

On a parthenogenetic population of *Artemia* (Crustacea, Branchiopoda) from Algeria (El-Bahira, Sétif)

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Abstract

The brine shrimp *Artemia* is a small crustacean of hypersaline lakes which is commonly used in larviculture. The parthenogenetic population of *Artemia* from El-Bahira Lake (10 ha area), situated in the High Plateaus of Northeastern Algeria (1034 m alt), has been characterized and surveyed during two hydroperiods of 2009 and 2013. Contrary to other known parthenogenetic populations, which develop in hot seasons and reproduce by ovoviviparity, *Artemia* from El-Bahira was found to develop only in cold seasons (winter and spring), even if the lake doesn't dry in summer. It reproduces by oviparity and produces few cysts (5.69 ± 3.6 and 98.00 ± 28.32 offsprings/brood). Individual density was much lower during the hydroperiod of 2013, whereas fecundity was higher than in the previous hydroperiod (2009). Cyst reserve was estimated at 133.13 kg of dry weight which corresponds to a rate of $13.31 \text{ kg} \cdot \text{ha}^{-1}$.

Keywords : *Artemia* parthenogenetic, cold seasons, oviparity, cyst reserve

Resumen

El camarón de salmuera *Artemia* es un pequeño crustáceo de lagos hipersalinos que se utiliza comúnmente en la larvicultura. La población partenogenética de *Artemia* de El-Bahira Lake (área de 10 ha), situado en las altas mesetas del Noreste de Argelia (1034 m alt), se ha caracterizado y estudiado durante dos hidroperiodos de 2009 y 2013. A diferencia de otras poblaciones partenogenéticas conocidos, que se desarrollan en las temporadas de calor y se reproducen de manera ovípara, se encontró *Artemia* de El-Bahira desarrollar sólo en épocas de frío (invierno y primavera), aunque el lago no se seca en verano. Se reproduce de manera ovípara y produce unos quistes ($5,69 \pm 3,6$ y $98,00 \pm 28,32$ crías / cría). Densidad individual era mucho más bajo durante el período hídrico de 2013, mientras que la fecundidad era mayor que en el período hídrico anterior (2009). Reserva quiste se estimó en 133.13 kg de peso seco que corresponde a una tasa de $13.31 \text{ kg} \cdot \text{ha}^{-1}$.

Palabras clave: partenogenética *Artemia*, estaciones frías, ovípara, reservas de quistes

Introduction

The genus *Artemia* Leach, 1819 (Branchiopoda, Anostraca) is a small crustacean that lives in saline and hypersaline lakes all around the world except in Antarctica (Van Stappen et al. 2002). It was adapted to live in different variation of environmental factors, particularly the salinity; thanks to a highly efficient osmoregulation system. When conditions are favourable, females are able to produce either tens to hundreds of nauplii or cysts (to overcome drying of the environment and thus, ensure the continuity of the species) (Gajardo & Beardmore, 2012). The life cycle is usually short, but can last from 20 days to 3 months in some species. According to geographic origins and abiotic factors, populations have different life histories and development strategies as response to environmental conditions (Barata et al. 1995). The genus *Artemia* comprises groups of bisexual and parthenogenetic forms which are the most widespread. Three bisexual species are endemic to the New World and all the other species, except *Artemia monica* Verrill, 1869 (Mono Lake, California) and the South American *Artemia persimilis* Piccinelli & Prosdocimi, 1968 (De Los Rios-Escalante & Salgado, 2012), are found in the Old World. The latter is the most ancient (80-90 MYA) and common ancestor of all *Artemia* species (Baxevanis et al. 2006). The

American *Artemia franciscana* Kellogg 1906, considered as invasive, recently invaded several biotopes in the Old World (Amat et al. 2005). Except the reported presence of parthenogenetic cysts in commercial tanks mixed to *A. franciscana* strains from Great Salt Lake, this form is absent in American continent (Campos-Ramos et al. 2003, Endebu et al. 2013). Thus, the brine shrimp *Artemia* is widely commercialized for use as food in aquaculture, its nauplii represent 85 % of live preys which are routinely used in finfish and shellfish larval rearing (Dhont & Sorgeloos, 2002). They are sold in form of cysts which are collected in hypersaline environments. A great part of the marketed products comes from the Great Salt Lake (Utah, USA) which produces thousands tons per year of cysts (Utah DNR-DFFSL, 2011). Even if over 700 biotopes lodge this brine shrimp (Van Stappen et al. 2002, Litvinenko et al. 2014), the demand of aquaculturists is continually increasing, which favours the research of new strains and new deposits.

