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Preliminary characteristics (biometry, sexual dimorphism and fatty acid profile) of the brine shrimp *Artemia salina* (L., 1758**)** (Crustacea: Anostraca) from Sabkhet Boujmal, Tunisia

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Abstract

The brine shrimp *Artemia* is considered as an irreplaceable live food for the larval rearing of most marine fish and crustacean larva. In this work we report preliminary characteristics of a new population of *Artemia salina*, identified in Sabkhet Boujmal, (BJ, Tunisia). These strain characteristics included cysts and nauplii biometry, sexual dimorphism and fatty acids profile. The non-decapsulated (246.8 ± 13.1 µm) and decapsulated cysts (235.4 ± 10.5 µm) diameters and nauplius length (457.5 ± 30.2 µm) and chorion thickness (5.7 µm) were measured. Sexual dimorphism showed that the main morphological differences between males and females observed were the maximal distance between compound eyes, length of first antenna, width of third abdominal segment, total length, diameter of compound eyes and abdominal length. Fatty acid composition of *Artemia salina*'s decapsulated cysts collected from BJ revealed that only 46.61 % of the peaks were identified. The predominant fatty acids were the palmitic acid [C16:0] with 4.67%, palmitoleic acid [C16:1n-7] with 3.45 %, oleic acid [C18:1n-9] with 6.46 %, cis-vaccenic acid [C18:1n-7] with 3.60 % and eicosadienoic acid [C20:2n-6] with 10.10 %. Nevertheless, eicosapentaenoic acid and linolenic acid were found in the decapsulated cysts from BJ at about the same rate (3.29% and 2.83 %, respectively). Docosahexaenoic acid (DHA, 22:6n-3) as well as arachidonic acid (ARA, 20:4n-6) were found in lower quantities with 1.02 and 1.49 mg.g-1 dry weight, respectively.

Key words: *Artemia salina*, biological characteristics, Sabkhet Boujmal, Tunisia

Introduction

The brine shrimp *Artemia* are found in a variety of harsh environments in many parts of the world (Triantaphyllidis et al*.,* 1998; Van Stappen, 2002). The genus *Artemia* is a complex of bisexual and parthenogenetic species, and probably superspecies, which diverged about five to six million years ago from an ancestral form living in the Mediterranean area (Abreu-Grobois & Beardmore, 1982; Badaracco *et al.,* 1987). 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), differentiated on the basis of the criterions such as reproductive isolation (e.g. Abreu-Grobois, 1987; Browne & Bowen, 1991), biometrical and morphometrical variability (e.g. Hontoria & Amat, 1992; Triantaphyllidis et al., 1997a; Mura et al., 2005), genetic characterization (e.g. Triantaphyllidis et al., 1997b; Kapas et al., 2004; Tizol-Correa et al., 2009) and morphological differentiation according to the SEM observations (e.g. Mura & Nagorsskaya, 2005; Mura et al., 2005). All bisexual species are diploid while asexual populations may be diploid, polyploid or a mixture of different ploidies (Abatzopoulos et al*.*, 2003). Gajardo et al*.* (2002) indicated the existence of seven bisexual species: *Artemia salina, Artemia urmiana, Artemia sinica, Artemia* sp. (Kazakhstan), *Artemia tibetiana, Artemia persimilis,* and *Artemia franciscana.* Triantaphyllidis et al*.* (1997b), using morphological and molecular (allozyme and Amplified Fragment Length Polymorphism 'AFLP') characteristics determined that *A. tunisiana* and *A. salina* were the same species; hence, the binomen *A. salina* had priority over *A. tunisiana* (the Principle of Priority Code, Article 23). Furthermore, *Artemia salina* is now recognized as the only autochthon bisexual species located in the Mediterranean area. The presence of *Artemia* in Tunisia was first reported by Seurat (1921) and Gauthier (1928) in Chott Ariana and Sabkhet Sidi El Hani, respectively. Later, Ben Abdelkader (1985), Sorgeloos et al. (1986), Romdhane (1994), Triantaphyllidis et al. (1998) and Romdhane et al. (2001) announced the occurrence of *Artemia* populations in 10 other sites. Most recently Ben Naceur et al. (2009, 2010a) reported *Artemia* populations from 22 biotopes in Tunisia categorized such as temporal and ephemeral salt lakes, distributed from semi-arid to Saharan hydrogeographical zones.

