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Determination of biological characteristics of *Artemia salina* (Crustacea: Anostraca) population from saline Bethioua (Oran, Algeria)

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Abstract

This study is devoted to some aspects of the morphology and estimates the quality of *Artemia* cysts (branchiopod, Anostraca) from saline Bethioua (Oran, Algeria). The standard procedure used for the outbreak of cysts is that of Sorgeloos *et al.* (1986) and for decapsulation the method of Vanhaecke *et al.* (1980).The results allowed us to detect the existence of natural population at the site, with decapsulated cysts averaging 217.66 ± 17.5µm in diameter; the length of freshly hatched nauplii was 522.36±32,4µm. Concerning the study of the quality of cysts, our results have identified efficiency outbreaks of 93120 nauplii/ g., and hatching rate of 74.34 % which is an excellent quality compared to other cysts already studied in Algeria. For the synchronization time Ts = 7h. For the determination of proteins and lipids results it show very acceptable rates especially for proteins with 55.5 % and 41 % and 8 % and 18.5 % for the lipids in the decapsulated cysts and nauplii respectively. It is concluded that cystes and nauplii of *Artemia* produced in the saline of Bethioua may be an excellent nutritional source for the larval stage of fish and crustaceans.

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Introduction

Artemia salina (L.) is an anostracan branchiopod of the Artemiidae family with an age of about 100 million years. Linný (1758) described it as *Cýncer salinus* but 61 years later, Leach (1819) transferred it to *Artemia salina*. *Artemia* Leach (1819) is living in tropical, subtropical, and temperate hypersaline habitats (Persoone et Sorgeloos, 1980). *Artemia salina* lives only in lakes and ponds with high salinity, which varies between 60-300 ppt. It is a species endemic to the Mediterranean, but is found on all continents. It has developed physiological mechanisms of adaptation that enable it to survive in sites almost inaccessible to predators and competitors. Females can reproduce by both ovoviviparity and oviparity in order to ensure the perennial continuity of the species (Browne, 1980; Amat *et al*., 1995; Barata *et al*., 1995). The brine shrimp can live for at least four months. It matures in 10 days when the optimum conditions: temperature (28°-30°C), salinity, food and population and density are reunited. The medium should be very airy and carbonated to buffer the pH (7.8 to 9.5). If the pH drops below 7.5, it is a sign of a strong bacterial fermentation. The brine shrimp do not stand at this pH and especially the lack of oxygen. *Artemia* cysts are metabolically inactive and even in the case when the lakes are dried up (Ono *et al*., 2016).

When the middle of the living conditions are not favorable, the brine shrimp can produce cysts, which have the ability to power after rehydration and give birth to a larva called nauplius (nauplii plural), and sometimes even years after. These cysts are available throughout the year in large quantities along the shorelines of hypersaline lakes, coastal lagoons scattered over the five continents. 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).

The Artemia nauplii are an indispensable link in trophic feed for more than 80% of fish fry and crustaceans larvae. This importance is due to the availability, simplicity and nutritional value compared to other foods.

Artemia has been the object of many investigations in relation to its use in aquaculture (Bengtson *et al*., 1991; Vanhaecke *et al*., 1995; Sorgeloos *et al*., 2001). In Algeria, 11 sites with *Artemia* have been located (Sorgeloos *et al*., 1986; Zemmouri, 1991; Gagneur et Kara, 2001). These stocks have been the subject of qualitative and/or quantitative studies, those of Haddag, 1991; Kara, 1998; Amarouayache, 2002; Amarouayache *et al*., 2009, Kara *et al*., 2004 and Kara *et al*., 2012.

In the present work, we were interested to the biometric characterization of nauplii, analysis of the protein and lipid profile of decapsulated cysts and quality hatching Artemia cysts of saline Bethioua.

Materials and methods

Site description

The saline Bethioua is distant 15 Km from the Mediterranean coast. It is located 20 Km south of the city of Arzew and 50 km from the capital Oran. It is located at an altitude of 58.6 m from the sea, at a latitude of 35 ° 43 North and a longitude of 00 ° 08 west. It is fed by rainwater and runoff, and reaches a maximum depth of about 1.20m average.

Sampling

The cysts were collected directly at different points on the mother's pelvis of the shore with the aid of a scoop and on water surface using a sieve of 100 µm mesh. Regarding biomass Artemia (adult), it was collected essentially in the main channel, at a depth of 30 to 70 cm; placed in plastic bottles of 1 liter and transferred to the laboratory for analyzes.

