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Biological Characteristics of *Artemia* Parthenogenetic Populations (Crustacea *Anostraca*) from Algeria: Survival, Growth and Reproduction

Amorouayeche Abdelkader, Belksier Mohamed Hacen, Amarouayache Mounia*

Marines Bioresources Laboratory, Annaba University BadjiMokhtar, Algeria

***Corresponding author:** m.derbal@yahoo.fr

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ABSTRACT

The brine shrimp *Artemia* is an important live feed used worldwide in shrimp and fish larviculture. Parthenogenetic strains are less known and less used, compared to bisexual, owing to their larger size and their dominant ovoviviparous mode of reproduction. The life cycles of *Artemia* parthenogenetic population from two Algerian salterns (Sidi Bouziane and Bethioua) were studied under laboratory conditions in order to provide additional data for future management of aquaculture farming. Individuals were reared at a salinity of 120g l^{-1}and a temperature of 24°C and fed with baker's yeast and a vitamin complex. Survival rate, growth, adult size and reproduction traits were determined. Survival rate of 50% was attained after 10 days, and the growth rate was 0.19mmday^{-1} for both populations. Adults lengths were 8278± 489 and 8710± 551.0 m for Sidi Bouziane and Bethioua, respectively. Females attained their first sexual maturity at 28 to 30 days of experiments. Ovoviviparity was the dominant reproduction mode (>75% of all cases) in both populations. Mean fecundity was 23.5± 14.9 and 24.82± 12.06 offsprings per female for Sidi Bouziane and Bethioua, respectively. Overall, the two populations showed similar life history traits. The suitability and the possible use of these parthenogenetic populations in aquaculture were discussed.

INTRODUCTION

Artemia **(Leach, 1819)** is a small brine shrimp (Branchiopoda, Crustacea) that lives in saline and hypersaline lakes, distributed in arid and semi-arid regions worldwide, except in the Antarctica **(Abatzopoulos *et al.*, 2002)**. It is an extremophile and cosmopolitan with two modes of reproduction: ovoviviparity when environment conditions are favorable and oviparity by producing resting eggs to ensure the perennity of the species during drought **(Gajardo & Beardmore, 2012)**. Actually, there are nine recognized bisexual species, including *Artemia salina* **(Leach, 1819)** from Europe and North Africa **(Asem *et al.*, 2023)**. In addition to the aforementioned locations,, the parthenogenetic *Artemia* populations with different ploidy are found with rare exceptions for the New world **(Endebu *et al.*, 2013)**, and in the Old World **(Nougué *et al.*, 2015)**.

Bisexual females of *Artemia* do not borrow parthenogenesis as the case in other branchiopods **(Maniatsi *et al.*, 2011)**. However, both bisexual and parthenogenetic species can co-habit together **(Amat *et al.*, 1995; Lantushenko *et al.*, 2022)**. These forms have different life history strategies when living in the same biotope and have developed different tolerance degrees toward the environmental conditions **(Ghomari & Amat, 2014)**. **Browne and Wanigasekera (2000)** considered parthenogenetic forms as niche specialists because of their narrowest tolerance range for salinity and temperature in comparison with bisexual species; thus, they prefer more stable habitat conditions. **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 and should be oviparous. Thanks to resting eggs' production, *Artemia* can overcome the dry season in these environments.

In addition to field works, experimental studies have been conducted on the effect of temperature and salinity on survival and lifespan of different populations of both reproductive forms of *Artemia* in controlled conditions **(Vanhaecke *et al.*, 1984; Larti *et al.*, 2012)**. It has been found that parthenogenetic populations outcompeted all the other species; they reproduce longer, have higher fecundity and larger size, and prefer ovoviviparity, which allows high *Artemia* densities in the environment **(Browne & Wanigasekera, 2000)**.

Artemia nauplii and other developing stages are currently used in aquaculture as food for young larval stages in shrimp industry (80%) and fish (12%), thanks to their nutritional value **(Léger *et al.*, 1986)** and since other numerous advantages of their utilization were highlighted **(Sorgeloos *et al.*, 2001)**. The bisexual American species *Artemia franciscana* (Kellog, 1906) from Utah was and remains the most marketed worldwide, with more than 4000t/ year of dry cysts in 2010 **(Van Stappen *et al.*, 2020)**. During the last decade, the Chinese and Russian/Kazakhstan cyst production increased drastically to represent around 30% each of the world market **(Litvinenko *et al.*, 2015)**. In addition to aquaculture, human consumption in Asian countries has been developing in recent years **(Van Stappen *et al.*, 2020)**. This fact presents an opportunity to utilize and value adult biomass alongside cysts in *Artemia* farming.