Artemia have been widely used as a model organism for biochemical, physiological, genetic and ecological studies with more than 5000 published papers (McCourt & Lavens, 1985). However, the most common feature of *Artemia* in the literature is for their importance in aquaculture. In fact, the brine shrimp *Artemia* is of considerable economic importance in fish and shellfish larviculture (Bengtson *et al.,* 1991). The use of *Artemia* nauplii is well established due to its many advantages: year-round availability as on-the-shelf cysts, good nutritional value for some fish and relatively easy improvement through simple enrichment techniques (Léger et al., 1986). Annually, more than 1,500 metric tonnes of dry *Artemia* cysts are marketed worldwide to feed fish and shellfish (Dhont & Sorgeloos, 2002). During the last 25 years, the Great Salt Lake (GSL) has been the premier supplier of *Artemia* cysts to the world aquaculture market and the subject of numerous speculations regarding its capacity to sustain a growing aquaculture industry (Lavens & Sorgeloos, 2000). The decline of *Artemia* cyst harvests from the Great Salt Lake (Utah, USA) since 1977 (Lavens & Sorgeloos, 2000) has intensified the search for alternative resources, especially in inland lakes that are sufficiently large and productive to justify commercial exploitation.

This study aims to provide a preliminary biological and biochemical characteristics of *Artemia* of Sabkhet Boujmal (Tunisia). The cysts and nauplii biometry, sexual dimorphism and fatty acids profile will be examined and compared to existing stocks.

Materials and methods

Site description:

Sabkhet Boujmal (BJ) is an inland site (34°57' N – 10°24' E) situated 40 km northwest of Sfax city (Fig 1). Its surface area is about 1100 ha. Its length

is about 6 km and its width is approximately 3 km. The most important source of water feeding the Sabkha is the El-Hallouf wadi. The maximum and the average depths of BJ are 1 m and 0.5 m, respectively. The site is generally filled by rain water from November to May, and dried up from June to October. The presence of *Artemia* in BJ was repoted for the first time by Ben Naceur *et al*. (2009). During our visit the site was dry and covered by salt layer, where cysts were harvested. After laboratory culture and based on laboratory morphometrical characteristics and SEM observation, *Artemia* from BJ was identified as *Artemia salina* species (unpublished data).

Cysts and nauplii biometry:

Cysts were sampled on the Sabkha banks and treated following the protocol described by Sorgeloos *et al*. (1986). One hundred cysts were hydrated during 2 h in 10 $g.l^{-1}$ sea water and fixed with 1% Lugol overnight to measure their diameter. Also, 100 cysts were decapsulated with hypochlorite according to the methodology described in Sorgeloos et al. (1986) and hydrated during 2 h in 10 $g.l^{-1}$ sea water and fixed within 1% Lugol overnight (Sorgeloos, 1997). Both diameter values were measured according to Sorgeloos (1997) with a microscope provided with calibrated micrometer eye pieces. Chorion thickness was calculated according to the methodology described in Sorgeloos (1997).

To analysis the biometrical characteristics of nauplii instar-I, cysts were hatched in natural seawater (32 ± 1 psu) at 28 ± 1 °C, under continuous illumination (2000 lux) and aeration, while the pH was maintained above 8,. The length of nauplii instar-I (n=100) was measured under a microscope equipped with a calibrated micrometer.

