Biometrical Comparison of *Artemia urmiana* Günther, 1899 (Crustacea: Anostraca) Cysts between Rainy and Drought Years (1994-2003/4) from Urmia Lake, Iran

A. Asem, N. Rastegar-Pouyani, P. De Los Rios, R. Manaffar and F. Mohebbi

***Abstract*—**Nowadays, biometrical characterizations of *Artemia* cysts are used as one of the most important factors in the study of *Artemia* populations and intraspecific particularity; meanwhile these characters can be used as economical indices. For example, typically high hatching efficiency is possible due to the small diameter of cysts (high number per gram); therefore small diameter of cysts show someway high quality of cysts. This study was performed during a ten year period, including two different ecological conditions: rainy and drought. It is important from two different aspects because it covers alteration of *A. urmiana* during ten years also its variation in the best and worst environmental situations in which salinity increased from 173.8 ppt in 1994 to 280.8 ppt in 2003/4. In this study the biometrical raw data of *Artemia urmiana* cysts at seven stations from the Urmia Lake in 1994 and their seven identical locations at 26 studied stations in 2003/4 were reanalyzed again and compared together. Biometrical comparison of untreated and decapsulated cysts in each of the seven similar stations showed a highly significant variation between 1994 and 2003/4. Based on this study, in whole stations the untreated and decapsulated cysts from 1994 were larger than cysts of 2003/4 without any exception. But there was no logical relationship between salinity and chorion thickness in the Urmia Lake. With regard to PCA analyses the stations of two different studied years certainly have been separated with factor 1 from each other. In conclusion, the interaction between genetic and environmental factors can determine and explain variation in the range of cysts diameter in *Artemia*.

***Keywords*—** *Artemia urmiana*, Biometry, Cyst, Urmia Lake

I. INTRODUCTION

THE different species and populations of the Anostracan *Artemia* located in tropical and temporal regions in the salt water in five continents of the world [1]-[2]. Up to now more than 500 sites have been recoded as *Artemia* habitats in all over the world [2]. The brine shrimp can be found alive in high range salinity variations, between 45 ppt to supersaturate as high as 340 ppt [3]-[4].

More than one thousand years ago, in 982 an unknown Iranian geographer reported for the first time the presence of *Artemia* as Brine Worm from Urmia Lake at his unique geography book: *The Limits of the World from the East to the West* [5]. Günther during his grand tour accentuated that this species belonged to the genus *Artemia* (see [6]: page 509). After that, Günther denominated this species as *Artemia urmiana* (see [7]: page 395). Clark and Bowen [8] have confirmed its biological species concept because they proved that there is reproductive isolation between *A. urmiana*, *A. salina* and *A. franciscana* under laboratory conditions.

The Urmia Lake is one of the largest permanent hyper-saline lakes in the world [9]-[10]. The total surface area ranges between 4000 km^2 and 6000 km^2 depending on evaporation and water influx [10]. Up to now, the Urmia Lake has sustained different ecological situations. Figure 1 shows the surface level of the Urmia Lake fluctuations from 1965 to 2007. This diagram draws the variation of ecological conditions in this lake. Salinity directly depends on the surface level than other factors such as food availability and Oxygen will be changed with salinity alterations.

The genus *Artemia* shows a wild variation of ecological effects on its biological processes. The laboratory examinations can prove the effect of salinity and temperature on survival, growth and reproductive characters of *Artemia* [11]-[12]-[13]-[13]-[15]-[16]. as well as, ionic composition of the habitat can produce ecological isolation and can lead to morphological and biometrical variations [17]-[18]-[1]. All these evidences show that ecological speciation is the main mechanism in *Artemia* speciation.

Artemia urmiana Günther, 1899 can be a useful taxon of genus *Artemia* for intra-species variation studies. This species is endemic to Urmia Lake. This is a large saline lake and different studies have demonstrated its intra-specific variation with genetic and morphologic characterizations [19]-[20].