In Algeria, the occurrence of *Artemia* has been reported in at least 24 sites. Fourteen of them have been characterized by morphometry (Ghomari et al. 2011, Kara & Amarouayache 2012), but only two populations of *Artemia salina* are well-known; chott Marouene in the Sahara (Kara et al. 2004, Amarouayache et al. 2009ab, Amarouayache and Kara, 2010) and sebkha Ez-Zemoul (Amarouayache et al. 2009b, 2010, 2012). This work is a contribution to the characterization of Algerian populations of *Artemia*. It aims to obtain qualitative and quantitative data on a new parthenogenetic population and its cyst reserve for aquaculture and/or ecological purposes.

Materials and methods

Description of the study site

El-Bahira Lake (also called Bahr Lehnamide, Rasfa) is a small salt lake (10 ha) situated in Sétif district (35°50'N; 05°15'E) in the Eastern High Plateaus (1034 m, alt.). The semi-arid climate in this region is characterized by a cold winter with 14 days of snow, an average of 414.5 mm/year of annual rainfall, and a dry and hot summer. Water column is represented by a thin layer near the banks and a maximum depth of 2 m into the center. Thus, water temperature follows atmospheric temperature (see Table 1). Annual precipitations were of 403 mm/year in 2009 and 492.6 mm/year in 2013. Except for some helminthes parasitizing *Artemia*, this crustacean is the only representative of the aquatic fauna.

Table 1. Monthly atmospheric temperatures (T°C) during the study periods 2009 and 2013.

Months	J	F	M	A	M	J	J	A	S	O	N	D
T(°C) 2009	5.1	4.6	8.6	9.2	18.0	23.6	28.7	26.2	19.4	15.1	11.2	7.9
T(°C) 2013	5.5	4.0	9.5	3.2	15.1	20.3	25.8	24.7	21.3	20.1	9.2	6.4

Sampling and analysis

The study of density and demographic structure of *Artemia* population from El-Bahia Lake was carried out during two humid cycles. The site was frequently visited from the beginning of the filling to the total disappearance of the population, or complete drought of the biotope. Samples have been collected all around the lake in 5 different sites, bimonthly in 2009 from February to June and monthly in 2013 from January to June. We should note that brine shrimps were totally absent during two successive hydroperiods of 2014 and 2015, when salinity was near saturation and water level was very low. According to animal density, 1 to 10 L of *Artemia* biomass were filtered on a sieve of 125 µm mesh opening then fixed in 4% formalin. Water salinity was measured during sampling.

The number of individuals has been counted under binocular, using Cuve Dollfus for density. They were separated into developing stages according to morphological criteria described by Sorgeloos et al. (1986) as follows: (1) nauplii and metanauplii (instar I - instar IV), (2) juveniles and pre-adults (instar V - instar XIV), and (3) adults.

Mature females have been considered for biometry and reproduction. Individuals were measured from the naupliar eye to anus using a compound microscope equipped with a micrometer (Amat 1980). Ovisacs have been dissected and their content, cysts or nauplii have been counted to

determine reproduction mode oviparity or ovoviviparity, and the fecundity which was determined by number of offsprings per brood (Amat 1982).

