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Preliminary characterization of two Tunisian *Artemia salina* populations

Caractérisation préliminaire de deux populations Tunisiennes d'*Artemia salina*

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Abstract

Mahdhi A., K. Chaieb, R. Charfeddine, H. Laachkar, F. Kammoun, A. Bakhrouf – Preliminary characterization of two Tunisian *Artemia salina* populations. *Mar. Life*, 17 (1-2) : 3-11.

Ecological parameters of two *Artemia* populations collected in the Sahel of Tunisia at the Saltworks of Sahline (S1) and the Sebkha of Moknine (S2) were investigated. The presence of *Artemia* specimens is limited to the period between December and May when water temperature was between 8 and 24° C, the salinity was between 128 and 242 psu, and the pH ranged from 7.3 and 8.1. In S1, ovoviviparity dominated the oviparity during the first period of abundance; however the oviparity is the dominant reproduction mode in S2. Concerning the total body length of adult specimens collected *in situ*, results show that females are larger than males at the two study sites. Biometrical analysis revealed that the mean values for the untreated cysts from S1 and S2 measured 222.66 µm and 219.64 µm respectively. Decapsulated cysts have a diameter of 205.37 µm for S1 and 199.89 µm for S2 and the chorion thickness ranged from 8.64 µm in S1 to 9.87 µm in S2. The freshly hatched nauplii Instar-I from S1 measured 445.22 µm and those obtained from S2 measured 451.05 µm.

KEY-WORDS :
Artemia, Saltworks, Sebkha, Ecology, Cysts quality.

Résumé

Mahdhi A., K. Chaieb, R. Charfeddine, H. Laachkar, F. Kammoun, A. Bakhrouf – Caractérisation préliminaire de deux populations Tunisiennes d'*Artemia salina*. *Mar. Life*, 17 (1-2) : 3-11.

Les paramètres écologiques de deux populations d'*Artemia salina* collectées au niveau de deux milieux hypersalés localisés au niveau du Sahel tunisien : Saline de Sahline (S1) et sebkhat de Moknine (S2) ont été étudiés. Les résultats obtenus montrent que la présence du branchiopode *Artemia* est limitée à la période comprise entre décembre et mai où la température de l'eau était entre 8 et 24 °C, la salinité entre 128 et 242 psu et le pH entre 7.3 et 8.1. Dans le site S1, l'ovoviviparité domine l'oviparité au cours de la première période, alors qu'au niveau du site S2 c'est l'oviparité qui domine le mode de reproduction des femelles. L'étude de la longueur totale des spécimens adultes collectés *in situ*, montre que ce sont les femelles qui présentent la taille la plus grande au niveau des deux sites S1 et S2. L'analyse biométrique a montré que les valeurs moyennes du diamètre pour les cystes non traités des sites S1 et S2 sont respectivement de l'ordre de 222.66 µm et 219.64 µm. Les cystes décapsulés présentent un diamètre de 205.37 µm pour le site (S1) et 199,89 µm pour le site (S2), alors que l'épaisseur de chorion était de 8,64 µm pour les cystes collectés au site S1 et de 9.87 µm pour le site S2. La taille des nauplii 'stade I' obtenus par l'éclosion des cystes collectés au niveau des berges des deux sites, est de l'ordre de 445.22 µm pour le site S1 et de 451.05 µm pour le site S2.

MOTS CLÉS :
Artemia, Saline, Sebkhat, écologie, qualité de cystes.

