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RESEARCH

First biocharacterization of *Artemia* populations from western and northwestern Algeria

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Abstract

Morphological and reproductive characteristics of brine shrimp *Artemia* from two western Algerian populations (El Melah and Timimoun) were studied for the first time alongside the northwestern population (Bethioua). Sexual, survival, growth, and reproduction were recorded for each population raised under standardized culture conditions. The morphological results showed that *Artemia* from the Timimoun and El Melah populations are bisexual, while the Bethioua population is parthenogenetic. After 20 days at salinity of 80 PSU, higher survival rates were found for the El Melah (85.5%) and Timimoun (72.0%) populations, while the Bethouia population showed the lowest survival rate (30.2%). Total body lengths of naupliar stages of the parthenogenetic populations were significantly longer than those of the bisexual populations. The Bethouia population displayed the highest absolute growth rate resulting in the largest adults compared to the bisexual populations. El Melah and Timimoun females were significantly larger morphologically in seven of the nine morphological characteristics compared to their respective males. The Bethouia females had smaller total and abdominal lengths compared to the females from the bisexual populations. The bisexual strains were significantly different from the parthenogenetic population in 9 of the 11 reproductive characteristics. Bethouia females, however, had the highest offspring/day/female and longest post-reproductive period compared to bisexual females. The Timimoun population adapted reproductive strategies differently compared to the other two populations: a late maturity (21.6 days), and more offspring/females (65.1). The data from the study will help future management and potential development of the Algerian brine shrimp populations.

Keywords Aquaculture · Brine shrimp · Bisexual · Parthenogenetic · Reproduction · Future management

Introduction

The brine shrimp *Artemia* Leach, 1819, a keystone species in hypersaline food webs, is the most intensively studied aquatic organism, due to its importance in the aquaculture industry (Sorgeloos 1980; Bengtson et al. 1991; Sorgeloos et al. 1998; Lenormand et al. 2018; Van Stappen et al. 2020).

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In aquaculture hatcheries, *Artemia* is an important live food source in larviculture (Sellami et al. 2020). With a basic nutritional content like fatty acids and its small naupliar size that perfectly matches the mouth sizes of the early-stage crustacean and fish larvae, *Artemia* presents a better live food source for the latter (Sorgeloos et al. 2001; Van Stappen et al. 2020; Chabet dis et al. 2021; Sellami et al. 2021).

Kara and Amarouayache (2012) prepared the final checklist of distribution and zoogeography of *Artemia*. In their review, many *Artemia* specimens were introduced as unknown populations and labeled by "?" symbols. They showed that the taxonomy and systematics of Algerian *Artemia* populations are unresolved despite many of the populations having been characterized morphologically, showing the presence of *Artemia salina* as well as 2n and 4n parthenogenetic populations (Ghomari et al. 2011). In addition, only three of the recorded populations were the subject of several ecological and biological studies: (1) i Marouane (Kara et al. 2004; Amarouayache et al. 2009; Amarouayache and Kara 2017); (2) Sebkha Ez-Zemoul (Amarouayache et al. 2010; Amarouayache and Kara 2015); and (3) and Bethioua Sebkha (Ghomari et al. 2011; Amarouayache et al. 2017; Chabet dis et al. 2021).

Due its size and climatological conditions, Algeria has a production potential for *Artemia* allowing its survival in the country's ecosystems, including continental salt lakes, ponds, chott, sebkha, and hypersaline areas (FAO 2018). However, the studies already carried out on *Artemia* populations and strains in Algeria are still in the initial phase and few scientific data are available (FAO 2018). Studies in natural conditions must be accompanied by laboratory studies which makes it possible to obtain more information on the characteristics of local strains (FAO 2018), studying the biometry of the cysts, nauplii to adults, nutritional content, hatching, growth, life parameters, and reproduction at different abiotic factors. Data will possibly help to verify the production feasibility of the different Algerian brine shrimp populations.

The aim of this study is to carry out a careful analysis of three *Artemia* populations in order to provide more insights into possible approaches for research and management of *Artemia* productivity in Algerian habitats. A survey was set up to sample brine shrimp for the first time in El Melah and Timimoun Sebkha. Amarouayache and Kara (2017) reported that such studies might be helpful in future aquaculture management for both ecological and exploitation purposes. Cysts were harvested and exposed to standard laboratory tests to define the taxonomic classification of the *Artemia* populations and to study their population dynamics.

Materials and methods

Study sites and sampling

Artemia cysts were collected from three sites (Fig. 1): Bethioua Sebkha (35.73833°N 000.26480°W) with a salt lake's surface of 29 km^2; El Melah Sebkha (29.05601°N 001.02925°W) with a salt lake's surface of 176 km^2; and Timimoun Sebkha (29.26109°N 000.18836°E) with a salt lake's surface of 768 km^2; during February 2017. In the laboratory, the cysts were immersed in saturated brine to separate the cysts from debris, then transferred to a descending series of sieves (1000–80 µm), and finally cleaned by differential flotation in freshwater as described by Amat (1980). After the cysts were dried at 39 °C, they were immediately hatched.