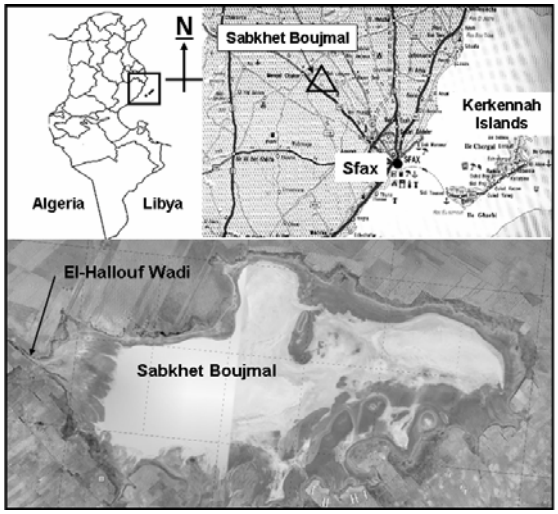

Figure 1. Location of the *Artemia salina* sampling site in Sabkhet Boujmal, Tunisia (34°57' N – 10°24' E).

Adult sexual dimorphism:

Cysts were hatched according to the method described by Sorgeloos et al. (1986). Nauplii obtained were transferred to 2 l cylindroconical plastic tubes, with 90 ± 10 g.l^{-1} filtered and autoclaved sea water plus crude sea salt (Amat et al., 2005). The temperature was maintained at 24°C under 16:8 h (light/dark) photoperiod. The animals were fed on the unicellular algae *Chlorella* sp (density: 100-200 10^3 cells.ml^{-1}). The medium was completely renewed twice a week with fresh microalgal culture. The animals were examined when most of them reached the adult stage (i.e. when offspring production observed). The animals were anaesthetized with few droplets of water saturated with chloroform and measured under a dissecting microscope provided with a calibrated micrometer eye pieces. The following biometric variables were examined on at least 20 individuals per sex: total length (tl), abdominal length (al), width of third abdominal segment (wts), length of furca (lf), number of setae inserted on left branch of the furca (nlf), number of setae inserted on right branch of the furca (nrf), width of head (wh), diameter for compound eyes (dy), maximal distance between compound eyes (dby) and length of first antenna (la), the abdomen length compared to the total body length ratio (ra, %) was also calculated for both sexes (Amat et al., 2005).

Fatty acids profile:

Prior to lipid extraction, cyst samples were hydrated in distilled water under strong aeration until cysts were observed to be completely spherical. They were then decapsulated with sodium hypochlorite (Sorgeloos et al., 1986). Lipid extractions and fatty acid analyses for decapsulated cysts were carried out as in Navarro et al. (1992a, b). Total lipids were extracted and stored in chloroform/methanol (ratio 2/1 v/v) with 0.01% butylated hydroxytoluene (BHT) (Sigma Chemical) as an antioxidant. Lipid aliquots were transmethylated overnight after the addition of nonad-ecaenoic fatty acid (19:0) (99% pure; Sigma Chemical) as an internal standard. Fatty acid methyl-lesters (FAMEs) were extracted with hexane/diethyl ether (ratio 1/1 v/v) and purified by thin-layer chromatography (silica gel G 60, Merck) using hexane/diethylether/acetic acid (ratio 85/15/1.5 by vol.) as the solvent system. Analyses of FAMEs were performed with a Fisons Instruments GC 8000 (Thermo Electron) gas chromatograph equipped with a fused silica 30 m×0.25 mm open tubular column (tracer, TR-WAX, film thickness: 0.25 µm; Teknokroma) and a cold on-column injection system, using helium as carrier and a 50 to 220°C thermal gradient. Peaks were recorded on a personal computer using the software Chrom-Card for Windows (Fisons CE Intstruments), and were identified by comparison with known standards.

Statistical analysis:

A frequency (%) and mean ± S.D. analysis technique with hydrated and decapsulated cysts as well as nauplii length were analysed using the statistical package SPSS for Windows version 10.0. Sexual dimorphism, between male and female individuals, was investigated by Principal Components Analysis (PCA) using XLSTAT-Pro 7.5 computer program.