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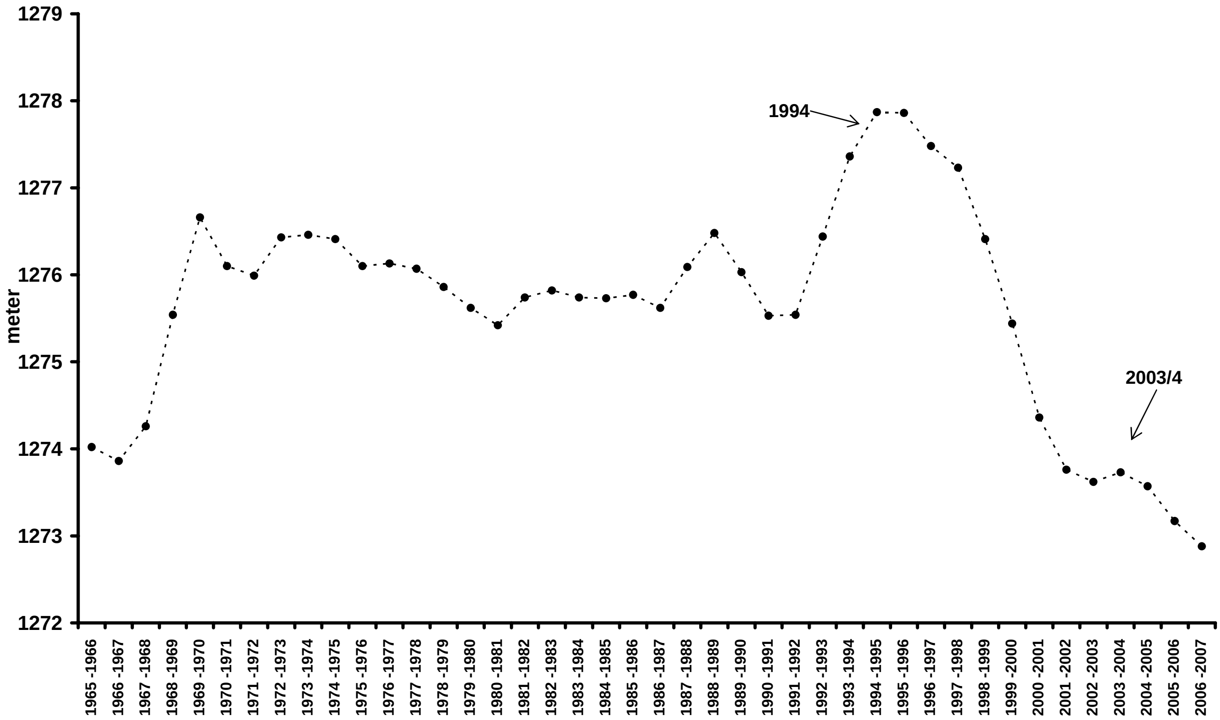

Fig. 1 Annual variation of the surface elevation of south arm of the Urmia Lake (1965-2007)

The aim of this review was to determine the ecological effects on biometrical alteration of *A. urmiana* cysts from the Urmia Lake with rainy and drought environmental conditions during a decade in 1994 and 2003/4. This review can make a manifest the relationship between ecological situations and biometrical variation of *Artemia* cysts.

II. Material and Methods

A. Biometrical data

In this study, the biometrical raw data of *Artemia urmiana* cysts have been reanalyzed and compared at seven stations in 1994 [21] and their identical locations in 2003/4 [22] from the Urmia Lake. Figure 2 shows the seven stations from Urmia Lake.

B. Statistical analyses

One-way ANOVA (Tukey), T-test and Principal Component Analysis (PCA) and also Discriminant Function Analyses (DA) were used. Also, the SPSS 13 was employed to run all statistical analyses.

The statistical analysis was practiced for study of significant interaction between years and stations. With regard to One-way ANOVA (Tukey), biometrical variation of cyst chatacters (diameter of untreated and decapsulated cysts) among different stations were studied in each year. Then significant differences were analyzed between two years in each station by T-test. Finally total means of three characters were calculated from seven stations for whichever year and these data were analyzed via T-test.

