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# Determination of biological characteristics of Tunisian *Artemia salina* populations

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**Abstract:** In this study, *Artemia salina* cysts collected from four Tunisian hypersaline environments were characterized using biometrical, biological and biochemical descriptors. Biometrical analysis revealed that the mean diameter value ranged from 220.25 to 237.76 μm, for the untreated cysts, from 199 to 224.52 μm for decapsulated cysts and that the chorion thickness ranged from 6.62 to 10.58 μm. 48 h after hatching, *Artemia* from Sebkha El Meleh, Sebha Moknine, Sfax saltworks and Sahline saltworks presented a hatching percentage of 52.7%, 53.75%, 70.16% and 43.74%, respectively. The length of the freshly hatched instar I nauplii varied from 429 to 449.34 μm. The fatty acids profile showed that the n-3 series was strongly dominant in the samples collected from Sebkha Moknine and Sfax saltworks, representing a percentage ranging from 17.92% to 22.45% of the total fatty acids. The data collected in this study can be useful to add new information on biological and biochemical characterization of *Artemia* strains present in Tunisia. On the basis of fatty acid profile, the *Artemia* strains collected from Sahline saltworks, Sfax saltworks and Sebkha El Meleh can be as classified as "marine" type, and that from Sebha of Moknine as "freshwater" type.

**Key words:** *Artemia;* cysts quality; fatty acids; Tunisia

## Introduction

The brine shrimp genus *Artemia* belong to branchiopod crustaceans that inhabit hypersaline habitats and comprises seven recognized sexual species and several parthenogenetic lineages with different ploidy levels (Gajardo et al. 2002; Baxevanis et al. 2006). Populations of the brine shrimp *Artemia* are found in many inland salt lakes and coastal salterns distributed all over the world (Triantaphyllidis et al. 1998; Van Stappen 2002). The genus is cosmopolitan and comprises both sexually reproducing species and parthenogenetic populations. Salinity is the most important environmental factor governing *Artemia* distribution with populations being found in salt lakes and pans at salinity levels above approximately 40 g $L^{-1}$ (Vanhaecke et al. 1987) where fish and many predatory invertebrates are absent (Browne & MacDonald 1982). *Artemia* cysts can be naturally dispersed over long distances by becoming attached to the feathers of wading birds (Green et al. 2005) or being carried by wind. However, due to their high commercial value and their use as a live feed routinely used in fish hatcheries, *Artemia* cysts demand in aquaculture has increased. In order to sustain the fast growing aquaculture industry (Lavens & Sorgeloos 2000), natural resources other than Great Salt Lake in Utah (USA) should be explored as alternative commercial sources (Triantaphyllidis et al. 1994; Lavens & Sorgeloos 2000). The easiest solution was to inoculate *Artemia* cysts into salt pans throughout the world, for example in Kenya and Vietnam (Vu Do & Nguyen 1987). Unfortunately, inoculation harbors the danger of introducing invasive species that may establish themselves in the new environment and replace local species or create mixed populations which are undesirable for aquaculture purposes. For instance, Van Stappen (2002) suggested that *Artemia franciscana* may replace other species, such as *Artemia salina* (L., 1758) which is known to occur on the African continent from Tunisia to South Africa. For this reason, the genus *Artemia* are of interest to both biologists studying their evolution and developmental biology (Abatzopoulos et al. 2002) and aquaculturists for usage as live feed in fish and shrimp larviculture (Dhont & Sorgeloos 2002). In Africa, different countries host this valuable resource, but despite their wide distribution and the last studies (Muñoz et al. 2008; Ben Naceur et al. 2008a, b, 2009; Mahdhi et al. 2010), very little is known about the genus *Artemia* that remained uncharacterized. The aim of this study was to contribute to the characteriza-

tion of the brine shrimp *Artemia* populations collected from different Tunisian hypersaline biota utilizing the biometry, hatching quality and fatty acids profiles of cysts and instar I nauplii.

