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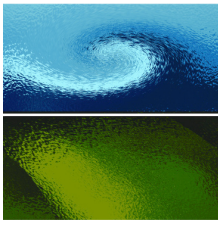

RESEARCH ARTICLE

Determination of biological characteristics of *Artemia salina* (Crustacea: Anostraca) population from Sabkhet Sijoumi (NE Tunisia)

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Abstract

1 - *Artemia* (Crustacea, Anostraca) are typical inhabitants of extreme environment, as inland salt lakes and coastal salterns, distributed all over the world. This species has been shown to be an irreplaceable live feed for marine and shrimp larvae, and can be used by biologist for studying their evolution and developmental biology.

2 - The aim of this paper is to determinate the biological characteristics of *Artemia salina* of Sabkhet Sijoumi (NE Tunisia) using cysts and naupliar biometrics, as well as lipid and fatty acid profiles and reproductive characteristics.

3 - The results showed that the hydrated cysts measured 260.9 ± 25.6 µm in diameter and the freshly hatched nauplii 436.7 ± 49.7 µm in length. The study of fatty acid methyl esters revealed that the Sijoumi *Artemia* population belongs to the "marine" type of *Artemia* showing a high level of eicosapentaenoic acid (C20:5n-3, 14.73 ± 0.78%). The reproductive characteristics revealed that the percentage of oviparous offspring was 28.42%.

4 - This study is a contribution to the characterization of *Artemia* population from the African coast of the Mediterranean region. Morover, *Artemia* from Sabkhet Sijoumi showed that this strain can be used as an acceptable food source in aquaculture hatcheries.

Keywords: *Artemia*, biological characteristics, Sijoumi, Tunisia.

Introduction

Anostraca are inhabitants of ephemeral coastal and inland waters. They produce latent eggs "cysts" containing a gastrula embryo with approximately 4000 cells for *Artemia* species. These cysts can resist to long drought periods at severe hypersalinity, air desiccation, high dose of ultraviolet radiation, varying degrees of hypoxia and extremes of temperature (Abatzopoulos et al., 2002; Kraus et al., 2004; Tanguay et al., 2004). The brine shrimp *Artemia* occurs in salt lakes or brine waters of solar saltwork (coastal or inland; chlorine, sulphate or carbonate water), even if these habitats are characterized by an unstable environment and varying abiotic and biotic conditions. Several authors have shown that the cysts and nauplii biometry and nutritional characteristics of *Artemia* may greatly vary from one batch to another and from different geographical regions (Johns et al., 1980; Léger and Sorgeloos, 1984; Van Stappen, 1996). The geographical isolation and the specific habitat conditions have led to various phenotypes with different biological, chemical and physiological characteristics (Vanhaecke and Sorgeloos, 1980).

Nutrition is a major problem of crustacean and marine fish larvae. Regardless of the vast

improvement in fish nutrition industry, there still no artificial feed formulation available to completely substitute for *Artemia*. In fact, *Artemia* nauplii are considered as an irreplaceable live feed for the larval rearing of most marine fish and crustacean larvae (Sorgeloos et al., 2001; Kolkovski et al., 2004). Annually, more than 1500 metric tonnes of dry *Artemia* cysts are marketed worldwide to feed fish and shellfish (Dhont and Sorgeloos, 2002). In the Mediterranean area, *Artemia* species have been investigated and characterized only in the European sites (Triantaphyllidis et al., 1993), while in the south of the Mediterranean basin, where promising *Artemia* sites exist as in Tunisia (Van Ballaer et al., 1987; Aloui, 2003; Ben Naceur et al., 2008), in Morocco (Sadkaoui, 2000), in Egypt (El-Bermawi, 2003), in Algeria (Kara et al., 2004; Samraoui et al., 2006) and in Libya (El-Magsodi et al., 2005), only few data about biology, nutritional quality and reproductive characteristics of *Artemia* are available. In Tunisia, the presence of *Artemia* was signalized for the first time by Seurat (1921) in Chott Ariana (NE Tunisia) and by Gauthier (1928) in Sabkhet Sidi El Heni (Tunisia Sahel). Other sites become later signalized by Ben Abdelkader (1985), Sorgeloos et al. (1986), Van Ballaer et al. (1987), Romdhane (1994) and Romdhane et al. (2001).