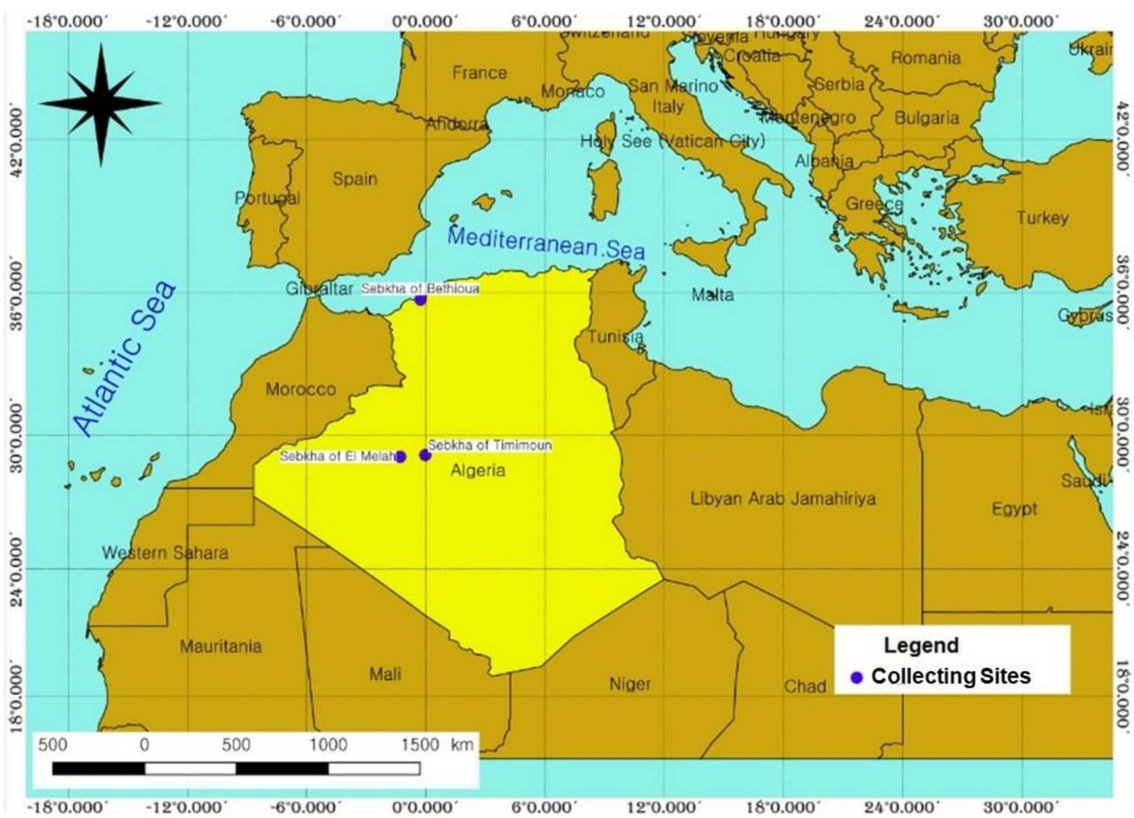

Fig. 1 Location of the three sampling areas of Algerian *Artemia* populations

Culture conditions

Artemia cysts from the three biotopes were hatched in continuous aerated seawater 35 PSU, at 24 ºC and pH 8, and under continuous fluorescent lighting (2500 lm). The nauplii were cultured to the adult stage in standard laboratory conditions as described by Hontoria and Amat (1992) to minimize the environmental effects on the phenotype (Gilchrist 1960). The marine green alga *Tetraselmis suecica* (Butcher 1959) was used as food for the experiments.

Survival and growth

Survival and growth experiments were conducted in three replicates at 24 ± 1 °C under constant light and algal food concentrations in separate 2 L glass containers. Initially, newly hatched nauplii from the El Melah, Timimoun, and from the Bethouia population were cultured in seawater 35 PSU, with a density of culture (ind/ml) 1:1, algae concentration 1.2*10^5 cell/ml for 5 days. Then each individual *Artemia* culture was continued for the three populations for another 5 days at 60 PSU with a density of culture (ind/ml) 0.1:1, algae concentration 5*10^4 cell/ml. The surviving *Artemia* individuals were then exposed to 80 PSU for 5 days with a density of culture (ind/ml) 0.1:1, algae concentration 5*10^4 cell/ml. Finally, the surviving *Artemia* individuals were cultured at 80 PSU for another 5 days. These stepwise increasing salinities conditions were adapted from modified protocol described by Ghomari et al. (2011) to minimize the environmental influence on morphology.

Survival was monitored in intervals of 24 h by direct count of three replicates for a period of 21 days. Dead individuals were removed during each observation. Survival percentage was calculated at each salinity in 5-day intervals.

At the beginning of the growth experiments, the total lengths of the randomly selected 30 newly hatched (emerging from shells) nauplii were recorded using an Optika dissecting microscope equipped with a calibrated ocular micrometer (Optika, Ponteranica, Italy). At 0, 5, and 15 days, measurements were made on lightly chloroform-anesthetized *Artemia* individuals, according to the method of Dana and Lenz (1986). After 20 days, the nauplii became adults. Absolute growth rate (AGR) was calculated using the following formula calculated by Wooton (1991): AGR = (final length—initial length)/total experimental days.

Morphometric analysis

The microscopic identification followed the morphological characters described by Amat (1980) and Mura and Brecciaroli (2004). The morphometrics of mature adult individuals cultured at 80 PSU were measured using an Optika dissecting microscope equipped with a calibrated ocular micrometer. Nine morphometric characters: total length (TL), abdominal length (AL); third abdominal segment width (AI); width of the ovisac/male genital segment (OW/ge); furca length (FL); head width (HW); first antenna length (antL); eye diameter (ED); and distance between compound eyes (DiY) were analyzed following the procedures outlined in Hontoria and Amat (1992).