According to value of the three traits, the PCA was performed to classify rainy (1994) and drought (2003/4) years stations.

III. Results

Table 1 shows the biometrical data of *A. urmiana* cysts at seven stations at two different years, 1994 and 2003/4 from Urmia Lake. It is possible to find the following results, the capsulated cyst diameter varied between 262.7 ± 15.2 and 286.6 ± 14.3 µm in 1994, whereas for the data collected in 2003/2004 the diameter varied between 248.32 ± 11.56 and 259.34 ± 11.36 µm. The decapsulated cyst diameter varied between 258.6 ± 17.4 and 274.4 ± 18.4 µm in 1994, whereas it varied between 249.50 ± 11.48 and 274.4 ± 18.4 µm in 2003/2004. The thickest and the thinnest chorions varied between 1.2 to 9.3 µm in 1994, whereas it varied between 3.3 to 9.3 µm in 2003/4.

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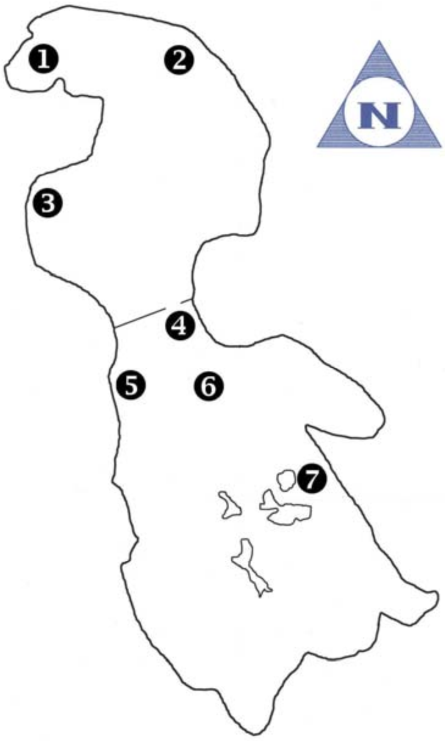

Fig. 2 Geographical stations of the studied area from the Urmia Lake.

The results of ANOVA denoted an interaction between years and stations that contained biometrical significant differences (years*stations, p<0.000) in variety of untreated and decapsulated cyst. In this context, the respective Tukey multiple comparison test showed that biometry of untreated and decapsulated cysts have significant differences among stations during one year (Table 2; p<0.05). The biometrical comparison of untreated and decapsulated cysts in each seven similar stations between 1994 and 2003/4 showed high significant variation (T-test, p<0.000). As it is shown, in whole stations, the untreated and decapsulated cysts were collected in 1994 were bigger than the cysts of 2003/4 broadside. The total means for untreated and decapsulated characters were calculated from stations of each year suggested a widely expressive variety between 1994 and 2003/4 (T-test, p<0.000) and the results indicated afresh that the biggest untreated and decapsulated cysts from the Urmia Lake belonged to 1994 whereas the total mean of chorion thickness from seven stations didn't show any significance difference between 1994 and 2003/4 (T-test, p>0.05). Figure

TABLE I
BIOMETRICAL DATA FOR THREE CHARACTERS OF *A. URMIANA* CYSTS FROM SEVEN STATIONS IN RAINY (1994) AND DROUGHT (2003-2004) YEARS IN THE URMIA LAKE (SAME LETTER IN INDIVIDUALLY ROW SHOWS SIGNIFICANT FOR EACH CHARACTERS BETWEEN DIFFERENT YEARS, P<0.0000)