North Africa is rich in saline lakes, locally called Chotts and Sebkhas, which harbor bisexual and parthenogenetic populations of *Artemia* **(Kara & Amarouayache, 2012)**. Unfortunately, being ephemeral and often inaccessible, the exploitation of their wild brine shrimps is complicated, despite several works which demonstrated the suitability of several strains for aquaculture use **(Kara *et al.*, 2004; Amarouayache & Kara, 2015; Amarouayache *et al.*, 2017; Chabet Dis *et al.*, 2021)**. Sixteen strains have been morphologically or genetically characterized, showing the presence of *A. salina*, and parthenogenetic populations with 2n and 4n ploidy **(Ghomari *et al.*, 2011)**. The main biological and ecological studies concerned bisexual populations from the eastern and southwestern Algeria **(Zemmouri, 1991; Kara *et al*., 2004; Amarouayache *et al*., 2009; Amarouayache *et al*., 2010; Amarouayache *et al*., 2012; Chabet Dis *et al*., 2021)**. Parthenogenetic populations are still less studied and little is known in Algeria or even elsewhere **(Saygi, 2004; Amarouayache & Belakri, 2015; Chabat Dis *et al*., 2023)**.

The aim of this work was to better understand the life history of two parthenogenetic populations from the Northwest of the country under experimental conditions, and to determine whether they are suitable for production for aquaculture and agri-food industries in general.

MATERIALS AND METHODS

Artemia rearing

Artemia's cysts were collected in Sidi Bouziane saltern (Rélizane district) at coordinates 35°50'37".08N-00°43'60.00"W and in Bethioua saltern (Arzew district) at coordinates 35°41N-00°17'W, situated in a semi-arid region in the northwest of Algeria, 140km apart from each other (Fig. 1). They were cleaned, separated, and stored according to **Sorgeloos *et al*. (1986)** protocol. A 0.2g cyst sample was incubated in 1L Zug bottles at standard conditions recommended by these authors: 37 psu sea water, temperature 24°C, continued aeration, 2000 lux light, pH 8. After 36h, the freshly hatched *nauplii* samples were immediately transferred, after count, in 1L glass tanks where were reared, as described in **Amarouayache and Kara (2017)**. The starting density was of 1000ind.L^{-1} for each population. A brine of 120psu concentration was prepared for rearing using distilled water and industrial salt. The choice of this salinity is justified by the approximate upper limit of the presence of other halophilic organisms for future managements. Temperature of water was maintained by thermostatic control 24± 1°C, photoperiod was of 16 : 8 light : dark photoperiod, oxygen was maintained at saturation using an aerator. Brine shrimps were fed with 85% baker's yeast and 15% vitamin complex **(Barnabé, 1991)**. The yeast was prepared by dissolving an amount in a 50ml vial with distillated tepid water (30°C) according to doses recommended by **Coutteau *et al*. (1992)**. The yeast was daily distributed, while the vitamin complex was added twice a week with the water renewing. Each experiment was repeated twice for both populations.

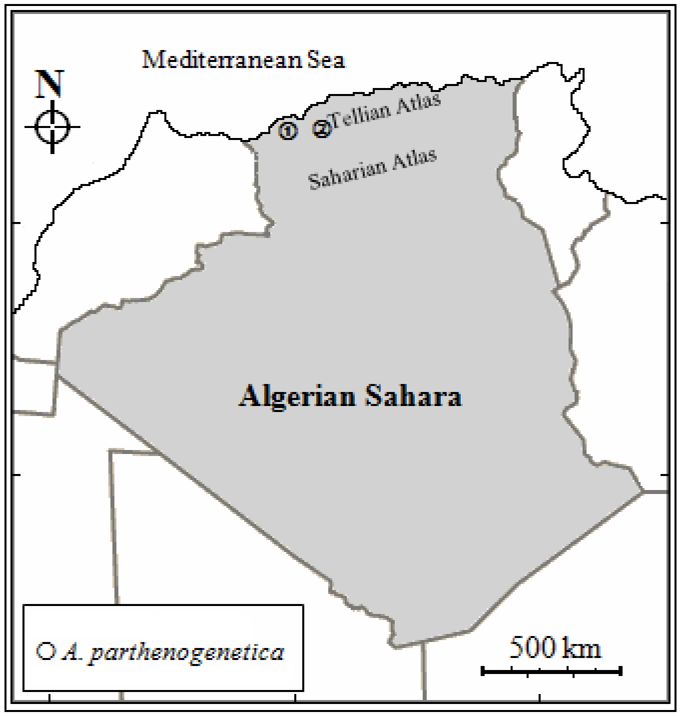

Fig. 1. Localization of study sites: 1. Bethioua saltern; 2. Sidi Bouziane saltern