Results

Cysts and nauplii biometry:

Size frequency of hydrated and decapsulated cysts diameters, and nauplii instar-I length are shown in Fig. 2. The principal frequency in hydrated cysts was 240-250µm with 38%, in decapsulated cysts (220-230 and 230-240µm) with 32 and 33%, respectively, and nauplii frequency (460-480 and 480-500µm) with 23 and 22%, respectively. The mean value ± S.D. for cysts, chorion thickness and naupliar length are shown in Table 1.

Adult sexual dimorphism:

Principal Component analysis (PCA) revealed two main directions of variation for adult *A. salina* from Sabkhet Boujmal (Table 2), with axis 1 explaining 33.97 % and axis 2 explaining an additional 23.57 % of the total variance, revealing that variables were most correlated with factor 1

(horizontal axis) than with factor 2 (vertical axis). Relatively to the first component (PCA axis 1), maximal distance between compound eyes (dby), length of first antenna (la), width of third abdominal segment (wts), total length (tl), diameter for compound eyes (dy) and abdominal length (al) are the most important variables for males and females discrimination with a total contribution of 90.77 %. Whereas, for the second component (PCA axis 2), width of head (wh), diameter of compound eyes (dy), total length (tl) and abdominal length (al), are the most important variables with a total contribution of 77.85 %. The ACP divided adult specimens in two evident groups (Fig 3). Positive loadings of maximal distance between compound eyes *(*dby*)*, length of first antenna (la) and diameter of compound eyes *(*dy*)* were observed in relation to the principal component 1, whereas width of third abdominal segment *(*wts*)*, total length *(*tl*)* and abdominal length *(*al*)* indicated negative loadings.

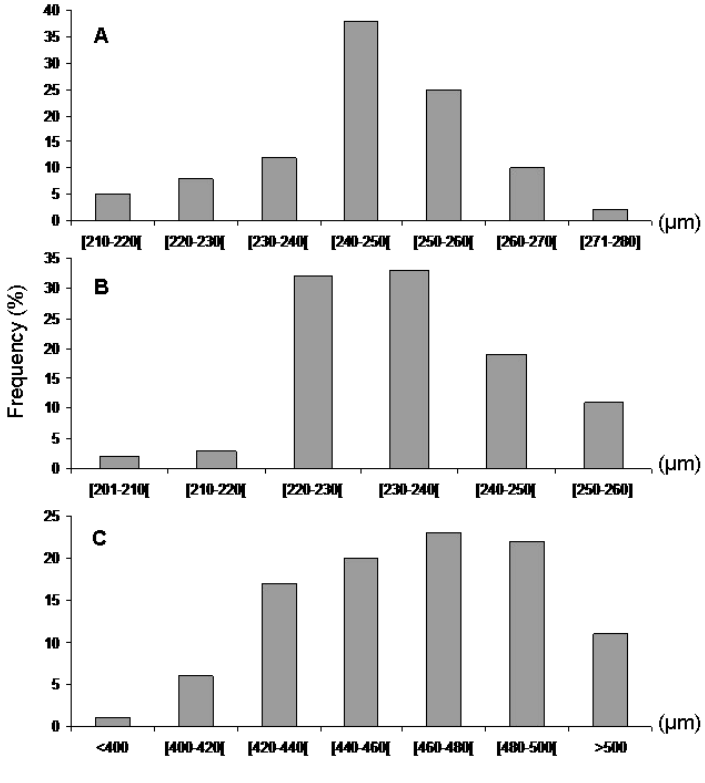

Figure 2. Size frequency distribution for *Artemia salina* from Sabkhet Boujmal (Tunisia)
A: hydrated non-decapsulated cysts; B: decapsulated cysts; C: nauplii.

Table 2. Contribution and portion of each morphometrical parameters used to the sexual dimorphism of Adults *Artemia salina* from Sabkhet Boujmal (Tunisia) in the two first components.