Reproductive experiments

Thirty female populations were raised separately at salinity of 80 PSU, 24 ºC, and 12 h light:12 h dark photoperiod. Only dead males were replaced. The presence of cysts or nauplii was checked daily and water was renewed after counting. According to Browne et al. (1984), the following reproductive characteristics: number of brood per female (NB); offspring per female (OF); offspring per brood (OB); offspring/day during the reproductive period (O/D); brood intervals (BI); percentage of cysts (OVI); percent of offspring for nauplii (OVO); life span (LS); pre-reproductive period (PRP); reproductive period (RP); and post-reproductive period (PSRP) were determined for each population.

Data analyses

Data analyses for the survival, growth, and reproduction of each population were performed with the Excel 2007 statistical program (Microsoft® Office Excel® 2007). The differences in the means of the morphological variables measured among the three populations and those of their reproductive performance were compared with analysis of variance ANOVA ($P < 0.05$) (VassarStats), and Principal Components Analysis (PCA). A correlation matrix of the variables was used to explore the data set for bisexual populations to determine sexual dimorphism. Morphological variation of the three *Artemia* populations from this study and the Mediterranean basin was investigated by hierarchical cluster analysis. The statistical analysis was done by the Statistical Environment R Version 3.6.1 (Team RC 2013) using the Vegan (Oksanen et al. 2013), ade4 (Dray and Dufour 2007), Mass (Ripley 1996; Venables and Ripley 2002) and factoextra (Kassambara and Mundt 2016) packages.

Results

Observations confirmed the presence of males with claspers and females with ovisacs for the El Melah and Timimoun populations. Both populations belong to *Artemia salina* (Linnaeus 1758) characterized by subconical frontal knobs. A parthenogenetic strain, however, was identified for the Bethioua population, according to the morphological characterization described by Amat (1980).

Survival and growth

Survival rate decreased with increasing salinity (Fig. 2). After 20 days at 80 PSU, the highest survival was observed for the El Melah population (85.5%), compared to Timimoun (72.0%) and Bethioua (30.2%) populations.

Results of the growth experiments (total body length Lt) for the three populations are presented in Fig. 3. At 0, 5, 10, and 20 days, the lengths were found to be significantly

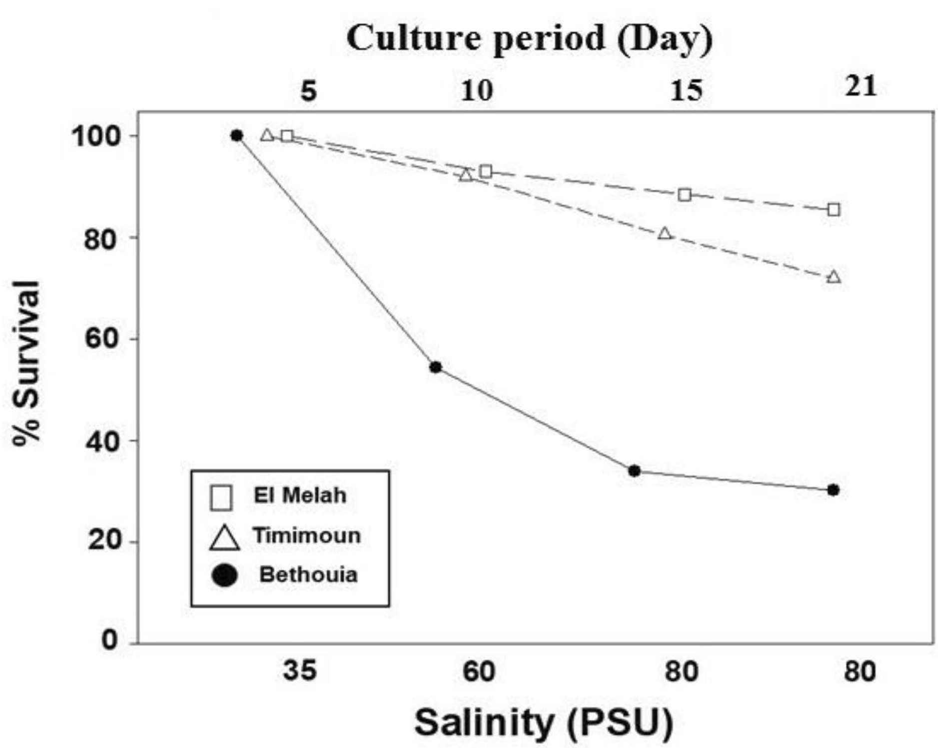

Fig. 2 Percent survival of the three *Artemia* populations on 5, 10, 15, and 21 days at 35, 60 and 80 PSU