Once laid by females, cysts float on the water surface and are carried away by winds, then they are deposited on the banks in the form of stripes and dehydrate forming a dark brown crust. Reserve of cysts has been estimated during the dry season of 2009. This estimation consists first to measure the area of the stripes of cysts appointed into 6 stations. Several samples of cysts have been collected within a square of 400 cm² along these stripes. Cysts have been cleaned, separated and dehydrated according to the protocol described by Sorgeloos et al. (1986), and then weighted. The mean dry weight of cysts in each square was then extrapolated to the total area of the stripe for each station. The total dry weight of cysts deposited on the banks of the lake was determined.

Results

Artemia developed in El-Bahira Lake from January-February to May-June when salinity was between 46 and 127 ppt (Tab. 2). However, during the second hydroperiod of 2013, adults dominated in January which means that *Artemia* appeared earlier. The younger stages (nauplii and metanauplii) were dominant at the beginning of the wet period during several months and became less represented or totally absent at the end of the wet period. Older stages were dominant at the end of both hydroperiods of 2009 and 2013 (Fig. 1).

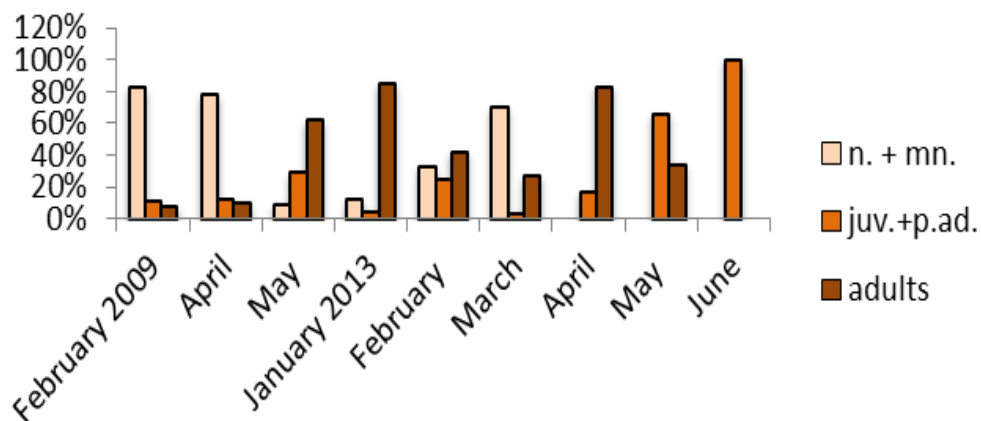


Figure 1. Demographic structure (n.+ mn.: nauplii and metanauplii, juv.+p.ad.: adults and pre-adults, adults) of *Artemia* in % during two hydroperiods of 2009 and 2013.

Individual as well as adult densities were much higher during the first hydroperiod, and high values were observed in April/May with 107.7 ind.L⁻¹ and 10.97 ind.L⁻¹ in 2009 and 2013 respectively (Tab. 2). The highest adult's density was of 11.12 ind.L⁻¹ in 2009 and 4.13 ind.L⁻¹ in 2013. Adults measured between 8.00 ± 0.01 and 12.15 ± 1.13 mm with a maximum individual size of 14.48 mm observed in April 2009 (Tab. 2). No males have been recorded. Females reproduced by oviparity during the two hydroperiods. Only few females <10% reproduced by ovoviviparity in 2009. Fecundity was between 5.69 ± 3.60 and 18.43 ± 9.67 offspring/brood (n = 80) in 2009 and between 15.66 ± 21.61 and 98.00 ± 28.32 offsprings/brood in 2013 with a maximum individual fecundity of 134 offsprings/brood observed in February 2013 (Tab. 2).

Table 2. Variations of water salinity (ppt), adult size, fecundity, adults and total population densities during the two hydroperiods of 2009 and 2013.