Introduction

The brine shrimp *Artemia* (Crustacea, Anostraca) have been found in hypersaline environments distributed all over the world (Van Stappen, 2002) at salinity levels ranging from 80 to 220 psu depending on the strain and/or species (Dana, Lenz, 1986; Sorgeloos *et al.,* 1986; Hammer, Hurlbert, 1992; Camargo *et al.,* 2003; Litvinenko *et al.,* 2007; Ben Naceur *et al.,* 2009 a, 2009 b). To date, several 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.,* 2002). Beside these species, numerous parthenogenetic populations with ploidy levels varying from di- to tri-, tetra- and pentaploid have been described in the Old World (Triantaphyllidis *et al.,* 1998). In the Mediterranean basin, sexual and diploid or polyploid parthenogenetic strains occur in inland and coastal salt lakes and the majority of these populations consist of one species (Browne, 1988, 1992). In order to sustain the fast growing aquaculture industry and meet the high demand for *Artemia* cysts (Dhont, Sorgeloos, 2002), natural resources other than Great Salt Lake in Utah (USA) should be explored as alternative commercial sources (Triantaphyllidis *et al.,* 1994; Lavens, Sorgeloos, 2000). In Tunisia *Artemia* occur in some athalasso and thalassohaline ponds. The presence of *Artemia* in Tunisia was first reported by Seurat (1921) in the chott of Ariana, Heldt (1926) in the old ports of Carthage and Gauthier (1928) in the sebkha of Sidi Elhani (in Ben Abdelkader, 1985). The main objective of this study was to undertake a preliminary characterization of *Artemia* populations in two hypersaline environments: the Saltworks of Sahline (S1) and the Sebkhat of Moknine (S2), on the basis of the population composition, abundance, reproduction, biometry of cysts and Instar-I nauplii as well as cyst hatching characteristics.

Material and methods

Study area and sampling

Sites were investigated in the period between September 2004 to August 2005 from the Saltworks of Sahline (S1) (35° 44' N, 10° 46' E) a thalassohaline environment with a total area of 12 km^2 and from an athalassohaline environment with area of 40 km^2, the sebkha of Moknine (S2) (35° 39' N, 10° 53' E) **(Figure 1)**. For the sebkha of Moknine (S2), the parameters were monitored only during the period between December and April in view of the early draining of the site. *Artemia* live specimens were sampled monthly from one sampling point at each site. *Artemia salina* cysts were collected once from the border of the ponds. Samples were 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°C and stored in the refrigerator (4°C). The two *Artemia* strains were identified as *Artemia salina* with a 97% identity after sequence analysis of the mitochondrial gene COI (unpublished data).

Determination of physico-chemical parameters

Temperature was measured using an ordinary thermometer (type, Handy lab 1) with a precision of 0.5°C, salinity was determined using a refractometer (ATAGO) and pH was assessed using a pH-meter (type, Handy lab 1) with a precision of 0.1.

Determination of bio-ecological parameters

Abundance of *Artemia,* composition of population, parthenogenesis and bisexuality and adult size were determined in one liter of water taken from each site. Abundance was determined by counting *Artemia* specimens using a magnifying glass. To determine the population composition, the nauplii (larvae without thoracopode), juveniles (larvae with thoracopode but not sexually differentiated), pre-adults (specimens sexually differentiated and not having reached the reproduction stage) and adults were numbered. The mode of reproduction (oviparity and ovoviviparity) has been determined from 100 females collected monthly and randomly *in situ*. The mean of male and female adult sizes collected from each site was determined monthly and the maturity size was calculated from the mean sizes. For fecundity analysis, females' ovigerous sacs (n = 20) were dissected and the number of cysts or nauplii was counted.

Biometry of cysts and nauplii and hatching characteristics

Cyst samples were characterized on the basis of their diameter and chorion thickness. For this purpose, a small sample of cysts was first hydrated in sea water (34 psu) at 25 ± 0.5°C and pH 7.99, and then fixed in 1% Lugol

solution (5%). Decapsulated cysts was obtained according to Sorgeloos *et al.* (1986) and fixed in 1% Lugol solution (5%) and then left overnight in the dark. Treated and decapsulated cyst 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 sea water (salinity 34 psu, temperature 27 ± 1°C, pH 7.99), Instar-I nauplii were harvested and fixed in 1 % Lugol solution and measured under a microscope equipped with a graduated micrometer. In order to determine the number of cysts.g^{-1}, 0.1 g of clean cyst was sampled, counted and the number was converted to 1 g of cyst. This procedure was carried out in triplicate for each sample. In order to examine the cysts' hatching quality, hatching percentage (H%), hatching efficiency (HE) and hatching rate (HR) were determined 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 total hatchable nauplii), and the synchronization time (t_s), which is the difference between t_{90} - t_{10}.

Statistical analysis

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

Results

Bio-ecological parameters

1. Physico-chemical parameters

During the period between September and November and May to August (2004-2005), the temperature varied between 20 to 31°C. In the period of abundance (December to April 2005), this parameter varied between 10 to 20°C. For the period of abundance, salinity was maintained at values higher than 200 psu in S2. While in S1, it ranged between 160 and 197 psu. The monitoring of pH fluctuation showed that it remains alkaline and ranges between 7.3 and 8.1 **(Table I)**.