Survival and growth

The survival rate, expressed in percentage of surviving *nauplii* compared to the initial density, was determined by subtracting the number of dead individuals from the total count twice a week from day 1 to day 45. Dead individuals were removed from the culture during each water change. For measurements, individuals were anaesthetized using diluted chloroform at a concentration of one drop in 4ml of water from the rearing medium **(Amat, 1980)**. From each tank, five individuals were measured, from the naupliar eye to the anus, under a compound microscope equipped with an ocular micrometer (x 10), every two days until the end of the experiment. Then, they were rinsed with fresh water to reanimate them and placed back in their rearing medium. The sizes (at the anus) of male and female adults were determined once the growth was completed (at death).

Reproductive parameters

Length and age at first sexual maturity were determined. Females were considered as mature once 50% of the population presented sign of reproduction, such as the presence of embryos or developed shell glands in the ovisacs **(Amat, 1982)**. Thirteen mature parthenogenetic females for each population were reared separately in small glasses filled with 100ml of brine (120 psu). The presence of cysts or *nauplii* was daily checked in each box, and water was renewed after their counting and removal. Eight reproductive parameters were determined according to **Browne *et al*. (1984)**: total fecundity (offsprings/females), brood number, fecundity (offspring/brood), oviparity percentage, pre-reproductive period (day), reproductive period (day), post- reproductive period (day) and females' longevity.

Statistical analyses

The survival curve and the regression line for the growth of the two populations of *Artemia* were realized in Excel 2007 statistical program. The corresponding regression equations and correlation coefficient for each of the two parameters were calculated using the same program. Growth rate was deduced from regression equation (b). Adults' size and life history traits were compared one by one using the Student's t-test ($P \leq 0.05$) implementing Prism software after a Shapiro-Wilk test of normality.

RESULTS

The survival rate of both populations of *Artemia* is expressed in Fig. (2). The survival curbs are similar in both populations, and 50% of survival was attained after 10 days, while 10% of survival was attained after 40 days. The latest individual of Bethioua survived until 107 days. The growth regression lines are illustrated in Fig. (3). The growth rate was of 0.19mm.day^{-1} for both populations of Sidi Bouziane and Bethioua, with respective correlation coefficient between size and days $R^2 = 0.97$ and $R^2 = 0.99$, respectively. It was noticed that growth continues throughout individual life. Mean sizes at the end of growth were 8278± 489.3 and 8710± 551.0µm for Sidi Bouziane and Bethioua, respectively, and were not significantly different (t = 0.478, P=0.64). The maximum individual size was 9.1mm for Sidi Bouziane and 11.5mm for Bethioua.

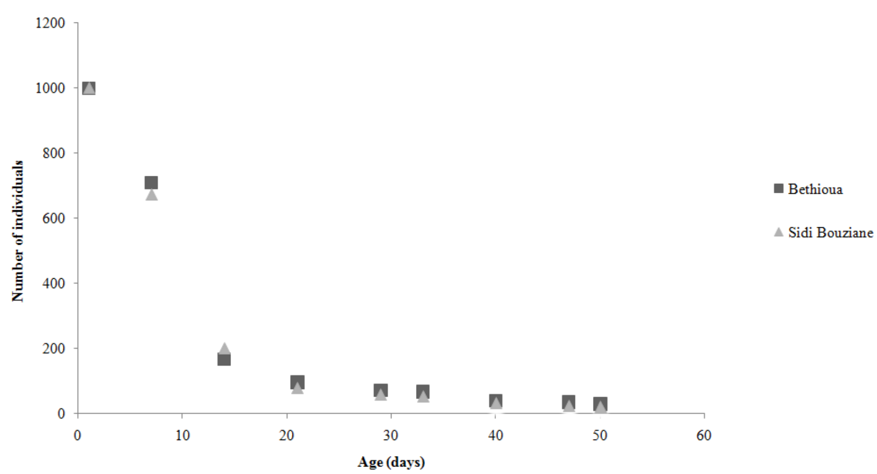

Fig. 2. Survival rate (% day^{-1}) of *Artemia* females from Sidi bouziane and Bethioua salterns