## Material and methods

*Study area and sampling*

*Artemia* cysts were collected from four hypersaline environments: Sebkha El Meleh (33°26′ N, 10°54′ E), Sebkha of Moknine (35°39′ N, 10°53′ E), Sahline saltworks (35°44′ N, 10°46′ E), and Sfax Saltworks (34°43′ N, 10°44′ E) (Fig.1) during the period June and August 2008. Sampled material was packed in polyethylene plastic bags for transportation and cysts were treated according to the procedure described by Sorgeloos et al. (1986). Briefly, separation of samples by density in the saturated brine in order to separate the heavy remains from the light ones. Washing with fresh water through a sieve of 70 µm during 5 to 10 minutes and separation by decantation in fresh water. Full cysts deposited at the bottom were dried during 48 h at 30 ℃ and stored in the refrigerator (4 ℃). Samples used for this study are *Artemia salina* according to the analysis of sequences of mitochondrial COI gene (Unpublished data).

*Biometry of cysts and nauplii and hatching characteristics*

The diameter of the hydrated and decapsulated cysts as well as the nuapliar length were measured during this study. For this purpose, a small sample of cysts was first hydrated in artificial seawater (34 g $L^{-1}$) at 25 ± 0.5 ℃ and pH 7.99, and then fixed in 1% Lugol solution (5%). Decapsulated cysts were acquired according to Sorgeloos et al. (1986) and fixed in 1% Lugol solution (5%) and then left overnight in the dark. The diameter of untreated and decapsulated cysts diameter (µm) was measured in 200 cysts with a precalibrated microscope (ZEISS) and chorion thickness was calculated using this formula: (cyst diameter – decapsulated cyst diameter)/2. Mean value and standard deviation were calculated. Concerning naupliar length, cysts were hatched in filtered artificial seawater (salinity was 34 g $L^{-1}$, temperature 27 ± 1 ℃, pH 7.99), instar I nauplii were harvested and fixed in 1% Lugol solution and measured under the microscope equipped with a graduated micrometer. In order to determinate the number of cysts per gram, we counted 0.1 g of clean cyst and the number was converted in to 1 g of cyst. This procedure was made in triplicate for each sample. In order to define the cysts hatching quality, hatching percentage (H%), hatching efficiency (HE) and hatching rate (HR) were performed according to Sorgeloos et al. (1986). The hatching kinetic allowed the determination of $t_0$ (incubation time at the appearance of the first nauplii), $t_{10}$ and $t_{90}$ (time at the appearance of 10% and 90% of totally hatched nauplii), and the synchronization time ($t_s$), which is the difference between $t_{90} - t_{10}$.

*Lipid analysis*

Cysts and instar I nauplii used for lipid analysis were collected after incubation of clean cysts in artificial seawater for 24 h. Total lipids were extracted from cysts and instar I nauplii according to Folch et al. (1957). Fatty acid methyl esters (FAMEs) were prepared by acid-catalysed transmethylation using tricosanoic acid (C 23:0) as internal standard (Lepage & Roy 1984). Methyl esters were extracted by n-hexane, then dried and re-suspended in c-hexane (1%). The analysis of FAMEs was performed using a gas chromatograph (model Autosystem XL, Perkin Elmer, USA) equipped with a flame ionization detector and fitted with a fused silica capillary column (Omegawax 320 Supelco; Bellefonte, PA, USA), using helium as a carrier gas. Injector temperature was 250 ℃, detector temperature was 300 ℃, and the column temperature was programmed at 200 ℃. Individual FAMEs were identified by comparison with known standard (PUFA mix of fish oil, Supelco, Bellefonte, PA, USA) and reported as percentage of total fatty acids.

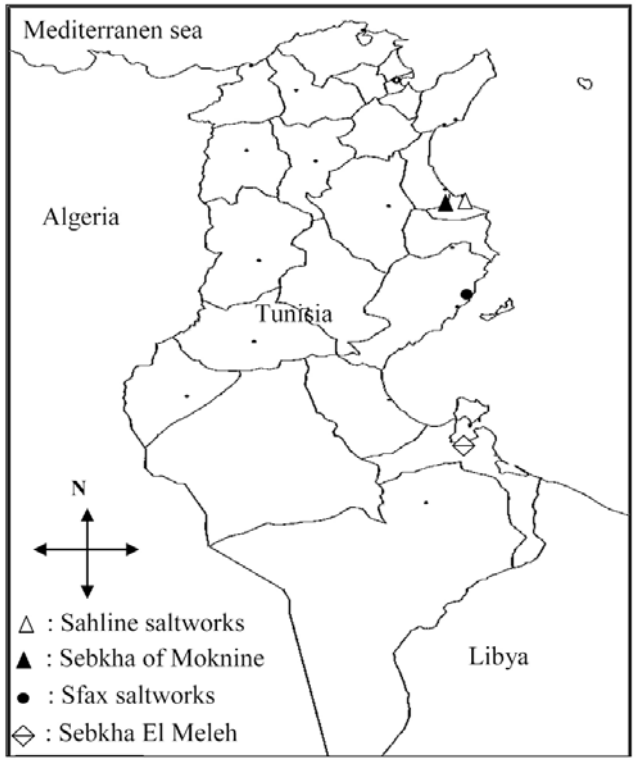


Fig. 1. Geographical location of the study sites.