Incubation and hatching of cysts

The standard procedure used is that of Sorgeloos *et al*. (1986) and Lavens and Sorgeloos (1996). It consists of incubating 250 mg of cysts in a conical-cylindrical glass container, containing 100 ml of filtered natural seawater (0.45 to 0.2µm).

Cysts need to be kept in suspension by applying aeration from below, the temperature maintained between 25 and 28°C, a salinity of 15 to 35 g/l; a pH around 8.0; an amount of oxygen of 2 mg/l as a minimum, preferably 5 mg/l; a maximum density of cysts not exceeding 2 g/l; ensure a constant illumination of 1000 to 2000 lux. (Two ampoules of 40 watts are sufficient for four containers incubations) (Granvil Treece, 2000). The larvae were fed every 48 hours. The renewal of the rearing environment is done at least once a week to remove dead larvae, uneaten food and larvae excrement.

Biometric measurements

To study the populations of *Artemia* from the saline Bethioua, we performed measurements on dry cysts, hydrated non decapsulated cysts (1h of hydration) and hydrated decapsulated cysts by the method of Vanhaecke *et al*. (1980). Other measurements were made on freshly hatched nauplii, and on male and female adults, according to the method of Amat (1980).

Morphological analysis of adults

After 15 days of rearing, adults specimens (n = 20) in each population were harvested, fixed with a few drops of iodine (Gilchrist, 1960). A set of 10 morphological characters, namely the total length, length of the abdominal part, fork length, bag width, width of queux, antennules length, eye diameter, distance between the eyes, number of rights and lefts bristles.

These measurements were taken under a microscope equipped with a calibrated micrometer in accordance with the method described by Hontoria and Amat (1992). The average for each parameter was calculated.

Study quality hatching of cysts

The study of the quality hatching cysts harvested at the saline Bethioua was investigated by determining the hatching Efficiency (HE) (Sorgeloos *et al.,* 1978), the hatching pourcentage (HP) (Bruggeman *et al.,* 1980) and hatching time (Vanhaecke and Sorgeloos, 1982) in order to determine time synchronization (Ts).

Biochemical analyzes

The biochemical content cysts, nauplii and adult *Artemia* is considered a useful parameter for determining the quality of different populations of *Artemia* (Cristopher *et al*., 2004). The dosage of the proteins was determined using the technique described by Lowry *et al*. (1957) and the determination of total lipids according to Folch *et al*. (1957) method. Biochemical analyzes have been brought on dry decapsulated cysts as well as on adult *Artemia*.

Results

Sampling

During our sampling in saline Bethioua, we found average densities Artemia of 52.5% (from 9-250 individuals per liter). The population consisted of 75% male and female adults, 21% of juveniles and 4% nauplii.

Table 1. Biometry of *Artemia* cysts of saline Bethioua (µm).

Source	CNH	CH	CD	Chorion thickness	nauplii length
saline Bethioua	220.41±12.43	237.18±17.92	217.66±17.5	10,26	522.36

Biometric measurements

The results are summarized in Table 1, the average length of the nauplii stage I is 522.36 ± 32.4 µm, the average diameters of the non hydrated cysts is 220.41 ± 12.43 µm and is 237.18 ± 17.92 µm for hydrated cysts, it is 217.66 ± 17.5 µm for the decapsulated cysts. The chorion has an average thickness of 10.26 ± 0.15 µm.

Morphological analysis of adults

The average size of adult Artemia (mm) is 7.66. + 1.11 for males and 11.55 + 1.02 for females. For other morpho-metric measurements, they are grouped in the table 2.

Study quality hatching of cysts

The results concerning the determination of parameters of hatching cysts from Bethioua show a hatching percentage of 74.34% (48h) and a hatching efficiency of 93120 nauplii/g.

The hatching time

The cysts hatching time (T0, T10, T50 and T90) are obtained by extrapolation from the corresponding curve of this population (Fig.1). From this curve were obtained T0 = 17 h, T10 = 18 h, T50= 21 h, T90 = 25h and the time synchronization Ts = 7h.

Table 2. Morpho-metric characters of *Artemia* adult females of saline Bethioua from breeding in the laboratory (n = 20; All measurements are in mm).

