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Influence of environmental factors on the life cycle and morphology of *Artemia salina* (Crustacea: Anostraca) in Sabkhet El Adhibet (SE Tunisia)

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Abstract: This study was aimed to examine in greater detail the influence of selected environmental factors on the life cycle and morphological characteristics of the brine shrimp *Artemia salina* (Linnaeus, 1758). During this follow-up, from November 2005 to April 2006 and from November 2006 to April 2007, Sabkhet El Adhibet (southeast Tunisia: 33°07'7.58"N, 11°24'8.69"E) was surveyed monthly to determine the impact of water salinity, temperature, pH, dissolved oxygen, and phytoplankton density and community structure on *Artemia* density, population structure, reproductive mode, and total offspring. Strong correlations were found between physicochemical parameters of water and *Artemia* reproduction characteristics. In contrast, no significant relationship was detected between physicochemical variables and *Artemia* population structure and density. Further, there were no correlations between phytoplankton density and the *Artemia* life cycle. Moreover, we observed relationships between physicochemical parameters and all morphological characteristics, especially between the width of 3rd abdominal segment and salinity (r_{xy} = 0.96), temperature (r_{xy} = 0.73), pH (r_{xy} = -0.77) and oxygen (r_{xy} = -0.92) for male specimens, and between the length of the furca and both salinity (r_{xy} = -0.76) and dissolved oxygen (r_{xy} = 0.74), and between the maximal diameter of compound eyes and temperature (r_{xy} = -0.56) for female specimens. Principal component analysis (PCA) shows that male and female specimens collected at different environmental conditions converge, which explains the morphological similarity between them according to salinity, temperature, and dissolved oxygen concentration as well as total phytoplankton, diatom, cryptophyte, and dinophyte density.

Keywords: *Artemia salina*, physicochemical parameters, phytoplankton density, life cycle, morphological characteristics

INTRODUCTION

Permanent salt lakes show an almost continuous range of salinity, from brackish to hypersaline, and all except those at the highest salinities share some features, and perhaps stressors (e.g. oxygen and temperature), with their freshwater counterparts (Jellison 2005). Inland hypersaline lakes in arid and semi-arid basins worldwide are

relatively simple ecosystems, which can be used to understand how their components interact (Gajardo et al. 2006). Studies on secondary production are essential for the evaluation of energy and matter transfer along the food web, as well as the rational management of aquatic ecosystems (Downing 1984). These challenging ecological settings make the genus *Artemia* useful model organisms for studies on evolutionary and ecological aspects of the stress response, at all levels of biological organization (Clegg & Trotman 2002). Moreover, *Artemia* is considered to be an irreplaceable live food for the larval rearing of most marine fish and shellfish species (Sorgeloos et al. 2001). Mariculture of finfish and crustaceans uses freshly-hatched nauplii of brine shrimp as part of the live food chain. In fact, the demand for *Artemia* cysts has gradually increased from a few metric tons to approximately 800 metric tons per annum, representing approximately 40% of the total aquaculture demand for early-stage feeds (Sorgeloos et al. 2001).

Organisms living in temporary inland and costal saline lakes (e.g. copepods, ostracods, rotifers, and branchiopods) have specific adaptive strategies for survival in high salinity conditions and for preventing the loss of cellular water under high osmolarity in hypersaline conditions. These aquatic invertebrates produce diapausing resistant stages in their life cycle, which allows them to survive during adverse periods or drought. While lower and intermediate salinity habitats are populated by various groups of invertebrates, hypersaline environments are characterised by monocultures of *Artemia* as major zooplankton (Van Stappen 2002). In the natural environment, temperature, feeding conditions, and salinity are important factors influencing *Artemia* populations (Browne 1982; Wear & Haslett 1987; Camargo et al. 2004; Litvinenko et al. 2007; Arashkevich et al. 2008). The impact of these ecological parameters can be explained through different *Artemia* responses, e.g. different reproductive strategies, life span, and morphological appearance.

Considering the significant role of *Artemia* in the food chain, as well as its importance in aquaculture, it was desirable to improve the understanding of the life cycle of the brine shrimp *Artemia salina* (Linnaeus, 1758). Hence, this research was aimed to identify the impact of some abiotic and biotic parameters on the density, reproductive mode, total offspring, and morphological parameters in the *Artemia salina* population from Sabkhet El Adhibet (SE Tunisia).