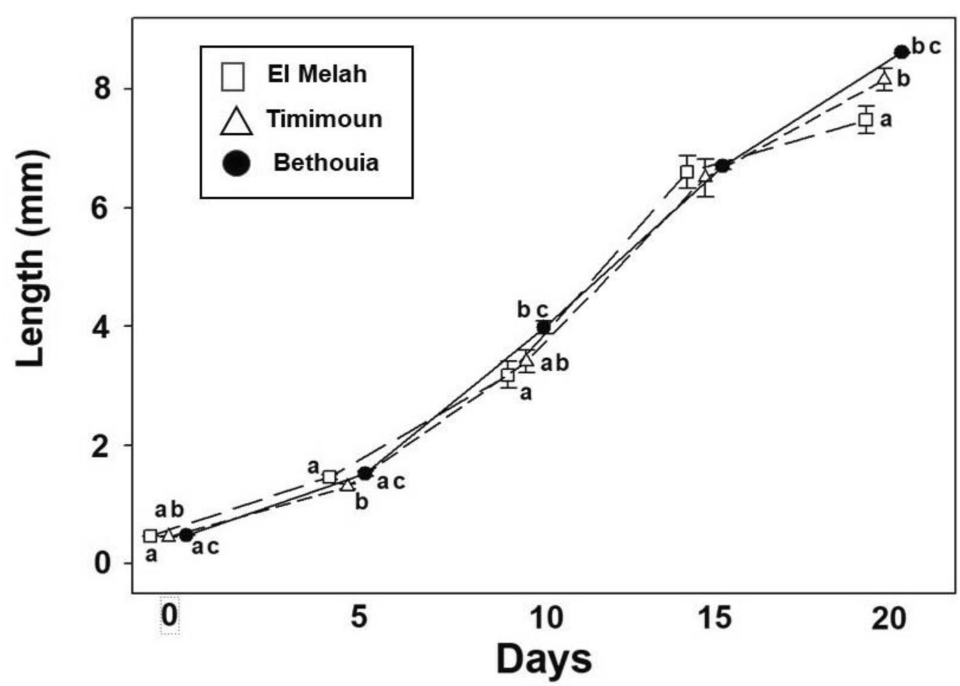

Fig. 3 Growth expressed as mean total length (mm) of the three Algerian *Artemia* populations ($n=30$). For the same time point, different letters denote significant differences ($p<0.05$)

different ($P<0.05$) among the three populations, especially when comparing the bisexual and parthenogenetic populations. At 0 day, the nauplii from the Bethouia population was significantly longer ($P<0.05$) 0.48 mm compared to the Timimoun population (0.46 mm). No significant differences in lengths were observed between El Melah and Timimoun populations. At 5 days, *Artemia* individual lengths of the El Melah population (1.46 mm) were significantly longer ($P<0.05$) compared to that of Timimoun population (1.30 mm) but not different than the lengths of the Bethouia population (1.52 mm). The lengths between Timimoun and Bethouia populations, however, were significantly different ($P<0.05$). At 10 days, *Artemia* individual lengths from El Melah population (3.19 mm) were significantly shorter ($P<0.05$) compared to those of the Bethouia population (3.98 mm). The lengths of Timimoun population (3.41 mm) were not different from either of the other two populations. No differences were observed for the *Artemia* individual lengths at 15 days. At 20 days, adults from the Bethouia (6.70 mm) were significantly longer ($P<0.05$) compared to those from El Melah (6.60 mm). Adult lengths from Timimoun (6.50 mm) were not different from those of either of the two other populations.

The parthenogenetic Bethioua population showed the highest AGR (0.39 mm/day). The two bisexual populations had lower AGRs, 0.33 and 0.37 mm/day for the El Melah and Timimoun populations, respectively.

Morphometric study

The means of morphometric characters of all three Algerian populations varied according to population and to sex (Table 1). For the El Melah population, the lengths of the total body (TL) and abdomen (AL), widths of the ovisac/male genital segment (OW/ge), and third abdominal segment (AI) were significantly greater ($P<0.05$) in the females than the males. The lengths of the furca (FL) and first antenna (antL), eye diameter (ED) and distance between the compound eyes (DiY) were, however, significantly greater ($P<0.05$) in the males compared to the females. The head widths (HW) showed no significant differences ($P>0.05$). For the Timimoun population, the females showed similar morphometrics to the El Melah females except for the furca length which was not significantly different ($P>0.05$) compared to the males. The males also displayed similar characters to those of the El Melah males except their head widths were significantly larger ($P<0.05$) than those of the females.

The males from El Melah and Timimoun population showed differences in three of the nine characters. The males from Timimoun had significantly ($P<0.05$) larger widths of the genital segment and diameters of the head and eye compared to the males from El Melah.

The parthenogenic Bethouia female population showed significant differences ($P<0.05$) in six of the nine characters compared to the El Melah and Timimoun female populations. The Bethouia females showed significantly ($P<0.05$) smaller total and abdominal lengths, ovisac widths and eye diameters compared to the bisexual females. The Bethouia females, however, had significantly ($P<0.05$) longer lengths of the furca and first antenna compared to females from the bisexual populations.

The coefficient of correlation r between morphometric characters of the *Artemia* populations is presented in Table 2. Strong positive relationships were recorded between total lengths and abdominal lengths ($r=0.95$), ovisac widths ($r=0.88$), and third abdominal segment widths ($r=0.75$). Positive relationships between abdominal lengths and widths of ovisac ($r=0.90$) and third abdominal segment

Table 1 Biometric characters of female and male *Artemia* populations from northwestern and western Algeria

