mar. Biol. Ecol.* 183, 273–282.
- Triantaphyllidis, G.V., Pouloupoulou, K., Abatzopoulos, T.J., Pinto Perez, C.A., Sorgeloos, P., 1995. International study on *Artemia*: XLIX. Salinity effects on survival, maturity, growth, biometrics, reproductive and lifespan characteristics of a bisexual and a parthenogenetic population of *Artemia*. *Hydrobiologia* 302, 215–227.
- Triantaphyllidis, G.V., Abatzopoulos, T.J., Miasa, E., Sorgeloos, P., 1996. International study on *Artemia*: LVI. Characterization of two *Artemia* populations from Namibia and Madagascar: cytogenetics, biometry, hatching characteristics and fatty acid profiles. *Hydrobiologia* 335, 97–106.
- Triantaphyllidis, G.V., Criel, G.R.J., Abatzopoulos, T.J., Thomas, K., Peleman, J., Beardmore, J.A., Sorgeloos, P., 1997a. International study on *Artemia*: LVII. Morphological and molecular characters suggest conspecificity of all bisexual European and North African *Artemia* populations. *Mar. Biol.* 129, 477–487.
- Triantaphyllidis, G.V., Criel, G., Abatzopoulos, T.J., Sorgeloos, P., 1997b. International study on *Artemia*: LIII. Morphological study of *Artemia* with emphasis to Old World strains: I. Bisexual populations. *Hydrobiologia* 357, 139–153.
- Triantaphyllidis, G.V., Abatzopoulos, T.J., Sorgeloos, P., 1998. Review of the biogeography of the genus *Artemia* (Crustacea, Anostraca). *J. Biogeogr.* 25, 213–226.
- Vanhaecke, P., Sorgeloos, P., 1980. International study on *Artemia*: IV. The biometrics of *Artemia* strains from different geographical origin. In: Persoone, G., Sorgeloos, P., Roels, O., Jaspers, E. (Eds.), *The Brine Shrimp Artemia*: Vol. 3. Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, Belgium, pp. 393–405.
- Vanhaecke, P., Tackaert, W., Sorgeloos, P., 1984. International study on *Artemia*: XXXII. Combined effects of temperature and salinity on the survival of *Artemia* of various geographical origin. *J. Exp. Mar. Biol. Ecol.* 80, 259–275.
- Van Stappen, G., 2002. Zoogeography. In: Abatzopoulos, Th.J., Beardmore, J.A., Clegg, J.S., Sorgeloos, P. (Eds.), *Artemia: Basic and Applied Biology*. Kluwer Academic Publishers, Dordrecht, pp. 171–224.
- Van Stappen, G., Fayazi, G., Sorgeloos, P., 2001. International study on *Artemia*: LXIII. Field study of *Artemia urmiana* (Günther, 1890) population in Lake Urmiah, Iran. *Hydrobiologia* 466, 133–143.