MATERIAL AND METHODS

Study area

Sabkha is a local Gulf Arabic word for a salt flat, and its geological usage implies intrasediment evaporate growth beneath a flat geomorphic surface with an elevation that is dictated by the top of the capillary fringe (Warren & Kendall 1985). The Tunisian territory contains a great number of sabkhas, especially in the centre and the south, representing 22% of the total wetland area (e.g. Sabkhet Sijoumi, Sabkhet El Kalbia, Sabkhet Sidi El Heni, Sabkhet El Melah of Zarzis, Sabkhet El Adhibet, and Sabkhet El Briga).

Sabkhet El Adhibet (33°07’7.58”N–11°24’8.69”E) is an inland site, located in SE Tunisia, 16 km from the Tunisian-Libyan frontier. Its total surface is 12 500 hectares,

including 500 hectares occupied by industrial salt works. Trenches delimiting saltworks were dug artificially to consolidate the dividing dykes of the basins, with an average depth of 0.75 m. These artificial canals in the borders of saltworks are filled by rainwater accumulated during the rainy season until May or June, and *Artemia* occurs there. In these canals, water salinity depends on precipitation and evaporation rate. The saltern is generally filled with rainwater from December to February. In Sabkhet El Adhibet, *Artemia* sp. was reported for the first time by Romdhane et al. (2001). Lately, Romdhane et al. (2004) used discriminant analysis to compare the morphometry of *Artemia* males and females with other *Artemia* populations, and they concluded that Sabkhet El Adhibet is inhabit by *Artemia salina* (Linnaeus, 1758). Muñoz et al. (2008) basing on the mitochondrial genetic diversity, confirmed this result.

Sabkhet El Adhibet is located in an upper-arid area. Maximum length and width are 8 and 7 km, respectively. In the saltworks, water is pumped from the underground brine reservoir (salinity about 280 g L^{-1}) and directly administered in the crystallizer. Three stations were selected (at the central parts of 3 sides of the saltwork, see Fig. 1) in artificial canals at the border of the saltworks (*Artemia* is absent in the crystallizers).

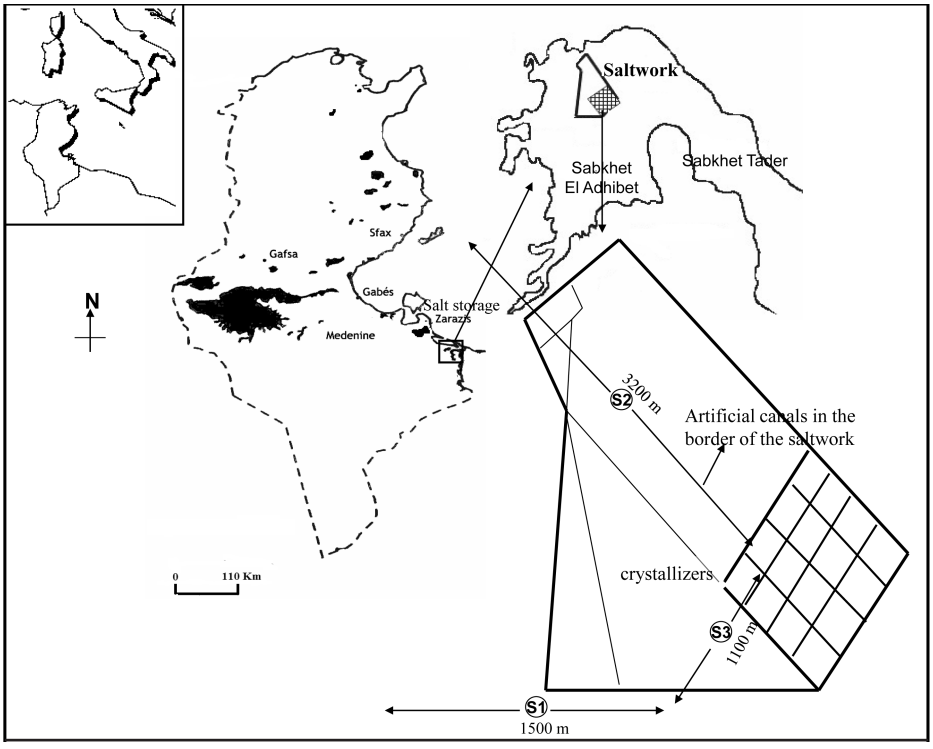

Fig. 1. Location of Sabkhet El Adhibet and sampling stations (S1-S3)