Artemia was signalized in the Sabkhet Sijoumi (NE Tunisia) for the first time by Romdhane et al. (2001). Besides, another investigation made in Sabkhet Sijoumi by Romdhane and Ghlala (2002) pointed out that only *Artemia* parthenogenetica population was observed and sampled. Nevertheless, in our several visits effectuated since December 2003, we identified bisexual specimens of *Artemia* and no parthenogenetic population was found with direct sampling in this area. This observation is confirmed after results obtained by laboratory culture. Moreover, Romdhane et al., 2004 compared, by a discriminant analysis, the morphometry of *Artemia* males with other *Artemia* populations, and they concluded that *Artemia* from Sabkhet Sijoumi is belonging to *Artemia* salina species.

In this work, *Artemia* population from Sabkhet Sijoumi (NE Tunisia) is characterized by studying the cysts and nauplii biometrics. The fatty acid profile of decapsulated cysts and reproductive characteristics were also analyzed.

Materials and methods

Site description and cysts sampling

Sabkhet Sijoumi is located in the south western of Tunis (36°46'49"N, 10°07'16"E), with an area of 3000 hectares. The holding capacity of the sabkha is estimated at approximately 45 million m^3. The geology of this site is characterized (from north-west to south-east) by an argillaceous substratum encrusted with sandy lenses supported on the south of the alternate marl and limestone benches. The presence of a not very permeable clay and a muddy clay, strongly charged with salts, is the result of the geological evolution of the sabkha and the process of sedimentation which affects the bottom of this basin under the current climatic conditions. The cysts were sampled on the sabkha banks in 2003, and then treated following the protocol described by Sorgeloos *et al*. (1986).

Biometrics of cysts and nauplii

The diameter of the hydrated and decapsulated cysts (n=100) was measured under a microscope equipped with a calibrated micrometer, according to Vanhaecke and Sorgeloos (1980). The chorion thickness was also measured (Vanhaecke and Sorgeloos, 1980).

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

Fatty acid analyses

Lipids were extracted using the method by Folch *et al*. (1957), and transmethylated overnight (Christie, 1982) after the addition of C19:0 (99% pure, Sigma Chemical Co., Poole, Dorest, UK) as an internal standard. Methyl esters were extracted with hexane: diethyl ether (1:1, v/v), and purified by thin layer chromatography (Silica Gel G 60, 20 X 20 plates, Merck) using hexane: ether:acetic acid (85:15:1.5, v/v/v) as solvent.

The analyses of the methyl esters were performed on a PACKARD gas chromatograph (Packard Instrument Inc., Caversham, UK) equipped with a fused silica 50 m x 0.22 mm, an open tubular column coated with FFAP (film thickness: 0.25µm, SGE, UK, Ltd., London) and, an on-column injection system, using hydrogen as a carrier gas and a thermal gradient from 50 to 235°C. Peaks were recorded in a Shimadzu C-R 6 A Chromatopac recording integrator, identified by comparison with known standards, and quantified by means of the response factor of the internal standard.

Reproductive characteristics

After hatching, nauplii were transferred to 5 litres containers filled with sea water (38 psu salinity) at 25°C, under mild aeration at a photoperiod 16h light: 8 h dark, and supplied with moderate aeration from the bottom, this sets the oxygen concentration to saturation. The culture densities were never allowed above 50 animals l^{-1}. Cultures were fed on a mixed diet of algae (*Tetraselmis suecica* and *Dunaliella salina*, 1:1) in an approximate density of 100 000 cells ml^{-1}. The medium was completely renewed twice per week with fresh seawater and microalgae cultures. Virgin females (as soon as sexual differentiation occurred) were isolated from the stock cultures before they reached sexual maturity also under the same culture condition. Males were collected directly from the stock culture. Both males and females (n=10 couples) were transferred to 20 ml falcons, and intra-cross fertility tests were performed at the same culture conditions. New males or virgin females replaced dead individuals. The culture medium was changed once a week to ensure the waste disposal and micro algae agglomeration. Total offspring, days between broods, number of cysts and number of nauplii were determinate. Observations of the offspring were studied for a maximum period of 30 days.

Results

Biometrics of cysts, nauplii and adults

Table 1 shows the essential biometric characteristics of cysts and nauplii. The mean diameter of hydrated and decapsulated cysts and the length of freshly hatched *Artemia* nauplii produced from hydrated cysts were 260.9±25.6µm, 233.8±23.3µm and 436.7±49.7µm, respectively. The size frequency distributions of hydrated and decapsulated cysts (diameter) and of freshly hatched nauplii produced by hydrated and decapsulated cysts (length) are shown in Fig. 1.