	Variable contribution		Component	
	F1	F2	F1	F2
Total length	13.673	15.428	-0.715	0.632
Abdominal length	11.096	12.297	-0.644	0.565
Width of 3th abdominal segment	15.883	5.870	-0.770	0.390
Length of furca	8.312	1.942	0.557	0.224
Number of setae inserted on left branch of the furca	0.073	1.256	-0.052	0.180
Number of setae inserted on right branch of the furca	0.074	0.172	-0.053	0.067
Width of head	0.279	32.852	-0.102	0.923
Diameter for compound eyes	11.455	17.282	0.654	0.669
Maximal distance between compound eyes	19.373	7.552	0.851	0.443
Length of first antenna	19.333	4.790	0.850	0.352
Abdomen length compared to the total body length ratio (%)	0.449	0.558	0.130	-0.120
Total variance explained				
Variance %	33.972	23.572		
Cumulative %	33.972	57.543		

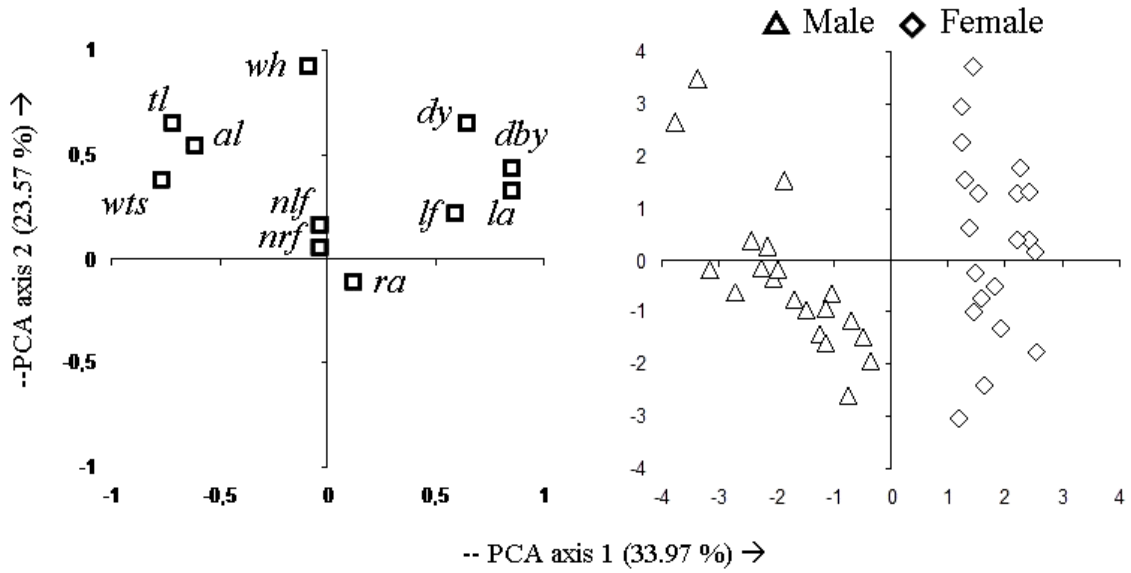

Figure 3. Results obtained from the application of PCA to compare the morphological traits of adult *Artemia salina* male and female specimens from Sabkhet Boujmal (Tunisia). Total length (tl), abdominal length (al), width of third abdominal segment (wts), length of furca (lf), number of setae inserted on left branch of the furca (nlf), number of setae inserted on right branch of the furca (nrf), width of head (wh), diameter for compound eyes (dy), maximal distance between compound eyes (dby), length of first antenna (la) and the abdomen length compared to the total body length ratio (ra, %).

Fatty acids profile:

Table 3 shows the fatty acid composition of *Artemia salina* decapsulated cysts collected from BJ. Result indicated that only 46.61 % of the peaks were identified. The predominant fatty acids were the palmitic acid [C16:0] with 4.67%, palmitoleic acid [C16:1n-7] with 3.45 %, oleic acid [C18:1n-9] with 6.46 %, cis-vaccenic acid [C18:1n-7] with 3.60 % and eicosadienoic acid [C20:2n-6] with 10.10 %. Nevertheless, eicosapentaenoic acid (EPA, C20:5n-3) and linolenic acid (LLA, C18:3n-3) were found in the decapsulated cysts from BJ at about the same rate (3.29 and 2.83 %, respectively). Docosahexaenoic acid (DHA, 22:6n-3) as well as arachidonic acid (ARA, 20:4n-6) were found in low quantity with 1.02 and 1.49 $mg.g^{-1}$ dry weights, respectively.