Sampling and measurements

Variation in water temperature, salinity and pH was measured in situ by using a WTW handheld Multi-Parameter Instrument (Multi/340i/SET). These variables were monitored in the morning between 07:00 and 11:00 a.m., and sampling was performed monthly from November 2005 to April 2006 and from November 2006 to April 2007 (because the site was dry since May to late October). Dissolved oxygen concentration was determined by the Winkler test. During the investigation period, 1 L of water was sampled from the surface at each station and preserved with Lugol's solution and a neutralised formaldehyde solution for the determination of densities of microalgae. Their community structure was assessed in sedimentation chambers under an inverted microscope (Leitz).

Artemia samples were collected by filtering 100 L of water from each station through a plankton net (120 µm mesh size) and preserved in situ with neutralized 5% formalin solution. *Artemia* density and population structure, fecundity, and type of offspring output (after dissecting ovigerous sacs) were assessed with a magnifying glass.

For the morphological analysis, male and female specimens were randomly collected by means of hand plankton nets (120 µm mesh size), only during the first study period (November 2005–April 2006) from station S3, in order to minimize differences in environmental influences (between the 3 stations) on morphological characteristics. *Artemia* biomass collected was stored in plastic containers and transferred to the laboratory for analysis. A random sample of 20 adult male and female specimens (i.e. well-developed antennae for males, and full and well-developed brood pouch, i.e. ovisac, for females) were removed and anesthetized with some droplets of water saturated with chloroform. The following morphological parameters were quantified in each *Artemia* sample: total length (*tl*), abdomen length (*al*), width of 3rd abdominal segment (*wts*), length of the furca (*lf*), number of setae on the left furcal branch (*nlf*), number of setae on the right furcal branch (*nrf*), width of the head (*wh*), maximal diameter of compound eyes (*dy*), maximal distance between compound eyes (*dby*), length of 1st antenna (*la*), width of the ovisac (*wo*) (for each female), width of 2nd abdominal segment (*wss*) and width of the frontal knob (*fk*) (for each male).

Statistical methods

The effect of the measured environmental parameters on the *Artemia* life cycle was analyzed using Pearson correlation coefficients calculated by XLSTAT-Pro 7.5 software.

Morphological characteristics were subjected to one-way ANOVA with *post hoc* Least Significant Difference (LSD) test using Statistica 5.0 software. Significance was accepted at $P<0.05$. Principal component analysis (PCA) was carried out using XLSTAT-Pro 7.5 software.

RESULTS

Results of physicochemical water parameters, phytoplankton characteristics, and the *Artemia* life cycle in Sabkhet El Adhibet used in this work were first pre-

sented by BEN NACEUR et al. (2009). Table 1 reports the main information about biotic and abiotic conditions at this site.

Impact on the Artemia *life cycle*

Correlation analyses (Table 2) between environmental parameters (physicochemical parameters and phytoplankton density; X_1-X_{11}) and parameters of the *Artemia salina* life cycle (*Artemia* population structure and density, reproductive mode, and offspring output; Y_1-Y_9) in Sabkhet El Adhibet revealed:

(1) a high negative correlation between:
– salinity (X_1) and offspring output (Y_6 and Y_7): number of cysts (r_{xy} = -0.75) and of nauplii per female (r_{xy} = -0.77);
– temperature (X_2) and offspring output (Y_6 and Y_7): number of cysts (r_{xy} = -0.69) and of nauplii per female(r_{xy} = -0.81);
– pH (X_3) and oviparous reproduction mode (Y_8; r_{xy} = -0.71);
(2) a high positive correlation between:
– pH (X_3) and ovoviviparous reproduction mode (Y_9; r_{xy} = 0.71);
– cyanobacteria (X_{11}) and nauplii, juvenile and adult *Artemia* density (Y_1, Y_3, and Y_4; r_{xy} = 0.71, 0.84 and 0.90, respectively);
(3) a moderate negative correlation between:
– salinity (X_1) and ovoviviparous reproduction mode (Y_9; r_{xy} = -0.67);
(4) a moderate positive correlation between:
– salinity (X_1) and oviparous reproduction mode (Y_9; r_{xy} = 0.67);
– pH (X_3) and offspring output (Y_6 and Y_7): number of cysts (r_{xy} = 0.65) and nauplii per female (r_{xy} = 0.70);
– dissolved oxygen (X_4) and offspring output (Y_6 and Y_7): number of cysts (r_{xy} = 0.69) and of nauplii per female (r_{xy} = 0.64).

Impact on Artemia *morphology*

The population sampled in April had significantly lower values of all morphometric characters (except for width of the ovisac in females), as compared to the other samples. The frontal knob of males and the diameter of compound eyes of both sexes, did not differ between samples (except for the April sample). For the other morphological characters, the statistical comparison (ANOVA, LSD test) shows different degrees of variation among samples, and did not show any particular similarity between *Artemia* specimens collected at different times (Table 3).

Table 4 shows the results of correlation analysis of the male morphological characteristics and physicochemical variables as well as phytoplankton density. The strongest correlation between morphological characteristics and physicochemical parameters was revealed between the width of 3rd abdominal segment and salinity (r_{xy} = 0.96), temperature (r_{xy} = 0.73), pH (r_{xy} = -0.77), and oxygen (r_{xy} = -0.92). For phytoplankton density, the strongest correlation was between the length of the furca and density of total phytoplankton (r_{xy} = 0.69), diatoms (r_{xy} = 0.73), dinophytes (r_{xy} = 0.73) and cryptophytes (r_{xy} = 0.73). PCA shows according to axis 1 (with 53.06 % of the total variance) that all specimens collected at different environmental conditions converge and show a morphological similarity in total length (*tl*), maximal distance

Table 1. Biotic and abiotic parameters (mean±SD) for the 3 stations selected in Sabkhet El Adhibet (Ben Naceur et al. 2009)

		2005–2006					2006–2007					
Parameters	symbols	November	December	January	March	April	November	December	January	February	March	April
Environmental parameters												
Salinity (psu)	X_1	57.3±2.5	32.2±7.6	38.7±2.9	76.7±3.1	281.7±10.4	52.3±9.1	64.3±5.1	117.7±59.5	156.7±25.2	177.7±8	276.3±3.8
Temperature (°C)	X_2	23.6±2.7	12.3±1.6	16.6±0.5	12.1±1.2	25.4±1.3	17.2±0.5	15.2±0.3	16.6±0.7	15.7±0.2	17.1±0.1	18.7±1.3
pH	X_3	9±0.2	8.7±0.2	8.4±0.1	8.4±0.1	7.6±0.1	8.8±0.2	8.7±0.2	8.4±0.3	8.3±0.3	8.1±0.1	7.6±0.1
Dissolved oxygen (mg L^{-1})	X_4	13.5±1.8	15.4±1.2	16.3±0.8	12.8±0.5	3.4±0.1	8.5±0.3	17.5±5.2	8.5±3.3	5.2±1.3	4.2±0.3	4.3±1.3
Total phytoplankton density (10^3 cell L^{-1})	X_5	563±131	8628±3210	14582±4776	3159±990	1939±693	188±74	1963±443	476±184	3060±913	2028±648	850±217
Chlorophyte density (10^3 cell L^{-1})	X_6	292.5	7971.9	315.9	2517.8	1731.6	2.8	388.4	456.3	238.6	368.5	487.6
Diatom density (10^3 cell L^{-1})	X_7	229.3	629.4	12151.6	449.2	187.2	183.9	1162.9	18.2	2358.7	1628.6	343.5
Dinophyte density (10^3 cell L^{-1})	X_8	23.4	0	638.8	173.1	11.7	0	343.9	0.4	294.8	17.5	0
Cryptophyte density (10^3 cell L^{-1})	X_9	0	4.6	365.0	9.3	0	0	35.1	0.9	0	0	0