Biochemical analysis

Total lipids and the major fatty acid content are reported in Table 2. Palmitic acid (16: 1n-7) was the most abundant fatty acid (31.28 mg g^{-1}dry weight). Eicosapentaenoic acid "EPA" (20: 5n-3) was present in very high amounts (22.48 mg g^{-1} dry weight). On the other hand, the level of linolinic acid "LLA" (18: 3n-3) was low (4.33 mg g-1 dry weight). However, the other essential fatty acid docosahexaenoic acid "DHA" (22: 6n-3) has not been detected.

Reproductive characteristics

Table 3, shows the reproductive performance of the Sijoumi's *Artemia* population. The number of produced nauplii (F_1) was 1856, representing 71.58% of the total offspring and the percentage of the oviparous mode was 28.42%. Broods per female and time between broods were 3.6 and 5.8, respectively.

Discussion

The use of *Artemia* as food for a number of aquatic organisms became highly increased in aquaculture. The biological variations of different populations led researchers to characterize the different strains by various methods (Hontoria and Amat, 1992). Thus, in response to the increasing demand of *Artemia* cysts by the aquaculture industry, the investigation of a new *Artemia* populations and its biometrics, hatching characteristics as well as biochemical characteristics are suggested (Triantaphyllidis *et al*., 1998). The studies reported by Vanhaecke and Sorgeloos (1980) revealed significant differences in the size of the cysts among different *Artemia* strains. These authors showed that considering cyst diameter, the American *Artemia* (belonging to the *A. franciscana* species) is relatively small when compared with those from other *Artemia* sources belonging to the *A. salina* or *A. tibetiana* species. From the results obtained herein, cysts collected from Sabkhet Sijoumi belong to the big egg class as classified by Bengtson *et al*. (1991) with a mean diameter of 260.9 µm, which can be compared with the cyst diameter reported by Vanhaecke and Sorgeloos (1980). On the other hand, it

Table 1. Biometrics of cysts and nauplii *Artemia* from Sijoumi population

Characteristics	Range	Mean ± SD
Diameter of hydrated cysts (µm)	203.2 – 338.7	260.9 ± 25.6
Diameter of hydrated decapsulated cysts (µm)	196 – 290.3	233. 8 ± 23.3
Chorion thickness (µm)	-	13.5
Length of nauplii produced from hydrated cysts (µm)	348.7 -553.8	436.7 ± 49.7
Length of nauplii produced from decapsulated cysts (µm)	341.7 – 509.2	428.7 ± 36.8