*Statistical analysis*

The diameter of untreated and decapsulated cysts, as well as the length of instar I nauplii and fatty acid profiles were analyzed by a one-way ANOVA (Duncan's test, $P < 0.05$). Data were analyzed using Statistica (version 5.5) software.

## Results

*Biometry of cysts and nauplii and hatching characteristics*

Hydrated and decapsulated cysts diameter, chorion thickness and length of instar I nauplii were measured. The results are summarized in Table 1. The mean diameter values were 237.76, 220.25, 228.1, 221.75 µm of non-decapsulated cysts obtained from Sebkha El Meleh, Sebkha Moknine, Sfax salworks and Sahline salworks, respectively. Decapsulated cysts diameter ranged from 199 to 224.52 µm. Statistical analysis showed a significant difference between the mean diameter of capsulated and decapsulated cyst from different studied sites ($P < 0.05$). In addition, mean values of chorion thickness ranged from 6.62 to 10.58 µm (Table 1). Total naupliar length was measured from 429 to 449.34 µm showing significant differences ($P < 0.05$).

Table 1. Biometry and hatching description (mean ± SD) of *Artemia salina* cysts from Sebkha El Meleh, Sebkha Moknine, Sfax salworks, Sahline salworks. Hatching percentage and hatching efficiency were determined after 48 h incubation under standard conditions.

| Measured parameters | Sebkha El Meleh | Sebkha Moknine | Sfax salworks | Sahline salworks |
|---|---|---|---|---|
| Untreated cysts diameter (µm) | 237.76 ± 31.62 | 220.25 ± 12.84 | 228.1 ± 26.18 | 221.75 ± 11.69 |
| Decapsulated cyst diameter (µm) | 224.52 ± 21.95 | 199.09 ± 15.08 | 211.3 ± 19.21 | 204.62 ± 13.84 |
| Chorion thickness (µm) | 6.62 | 10.58 | 8.4 | 8.56 |
| Nauplii length (µm) | 29 ± 16.40 | 449.34 ± 11.68 | 438.85 ± 15.91 | 445.57 ± 10.61 |
| Hatching percentage ($H$%) | 52.37 ± 1.33 | 53.75 ± 0.12 | 70.16 ± 1.11 | 43.74 ± 0.70 |
| Hatching Efficiency HE (nauplii/g of cyst) | 59333.33 | 62993.33 | 76666.66 | 52000 |
| $T_0$ (h) | 18 | 15 | 13 | 14 |
| $T_{10}$ (h) | 23 | 20 | 15 | 21 |
| $T_{90}$ (h) | 65 | 59 | 59 | 69 |
| $T_s$ (h) | 42 | 39 | 44 | 48 |