Euglenophyte density (10^3 cell L^{-1})	X_{10}	0	0	1104.4	0	4.6	0	0	0	0	0	0
Cyanobacteria density (10^3 cell L^{-1})	X_{11}	18.7	22.4	7.0	9.3	4.6	1.4	32.7	0.9	168.4	14.0	19.6
Artemia salina parameters												
Total density (ind L^{-1})	Y_1	3058±2924	0.22±0.12	7.60±1.40	21.87±14.25	0.38±0.22	7.40±6.73	10.17±1.13	7.16±5.62	39.57±15.03	7.37±2.53	2.74±3.17
Nauplii density (ind L^{-1})	Y_2	2567±2715	0.05±0.07	2.19±0.35	0.08±0.15	0.05±0.08	5.43±5.84	4.59±0.89	1.11±1.12	7.18±4.39	1.54±1.04	0.14±0.19
Metanauplii density (ind L^{-1})	Y_3	176±156	0.07±0.12	0.06±0.11	0.88±0.92	0.04±0.06	0.14±0.13	1.85±0.55	1.29±1.22	4.10±3.79	1.54±0.79	0.29±0.27
Juvenile density (ind L^{-1})	Y_4	148±141	0.03±0.03	0.26±0.30	5.18±5.24	0.11±0.05	0.27±0.14	0.86±0.78	3.65±4.01	16.36±9.83	1.69±0.59	0.45±0.51
Adult density (ind L^{-1})	Y_5	165±141	0.06±0.07	5.06±1.86	15.71±8.40	0.17±0.04	1.55±0.64	2.89±0.29	1.10±1.04	11.92±2.24	2.93±1.59	1.85±2.20
Average number of cysts/female	Y_6	44.8±23.1	70.2±27.2	55.6±23.9	58±24.6	33.1±13.3	53.2±15.8	64.5±34.8	40±23.7	63.8±24.1	37.3±24.2	29.4±11.8
Average number of nauplii/female	Y_7	55.4±24.6	69.8±24.1	61.3±29.8	67.4±22.7	17.8±8.3	55±20.7	66.2±28.8	54.4±25.1	69.6±26.2	50.8±23.3	42.1±12.1
Offspring encysted (%)	Y_8	76	73	77	88	95	75	76	70	75	73	93
Offspring nauplii (%)	Y_9	24	27	23	12	5	25	24	30	25	27	7

Table 2. Pearson correlation matrix (r_{xy}) between environmental parameters and life cycle parameters (density, population structure, and reproduction) of *Artemia salina*. Bold numbers are significant at P=0.05. X_1: salinity; X_2: temperature; X_3: pH; X_4: dissolved oxygen; X_5: total phytoplankton density; X_6: chlorophyte density; X_7: diatom density; X_8: dinophyte density; X_9: cryptophyte density; X_{10}: euglenophyte density; X_{11}: cyanobacteria density; Y_1: total *Artemia* density; Y_2: nauplii density; Y_3: metanauplii density; Y_4: juvenile density; Y_5: adult density; Y_6: average number of cysts/female; Y_7: average number of nauplii/female; Y_8: percent offspring encysted; Y_9: percent offspring nauplii

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
Y_1	-0.20	-0.03	0.35	0.04	-0.17	-0.31	-0.02	0.23	-0.13	-0.12	**0.71**
Y_2	-0.31	0.45	0.58	0.22	-0.25	-0.29	-0.10	-0.10	-0.10	-0.10	0.16
Y_3	0.00	-0.10	0.18	-0.14	-0.29	-0.34	-0.12	0.18	-0.27	-0.28	**0.84**
Y_4	0.08	-0.24	-0.01	-0.27	-0.12	-0.18	-0.03	0.21	-0.19	-0.17	**0.90**
Y_5	-0.13	-0.47	0.02	0.06	0.10	-0.10	0.15	0.41	0.07	0.06	0.48
Y_6	**-0.75**	**-0.69**	**0.65**	**0.69**	0.44	0.44	0.21	0.47	0.18	0.13	0.39
Y_7	**-0.77**	**-0.81**	**0.70**	**0.64**	0.33	0.23	0.21	0.41	0.16	0.12	0.37
Y_8	**0.67**	0.39	**-0.71**	-0.36	-0.14	-0.02	-0.15	-0.12	-0.09	-0.08	-0.17
Y_9	**-0.67**	-039	**0.71**	0.36	0.14	0.02	0.15	0.12	0.09	0.08	0.17

between compound eyes (*dby*), diameter of compound eyes (*dy*), width of 3rd abdominal segment (*wts*), width of the head (*wh*) and width of 2nd abdominal segment (*wss*) with a cumulative contribution of 62.7% (Table 5; Fig. 2).