	TL (mm)	AL (mm)	OW/ge (mm)	AI (mm)	FL (mm)	HW (mm)	antL (mm)	ED (mm)	DiY (mm)
El Melah females	8.45 (0.88)*[a]	4.58 (0.57)*[a]	1.59 (0.32)[a]	0.36 (0.07)*	0.21 (0.04)*[a]	0.65 (0.10)[a]	0.58 (0.11)*[a]	0.20 (0.03)*[a]	1.18 (0.14)*[a]
El Melah males	6.79 (0.43)	3.30 (0.24)	0.40 (0.08)*	0.23 (0.06)	0.26 (0.06)	0.66 (0.14)	0.81 (0.08)	0.23 (0.03)	1.29 (0.11)
Timimoun females	8.60 (0.59)*[a]	4.53 (0.38)*[a]	1.57 (0.24)*[a]	0.35 (0.06)*	0.25 (0.05)[a]	0.64 (0.07)[a]	0.61 (0.10)*[a]	0.21 (0.03)*[a]	1.21 (0.08)*[a]
Timimoun males	6.95 (0.59)	3.37 (0.34)	0.46 (0.09)[+]	0.24 (0.08)	0.27 (0.05)	0.76 (0.19)[+]	0.84 (0.11)	0.26 (0.04)[+]	1.34 (0.17)
Bethioua females	7.34 (0.60)[b]	3.69 (0.41)[b]	0.88 (0.25)[b]	0.37 (0.06)	0.44 (0.11)[b]	0.62 (0.07)[b]	0.80 (0.10)[b]	0.18 (0.03)[b]	1.20 (0.09)[b]

Values are means +/– (SE); $N=30$ individuals/population

[a,b,c]Represent significant difference ($P\leq0.05$, ANOVA) among three female biometrics

[+]Between the two male biometric

*Represents significant difference ($P\leq0.05$, Welch Two Sample t test) between intrapopulation of the female and male biometrics

Table 2 Coefficient of correlation (r) between morphometric characters of *Artemia* populations

	TL	AL	OW/ge	AI	FL	HW	antL	ED
TL								
AL	**0.95**							
OWge	**0.88**	**0.90**						
AI	**0.75**	**0.75**	**0.70**					
FL	–0.07	–0.11	–0.23	–0.06				
HW	0.14	0.04	–0.07	–0.02	0.14			
antL	–0.43	–0.56	–0.62	–0.40	0.32	0.33		
ED	–0.16	–0.21	–0.32	–0.10	0.29	0.23	0.53	
DiY	0.06	–0.06	–0.21	–0.04	0.44	0.56	0.64	0.59

Numbers in bold indicate significant correlation ($P \leq 0.05$)

$N=30$ individuals/population

widths ($r=0.75$) were also found. Strong negative relationships were found between ovisac widths and first antenna lengths ($r=-0.62$), abdominal and first antenna lengths ($r=-0.56$), total and first antenna lengths ($r=-0.43$), and third abdominal and first antenna lengths ($r=-0.40$).

PCA showed that according to males and females segregated along factor 1 (Fig. 4). Separation along factor 2 was very small in comparison. Both components accounted for 70.7% of the variance. The Pearson correlation between morphometric characters (Table 2) revealed a significantly ($P<0.0001$) strong positive correlation ($r=0.7$–0.95) between: (1) total and abdominal lengths, width of the ovisac/male genital segment, width of the third abdominal segment; (2) width of the ovisac/male genital segment and abdominal length, width of the third abdominal segment; and (3) width of the third abdominal segment and abdominal length. A significantly ($P<0.0001$) positive correlation ($r=0.5$–0.64) was observed between: (1) eye diameter and length of first antenna; and (2) distance between compound eyes and head width, eye diameter and length of first antenna. A significantly ($P<0.0001$) negative correlation ($r=0.5$–0.62) was noted between length of first antenna and abdominal length and width of the ovisac/male genital segment.

Reproductive characters

The reproductive characters are shown in Table 3. The parthenogenetic females from the Bethouia population showed significant differences ($P<0.05$) compared to the bisexual females from the El Melah and Timimoun populations in seven out of the nine reproductive characters, excluding percentage of cysts (OVI) and percent of nauplii (OVO). The Bethioua females had lower offspring/female (OF), numbers of brood/female (NB), life span (LS), pre-reproductive period (PRP) and reproductive period (RP) compared to the bisexual populations. The Bethioua females, however, had significantly higher offspring/day/female and female post-reproductive period (PSRP) compared to the females from the two bisexual populations.

Coefficients of correlation *r* between reproductive performances of the three *Artemia* populations are presented in

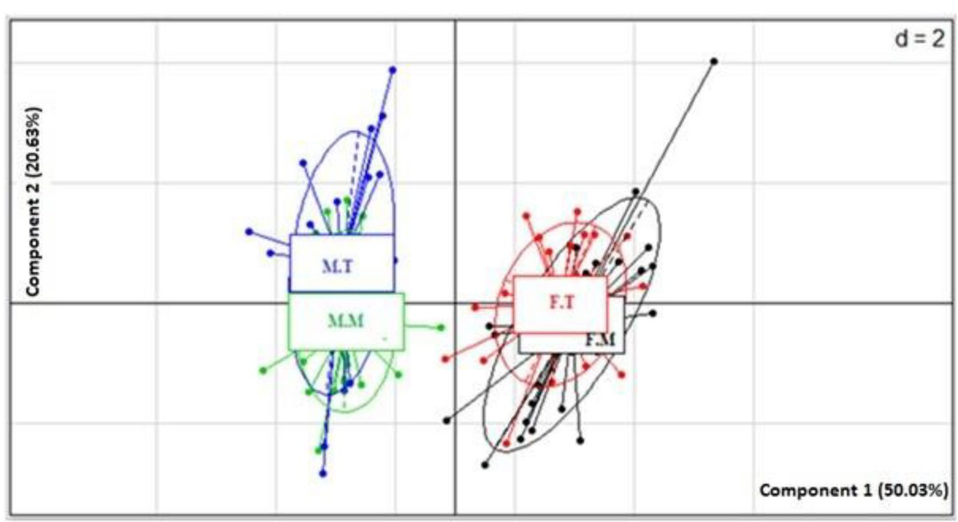

Fig. 4 Differentiation among female and male samples of studies populations. Timimoun female population (F.T); Timimoun male population (M.T); El Melah female population (F.M); El Melah male population (M.M); d = Euclidean distance