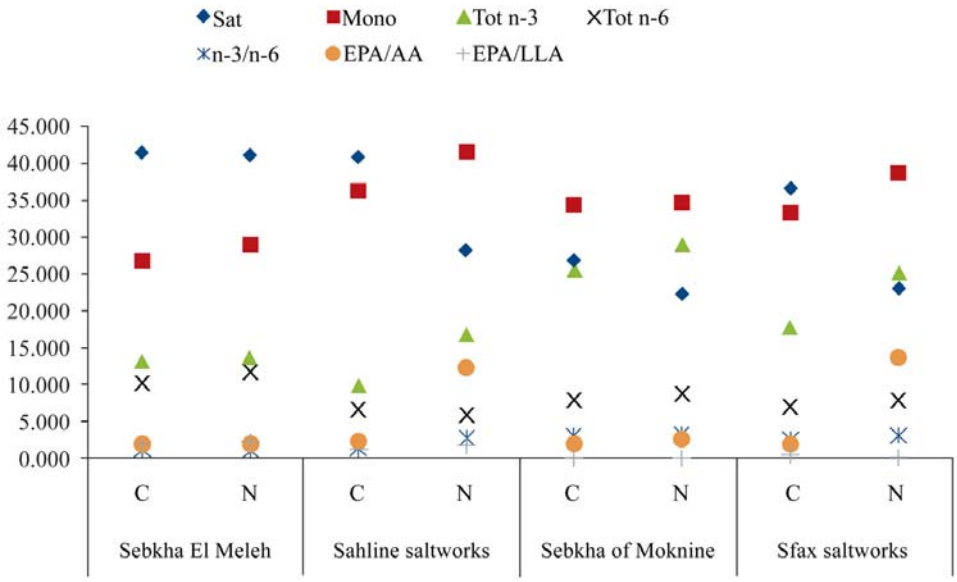


Fig. 2. Fatty acids classes (area %) of *Artemia* cysts and nauplii sampled from the different ponds. Sat – saturated; Mono – Monounsaturated; tot n-3 – total of fatty acids of n-3 series; Tot n-6 – total of fatty acids of n-6 series; LLA – linolenic acid; EPA – eicosapentaenoic acid; AA – arachidonic acid.

The hatching percentages of the studied samples were not satisfactory (52.37, 53.75, and 43.74% for Sebkha EL Meleh, Sebkha Moknine and Sahline salworks respectively after 48 h of incubation, see Table 1), with the exception of those collected from Sfax Saltworks (70.16%).

Hatching efficiency of the cysts harvested in the studied sites varied from 52,000 to 76,666 nauplii $g^{-1}$. The hatching rate and hatching synchrony were not satisfactory. In fact, cysts recovered from different localities required between 59 and 69 h for the hatching of 90% of the nauplii, and that affect synchronization time that ranged between 39 and 48 h (Table 1).

*FAME analysis*

Fatty acid composition of the different *A. salina* populations harvested from Tunisian ponds is summarized in Tables 2, 3 and Figure 2. All samples (cysts and nauplii) demonstrated a different incidence of fatty acid classes in relation to its origin (Fig. 2). Samples of cysts from Sebkha El Meleh, Sahline saltworks and Sfax saltworks are characterized by the higher levels of saturated fatty acids (41.49, 40.83, 36.7%), mainly represented by palmitic acid (C16:0) and stearic acid (C18:0) (Table 2; Fig. 2), followed by monounsaturated fatty acids. This last class is the most profound in fatty acid profile of cyst collected from Sebkha of Moknine (34.35%), in which oleic acid (C18:1 n-9) reach the 17.97% (Table 2). The third class, in order of abundance, is that of n-3 PUFAs, which are more represented in cysts from Sebkha of Moknine (25.6%) and from Sfax saltworks (17.94%) (Fig. 2). In these lots we have observed the highest levels of linolenic acid "LLA" (18:3n-3), compared with the rest of the samples ($P < 0.05$) (Table 2). Levels of eicosapentaenoic acid "EPA" (C20:5 n-3) are similar among samples (4.35–5.1%) except for the cysts from Sebkha of Moknine in which we recorded the lowest value (1.18%) (Table 2). As it regards the n-6 PUFAs, the higher level (10.34%) was recorded in samples from Sebkha El Meleh, while in other samples this class is present at similar level (6.7–7.9%) (Table 2; Fig. 2). Linoleic acid (18.2 n-6) is the most abundant, being arachidonic acid "AA" (C20:4n-6) low in all samples (Table 2). The ratio n-3/n-6 was higher in samples

Table 2. Fatty acid (expressed as area %) profiles of *Artemia* cysts recovered from Sebkha El Meleh, Sebkha Moknine, Sfax salworks, Sahline salworks.