2. Abundance and composition of *Artemia* population

Artemia population abundance at the two sites showed wide variations. In S1, the first specimens appear in

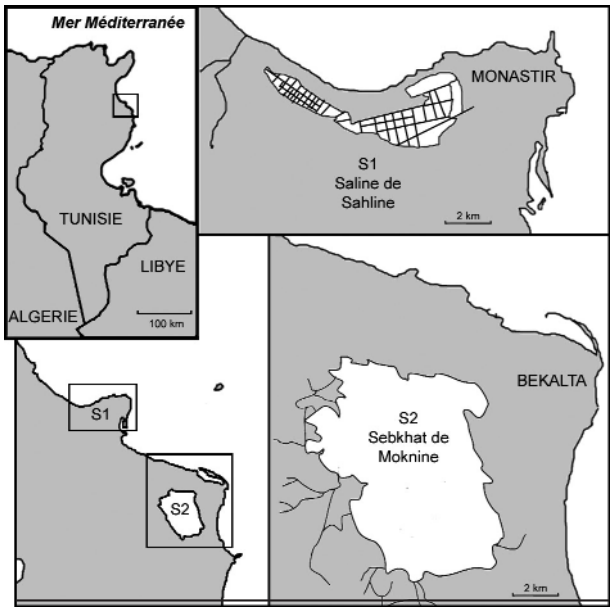

Figure 1
Geographical location of the study sites: Saltworks of Sahline (S1), Sebkha of Moknine (S2).

Localisation géographique des deux sites d'étude : Saline de Sahline (S1) et Sebkha de Moknine (S2).

Abiotic parameters	Temperature (°C)		Salinity (psu)		pH	
Site	S1	S2	S1	S2	S1	S2
September	26	nd	214	nd	7.3	nd
October	20	nd	203	nd	7.88	nd
November	19	nd	194	nd	7.75	nd
December	11	15	160	200	7.9	7.93
January	10	13	137	217	7.82	7.6
February	08	10	128	210	7.97	7.86
March	14	17	170	234	8.1	8.1
April	17	20	197	242	7.6	7.81
May	24	nd	210	nd	7.76	nd
June	26	nd	224	nd	7.42	nd
July	27	nd	246	nd	7.8	nd
August	31	nd	253	nd	7.7	nd

Table I
Abiotic parameters during the investigation period; S1: Saltworks of Sahline; S2: Sebkha of Moknine; nd: not determined.

Les paramètres abiotiques durant la période d'étude ; S1 : Saline de Sahline ; S2: Sebkha de Moknine ; nd : indéterminé.

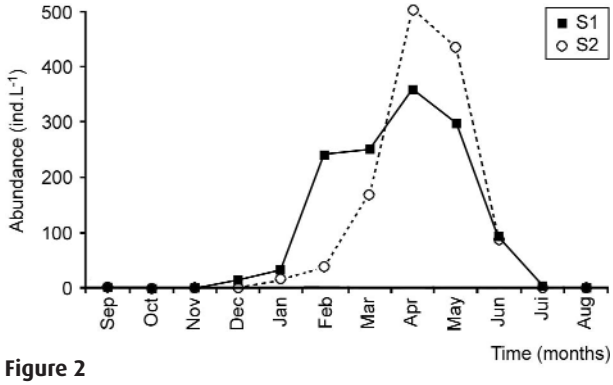

Figure 2
Abundance of *Artemia* populations at the two sites of study, Saltworks of Sahline (S1) and Sebkha of Moknine (S2).

*Abondance des populations d'*Artemia *dans les deux sites d'étude, Saline de Sahline (S1) et Sebkha de Moknine (S2).*