Station	Untreated cyst (µm)		
	1994	2003/4	Sig.
1	262.7±15.2	249.8±12.1	p<0.000
2	286.5±12.3	248.3±11.5	p<0.000
3	276.7±18.3	259.3±11.3	p<0.000
4	286.6±14.3	253.5±13.9	p<0.000
5	273.4±16.6	253.2±11.9	p<0.000
6	280.8±19.5	256.1±13.1	p<0.000
7	274.5±14.9	254.9±16.6	p<0.000
Total mean	**277.3±8.3**	**253.6±3.7**	**p<0.000**
Station	Decapsulated cyst (µm)		
	1994	2003/4	Sig.
1	258.6±17.4	231.2±10.4	p<0.000
2	267.8±15.7	231.6±11.8	p<0.000
3	274.4±18.4	245.5±10.6	p<0.000
4	273.9±16.6	238.4±11.1	p<0.000
5	266.9±15.1	246.7±12.1	p<0.000
6	264.3±14.1	249.5±11.4	p<0.000
7	270.3±14.8	246.7±11.9	p<0.000
Total mean	**268.0±5.5**	**241.4±7.5**	**p<0.000**
Station	Chorion thickness[a] (µm)		
	1994	2003/4	Sig.
1	2.1	9.26	-[b]
2	9.3	8.33	-
3	1.2	6.88	-
4	6.4	7.52	-
5	3.2	3.25	-
6	8.3	3.33	-
7	2.2	4.11	-
Total mean	**4.67±3.2**	**6.10±2.4**	**p>0.05**

a) This value is presented without standard deviation and b) according to its calculation method; there is no statistical analyses for Chorion thickness in each unless for total mean.

3 shows total means of three characters with maximum and minimum for 1994 and 2003/4. Maximum and minimum of untreated and decapsulated cysts didn't overlap between 1994 and 2003/4 but variations of chorion thickness covered each other in two different periods (Fig. 3). The Figure 4 delineates aggregation of stations with regard to the diameter of untreated cysts, decapsulated cysts and chorion thickness from the Urmia Lake by PCA.

TABLE II
STATISTICAL COMPARISON (ANOVA, P<0.05) OF THE UNTREATED AND DECAPSULATED CYSTS OF *A. URMIANA* FROM SEVEN STATIONS IN THE URMIA LAKE (SYMBOL + SHOWS SIGNIFICANT DIFFERENCES BETWEEN MEANS)

Cyst sample in 1994													
Untreated Cyst							Decapsulatied Cyst						
Station	1	2	3	4	5	6	Station	1	2	3	4	5	6
7	+	+	-	+	-	-	7	+	-	-	-	-	-
6	+	-	-	-	+		6	-	-	+	+	-	
5	+	+	-	+			5	+	-	+	+		
4	+	-	+				4	+	-	+			
3	+	+					3	+	-				
2	+						2	+					
Cyst sample in 2003-2004													
Untreated Cyst							Decapsulatied Cyst						
Station	1	2	3	4	5	6	Station	1	2	3	4	5	6
7	-	+	-	-	-	-	7	+	+	-	+	-	-
6	+	+	-	-	-		6	+	+	-	+	-	
5	-	-	+	-			5	+	+	-	+		
4	-	-	+				4	+	+	+			
3	+	+					3	+	+				
2	-						2	-					