| | Sebkha El Meleh | Sahline saltworks | Sebkha of Moknine | Sfax saltworks |
|---|---|---|---|---|
| Fatty acids | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| 14:0 | 1.99 ± 0.01 | 2.91 ± 0.08 | 1.46 ± 0.13 | 2.65 ± 0.49 |
| 16:0 | 21.37 ± 0.53 | 25.00 ± 1.16 | 18.17 ± 0.39 | 20.55 ± 2.19 |
| 16:1n-7 | 7.90 ± 0.14 | 10.92 ± 0.59 | 7.32 ± 0.28 | 6.20 ± 0.28 |
| 16:2n-4 | 2.95 ± 0.08 | 2.93 ± 0.10 | 2.06 ± 0.61 | 3.67 ± 0.60 |
| 16:3n-4 | 1.00 ± 0.00 | 3.81 ± 0.11 | 2.00 ± 0.13 | 2.78 ± 0.44 |
| 18:0 | 18.12 ± 0.17 | 12.90 ± 1.13 | 7.25 ± 0.07 | 13.50 ± 0.70 |
| 18:1n-9 | 9.97 ± 0.17 | 14.13 ± 2.45 | 17.97 ± 0.17 | 11.78 ± 2.39 |
| 18:1n-7 | 8.02 ± 0.06 | 7.45 ± 0.02 | 7.46 ± 0.211 | 9.87 ± 1.38 |
| 18:2n-6 | 7.74 ± 0.49 | 4.83 ± 0.69 | 7.404 ± 0.45 | 4.55 ± 0.77 |
| 18:3n-4 | 0.22 ± 0.10 | 0.00 ± 0.00 | 0.61 ± 0.11 | 0.40 ± 0.11 |
| 18:3n-3 | 2.45 ± 0.21 | 3.60 ± 0.56 | 19.33 ± 0.90 | 11.10 ± 1.55 |
| 18:4n-3 | 5.01 ± 0.12 | 1.87 ± 0.16 | 5.10 ± 0.04 | 2.10 ± 0.14 |
| 20:1n-9 | 0.99 ± 0.14 | 1.88 ± 0.44 | 1.14 ± 0.07 | 5.15 ± 0.21 |
| 20:4n-6 | 2.60 ± 0.09 | 1.91 ± 0.15 | 0.58 ± 0.08 | 2.55 ± 0.77 |
| 20:4n-3 | 0.66 ± 0.05 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 20:5n-3 | 5.10 ± 0.28 | 4.35 ± 0.37 | 1.18 ± 0.25 | 4.74 ± 0.36 |
| Sat | 41.49 ± 0.69 | 40.83 ± 2.20 | 26.89 ± 0.34 | 36.70 ± 0.99 |
| Mono | 26.89 ± 0.11 | 36.41 ± 1.33 | 34.35 ± 0.08 | 33.35 ± 2.78 |
| Tot n-3 | 13.22 ± 0.67 | 9.82 ± 0.77 | 25.62 ± 0.70 | 17.94 ± 1.33 |
| Tot n-6 | 10.34 ± 0.39 | 6.74 ± 0.84 | 7.98 ± 0.37 | 7.10 ± 1.55 |
| n-3/n-6 | 1.28 ± 0.11 | 1.46 ± 0.06 | 3.21 ± 0.06 | 2.56 ± 0.37 |
| EPA/AA | 1.95 ± 0.03 | 2.27 ± 0.01 | 2.02 ± 0.13 | 1.97 ± 0.74 |
| EPA/LLA | 2.08 ± 0.06 | 1.21 ± 0.08 | 0.06 ± 0.01 | 0.43 ± 0.09 |

Explanations: Sat – saturated; Mono – Monounsaturated; LLA – linolenic acid; EPA – eicosapentaenoic acid; AA – arachidonic acid; tot – Total.

Table 3. Fatty acid (expressed as area %) profiles of *Artemia* instar I nauplii recovered from Sebkha El Meleh, Sebkha Moknine, Sfax salworks, Sahline salworks.