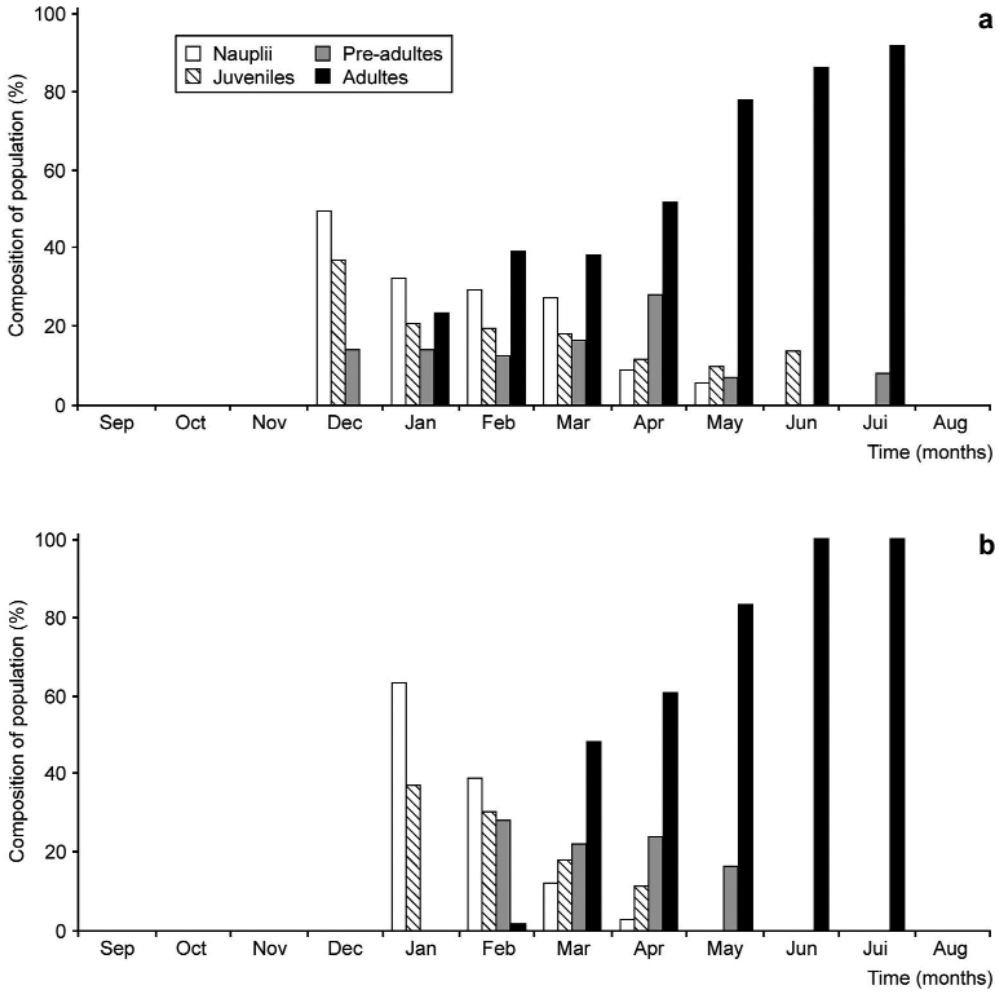

Figure 3
Composition of *Artemia* population in **a**: Saltworks of Sahline (S1) and **b**: Sebkha of Moknine (S2).

*Composition de la population d'*Artemia *dans **a** : le site Saline de Sahline (S1) et **b** : le site Sebkha de Moknine (S2).*

December. From January until February, the number of *Artemia* specimens increases and the maximum was reached in April (357.25 individual per liter: ind.L^{-1}). For S2, the first specimens appeared during January and the density reached its maximum during April (504 ind.L^{-1}) **(Figure 2)**.

A massive abundance of the cysts was noted at the two sites excluded of the two months of February and March. Nauplii, juveniles and pre-adults were observed in S1 during the studied period between December and May and were limited to March and April in S2. The adult stage was reached in January to be continued until July in S1. However, in S2 the adults started to appear in February **(Figure 3 a and 3 b)**.

3. Reproduction of *Artemia* populations

The analysis of all the samples taken from the two studied sites (S1 and S2) showed that the two populations of *Artemia* were composed of both males and females. A high percentage of ovoviviparous females at S1 were noted during the month of February. The oviparous females were abundant in March with a net dominance (100%) in May. At S2, predominance of the oviparity was noted during the whole period of study **(Figure 4)**. The fecundity of the females (number of cysts or nauplii per female) obtained from S1 was higher than that obtained from S2. Indeed, at S1, the average fecundity for the oviparous females was 41.84 cysts / females and 60.23 nauplii / females for the ovoviviparous females, whereas at S2, the average fecundity is about 34 cysts / females for the oviparous and 17.85 nauplii / females for the ovoviviparous ones **(Figure 5 a and 5 b)**.