Morphometric characters	Mean ± SD	Range
Total length	9.53 ±0.97	8.25-10.56
Abdomen length	5.08 ±0.44	4.09-5.41
Width of the egg sac	1.18 ±0.22	0.85-1.6
Fork length	0.27 ±0.17	0.225-0.45
Distance between eyes	1.25 ±0.15	1.05-1.6
Eye diameter	0.224 ±0.015	0.2-0.24
Width of queux	0.5 ±0.028	0.47-0.55
Antennules length	0.825 ±0.08	0.7-1
Number of right bristles	6.6 ±2.31	5-13
Number of left bristles	5.6 ±1.95	4-11

Biochemical analyzes

The data in table 2 show that the highest value of the protein was 55% recorded with the decapsulated cysts of the *Artemia* population of Bethioua. The levels of proteins found on the samples are acceptable, unlike those of lipids which are presumably low, not exceeding 8% for cysts.

Discussion

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).

Sampling and Biometric measurements

Artemia salina from saline Bethioua had an average density of 52.5% (from 9-250 individuals per liter). Clegg *et al.* (2001) argue that factors such as temperature, light intensity, and availability of food can affect the quantitative aspect and even cause temporary absence of a population of Artemia, for example densities of *Artemia* at Mono Lake (USA) according to (Lenz, 1980) range from 1 to 400 ind/l, while Mason (1967) reports that they are a few individuals per liter at the surface of water.

The data of measurements on Artemia cysts show that the diameters vary between 210 and 263.9 µm for hydrated non decapsulated cysts (CHND) and between 168 and 234 µm for hydrated decapsulated cysts (CHD). In another study on the same site Haddag (1991) reported that the cysts of bisexual strain (*A. tunisiana*) measured 239.1µm. These averages vary widely from one population to another and from one sampling period to another, even between samples from the same population (ref.) According to Kara *et al.* (2004) the hydrated cysts of Chott Merouane (Oued Souf, Algeria) had an average diameter of 236.5 µm. These findings join the results achieved by Vanhaecke and Sorgeloos (1980) carried on cysts of different geographic origins were the average diameter of hydrated non decapsulated cysts generally ranges from 220.1 µm to 330 µm, while that of decapsulated cysts varied from 200µm to 266.3µm. Regarding the thickness of the chorion, it ranges from 4.85 to 18.25 µm. This gap is smaller between samples cysts of the same population.

According to Vanhaecke and Sorgeloos (1980), the thickness of the chorion is, in no way depending on the diameter of the cysts. In other words, large cysts may have a thin chorion and vice versa.

Table 3. Biochemical composition of cysts and wild adult *Artemia*.

	Decapsulated cysts	Adult *Artemia*
Protein (%)	55,5	41,5
Lipids (%)	8	18.5

The nutritional efficiency of food prey is initially determined by its digestibility and therefore by its size and shape. Nauplii sizes vary considerably from one geographic source to another and often do not pose a problem for shellfish larvae capable of capturing and tearing food particles using its Appendices. By against, marine fish larvae, have very small oral cavities, the choice of the size of the nauplii is thus of great importance (Sorgeloos and Agh, 2005). The size of Bethioua nauplii is located between that of the San Francisco Bay strain *A. franciscana* (428 µm) and strain of Tibet (China) *A. tibetiana*. According to Ghomari (2012), the latter strain is characterized by the size of the nauplii (667 ± 32.7µm), and the diameter of these cysts (323 ±17.2 µm to 330 ± 14.6 µm) considered the greatest compared to all the bisexual and parthenogenetic other known strains. According to Agh and Sorgeloos (2005), as the size of the prey does not pose a digestive problem predatory, the use of large nauplii with higher individual energy content will be beneficial because, the predator organism will spend less energy by taking a smaller number of larger nauplii to cover its food needs.