Table 3 Reproductive performance of the three *Artemia* populations

POPULATION	OB	OF	B	O/D	BI	OVI	OVO	LS	PRP	RP	PSRP
El Melah	27.8 (17.0)	58.7 (56.5)^{a}	1.7 (1.2)^{a}	14.2 (9.6)^{a}	2.2 (2.0)	100	0	26.1 (3.6)^{a}	19.7 (7.9)^{a}	3.5 (3.3)^{a}	1.1 (1.1)^{a}
Timimoun	30.8 (16.7)	65.1 (58.9)^{a}	1.9 (1.5)^{ab}	29.2 (22.7)^{b}	1.6 (1.2)	100	0	24.5 (4.4)^{ab}	21.6 (1.2)^{ab}	2.9 (3.9)^{ab}	1.1 (0.9)^{a}
Bethioua	24.3 (13.4)	28.0 (15.0)^{bc}	1.2 (0.5)^{ac}	18.0 (14.2)^{ac}	1.3 (1.1)	0	96.7 (18.3)	23.6 (2.2)^{bc}	17.1 (5.7)^{ac}	1.3 (1.1)^{bc}	2.4 (1.9)^{c}

Numbers in bold indicate significant correlation ($P \leq 0.05$)

Values expressed as means +/– (SD). $N = 30$ individuals/population

^{a,b,c}Represent significant differences ($P \leq 0.05$, ANOVA) among the three female biometrics

Table 4. A very strong positive relationship ($r = 0.70$–0.91) was recorded between number of brood/female and reproductive period, offspring/female and number of brood/female, offspring/female and reproductive period, brood intervals and reproductive period, life span and pre-reproductive period, and brood intervals and reproductive period. A positive relationship ($r = 0.52$–0.67) was also observed between offspring/brood and offspring/female, offspring/female and brood intervals, number of brood/female and brood intervals, and offspring/day during the reproductive period and percent of offspring for nauplii. A strong negative relationship ($r = -0.97$) was found between percentage of cysts and percent of offspring for nauplii.

Discussion

Climate change has affected globally the dynamics of the *Artemia* population cycles, resulting in the difficulty of management and predictability of cyst harvests, even for Great Salt Lake (Eimanifar et al. 2015; Van Stappen et al. 2020). Consequently, new locations like commercial saltworks and ponds have been exploited with varying success for several decades in order to overcome cysts shortages (Van Stappen et al. 2020). The introduction of *Artemia franciscana* in salt ponds was scientifically supported due to its high productivity, fast growth, and reproduction which have been demonstrated by Triantaphyllidis et al. (1995). The use of this species, however leads to the extinction of the native species in the long term and consequently to the loss of biodiversity (Amat et al. 2007). The use of local strains, therefore, may be a more appropriate solution to maintain biodiversity.

In this study, the morphological characteristics revealed that both the El Melah and Timimoun populations belong to the native autochthonous sexual species *A. salina*, while the Mediterranean Bethioua population is parthenogenetic. The biodiversity of the *Artemia* populations from this study is similar to the Algerian populations previously reported by Ghomari (2013), Amarouayache and Kara (2010), Amarouayache and Kara (2015), Amarouayache et al. (2017) and to the other Mediterranean populations by Amat et al. (1995).

In this study, different survival, growth, and reproduction of three *Artemia* populations to increasing salinities were observed. At the highest salinity of 80 PSU, Bethioua population showed the lowest survival rates (30%) compared to > 70% for the two sexual populations. Differences in the survival rates between the parthenogenetic and sexual populations could be attributed to the adaptations of the two *A. salina* populations to extreme conditions of the western sebkhas compared to the Mediterranean sebkha for the Bethouia population. The Bethouia Sebkha located near the Mediterranean coast is exposed to more precipitation which can

Table 4 Coefficients of correlation (r) between reproductive performances of the three *Artemia* populations

	OB	OF	NB	O/D	BI	OVI	OVO	LS	PRP	RP
OF	**0.67**									
NB	0.43	**0.88**								
O/D	0.36	0.24	0.16							
BI	0.32	**0.53**	**0.58**	0.11						
OVI	0.16	0.32	0.23	0.49	0.12					
OVO	–0.12	–0.3	–0.2	**0.52**	–0.10	**–0.97**				
LS	0.02	0.16	0.21	0.23	0.36	–0.33	0.33			
PRP	0.17	0.05	0.15	0.36	0.30	–0.24	0.28	**0.70**		
RP	0.37	**0.86**	**0.91**	0.12	**0.71**	0.24	–0.23	0.36	0.16	
PSRP	0.27	0.01	–0.001	0.17	–0.04	–0.41	0.44	0.29	0.12	–0.04

Numbers in bold indicate significant correlation ($P \leq 0.05$)

$N = 30$

decrease salinity and cooler temperatures compared to other two harsher Saharan sebkhas.

Ghomari (2013) reported similar survival rates of 35.6% and 29.9% for the 2n and 4n strains, respectively. He reported, however that survival rates of parthenogenetic populations were higher compared to that of *A. salina* unlike our findings.

Reduced survival and reproductive potential in *Artemia* can be the affected by stress. Consequently, the population structure can change afterwards, even though the mechanisms that may allow the organisms to survive are retained (Sserwadda et al. 2018).