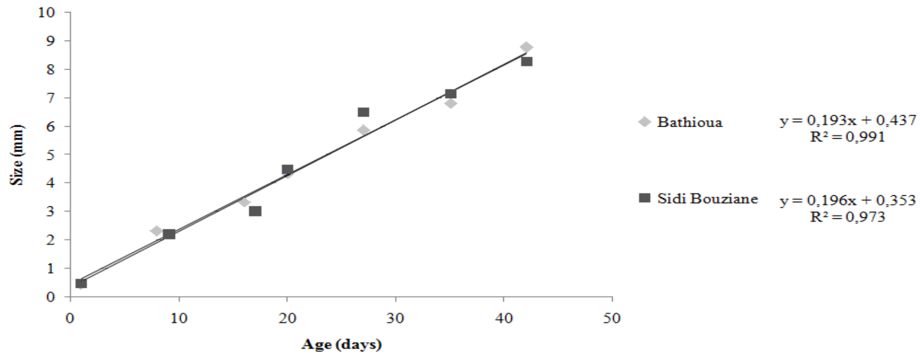

Fig. 3. Calculated growth curve (regression line) (mm day^{-1}) of parthenogenetic *Artemia* from Sidi Bouziane and Bethioua salterns

All *nauplii* samples reared in this study were differentiated into females, which confirms the parthenogenetic mode of life, except one observed male. Reproductive parameters are summarized in Table (1). Maturity was attained after 28.1± 1.6 and 31.3± 2.1 days for Bethioua and Sidi Bouziane females, respectively. Mean reproductive period was between 1.3 and 6.9 ± 4.5 days for Bethioua and Sidi Bouziane, respectively. Mean post reproductive period lasted for 1 to 3 days. Among reared females, 66.7% of Bethioua individuals were productive against 70% for Sidi Bouziane. Ovoviviparity was the dominant reproduction mode (>75%) in both populations. Mean fecundity was relatively low (23.5- 24.8 offsprings/female), with a maximum individual fecundity of 41 and 83 offsprings/female for Bethioua and Sidi Bouziane, respectively. Overall, the Student's t-test showed no difference between all the reproductive parameters, except the reproductive and post-reproductive periods (Table 1).

Table 1. Mean (± SD) of reproduction parameters in *Artemia* from parthenogenetic populations of Bethioua and Sidi Bouziane salterns

Parameters	A	B	C	D	E	F	G	H
Bethioua population	23.5 ±14.9	1.2 ±0.44	23.5 ±14.29	16.66	43.16 ±7.90	1.33 ±1.50	4.66 ±3.07	47.66 ±9.64
Sidi Bouziane population	36.57 ±23.37	1.42 ± 0.72	26.16 ±11.58	28.57	41.14 ±4.33	6.85 ±4.52	11.28 ±12.53	52.42 ±12.76
Student t-test (*P*)	1.188 (0.25) ns	0.825 (0.42) ns	0.181 (0.85) ns	-	0.584 (0.57) ns	2.955 (0.01)*	3.162 (0.009)**	0.747 (0.47) ns

Student t-test was used for comparison between the two populations. A : Total fecundity, B : Brood number, C : Fecundity, D : Oviparity, E : Pre-reproductive period, F : Reproductive period, G : Post-reproductive period, H : females longevity, ns : non significative difference, * : highly significative difference.** very highly significative difference.

DISCUSSION

Algerian parthenogenetic strains showed a relatively good survival performance compared with bisexual populations **(Amarouayache & Kara, 2017)**. **Ogello *et al*. (2014)** tested populations of the bisexual commercial strain of *Artemia franciscana* from Kenya, Vietnam and San Francisco and obtained 50% survival between 6 and 10 days and 100% mortality after 24 days. In a recent similar study on Bethioua population, **Chabet Dis *et al*. (2023)** concluded that this parthenogenetic species showed a better survival performance at low salinities. However, among the advantages of the brine shrimp farming, the high salinities avoid the appearance of other alive organisms in the rearing medium.

Experimental studies on the growth of *Artemia* have been multiplied in the last years **(Kristanti *et al*., 2020; Kundu *et al*., 2021, Li *et al*., 2023)**. In this study, we observed that growth continued until death as in **Anufriieva and Shadrin (2014)**, which means that brine shrimps must moult several times and throughout their life. The two studied parthenogenetic populations grow at rate of $0.19 mm.day^{-1}$, which is close to values obtained by **Correa-Sandoval *et al.* (1994)** with 0.159 and $0.183 mm.day^{-1}$, respectively, for *A. franciscana* from Mexico and San Francisco and lower than values obtained by **Amarouayache and Kara (2017)** with $0.25 mm.day^{-1}$ for *A. salina* populations from Chott Marouene, which has short lifespan. Limnological parameters of the biotope affect the development of brine shrimps, especially salinity **(Larti *et al*., 2012; Amarouayache & Kara, 2017)** and temperature. This parameter plays an important role in the acceleration of growth by enhancing food filtration **(Li *et al*., 2023)**. In crustaceans, the molting cycle is shortened when the temperature increases **(Hentsghel, 1968)**. In addition to these parameters, growth is faster in *Artemia* when grown in the dark **(Sorgeloos, 1973)**.