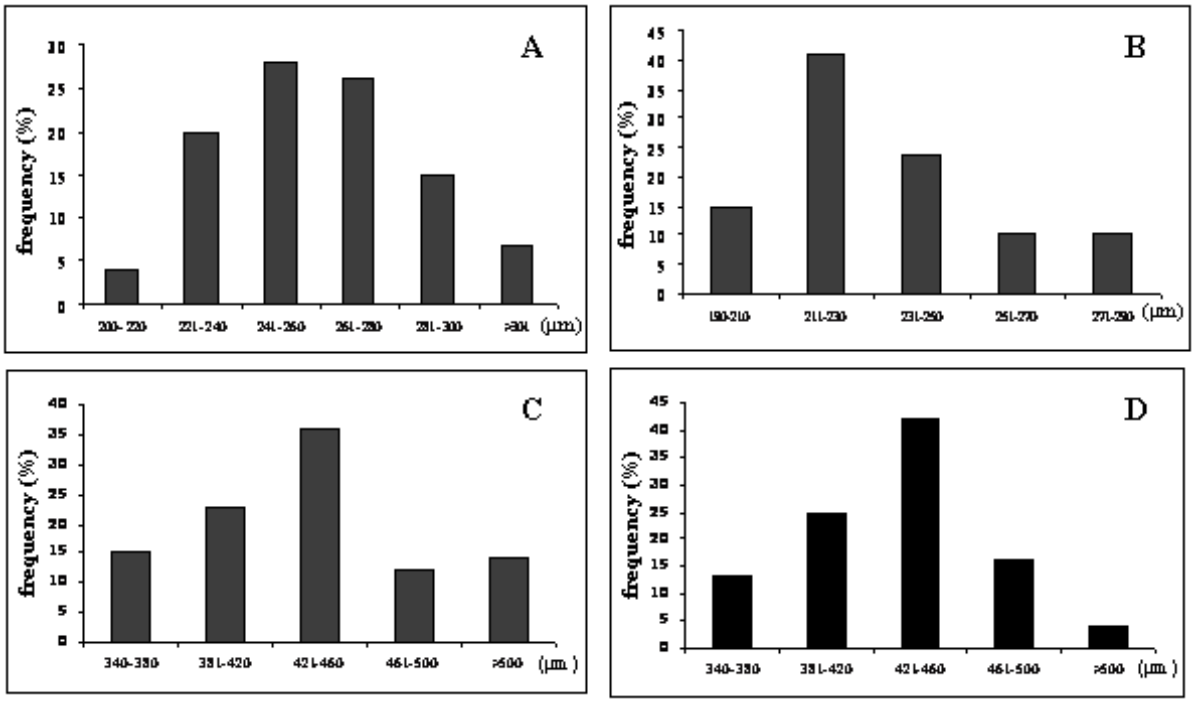

Figure 1- Size frequency distribution, A: untreated cysts; B: decapsulated cysts; C: nauplii (untreated cysts); D: nauplii (decapsulated cysts).

was clear that Cysts from Sabkhet Sijoumi are bigger than cysts from Great Salt Lake (244.2-252.5 µm) and San Francisco Bay (223.9-228.7 µm) reported by Sorgeloos *et al*. (1986).

Since artificial feed formulation is not yet available to completely substitute *Artemia*, young feeding fish larvae with live prey remains essential in commercial hatchery operations (Sorgeloos *et al*., 2001). Nauplii size appears to be the first criterion that (at least for some predator species) determines the ingestibility of specific *Artemia* nauplii (Beck and Bengtson, 1982). The biggest size of nauplii reported so far is that of the Lagkor Co Lake (Tibet, PR China) (Abatzopoulos *et al*., 1998) and the Jingyu Lake (Qinghai-Tibet Plateau, PR China) (Van Stappen, 2003) with a mean length of 667 µm and 607.1µm, respectively, (though bigger nauplii from Margherita di Savoia (517 µm) are mentioned by Vanhaecke and Sorgeloos, 1980). The fresh nauplii produced by cysts collected from Sabkhet Sijoumi, had an average length of 436.7 µm, and were much smaller than the nauplii from the Great Salt Lake, Utah (486 µm) (Van Stappen, 1996), but bigger than

Table 2. Qualitative and quantitative fatty acid profile of *Artemia* Sijoumi cysts

FAME	Percent (from total fatty acid)	mg g^{-1} (dry weight)
14:00	2.35 ± 0.1	3.57 ± 0.1
16:00	16.88 ± 0.63	25.73 ± 1.2
16:1n-7	20.55 ± 1.25	31.28 ± 0.6
18:00	4.28 ± 0.24	6.52 ± 0.21
18:1n-9	13.82 ± 0.52	21.05 ± 0.33
18:1n-7	12.06 ± 0.76	18.36 ± 0.9
18:2n-6	3.27 ± 0.31	4.97 ± 0.32
18:3n-3	2.84 ± 0.18	4.33 ± 0.15
18:4n-3	1.25 ± 0.09	1.91 ± 0.2
20:4n-6	1.16 ± 0.04	1.76 ± 0.07
20:5n-3	14.73 ± 0.78	22.48 ± 1.84
22:6n-3	N. D.	N. D.
Total lipids (% on dry weight basis)	18 ± 1.4	-

* N. D: Not Detected

Table 3. Reproductive performance of Sijoumi's *Artemia* population over 30 days of observation

Reproductive characteristic	Mean (Range)
Sum of offspring produced by the ten crosses	2593
Total offspring / female	259.3 (54 – 374)
Mean of cysts or nauplii / female	72 (12– 172)
Sum of cysts produced by the ten crosses	737
Cysts / female	67 (15 – 125)
Percentage of oviparous offspring	30.55
Sum of nauplii produced by the ten crosses	1856
Nauplii / female	74.24 (12 – 172)
Percentage of oviviparous offspring	69.45
Days between broods	5.8 (2 – 11)
Broods / female	3.6 ± 0.7

the nauplii from the San Francisco Bay, California (428µm) (Van Stappen, 1996). The comparison between nauplii length from Sabkhet Sijoumi and those from other *Artemia salina* strains revealed that nauplii from Sabkhet Sijoumi is larger than that there from Chott Marouane, Algeria (428.7 µm, Kara *et al*., 2004) and from Saline de Sahline, Tunisia (432.8 µm, Ben Naceur *et al*., 2008) but smaller than there harvested from Abu Kammash, Libya (468.2 µm, El-Magsodi *et al*., 2005).