Discussion

The brine shrimp *Artemia* are of interest to both biologists in a diversity of research fields, such as genetics, toxicology, biochemistry, radiobiology, physiology (Dhont & Sorgeloos, 2002) and aquaculturists where the brine shrimp *Artemia* is of considerable economic importance in fish and shellfish larviculture (Bengtson *et al.,* 1991). Several methods can be used to describe *Artemia* strains, the most common being: cysts and nauplii biometry, biochemical and physiological study, morphometrical measurements and reproductive characteristics.

Table 3. Fatty acid methylester analysis of the *Artemia salina* decapsulated cysts from Sabkhet Boujmal (Tunisia).

FAME	Area (%)	$mg.g^{-1}$ dry weight	FAME	Area (%)	$mg.g^{-1}$ dry weight
C14	0.73	1.01	C20	0.26	0.36
C14:1n-5	0.51	0.71	C20:1n-11	Tr	Tr
C15	0.55	0.76	C20:1n-9	0.29	0.40
C15:1	0.15	0.21	C20:1n-7	0.30	0.42
C16	4.67	6.51	C20:2n-6	10.10	14.09
C16:1n-7	3.45	4.81	C20:3n-6	0.56	0.79
C16:2	0.12	0.17	C20:4n-6	0.73	1.02
C17	Tr	Tr	C20:3n-3	1.56	2.18
C16:3	0.20	0.27	C20:4n-3	0.63	0.88
C16:4	0.46	0.65	C20:5n-3	3.29	4.59
C18	2.27	3.17	C22:5n-3	0.18	0.25
C181n-9	6.46	9.00	C22:6n-3	1.07	1.49
C18:1n-7	3.60	5.02	Somme	46.61	65.00
C182n-6	1.00	1.39	Total n-3	10.00	11.77
C18:3n-6	Tr	Tr	total n-6	11.90	17.39
C18:3n-3	2.83	3.94	n-3/n-6	0.84	0.68
C18:4n-3	0.45	0.62	16:0/16:1	1.35	1.35

*Tr: trace

Vanheacke and Sorgeloos (1980) reported that the diameters variations of *Artemia* cysts observed between different strains and species , in relation to the geographical origin might have genetic explanations. Nevertheless, Abatzopoulos *et al.* (2006) reported that although these criteria have been utilized in the past for strain characterization, they cannot be considered as reliable for defining the origin of unspecified cysts samples. Ben Naceur *et al*. (2010b) confirmed this result and showed a significant difference between *Artemia* cysts harvested from the same site (Sabkhet El Adhibet) but in different years (from 2002 to 2007). However, cysts diameter is an important parameter to characterize *Artemia* for aquaculture use. In fact, cyst biometry assists in the determination of number of $cysts.g^{-1}$. Generally, 1g from strains that produce small cysts contain more $cysts.g^{-1}$, thus usually