This study showed the effects of gradually increasing salinity in the three populations. Ben Naceur et al. (2009) reported that bisexual *Artemia* populations adapted well when the salinity was increased gradually. Triantaphyllidis et al. (1995) found, however, high mortality in *Artemia* when directly transferred or cultured in high salinities.

El-Bermawi et al. (2004) studied the salinity effects on survival, growth, and morphometry of four Egyptian *Artemia* populations. These populations included a bisexual species from Wadi El-Natrun Lake and three parthenogenetic species from Borg El Arab, El-Max saltworks and Qarun Lake. They found the bisexual population showed a maximum survival rate at 80 PSU which agreed with our findings of higher survival rates observed for the two bisexual populations at 80 PSU.

The hatching nauplii exhibited significant differences in their final lengths: 450 µm and 470 µm for the bisexual Timimoun and parthenogenetic Bethouia populations, respectively. These values are in the range of 400–500 µm recommended by Van Stappen (1996) for the use of first instar I in aquaculture. The rearing methods used in this study could be useful to provide food for the Algerian aquacultural industries.

Amat (1983) reported that the parthenogenetic strains were better adapted to harsh environmental conditions. In this study, the parthenogenetic population (Bethioua) showed the longest body lengths and highest absolute growth rates compared to the two bisexual populations exposed to increasing salinities. Our results are supported by the findings of Dhont and Lavens (1996) and Støttrup and McEvoy (2008) who reported that the parthenogenetic strains were better adapted to higher salinities, while the bisexual strains were ecologically adapted to low salinities.

In this study, survival and growth patterns between the bisexual populations (El Melah and Timimoun) showed significant differences. Castro-Mejía et al. (2011) also found similar differences in the survival and growth patterns of five bisexual *Artemia franciscana* populations from the Mexican Pacific Coast.

The PCA showed no differences between El Melah and Timimoun populations (factor 2) since they belong to the sexual strain *A. salina* but the factor 1 separated the males from females. Camargo et al. (2003) studied morphometric characterization of thalassohaline *A. franciscana* populations from the Colombian Caribbean. They reported that male morphometric characters separated the type of population groups more clearly than the female characters. All Colombian populations were, furthermore, correctly positioned in the Caribbean coastal group and the San Francisco Bay population in the North American group, with no overlapping between the two types, as was the case for the female individuals.

The hierarchical cluster analysis used the morphometric characters from this present study (Fig. 5) and those reported by Ghomari (2013) who also used the same conditions and morphometric characters. The 14 populations were divided into 3 main groups. The first group included the parthenogenic populations PT from El Gholea, Relizane, and Setif (Ghomari 2013). The second, regrouping also included the parthenogenic populations PD from Relizane, Setif, and Bethioua (Ghomari 2013) and the parthenogenetic population from Bethioua from the present study. The last group

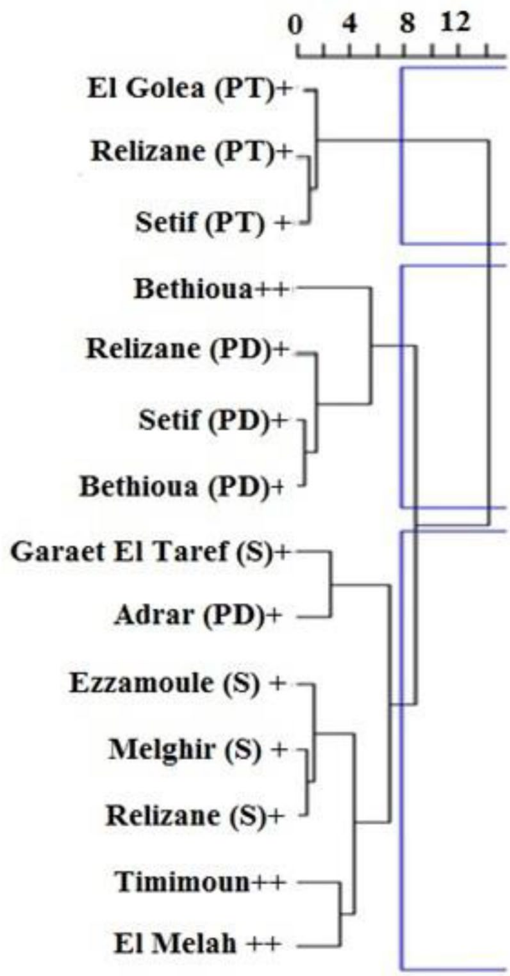

Fig. 5 Dendrograms from Hierarchical Clustering (Ward's method, Euclidean distance) of morphometrics parameters of *Artemia* population. Parthenogenetic diploid (PD); Parthenogenetic tetraploid (PT); *Artemia salina* (S), + +: present study, +: population studied by Ghomari (2013)

included the parthenogenic population PD from Adrar (Ghomari 2013) and *A. salina* populations from Gareraet El Taref, Ezzamoule, Melghir and Relizane (Ghomari 2013) and from the present study (El Melah and Timimoun).

The present study offered an opportunity to evaluate prime reproductive characteristics for populations for first time. Cuccu et al. (2011) reported, firstly, that reproduction is an important phase in the life history of living organisms. They stressed the importance of understanding the reproductive strategies of each species as a key to shedding light on their entire life cycle. Analysis of the reproductive characteristics revealed differentiation among the *Artemia* strains, not only between parthenogenetic and bisexual strains, but also between the bisexual populations. Compared to other Algerian populations from Ghomari (2013), the three populations showed lower reproductive characteristics. These characters included pre-reproductive, reproductive, post-reproductive periods; brood intervals; number of broods, offspring/brood, offspring/day, offspring/female; and life span.