Parameters	Feb. 2009	Apr.	Jun.
Salinity (ppt)	49	80	120
Adult size (mm) ± SD	9.06 ± 0.83	9.72 ± 1.00	12.15 ± 1.13
Fecundity (offspring/brood)	5.69 ± 3.60	11.43 ± 8.23	18.43 ± 9.67
Adults density (ind.L ⁻¹)	4.56 ± 0.41	11.12 ± 1.37	7.64 ± 0.24
Population density (ind.L ⁻¹)	63.8 ± 3.22	107.7 ± 7.78	12.38 ± 1.60

Parameters	Jan.2013	Feb.	Mar.	Apr.	May	Jun.
Salinity (ppt)	46	93.2	102	113	109	127
Adult size (mm) ± SD	8.00 ± 1.01	11.02 ± 0.72	10.4 ± 1.29	10.66 ± 1.26	9.06 ± 0.90	-
Fecundity (offspring/brood)	15.66 ± 21.61	98.00 ± 28.32	30.33 ± 29.79	19.60 ± 22.29	25.86 ± 19.38	-
Adults density (ind.L ⁻¹)	1.47 ± 0.02	0.50 ± 0.00	0.63 ± 0.00	4.13 ± 0.93	3.73 ± 0.74	-
Population density (ind.L ⁻¹)	1.73 ± 0.32	1.20 ± 0.02	2.33 ± 0.07	4.97 ± 0.96	10.97 ± 1.66	0.3 ± 0.01

Table 3 shows cyst amounts around El-Bahira Lake. Their distribution on the banks is heterogenic. They are concentrated in station 2 (92.6 % of total reserve) situated in the southwest with a production of 123.30 kg. Total cyst production of the lake was estimated at 133.13 kg, which corresponds to a production rate of 13.31 kg.ha⁻¹.

Table 3. Cyst reserves (kg) on the banks expressed by dry weight (DW). St: stations.

Parameter	St.1	St.2	St.3	St.4	St.5	St.6	Total
Cyst's strip area (m ²)	46	262.5	82.5	33	30	14	468
Cyst's DW (kg)	0.96	123.3	2.29	1.44	0.24	4.9	133.13
% cyst's DW/total reserve	1.08	92.6	1.72	1.08	0.18	3.68	100

Discussion

In this study we observed that *Artemia* appeared in the lake in January when salinity was at its lowest level. It is admitted that cysts can hatch in high salinities up to 70 ppt (Sorgeloos et al. 1986). When salinity increases above that level, a temporal stratification can take place allowing hydration and hatching of cysts floating on the surface within the thin layer of water which is less saline. Recruitment of young stages was observed during the first months at the beginning of the wet period and adults were always present. This means that more than one generation could develop in the site.

The population of *Artemia* from El Bahira Lake is exclusively represented by females; this means that the population is parthenogenetic. According to Maniatsi et al. (2010) bisexual females of *Artemia* can't borrow parthenogenesis and these two forms are clearly separated, but both can co-occur together (Amat 1983). However, it has been found that the bacteria *Wolbachia* could manipulate females and induce the feminization of a bisexual population (Maniatsi et al. 2010). During these two study periods over 9 months of the presence of *Artemia*, males have never been observed. Even if the lake was full of water, the occurrence of *Artemia* was restricted to the cold seasons; winter and spring. This is the case for two other Algerian bisexual populations of *A. salina*, that of chott Marouane (Amarouayache et al. 2009a) and that of sebkha Ez-Zemoul (Amarouayache et al. 2010). However, this finding doesn't corroborate with conclusions of Ghomari & Amat (2014), about parthenogenetic populations from the west of Algeria that prefer hot seasons. Van Stappen et al. (2001) found in Urmiah Lake that a parthenogenetic population co-occurred with the bisexual *A. urmiana* which was present only in cold seasons, while the parthenogenetic populations occurred year around. According to Browne and Wanigasekera (2000), parthenogenetic forms