4. Size of maturity and biometry of cysts and nauplii

The size of maturity is summarized in table II. The results showed that the female's size was greater than that of the males at the two localities, and the results showed a statistically significant difference **(Table II)** ($P < 0.05$).

Hydrated and decapsulated cyst diameter, chorion thickness and length of Instar-I naupliar were measured. The results are summarized in Table III. The mean diameter values were 222.66 µm of non-decapsulated

Table II

Site	Males Size (mm)	Females Size (mm)
S1	7.72 ± 0.46 a	8.62 ± 0.41 a
S2	7.32 ± 0.43 b	8.95 ± 0.31 b

Mean values ± standard deviation of size of adults *Artemia* at the two studied sites; superscripts (a, b) per line show significant differences ($P < 0.05$); S1: Saltworks of Sahline; S2: Sebkha of Moknine.

Taille moyenne ± écart-type des Artemias *dans les deux localités ; les exposants (a, b), par ligne, indiquent des différences significatives ($P < 0.05$) ; S1 : Saline de Sahline ; S2: Sebkha de Moknine.*

Table III

Sites	Cysts diameter (µm)	Decapsulated cyst diameter (µm)	Chorion thickness (µm)	Nauplii length (µm)
S1	7.72 ± 0.46 a	8.62 ± 0.41 a	8.64	445,22 ± 10.58 c
S2	7.32 ± 0.43 b	8.95 ± 0.31 b	9.87	451,05 ± 9.71 c

Cyst diameter, chorion thickness and length of Instar-I nauplii for the two *Artemia* population studied; superscripts (a, b, c) per column show significant differences ($P < 0.05$); S1: Saltworks of Sahline; S2: Sebkha of Moknine.

Diamètre des cystes, épaisseur du chorion et taille des nauplii au stade - I des deux populations étudiées ; les exposants (a, b, c), par colonne, indiquent des différences significatives ($P < 0.05$) ; S1 : Saline de Sahline ; S2 : Sebkha de Moknine.

Table IV

Sites	Number of cysts.g^{-1}	Hatching percentage (H%)	HE (nauplii/g of cyst)	HR (hrs) T_0	T_{10}	T_{90}	T_s
S1	215.673 ± 7.54 a	25.74 ± 0.14 b	22.106.66 ± 184.75 c	14	21	69	48
S2	240.824 ± 9.64 a	33.33 ± 2.98 b	27.286.66 ± 2331.20 c	15	20	59	39

Quality evaluation results for *Artemia* cyst from S1 and S2; H%: hatching percentage; HE: hatching efficiency; HR: hatching rate; Ts: hatching synchrony; superscripts (a, b, c) per column show significant differences ($P < 0.05$); S1: Saltworks of Sahline; S2: Sebkha of Moknine.

*L'évaluation de la qualité des cystes d'*Artemia *de S1 et de S2 ; H% : pourcentage d'éclosion ; HE : efficacité d'éclosion ; HR : taux d'éclosion ; Ts : Temps de synchronisation ; les exposants (a, b, c), par colonne, indiquent des différences significatives ($P < 0.05$) ; S1 : Saline de Sahline ; S2: Sebkha de Moknine.*

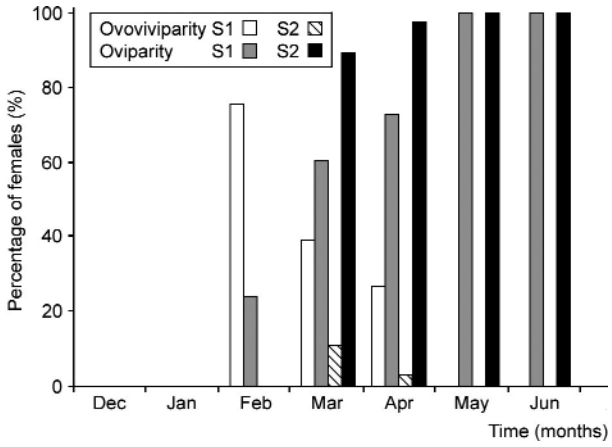

Figure 4
Percentage of the ovoviviparous and oviparous females at the two studied sites, Saltworks of Sahline (S1) and Sebkha of Moknine (S2).

Pourcentage des femelles ovovivipares et ovipares dans les deux sites d'études, Saline de Sahline (S1) et Sebkha de Moknine (S2).