produce more Nauplii.g^{-1} (Camargo *et al*., 2005). Moreover, decapsulated cysts can be used for the larviculture of various species like the fresh water African catfish (*Clarias gariepinus*) and common carp (*Cyprinus carpio*), marine shrimp and milkfish larvae (Verreth *et al*., 1987; Stael *et al*., 1995). In our case, the comparison between BJ cysts diameter and other Tunisian *Artemia salina* population showed that cysts harvested from BJ (246.8µm) were similar to cysts harvested from Bekalta saltworks (251.6µm, Van Ballaer et al., 1987), but smaller than cysts from Sabkhet Sijoumi (260.9µm, Ben Naceur *et al*., 2008a) and from Sabkhet El Adhibet (258.1-263.7µm, Ben Naceur et al., 2010b), and bigger than cysts from Sfax saltwork (235.4-239.7µm, Van Ballaer et al., 1987). On the other hand, the comparison between cysts from BJ and other *Artemia* species revealed that BJ cysts were similar to *Artemia franciscana* cysts from Salina Cero 'Colombian Caribbean' (249.8µm, Camargo et al., 2005), from Real de las Salinas 'Mexico' (249.2µm, Castro et al., 2006), from the Great Salt Lake 'Utah' (244.2-252.5µm, Sorgeloos et al., 1986) and *Artemia urmiana* 'Iran' (247.6-259.3µm, Asem *et al*., 2007), but smaller than cysts of *Artemia tibetiana* 'Qinghai-Tibet Plateau, PR China' (284.5-320µm, Van Stappen *et al*., 2003), and bigger than *Artemia franciscana* from San Francisco Bay, in USA (224-228.7µm, Sorgeloos *et al*., 1986) and from Halk El-Menzel in Tunisia (235.8µm, Ben Naceur *et al*., 2010a). Vanhaecke and Sorgeloos (1980) performed a comprehensive study on biometrical variations in *Artemia* strains from different geographical origins, and divided *Artemia* cysts into 3 groups: 1) the smallest cysts belonged to the Adelaide strain (Australia) and *Artemia* from the San Francisco Bay with 225.5 and 235.6µm, respectively; 2) the largest parthenogenetic cysts with diameter varying from 267.0 to 284.9µm; and 3) strains with cysts of intermediate size but with very thin chorion, which is characteristic for *A. franciscana* from Chaplin Lake and the Great Salt Lake with 240.0µm and 244.2-252.2µm, respectively. Based on this cysts classification, cysts harvested from BJ can be classified as a strain with cysts of intermediate size but with very thin chorion.

Since Seale (1933) and Rollefsen (1939) reported the high nutritional value of freshly hatched nauplii of *Artemia* as food for fry fish, the use of brine shrimp *Artemia* in aquaculture has increased exponentially (In Sorgeloos *et al*., 1986). The nutritional effectiveness of a food organism is in the first place determined by its ingestibility and, as a consequence by its size and form (Agh & Sorgeloos, 2005). Naupliar length was different from one geographical source of *Artemia* to another. Vanhaecke and Sorgeloos (1980) studied the nauplii size of 11 *Artemia* strains, and they reported a variation of this criterion from 429.0 to 517.0µm. The comparison between the mean nauplii length from BJ and other *Artemia salina* strains showed that nauplii size from BJ (457.5µm) were similar to nauplii from Sabkhet El Adhibet harvested in 2002 and 2007 (258.1 and 258.9µm respectively, Ben Naceur *et al*., 2010b), but bigger than nauplii from Sabkhet Sijoumi (236.7µm, Ben Naceur *et al*., 2008a), from Sahline saltwork (432.8µm, Ben Naceur *et al*., 2008b) and smaller than cysts from Megrine and Bekalta saltworks (258.8 and 251.6µm respectively, Van Ballaer *et al*., 1987). On the other hand, the comparison of nauplii from BJ and the two main *Artemia* srtains used in aquaculture, the Great Salt Lake 'GSL' and San Francisco Bay 'SFB', revealed that nauplii from BJ was smaller than those obtained from cysts harvested from the GSL (486.0-489.0µm, Sorgeloos et al., 1986) and bigger than those from SFB (428.0-433.0µm, Sorgeloos et al., 1986).

Many scientists are considering 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; Triantaphyllidis et al., 1995). Male and female morphometrical parameters are very useful and can be used for *Artemia* strain characterization in bisexual and parthenogenetic populations. Previous work on the morphology of *Artemia* has shown that *Artemia* population may undergo morphological changes according to the environmental condition (Gaevskaya, 1916; Litvinenko et al., 2007; Ben Naceur et al., in press). Furthermore, several works suggested that wide