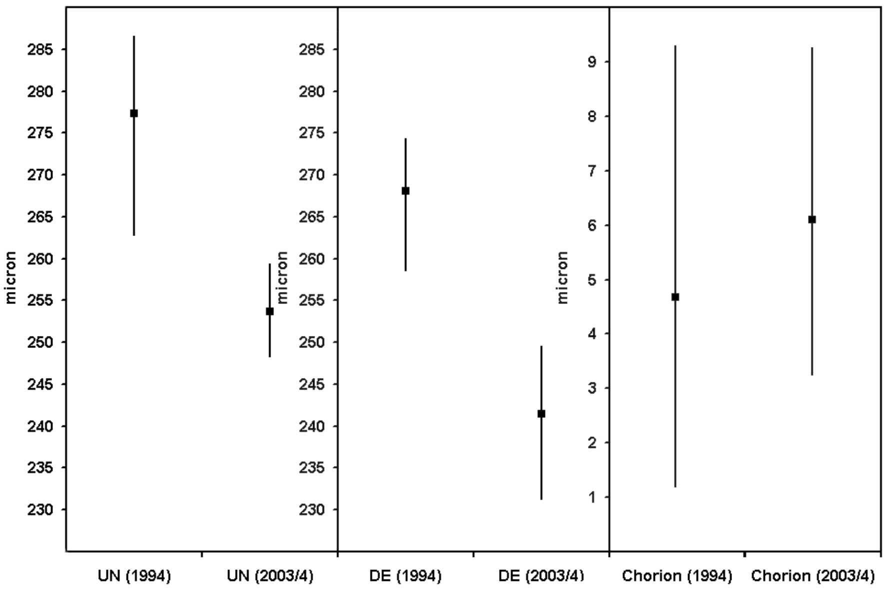

Fig. 3 Total means of three biometrical characters of cysts and their maximum and minimum from seven stations in 1994 and 2003/4. (UN: untreated cyst, DC: decapsulated cyst, CH: Chorion thickness)

IV. Discussion

A comparative study on biometrical variation of *Artemia* populations' cyst from different locations in the world could classify *Artemia* populations into three groups: I) the smallest cysts from the Adelaid population, San Francisco Bay area, Macau and Barotac Nuevo. II) The biggest size belonged to parthenogenetic populations from China, France, Italy and India. III) *A. franciscana* from Chaplin Lake and Great Salt Lake with medium cyst size and also very thin chorion [23]. The study of fourteen different populations of *A. franciscana* had shown a biometrical variation between 217 µm and 230 µm, with the exception of the Great Salt Lake (Utah, USA) and Galera Zamba (Colombia) cysts, with 242 µm and 245 µm [24]. Zhenqiu et al, [25] studied biometrical characterizations of parthenogenetic population of *Artemia* cysts from Xinjiana Uighur and Shandong. They confirmed that cysts diameter of parthenogenetic populations were larger than bisexual species. Triantaphyllidis et al, [26] studied characterization of two parthenogenetic populations from Namibia and Madagascar. Their results have shown that the biometry of untreated and decapsulated cysts (246.7 µm and 233.1 µm) from Namibia were significantly smaller than population from Madagascar (258.9 µm and 246.2 µm). These variations have been attributed to the ploidy level; because the population from Namibia was mainly diploid (2n= 42) but the other population from Madagascar was found to be triploid (3n= 63). These cysts are smaller than Vanhaecke and Sorgeloos [23] reports for parthenogenetic populations (284.9 µm for the Margheritic di Savoia population; Italy and 283 µm for the Toticorin population from India). Another study about parthenogenetic populations from Urmia Lake basin (costal lagoon and inside of the lake) have demonstrated that parthenogenetic populations' cyst have smaller diameter than bisexual species [27]. Cohen et al, [28] found a diameter ranging between 246.1±21µm and 230.3±1µm from four Argentinean *Artemia* populations. *A. tibetiana* has the biggest cyst ever recorded for both bisexual and parthenogenetic species (sample B: 323 ± 11.2 µm and sample C: 330 ± 14.6 µm) [29].

Comparison of untreated cysts for *A. urmiana*, *A. sinica* and *Artemia* sp. showed significant differences among the three populations (p<0.001) so cysts of *A. urmiana* are bigger (265.82±15.85) µm than two other spices: *Artemia* sp. and *A. sinica*: 230.05 ± 15.14 µm and 232.75 ± 11.22 µm [30].

Several studies have suggested the existence of biometrical variation of cysts between species of *Artemia* and also different populations of the same species in several locations. For example *Artemia franciscana* shows a variation from 220.5 to 241 µm for fully cyst and 5.4 to 7.9 µm for chorion thickness from Chile and also 200.4 to 292.3 µm for untreated cyst and 2.11 to 10.78 µm for chorion thickness from Mexico [31]. But comparing of diverse reports exhibit that cyst characterizations of *A. urmiana* has the widest range in its endemic habitat, the Urmia Lake:

Pilla and Beardmore [30] have reported 265.82±15.85 µm for untreated cysts of *A. urmiana*.