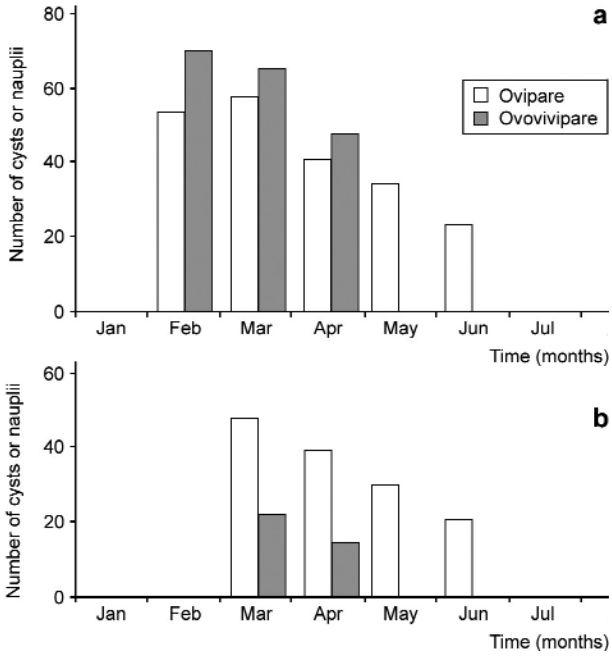

Figure 5
Monthly fluctuation of the number of cysts and nauplii per female in **a**: Saltworks of Sahline (S1) and **b**: Sebkha of Moknine (S2).

*Fluctuation mensuelle du nombre des cystes et des nauplii dans **a** : le site Saline de Sahline (S1) et **b** : le site Sebkha de Moknine (S2).*

cysts obtained from S1 and 219.64 µm for the cysts collected from S2. Decapsulated cysts from S1 measured 205.37 µm and 199.89 µm for S2 samples. Statistical analysis showed a significant difference between the mean diameter of capsulated and decapsulated cyst from S1 ($P < 0.05$). Samples of both untreated (non-decapsulated) and treated (decapsulated) cysts, obtained from S2, showed a significant statistical difference ($P < 0.05$). Comparison between the mean diameter of capsulated and decapsulated cyst collected from the two studied sites showed a significant difference ($P < 0.05$). In addition, mean values of chorion thickness were 8.64 and 9.87 µm for S1 and S2 respectively **(Table III)**. The mean values of total naupliar length were 445.22 µm for the S1 population and 451.05 µm for S2 and showed a significant difference ($P < 0.05$).

The hatching percentage and hatching efficiency of the cysts harvested at the two studied sites were 25.74 % and 22 106.66 nauplii.g^{-1} for S1 and 33.33 % and 27 286.66 nauplii.g^{-1} for S2. The hatching rate and hatching synchrony were also affected. Indeed, cysts recovered from the first site required 69 hours for the hatching of 90 % of the nauplii, while those obtained from the second required approximately 59 hours **(Table IV)**.