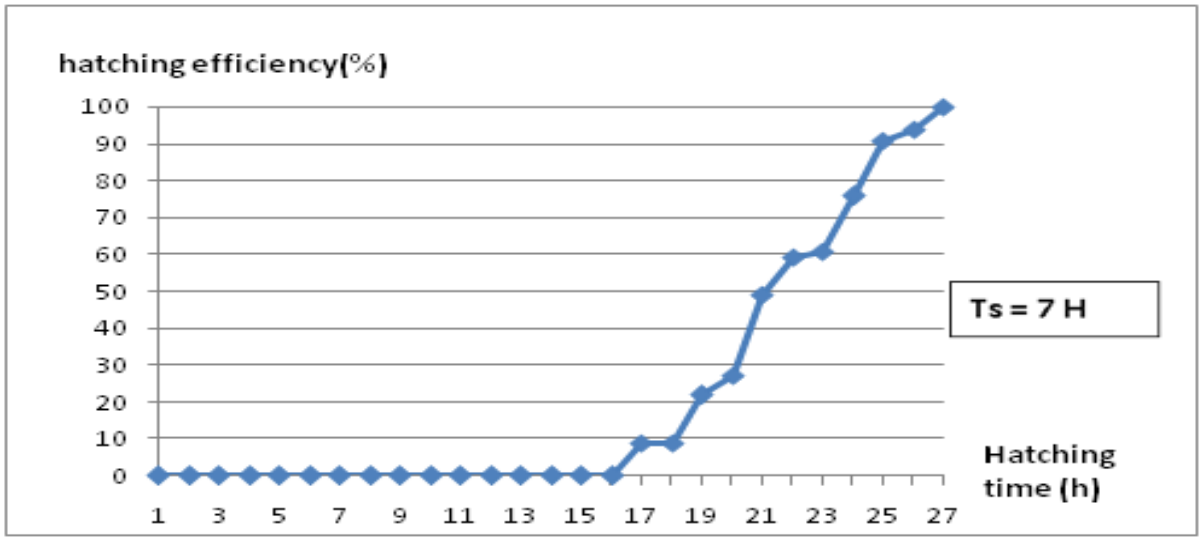

Fig. 1. Cyst hatching curve of the *Artemia* population of saline Bethioua.

The beneficial effect of feeding fish larvae with big nauplii is highlighted by experiences of Beck *et al.* (1980); Indeed, the growth of larvae *Menidia menidia* fed on large nauplii from Margherita of Savoia, GSL (489 µm) and Shark Bay (458µm) is significantly faster than that observed on the larvae fed with small nauplii SFB (428 µm) and Macau (447 µm). On the other hand, Cristopher *et al.*, 2004 reports that it is more appropriate to use the Big nauplii during advanced breeding stages, and adds they are more suitable for bioencapsulation which ensures hormones inducing ovulation (Touraki *et al.,* 1999) and the oral vaccination larvae economically sought. Our results suggest that, the point of view size, Artemia nauplii from saline Bethioua, could be used in larviculture.

Quality hatching of cysts

Our sample of cysts from the salt Bethioua shows excellent quality compared to other cysts of Algeria, with a hatching rate of 74.34% and an efficiency hatching of 93120 nauplii/g. of dry cysts. The comparison of the different results class our sample cysts in the same category as those corresponding to species of *Artemia* from San Francisco Bay (USA) (71.4%) and the Bohai Bay (China PR) (73.5%). We believe, however useful to mention that in general the hatching of *Artemia* cysts depends largely on the environmental conditions under which the cysts are produced (Bohra, 1980; Vanhaecke and Sorgeloos 1980).

The values obtained for the different hatching time suggests that for a 10% hatch rate and 90% of nauplii, it takes about 18 hours and 25 hours respectively. The duration hatching cysts Bethioua is shorter. The duration of an outbreak of other foreign strains, including those of San Francisco Bay (USA), San Pablo Bay (Canada) and Macau (Bezel) remain by far the best. The strain of San Francisco Bay, for example, requires about 17 hours and 20 hours for 50% and 90% of nauplii respectively. As for the population of *Artemia* Chott Merouane (Kara *et al.*, 2004), the T90 is 35 hours. Apparently hatching time of Bethioua population is approximate to those of cysts from Tuticorin (India) and Port Araya (Venzuela).

Regarding to the time synchronization (Ts), Dhont, Lavens and Sorgeloos, (1993) report that the latter should not be high. The appearance of the last nauplii should not exceed eight hours after the beginning of an outbreak. The time synchronization of our strain was Ts = 7H. This time is the same as the Great Salt Lake (USA) and Shark Bay (Australia).

Biochemical analyzes

The protein content varies considerably; the recorded value with decapsulated cysts of *Artemia* population of Bethioua is 55%. Our results agree with those of Amorouayeche *et al.* 2013 on the same species and give a grade of 58% protein. Sandoval (1993) indicated that the San Francisco cysts contain between 41.4 and 53.3% protein.

The resulting proteins rate is acceptable unlike the lipids which likely remain low not exceeding 8% for cysts. Our results of the levels of proteins and lipids in adult *Artemia* (41.5%) are similar to those reported by Leger *et al.* (1986), on wild *Artemia* GSL.

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Conclusion

View the results obtained in the context of our work, we can conclude and suggest that the salt lake of Béthioua (Oran) may be a potential candidate for the exploitation of *Artemia salina* in Algeria. Therefore, it is imperative to further this work and to insist that other studies initiated in this area to better manage the resources and so one day creating hatcheries establishments of fish larvae fresh water and marine in Algeria.

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