| | Sebkha El Meleh | Sahline saltworks | Sebkha of Moknine | Sfax saltworks |
|---|---|---|---|---|
| Fatty acids | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| 14:0 | 1.50 ± 0.06 | 2.23 ± 0.16 | 1.13 ± 0.23 | 1.89 ± 0.17 |
| 16:0 | 21.05 ± 0.21 | 18.62 ± 0.66 | 14.58 ± 0.68 | 14.92 ± 0.17 |
| 16:1n-7 | 8.05 ± 0.07 | 12.20 ± 1.10 | 7.76 ± 0.47 | 10.07 ± 0.17 |
| 16:2n-4 | 2.80 ± 0.07 | 3.99 ± 0.85 | 2.41 ± 0.50 | 2.38 ± 0.25 |
| 16:3n-4 | 1.05 ± 0.06 | 4.12 ± 0.16 | 1.66 ± 0.51 | 2.12 ± 0.24 |
| 18:0 | 18.57 ± 0.24 | 7.37 ± 0.44 | 6.67 ± 0.47 | 6.31 ± 0.19 |
| 18:1n-9 | 11.05 ± 0.21 | 17.12 ± 0.62 | 17.95 ± 1.06 | 15.57 ± 0.88 |
| 18:1n-7 | 8.97 ± 0.16 | 9.80 ± 0.91 | 8.00 ± 0.03 | 12.53 ± 0.68 |
| 18:2n-6 | 9.12 ± 0.11 | 5.37 ± 0.09 | 8.16 ± 0.19 | 7.61 ± 0.84 |
| 18:3n-4 | 0.00 ± 0.00 | 0.48 ± 0.25 | 0.60 ± 0.02 | 0.67 ± 0.03 |
| 18:3n-3 | 2.20 ± 0.14 | 4.68 ± 0.24 | 22.45 ± 0.76 | 17.92 ± 1.21 |
| 18:4n-3 | 5.29 ± 0.14 | 3.71 ± 0.42 | 4.49 ± 0.59 | 3.98 ± 0.17 |
| 20:1n-9 | 1.00 ± 0.13 | 2.44 ± 0.54 | 1.00 ± 0.28 | 0.55 ± 0.06 |
| 20:4n-6 | 2.67 ± 0.26 | 0.65 ± 0.07 | 0.59 ± 0.00 | 0.28 ± 0.11 |
| 20:4n-3 | 0.82 ± 0.07 | 0.46 ± 0.14 | 0.44 ± 0.14 | 0.00 ± 0.00 |
| 20:5n-3 | 5.17 ± 0.17 | 8.03 ± 0.46 | 1.63 ± 0.47 | 3.38 ± 0.90 |
| Sat | 41.13 ± 0.10 | 28.24 ± 0.04 | 22.38 ± 0.03 | 23.13 ± 0.19 |
| Mono | 29.08 ± 0.25 | 41.57 ± 0.26 | 34.72 ± 0.34 | 38.74 ± 1.80 |
| Tot n-3 | 13.49 ± 0.53 | 16.90 ± 0.06 | 29.03 ± 0.50 | 25.28 ± 0.14 |
| Tot n-6 | 11.79 ± 0.38 | 6.02 ± 0.16 | 8.76 ± 0.19 | 7.89 ± 0.96 |
| n-3/n-6 | 1.14 ± 0.00 | 2.80 ± 0.08 | 3.31 ± 0.13 | 3.22 ± 0.37 |
| EPA/AA | 1.94 ± 0.13 | 12.40 ± 0.63 | 2.73 ± 0.78 | 13.84 ± 8.84 |
| EPA/LLA | 2.35 ± 0.07 | 1.72 ± 0.18 | 0.07 ± 0.01 | 0.19 ± 0.06 |

For explanations see Table 2.

collected from Sebkha of Moknine and from Sfax saltworks. The ratio EPA/AA did not presents significant differences, while the quality index EPA/linolenic was higher in samples from Sebkha El Meleh and Sahline saltworks (Table 2). In instar I nauplii the most represented class of fatty acid is monounsaturated, except for Sebkha El Meleh lot, in which saturated FA are dominant (Table 3; Fig.2). Monounsaturated are characterized by the dominance of oleic and 16:1 n-7 fatty acids, followed by saturated fatty acids, such as palmitic and stearic (Table 3).

As in cysts, the higher percentage of n-3 PUFAs was recorded in nauplii of Sebkha of Moknine (29.03%) and Sfax saltworks samples (25.28%) (Table 3; Fig. 2)

because of the high percentage of linolenic acid (22.45 and 17.92%). In nauplii obtained from cysts of Sebkha El Meleh and Sahline saltworks, beside the lower level of n-3 PUFAs, the higher levels of EPA was recorded (5.17 and 8.03%). The index EPA/AA was significantly higher in nauplii of Sahline saltworks (12.40) and Sfax saltworks (13.84), compared to the value of other samples ($P < 0.05$) (Table 3). The ratio EPA/linolenic resulted higher in samples from Sebkha El Meleh and Sahline Saltworks ($P < 0.05$).