Artemia parthenogenetic individuals reared in this study are larger than other Algerian *A. salina* females **(Amarouayache *et al*., 2009; 2010)**, but are close to parthenogenetic females from El-Bahira Lake **(Amarouayache & Belakri, 2015)**. It is admitted that parthenogenetic populations are larger in size than bisexual and have also larger cells **(Amat, 1982)**. Overall, the results obtained are close to those of the respective wild populations **(Amarouayache *et al*., 2009, 2010; Amarouayache & Belakri, 2015)**, which indicates sufficient food rations and adequate rearing conditions (salinity, temperature, oxygen). Several studies showed the influence of salinity on the brine shrimp length **(Abatzopoulos *et al*., 2003; El-Barmawi *et al*., 2004; Agh *et al*., 2008)**. Thus, individuals save their energy for osmoregulation activity, which prevent them to reach big sizes compared with individuals living in lower salinities **(Amat, 1980)**.

Reproduction parameters are also important criteria for production estimation in *Artemia* farming. **Browne *et al.* (1984)** established several reproductive parameters in addition to fecundity to accurately describe life history and reproductive traits in *Artemia* populations. For the two studied populations, sexual maturity occurs at a relatively late age between 28 and 30 days. **Amarouayache *et al.* (2009, 2010)** and **Amarouayache and Kara (2017)** reported earlier maturity, between 18 and 22 days for bisexual populations, wild and tested in laboratory. Salinity plays a role in triggering reproduction **(Saygi, 2004; Abatzopoulos *et al*., 2003; Agh *et al*., 2008)**; consequently, females reach their first sexual maturity at an earlier age when salinity is higher. This “R strategy” is adopted when the saline biotopes dry out due to evaporation, allowing rapid development before the end of the hydroperiod. This observation is reinforced by the longer life span in parthenogenetic populations compared to bisexual **(Amarouayache & Kara, 2017)**.

Ovoviviparity is the dominant mode of reproduction, displayed at more than 75% in both parthenogenetic populations. According to **Barata *et al.* (1995)**, bisexual populations, generally develop in temporary saline lakes and are more likely to lay resting eggs to ensure the persistence of the species, while parthenogenetic populations are more likely to live in more suitable conditions and produce free *nauplii* larvae. Some exceptions can be observed, which raises questions about the genetic origin of some populations and possible bisexual ancestors **(Boyer *et al*., 2021, 2023; Huylmans *et al*., 2021)**. Thus, **Amarouayache and Belakri (2015)** reported exclusive oviparity as a mode of reproduction for the parthenogenetic population of El-Bahira in the wild. It should be noted that during our breeding, we obtained a male per thousand reared individuals in the population of Sidi Bouziane, and a male in the first generation (F1) of the population of Bethioua. In the literature, these males in parthenogenetic populations may be functional and are described as rare **(Maccari *et al*., 2013)**. In the other hand, several parameters can influence the mode of reproduction such as the physico-chemical parameters of the water, the age of the females **(Amat, 1982)**, and the quality of the food **(Wurtsbaugh & Gliwicz, 2001; Baxevanis *et al*., 2004)**.

Overall, even though reproductive traits show that both studied parthenogenetic strains are little fertile compared to other bisexual populations and species, they are interesting for farming, if we consider the fact that 100% of the population (100% females) participate to biomass production (the concept of Twofold cost of males). In addition, they live longer and attain large sizes compared with bisexual populations. The fact that the preferential mode of reproduction is ovoviviparity, this means that the free larvae will be active in the medium and participate to rapid increase of biomass, contrary to cysts. Indeed, the newly released cysts do not hatch immediately in the medium and should undergo several cycles of hydrations/dehydration before hatching to constitute a cyst bank **(Lavens *et al*., 1986)**, the concept of bet hedging **(Wang & Rogers, 2018)**. Further studies should be carried out to assess physico-chemical preferences in intensive aquaculture systems, the biochemical quality of parthenogenetic adults and how important is the mass production of adult *Artemia* for direct use as food for aquatic animals or human as well.

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