differences among populations and between males and females can be found, even when the animals were cultured in the same medium (Hontoria & Amat, 1992; Triantaphyllidis et al., 1995; Camargo et al., 2003). External sexual dimorphism in *Artemia* is seen in the larger size and modified shape of the male antennae. The male has a penis on each side of the body, whereas the female has one median ovisac. Body size comparison of male and female belonged to each bisexual *Artemia* species generally show a size sexually dimorphic which female individuals have larger body than males (Triantaphyllidis et al., 1997b). A size difference between sexes can be interpreted as a mating advantage because, so far as the *Artemia* breeding system is concerned, the female carries the male during copulation. For this reason the female needs a large body for this mating procedure and for surviving the mating process (Asem et al., 2010). Zhou et al. (2003) have shown that the overall percentage of correctly classified cases of females was a little higher than the male in *A. sinica* and *A. tibetiana* from China. According to Camargo et al. (2003) the classification based on male characters provides better group membership than females of *A. franciscana* populations from the Colombian Caribbean. Asem and Rastegar-Pouyani (2007) showed that morphological differentiation between male is higher than female samples of *A. urmiana*. Moreover, these authors reported that *A. urmiana* harvested from four different stations in Urmia Lake (Iran) was a sexually dimorphic, and where eyes diameter, length of antenna, total length, abdominal length, distance between compound eyes and length of furca were the most important characteristics in producing the sexual dimorphism. In our case, PCA showed that the main morphological differences between males and females of adults *Artemia* from BJ were the maximal distance between compound eyes (dby), length of first antenna (la), width of third abdominal segment (wts), total length (tl), diameter for compound eyes (dy) and abdominal length (al). Ben Naceur et al. (2010a) showed that for the invasive brine shrimp *Artemia franciscana* harvested from Sabkhet Halk El-Menzel (Tunisia) sexual dimorphism between adults male and female was based on maximal distance between compound eyes, diameter of compound eyes, length of first antenna and the abdomen length compared to the total body length ratio. The comparison between our results and those reported by Ben Naceur et al. (2010a) showed that except for abdomen length compared to the total body length ratio (ra, %), discrimination between male and female specimens were based on the same morphometrical parameters.

It is known that the fatty acid composition of *Artemia* nauplii can vary among strains and also from one batch to another within the same strain (Léger et al., 1986). This led Watanabe et al. (1978) to classify *Artemia* into two groups: freshwater-type *Artemia*, with n-3 unsaturated fatty acids such as linolenic acid (18:3n-3) but lacking eicosapentaenoic acid (20:5n-3), producing good survival and growth among freshwater animals, and marine-type *Artemia* whose lipids contain 20:5n-3, thereby making them suitable for feeding marine animals. Based on this classification, *Artemia* from BJ can be considered as an intermediate *Artemia* type with linolenic and eicosapentaenoic acid with approximately similar quantity. The same result was reported by Ruiz et al. (2007), where they observed that some *Artemia* sp. cysts from Argentina are an intermediate *Artemia* type. Docosahexaenoic acid (22:6n-3. DHA) is considered together with eicosapentaenoic (20:5n-3, EPA) and arachidonic (20:4n-6, ARA) acids as essential fatty acids (EFA) for marine fish (Sargent et al., 1997). In cysts harvested from Sabkhet Boujmal EPA, ARA and DHA were found in low quantity; however it's very important to mention that only 46.61% of the peaks were identified. For this reason it is necessary to repeat fatty acids analysis with freshly deposited cysts.

Conclusion

The preliminary description of the new reported *Artemia* from Sabkhet Boujmal showed that cysts harvested from this site can be classified together with cysts of intermediate size with very thin chorion. Considering the nauplii length of *Artemia* from BJ obtained in our study it is feasible to use it for feeding both fishand crustaceans larvae. Sexual

dimorphism showed that the main morphological differences between males and females were observed for the maximal distance between compound eyes (dby), length of first antenna (la), width of third abdominal segment (wts), total length (tl), diameter for compound eyes (dy) and abdominal length (al). Fatty acid profile showed that BJ cyst was identified such as an intermediate *Artemia* type, with a very low quantity of fatty acids. For this reason, it is necessary to repeat fatty acids analysis with freshly deposited cysts.

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