The fatty acid spectrum of cysts and nauplii is nearly identical (Van Stappen, 1996). Fatty acid content of *Artemia* nauplii is of major nutritional value for fish and crustacean larvae (Léger et al., 1986; Navarro, 1990). It is well know that marine fish and crustacean larvae have only a limited capacity to further desaturate and chain-elongate fatty acids18:3n-3, so that 20:5n-3 and 22:6n-3 are essential fatty acids for this marine animals (Bell et al., 1985; Léger et al., 1986; Watanabe, 1988). Watanabe et al. (1980) 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. The analyses of the fatty acid profiles of cysts collected from Sabkhet Sijoumi, showed a high content in 20: 5n-3 fatty acids (22.48 ± 0.78 mg g^{-1} dry weight) and a low one in 18:3n-3 (2.83 ± 0.18 mg g^{-1} dry weight), compared to commercial cysts reported by Dhont and Sorgeloos (2002) from GSL with 34.2-49.4 mg g^{-1} dry weight for 18:3n-3 fatty acid and 3.9-4.7 mg g^{-1} dry weight for 20: 5n-3 fatty acids. Based on the classification of Watanabe et al. (1980), cysts from Sabkhet Sijoumi are suitable for marine fish and crustacean larvae. Besides, arachidonic acid (20:4n-6) plays an important role in fish larvae growth and pigmentation (Koven *et al*., 2000), but Sijoumi's *Artemia* showed a low quantity of this fatty acid (1.16 ± 0.04 mg g-1 dry weight). Watanabe (1988) also reported that 20:4n-6 was associated with the "marine character". The presence of DHA in a low quantity is not surprising since the same results were observed in other *Artemia* stocks from different geographical regions (Webster and Lovell, 1990; Navarro *et al*., 1992; Camargo *et al*., 2005; Abatzopoulos *et al*., 2006). The level of eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) as well as their ratio (DHA/EPA) play a crucial role in aquaculture applications for the formation of biological membranes, neural tissues development, growth, stress resistance and pigmentation in cultured marine organisms, Particularly at the larval stage (Mourente *et al*., 1993; Bell *et al*., 1995; McEvoy *et al*., 1998; Harel *et al*., 2002). As in other natural *Artemia* populations, a low ratio of DHA/EPA is observed in *Artemia* cysts of Sabkhet Sijoumi. Therefore, special enrichment formulations had to be developed (Dhont and Sorgeloos, 2002).

The study of the reproductive characteristics of *Artemia* populations from different areas may reflect the adaptation pattern resulting from the compromise between the natural population and their local habitats, which are diverse in origin, water chemistry, salinity, temperature and stability (Abreu-Grobois, 1987). These adaptations can be expressed by several characteristics such as fecundity, variation in offspring quality (cysts or nauplii) for ensuring survival in populations exposed to unstable or stressful conditions. The *Artemia* of Sabkhet Sijoumi revealed under the laboratory conditions a tendency to reproduce by ovoviviparity. However, several works (Gajardo *et al*., 2001; El-Bermawi, 2003; Van Stappen *et al*., 2003; Ben Naceur *et al*., 2005) reported that in spite of these favourable conditions, some *Artemia* strains can follow an oviparous

reproduction mode.

Conclusion

This study is a contribution to the characterization of *Artemia* population from the African coast of the Mediterranean region. We dispose here the first data about biological characteristics of *Artemia* from Sabkhet Sijoumi. The nauplii instar-I showed a smaller length compared to those from GSL. Fatty acid profile suggest that *Artemia* from Sabkhet Sijoumi belonging to marine type *Artemia* with high level of EPA. For the reproductive characteristics *Artemia* from Sabkhet Sijoumi showed a tendency to reproduce by ovoviviparity. However, the quality of an *Artemia* strain is determined by both the nutritional value of the nauplii and the hatching quality of the cysts, for this reason its necessary to have freshly deposited cysts to perform hatching characteristics in order to evaluate the possibility to use *Artemia* from Sabkhet Sijoumi as food source in aquaculture.

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