In one of the reports *A. urmiana* cysts were studied in three stations from the Urmia Lake. The results showed that the diameter of the untreated cysts ranged from 249.8 to 280.7 µm, decapsulated cysts from 218.4 to 259.8 µm and the thickness of chorion ranged from 2.7 to 15.6 µm [32].

The other study has been done in 1994 by Abatzopoulos et al, [21] . In this report *A. urmiana* cysts were harvested from seven stations in the Urmia Lake. The diameter of the untreated cysts ranged from 262.7 to 286.6 µm, decapsulated cysts from 258.6 to 274.4 µm and the thickest chorion ranged from 1.2 to 9.3 µm [21].

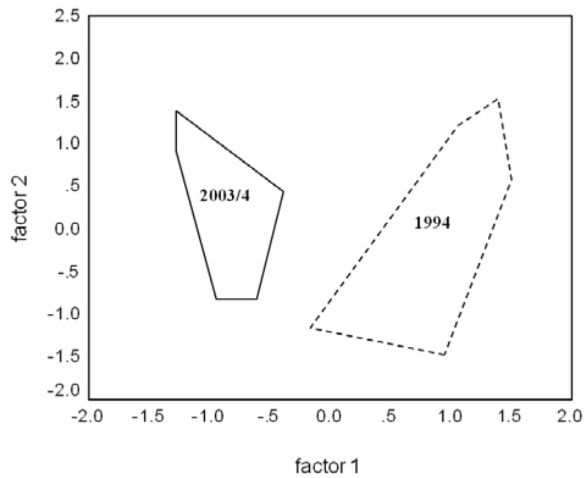

Fig. 4 Principal Component Analysis for different stations in 1994 and 2003/4

According to the last study, the diameter of the untreated cysts ranged from 247.6 to 259.3 µm, decapsulated cysts from 231.2 to 251.6 µm and the thickness of chorion ranged from 1.3 to 9.3 µm [22].

This study was carried out during a decade that also contained two different ecological conditions: rainy and drought. It is important from two different aspects because it covered alteration of *A. urmiana* during ten years also its variation in the best and the worst environmental situations for *A. urmiana* in which the salinity increased from 173.8 ± 1.1 ppt in 1994 [33] to 208.8 ± 6.7 ppt in 2003/4 [22].

According to statistical analyses, diameter of cysts and its decapsulated form have a wide variation between different stations in each year as well as in the rainy years (1994), the diameter of untreated and decapsulated cysts have significant variation among 13 and 9 pair stations respectively. In addition within the dry period (2003/4) the size of untreated and decapsulated cysts showed significant differences among 7 and 14 pair means respectively. These results express that through the time of rainy (1994) year the variation of untreated cysts was higher than its decapsulated form and conversely for the time of drought (2003/4) years the diameter of decapsulated cysts were more variant than its untreated cysts. This recent result can lead us to keep an economical management for exploitation in great *Artemia* habitats, so that biometrical characters of cysts may be considered in different stations of large habitats of *Artemia*. The total means of cyst size from seven stations for both rainy and drought years reflected that the biometry of untreated and decapsulated cysts decreased with salinity rise. Considering that in 1994 the total size of untreated cysts diminished from 277.33 ± 8.3 µm to 253.64 ± 3.72 µm in 2003/4, on the other hand, its decapsulated diameter decreases from 268.03 ± 5.54 µm to 241.43 ± 7.59 µm. Nevertheless, the total mean of chorion thickness didn't show any significant difference between rainy and drought periods. Firstly the thickness of chorion in seven stations of each period (1994 and 2003/4) indicated a high variation. In 1994, the maximum and minimum thickness of chorion were 9.26 µm and 3.25 µm respectively [21]; meanwhile in 2003/4 its maximum value was 9.3 µm and minimum was 1.9 µm [22]. Comparison of the results of chorion thickness between 1994 and 2003/4 offered an unclear and perplexing issue. Station 1, during the rainy year had chorion thickness of 9.26 µm and in the drought year its thickness decreases to 2.1 µm (Tab. 1), it means in this station the thickness of chorion decreased with salinity increasing. But there is a reverse situation in station 6 as in the rainy year, the chorion thickness was reported 8.3 µm and in the other times it was reported 3.33 µm. Therefore, salinity rising about 100 ppt in 2003/4 caused reduction of chorion thickness. Nevertheless station 5 didn't show any sensible difference in ten years when salinity had risen 100 ppt so its thickness was 3.2 µm in 1994 and 3.25 µm in 2003/4. The above discussion imply however the total mean of chorion thickness doesn't reflect significant difference between 1994 and 2003/4 but there is no reasonable relationship between salinity and its thickness so its pattern of variation is a puzzle at least under this conditions in the Urmia Lake.