Bisexual species present a typically oviparous mode unlike the parthenogenetic population. Abreu–Grobois (1987) reported that reproductive characteristics of *Artemia* populations from different locations are the results of the adaptation patterns between the natural populations and their local habitats, which are diverse in origin, water chemistry, temperature, salinity, and stability. The sebkhas of El Melah and Timimoun are located in Sahara desert, one of the driest and hottest in the world. The brine shrimp's adaptation to these environmental conditions at these sites can be expressed by several characteristics, such as offspring quality (cysts) for ensuring survival of the populations. Gajardo et al. (2001) and Van Stappen et al. (2003) also noted that despite these unfavorable conditions, some *Artemia* strains can follow an oviparous reproduction mode.

A native parthenogenetic population was found at the northwestern Bethioua Sebkha near the Mediterranean coast. This sebkha receives much water from rain and river flows. How has this parthenogenetic strain evolved in adapting to cooler temperatures and less fluctuating water conditions compared to the bisexual populations in the two Saharan sebkhas exposed to harsher environmental conditions.

The biogeographic distribution of the parthenogenetic strain is limited to the Old World and Australia (McMaster et al. 2007; Eimanifar et al. 2016). Allozymic and mtDNA studies show that all parthenogenetic populations arose from only one branch of an Old World sexual ancestor, possibly *Artemia urmiana* or *Artemia salina (*Browne 1992; Manaffar et al. 2011; Eimanifar et al. 2016). Asem et al. (2021) determined the evolutionary relationship and the genetic variation of bisexual and parthenogenetic *Artemia* using three mitochondrial and two nuclear markers, they find that the diploid parthenogenetic *Artemia* are closely related to *Artemia urmiana* and tetraploids share a common ancestor with *Artemia sinica*. With time, an unusually high degree of divergence has produced among populations because of considerable changes in environmental conditions, such as salinity and temperature which consequently generated variability in reproductive, lifespan and physiological traits, population size (Browne 1992; Eimanifar et al. 2020). Parthenogenetic populations have been predicted to respond less to environmental change which was observed in our study for reproduction and survival of the Bethioua population. The parthenogenetic *Artemia* populations from Portugal showed vulnerability and great variability in the physiological response to different abiotic conditions, suggesting possible local adaptations in response to different selective pressures experienced like other *Artemia parthenogenetica* populations (Pinto et al. 2013). The environmental parameters, such as salinity and temperature, play an important role in genetic structure and population size during evolution of local *Artemia* populations (Eimanifar et al. 2020).

Understanding of environmental factors is necessary to explain variability of physiological response. Future studies using biomarkers, e.g., fatty acids, may be able to elicit the understanding of the distribution of the Algerian brine shrimp and its sexual or parthenogenetic reproductive mode.

The results of these studies were confirmed by findings of Browne et al. (2002) who demonstrated that geographic strains show differences in the proportions of each type of offspring when allowed to reproduce in the laboratory. These differences suggest that inter-population differences for this trait may have a genetic basis. Gajardo et al. (2001) noted that variation in offspring quality (cysts or nauplii) reflects the important reproductive strategy of *Artemia*, ensuring survival in populations exposed to unstable or stressful conditions.

The brine shrimp has an efficient osmoregulatory system that allows it to maintain osmotic homeostasis at elevated salinities (Van Stappen 2003). This mechanism can increase energy cost and subsequently can affect other metabolic and physiological functions, such as growth and reproduction (Van Stappen 2003). The lower reproduction performance observed for all three populations may be the result of such increased energy costs for osmotic homeostasis. Among the three populations, the Timimoun population, however, displayed an adaptive strategy to increasing salinity. At 80 PSU, the females took a relatively longer time to produce their first offspring with more offspring/day, resulting in a potentially higher total offspring produced.

Van Stappen (2003) reported, moreover, that osmotic pressure depends on the salt composition; consequently, the osmoregulation cost of the *Artemia* populations may also depend on the ionic composition of the three sebkhas.

In Algeria, salt areas are managed only by salt production companies (ENSEL), which has slowed down the exploitation and development of brine shrimp *Artemia*. For *Artemia* pond culture, the best solution would be to establish by local saltworks, like in Mekong Delta, Vietnam (Van Stappen et al. 2020). Chabet dis et al. (2021) studied the nutritional quality of these strains and reported to be rich in 16:0, 18:1n-9, and 18:3n-3 which are more suitable for freshwater aquaculture. The present study will help for improved *Artemia* pond aquaculture, a practice that has been initiated with varying success in Vietnam, Thailand, Cambodia, Laos, and Myanmar (Van Stappen et al. 2020).

Conclusion

The parthenogenetic Bethioua population showed higher mortalities and grew faster than the bisexual populations. For reproduction, *Artemia* from El Melah and Timimoun Sebkhas showed oviparous reproduction. The reproductive potential of the populations is low. The Timimoun population appeared to exhibit the best strategy among the three studied populations based on late maturity, more offspring and increased longevity. By understanding how diverse the brine shrimp populations are from northwestern and western Algeria, this study will contribute to the assessment of the quality of these *Artemia* strains. The Saharan El Melah and Timimoun populations have evolved adaptations of increased survival rate and optimal reproductive performance, respectively.

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Conflict of interest The authors declare no competing interests.

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