prefer more stable habitat conditions and often reproduce by ovoviviparity, while bisexual species experience more harsh conditions and are rather oviparous. Barata et al. (1995) concluded from their ecological study that parthenogenetic populations were more adapted to high temperatures and low salinities, whereas the populations of *A. salina* live in ephemeral ponds that fill in winter and spring in the Mediterranean region. The parthenogenetic population of El-Bahira has the same behaviour as the Mediterranean bisexual species *A. salina*, it prefers cold temperatures of winter and spring and reproduces by oviparity even when the lake is filled in hot seasons. From the viewpoint of use in aquaculture, the production of cysts is more advantageous, but in ecological point of view, the production of nauplii enhances the population density in the site because of a direct development (Sorgeloos et al. 1986). Adults of El-Bahira Lake are larger than bisexual populations of Algeria (Amarouyache et al. 2009a, 2010). It is admitted that asexual forms are larger than sexual ones due to larger size of cells that constitute their tissue (Amat 1980b). In this study, females are considered as little fertile, in comparison with other parthenogenetic populations which can produce hundreds of offspring per brood (Browne & Vanigasekera 2000), but as fertile as Algerian bisexual populations which produce between 10 and 60 offsprings/brood (Kara & Amarouyache 2012). *A. franciscana* from the most commercialized strain of the world (Great Salt Lake) also produces some tens of offsprings/brood in natural conditions according to Gliwicz et al. (1995). As example, Van Stappen et al. (2001) considered fecundity comprised between 30 and 80 offspring/brood, produced by *Artemia* population of Urmiah Lake, as very high. The maximum of fecundity was attained in February then it decreased with increase of salinity during the second hydroperiod. This observation has been reported in many sites and it should be due to energy consumption in osmoregulation which prevents a high cyst production (Amat 1982). However, in 2009, this tendency was not observed. In another hand, it has been observed that fecundity was higher during the 2nd hydroperiod while densities were lower overall. Lenz & Dana (1987) concluded in their study that the decrease of fecundity when densities were high was a way to equilibrate the populations, which corroborates with our findings.

Despite the importance of quantitative aspect, studies on *Artemia* density and biomass in natural environments are scarce (Haslett & Wear 1985, Basbug & Demirkalp 1997, Van Stappen et al. 2001) because of the instability of the populations linked to climatic variations (Guermazi et al. 2009, Amarouyache et al. 2010). Indeed, a large decrease in density was observed between the two studied periods, with a maximum of 10.97 ind.L⁻¹ in 2013 against 107.7 ind.L⁻¹ observed in 2009. The same variations were found in *A. salina* of sebkha Ez-Zemoul (Amarouyache et al. 2010). These authors concluded that this situation was perhaps due to decrease of food availability, especially when other crustaceans co-occur, and predation by insects and water birds. Lenz and Browne (1991) reported maximum densities of 10 ind.L⁻¹ in hypersaline lakes of North-America, while average of about 50 ind.L⁻¹ was reported in Tunisian marsh of Sfax (Guermazi et al 2009) and in Western Siberia (Litvinenko et al. 2014). Lavens et al. (1986) have considered a natural density of 100 ind.L⁻¹ as very high.

Little is known about *Artemia* cysts production in saline lakes (Haslett & Wear 1985, Litvinenko et al. 2014). The greatest part of commercialized product (35-40 %) for aquaculture use is from the Great Salt Lake of Utah (USA), with an annual production between 3000 and 10,000 tons of raw cysts biomass recorded during this last decade (Utah DNR-DFFSL 2011). Recently, Litvinenko et al. (2014) reported statistics of *Artemia* biomass and cyst reserves from several Asiatic countries; saline lakes from Southern Siberia produce 1100 tons annually for local commercialization. In El-Bahira Lake, the cyst reserves were estimated at 133.13 kg of dry weight which corresponds to a rate of 13.31 kg.ha⁻¹. They are concentrated in the South-Eastern region opposed to dominant winds which generally blow from the Northwest. This value remains an underestimation since a fraction of newly layed cysts stays in water column. However, it can be considered as a good production according to Persoone & Sorgeloos (1980), who reported that a productive biotope gives 10 to 20 kg/ha/season. In Algeria, data on *Artemia*'s cysts production are scattered and exploitation is not organized. The chott Marouane (Eastern Algeria), a huge saline lake in the Sahara, is the most important and the biomass of cysts has been estimated at 7.6 tons at its Southern part (Amarouyache & Kara 2010).

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