## Discussion

Due to the economic importance of *Artemia* in aquaculture and to its presence in hypersaline environments distributed all over the world, new data and information concerning biological and biochemical characterization of new strains collected from different regions, may be useful for comparative assessments at global level. A series of study focused on description of *Artemia* strains collected from Africa (Triantaphyllidis et al. 1996; Kara et al. 2004; El-Magsodi et al. 2005) but few data about biological and biochemical descriptors are available for Tunisian *Artemia* populations (Guermazi et al. 2008; Ben Naceur et al. 2008a, b, 2009; Mahdhi et al. 2010). It is well known, in fact, that due to ecological concerns, many salt-works are destroyed or abandoned and *Artemia* populations risk to be extinct, therefore, data recording of the still existing populations can be useful for both fundamental and applied biology. Starting from biometry descriptors, in fact, it is possible to do a first preliminary differentiation and classification of various *Artemia* populations (Triantaphyllidis et al. 1996). Biometrical analysis revealed that the mean values for the untreated cysts from the different localities have a diameter ranging between 221 μm and 237 μm. Here, it seems important to note that biometry is usually inadequate for *Artemia* characterization since it is strongly affected by environmental parameters. According to Cole & Brown (1967) the ionic composition of the waters inhabited by *Artemia* varies more than in any other aquatic metazoan. Furthermore, the ionic composition of *Artemia* habitat can result in morphological differences (Hontoria & Amat 1992). Also, it has been demonstrated that the significant interactions between some physicochemical-biotic factors (salinity and chlorophyll a) and *Artemia* cyst production can affect the cyst quality (Camargo et al. 2003). Because of the worldwide distribution of *Artemia*, a high diversity of characteristics among these strains was observed. Some of these characteristics are phonotypical like the nutritional composition of the cysts (Léger et al. 1986). Others, such as, cysts diameter and resistance to higher temperature, are considered strain specific (Vanhaecke & Sorgeloos 1980). This parameter is smaller than that of Great Salt Lake (SGL) *Artemia franciscana* with a diameter ranging between 244.2 and 252.5 μm (Vanhaecke & Sorgeloos 1980) and those of Sebkhat Sijoumi with diameter of 260.9 μm (Ben Naceur et al. 2008a). Naupliar size appears to be the first criterion that (at least for some predator species) determines the ingestibility of specific *Artemia* nauplii (Beck & Bengtson 1982). The biggest size of nauplii was reported for that of Margherita di Savoia, Italy (517 μm) (Sorgeloos et al. 1983), the Jingyu Lake (607.1 μm) (Qinghai-Tibet Plateau, PR China) (Van Stappen 2003) and the Lagkor Co Lake (Tibet, PR China) (667 μm) (Abatzopoulos et al. 1998). According to our data, naupliar length shows a certain variation among the different sampling areas and can be classified into the smaller reported. In general, the naupliar length of the studied populations are larger than that of *A. salina* populations from Chott Marouane, Algeria (428.7 μm) (Kara et al. 2004), and smaller than those harvested from Abu Kammash, Libya (468.2 μm) (El-Magsodi et al. 2005) and from Sfax and Megrine saltworks (467.7 and 482.3 μm, respectively) (Van Baller et al. 1987). However, there are no differences between *Artemia* naupliar length studied in the current work and that obtained from cysts collected from Sabkha Sijoumi, Sahline salworks, and Sfax saltworks, Tunisia (436.7 μm, 432.8 μm, 422.2 μm, respectively) (Van Baller et al. 1987; Ben Naceur et al. 2008a, b). The hatching percentage (H %) of the *Artemia* populations after 48 h of incubation, was within the average of limits (between 20% and 90%) as reported by Vanhaecke & Sorgeloos (1980) but not quiet satisfactory especially for those collected from Sahline saltworks and sebkha of Moknine. Hatching efficiency (HE) is low for all the studied strains compared to that of San Francisco Bay strain (SFB) with a hatching efficiency of 127,222 nauplii $g^{-1}$ (Camargo et al. 2005). These two parameters might have been affected because of the cyst processing method (Sorgeloos et al. 1986) and maybe that collected cysts have been exposed to suboptimal conditions, e.g., repeated hydratation-rehydratation cycles or too long exposure to sunlight (Sorgeloos et al.1976), which can raise the mortality of embryos. On the other hand, these parameters depend strongly on the harvested period, improper processing and/or storage (Sorgeloos et al. 1986). The samples were collected at the end of the wet season (June), which maybe influenced the degree of diapause termination, cysts energy content and amount of dead/non-viable/abortic embryos. This may also be explaining the existence of a gelatinous substance in the unprocessed cyst material that could strongly affect cyst hatching ability (Abatzopoulos et al. 2006). *Artemia* cysts required between 13 and 18 h to hatch. This time was within the limits reported by Sorgeloos et al. (1986) ranged between 13.9 and 25.8 h. The slow hatching rate and poor hatching synchrony observed for the samples is a drawback for their eventual use in aquaculture hatcheries. This inconveniency can be resolved by applying two-step harvest. This strategy help to obtain good yields of instar I nauplii (Van Ballaer et al. (1987). Or by applying protocols of diapause deactivation, such as the use of hydrogen peroxide because these protocols can help to maximize the hatching yield and the variability of the hatching success (Van Stappen et al. 1998).