According to result of the PCA analyses, the stations of each year certainly were separated in two different groups (Fig 4). This shows biometrical characters have different patterns in 1994 and 2003/4. Two different years certainly have been separated with factor 1 from each other. The first and second components show 63.21% and 36.78% of the total variation respectively and in total, 100% of variation is presented in the first two components. According to the PCA, size of the untreated cysts (0.999) and decapsulated cysts (0.943) having the most role in differentiation of years but the chorion thickness (0.098) doesn't have any influential effect in this sequestration. In the DA, 100% of original grouped cases were correctly classified.

With regard to the above-mentioned results one could say that the ecological optimum conditions in the rainy time (1994) caused to produce untreated and decapsulated cysts with bigger size than the drought year (2003/4). The size of decapsulated cysts directly and its untreated cysts size indirectly depend on the embryo dimensions in ovisac. As well Abatzopoulos et al, [21] have concluded that the diameter of cysts can be attributed to seasonal fluctuations in physico-chemical parameters and food availability in different regions of the Urmia Lake. In hyper-saline ecosystems, the change of salinity can be a main factor that affects primary and secondary productivity, so the production decreases with rising salinity in such an ecosystem. Therefore, the salinity can influence *Artemia* biology via two ways: firstly it can limit its physiological process and secondly by effectuate food limitations. For the reason can be decided in rainy period the Urmia Lake had the best ecological condition then the protected embryo grows in ovisac and could obtain big size therefore had caused *Artemia* to produce big untreated and decapsulated cysts but in dry spell (2003/4) the high salinity had created a hard environmental and physiological limitations and the brine shrimp *A. urmiana* couldn't improve its embryo growth.

Each taxon in comparison with its closely related taxa has special characters but some of them may be changed under different ecological situations. This problem is considerable about genus *Artemia*; a ten year period in the Urmia Lake contain the best and the worst ecological situations and during ten years environmental factors may impose widely variations in cyst biometry. For example biometric determination of *Artemia franciscana* cysts samples from six locations in the Colombian Caribbean and from San Francisco Bay showed a wide variation for biometrical characterizations of cyst among different sites; the small records were: 201 µm (untreated cyst), 183.4 µm (decapsulated cyst), 3.1 µm (chorion) and the big records were: 252.9 µm (untreated cyst), 234.2 µm (decapsulated cyst), 13.2 µm (chorion) [34]. Same characters such as biometry of *Artemia* cyst if used cautiously can reflect invaluable taxonomical and biosystematical information about intra-specific and inter-specific variations. Otherwise these characters can misguide biosystematical results.

Here, the concept of Hatching Efficiency (H.E) is criticizable because with regard to definition of H.E; Hatching Efficiency refers to the number of nauplii hatched per gram dry weight of cysts [35]-[22]. Conventionally high H.E is possible due to the small diameter of cysts (high number per gram), therefore small diameter of cysts show someway higher quality of cysts [22]. With regard to definition of H.E and diameter of cysts in 2003/4, these cysts that had been produced in an unsuitable ecological condition must have high quality of H.E potential but doubtlessly the nutritional value of cysts in the drought year (2003/4) is less than rainy year (1994). With this approach, rely upon size of cyst and Hatching Efficiency can't reflex correct illustration of cyst quality so it will be intellectual to use the nutrition value of individual cyst and nauplii, size of untreated and decapsulated cyst and Hatching Efficiency together.

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