Discussion

Artemia showed abundance during the period between February and May 2005. This is in agreement with the variations of temperature (10°C and 26°C), salinity (128 to 242 psu) and pH (7.6 to 8.1) as represented in figure 2 and figure 3. *Artemia* tolerates temperatures which vary between 6°C (Relyea, 1937) and 35°C (Browne, Mac Donald, 1982; Wear *et al.,* 1986), salinity between 60 to 220 psu (Triantaphyllidis *et al.,* 1994; Camargo *et al.,* 2004; Dana, Lenz, 1986; Hammer, Hulbert, 1992; Litvinenko *et al.,* 2007; Ben Naceur *et al.,* 2009 a, 2009 b) and pH values generally neutral and/or basic between 7 and 8 (Ben Naceur *et al.,* 2009 a, 2009 b). The decrease of the abundance of *Artemia* apart from the period between February and May can be explained by a complexation of calcium ions with high levels of organic matter (Chave, Suess, 1970) causing calcium carbonate to precipitate at higher than expected salinities, brine turbidity with carbonates and gypsum and decreased evaporation. These phenomena create viscous brine and environments highly unfavorable to *Artemia*, resulting in increased calcium and sulfate concentrations in the harvested salt. The decrease in *Artemia* density in their environment can also be related to the abiotic parameters (*i.e.* salinity and temperature) (Abatzopoulos *et al.,* 2003; Camargo *et al.,* 2004). The predominance of ovoviviparity in S1 and the oviparity in S2 can be explained by the variations of the environmental factors (especially salinity) between the two sites **(Figure 4)**. This change of mode of reproduction, from ovoviviparity towards the oviparity can be also explained by the genetic control. Amat (1982) noted that under constant conditions, the females resulting from several parthenogenetic and bisexual strains tend to reproduce by ovoviviparity at the beginning then by oviparity. In the two localities, females showed larger sizes when compared to males. This difference of size has also been revealed in other studies. Triantaphyllidis *et al.* (1997), demonstrated that body size comparison of male and female belonging to each bisexual *Artemia* species generally show a sexually dimorphic size with female individuals having a larger body than males. The females from S1 were considered rather fertile in comparison with those obtained from S2. This could be explained by the effect of environment which varies from one site to another. This finding was confirmed by Abatzopoulos *et al.* (2003) who showed that high salinities induce a reduction in the fertility of the females. The diameter of cysts in S1 and S2 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.,* 2008 a). Considering the difference in cyst diameter, we also noted a difference in chorion thickness. The difference noted in the size of cysts and the thickness of chorion can be explained by some physico-chemical effects (*i.e.* salinity) (Camargo *et al.,* 2004). Nauplii 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 fresh nauplii (Instar-I) were among the smallest reported with a total mean length of 445.22 and 451.05 µm for the *Artemia* population at S1 and S2 respectively compared to the *Artemia* species (*tibetiana*) of Lagkor Co Lake (Tibet, PR China) (Abatzopoulos *et al.*, 1998) and the Jingyu Lake (Qinghai-Tibet Plateau, PR China) (Van Stappen *et al.*, 2003) with a mean length of 667 µm and 607.1 µm, respectively and those harvested from Abu Kammash, Libya (468.2 µm) (El-Magsodi *et al.*, 2005). But they are bigger than those of the San Francisco Bay, California (428 µm) (Van Stappen, 1996), than those from Chott Marouane, Algeria (428.7 µm) (Kara *et al.*, 2004) and from the Saline de Sahline, Tunisia (432.8 µm, Ben Naceur *et al.*, 2008 b). The hatching percentage (H%) of the two *Artemia* populations was within the average of limits (between 20 % and 90 %) as reported by Vanhaecke and

Sorgeloos (1980). This parameter might have been affected by the cyst processing method (Sorgeloos *et al.*, 1986) such as cyst treatment (some impurities remained present in samples) or decapsulation procedure. In fact in some methods such as hydrogen peroxide treatment, the effect was dependent on certain parameters such as hydration level of the cysts, H_2O_2 concentration and treatment time (Van Stappen *et al.*, 1998). Hatching efficiency (HE) is low for the two studied strains compared to that of San Francisco Bay strain (SFB) with a hatching efficiency of 127 222.2 nauplii.g^{-1} (Camargo *et al.*, 2005). This can be explained by some factors such as presence of other components (*i.e.* empty shells) and individual cyst weight (Camargo *et al.*, 2005). Moreover, Van Ballaer *et al.* (1987) gave a good illustration on the importance of the conditions before harvests. This explanation can be valid for *Artemia* strains studied during the present investigation because all samples were collected on the sebkha's banks. The high hatching synchrony of cysts, collected from S1 and S2, could be attributed to the method of treatment and environmental factors (*i.e.* salinity). In fact, the significant interactions between some physico-chemical-biotic factors (salinity and chlorophyll *a*) and *Artemia* cyst production can affect the cyst quality (Camargo *et al.*, 2003). Cysts from S1 and S2 hatched after 14 and 15 hours respectively. These times were within the limits 13.9 - 25.8 h as reported by Sorgeloos *et al.* (1986). While, the synchronization time was long with a value of 48 h for *Artemia* collected from S1 and 39 h for those from S2 and exceed the limits ranging from 4.4 - 17.3 h indicated by Sorgeloos *et al.* (1986).

In conclusion, the first experimental results presented in this paper have shown that the two studied populations of *Artemia salina* are of good quality in terms of cyst diameters and nauplii length, but their H% and HE were not satisfactory. Further studies, including the study of H% and HE of cysts recovered from water and biochemical analysis of cysts and nauplii, are recommended in order to confirm the suitability of this natural resource for aquaculture.

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