As for the biochemical descriptors of *Artemia*, the FAME analysis of freshly – hatched instar I nauplii and cysts revealed a high level of linolenic acid "LLA" (18: 3n-3) in the populations collected from Saltworks of Sfax and Sebhat of Moknine (22.45% and 17.92%). A high level of Eicosapentanoic acid (EPA) (20:5 $\omega$ 3), was recorded in *Artemia* of Sebkha El Meleh and Sahline Saltworks (15.17% and 8.03%).

According to Watanabe et al. (1980), who classified *Artemia* stocks of different geographical origins into two main categories: "marine type *Artemia*" with a high content in EPA and suitable for feeding marine fish and crustacean species, and "freshwater type *Artemia*" with a high concentration in LLA suitable for freshwater fishes, *Artemia* (cysts and nauplii) from Sebkha of Moknine can be suitable for freshwater fishes and those from Sebkha El Meleh, Sahline saltworks and Sfax saltworks for marine fish and crustacean species since the same results were observed in other *Artemia* stocks from different geographical regions (Navarro et al. 1992; Camargo et al. 2005; Abatzopoulos et al. 2006). The high amount of essential PUFAs recorded in *A. salina* from Tunisian hypersaline environments could be due to the presence of the Chlorophyceae *Dunaliella salina*, which is the most abundant phytoplankton species in the saltern (Ayadi et al. 2004) and known to be the most suitable food source for adult *Artemia* in extreme saline environments (Lavens & Sorgeloos 1996). Among saturated FAs dominated 16:0 and 18:0, in accord to data reported in other studies on the lipid composition of various *Artemia* species (Abatzopoulos et al. 2006). Based on the results obtained by Ben Naceur et al. (2008b) for *A. salina* cysts from Sahline saltworks of, and Guermazi et al. (2008) for Sfax saltworks, a variation on the fatty acid profile can be deduced. This find can be due to instability of nutritional value of the crustacean *Artemia* in Tunisian pounds. In fact, lipid profile is strongly dependent of different factors such as harvesting period, environmental conditions, and genetics aspects (Mura et al. 2000). The EPA/LLA ratio might well be used as an indicator of nutritional quality. The ratio of these two fatty acids is indeed a lipid quality index and it is employed to establish differences among the various strains of *Artemia*. High values of this index in *Artemia* are positively related to lipid quality that is directly affected by the fatty acid profile of the algae living in natural environments, which are the main live feed for the brine shrimp (Webster & Lovell 1991).

This study focused on *A. salina* strains collected from four different locations in Tunisia, provide information about two populations never studied before "Sebkha Moknine and Sebkha El Meleh" and other two populations previously partly studied "Sfax salworks of and Sahline salworks". The studied *Artemia* populations have a good quality in terms of cyst diameter, chorion tickness, naupliar length, but present low H% and HE. The biochemical profile of cysts and instar I nauplii demonstrate that the level of total $\omega$ 3, especially linoleic acid (18: 3 n-3) and EPA was within the limits and make possible to use of *A. salina* from Tunisian ponds as live feed for later developmental stages of cultured species. Their HUFA profile can be manipulated by utilising appropriate enrichment emulsions that aim to enrich and manipulate the lipid fraction, such as, the oil emulsions (Super Selco), microalgae and probiotic bacteria. Fatty acid profile suggest that *Artemia* from Sahline saltworks, Sebkha El Meleh and Sfax saltworks can be as classified as "marine" type, and that from Sebha of Moknine as "freshwater" type. To confirm the availability of this natural resource in aquaculture, further studies, including the study of H% and HE of freshly deposited cysts recovered from water are recommended. Also more information on both the availability and harvesting quantities of cysts should be given.

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