For females (Table 4), the strongest correlations between the morphological characteristics and physicochemical parameters were revealed between the length of the furca and salinity (r_{xy} = -0.76), diameter of compound eyes and temperature (r_{xy} = -0.56), number of setae on left and right branches of the furca and pH (r_{xy} = 0.74 and 0.67, respectively) and between dissolved oxygen and width of 3rd abdominal segment (r_{xy} = 0.7), length of the furca (r_{xy} = 0.74), number of setae on the left furcal branch (r_{xy} = 0.73), number of setae on the right furcal branch (r_{xy} = 0.72), width of the head (r_{xy} = 0.72), maximal diameter of compound eyes (r_{xy} = 0.71) and length of 1st antenna (r_{xy} = 0.72). For densities of individual phytoplankton groups, the strongest correlation was between total length and densities of total phytoplankton (r_{xy} = 0.62), diatoms (r_{xy} = 0.65), dinophytes (r_{xy} = 0.61), euglenophytes (r_{xy} = 0.60), and cyanobacteria (r_{xy} = -0.40) and between abdomen length and densities of total phytoplankton (r_{xy} = 0.70), diatoms (r_{xy} = 0.70), dinophytes (r_{xy} = 0.54), euglenophytes (r_{xy} = 0.63), and cyanobacteria (r_{xy} = -0.52).

For males, PCA shows according to axis 1 (with 62.47% of the total variance) that all specimens collected at different environmental conditions converge and show morphological similarity in total length (*tl*), maximal distance between compound

Table 3. Mean values (and SD in parentheses) of morphometric characters of male and female *Artemia salina*

Female

	tl	*al*	*wo*	*wts*	*lf*	*nlf*	*nrf*	*wh*	*dby*	*dy*	*la*
November 2005	9.8^{b} (1.0)	4.5^{b} (0.7)	1.5^{a} (0.3)	0.6^{bc} (0.1)	0.3^{b} (0.0)	6.9^{d} (1.7)	6.4^{c} (1.9)	0.9^{c} (0.1)	1.4^{b} (0.2)	0.3^{b} (0.0)	0.7^{b} (0.1)
December 2005	10.2^{b} (1.2)	5.6^{c} (0.6)	1.9^{a} (0.4)	0.6^{c} (0.1)	0.3^{b} (0.1)	5.8^{c} (1.7)	6.0^{c} (1.7)	0.9^{c} (0.1)	1.5^{b} (0.2)	0.3^{b} (0.0)	0.8^{b} (0.1)
January 2006	12.1^{c} (0.9)	6.5^{d} (0.7)	2.3^{a} (0.2)	0.7^{d} (0.1)	0.4^{d} (0.1)	5.6^{c} (1.7)	5.8^{bc} (1.9)	1.0^{d} (0.1)	1.7^{c} (0.1)	0.3^{b} (0.0)	0.8^{c} (0.1)
March 2006	9.8^{b} (1.8)	4.2^{ab} (1.2)	1.8^{a} (0.5)	0.5^{b} (0.1)	0.3^{c} (0.1)	4.5^{b} (1.8)	4.8^{b} (2.1)	0.7^{b} (0.1)	1.5^{b} (0.3)	0.3^{b} (0.1)	0.7^{b} (0.2)
April 2006	7.7^{a} (0.5)	3.9^{a} (0.4)	1.9^{a} (2.7)	0.4^{a} (0.1)	0.1^{a} (0.0)	1.5^{a} (0.8)	1.4^{a} (0.7)	0.6^{a} (0.0)	1.0^{a} (0.1)	0.2^{a} (0.0)	0.5^{a} (0.1)

Male

	tl	*al*	*wss*	*wts*	*lf*	*nlf*	*nrf*	*wh*	*dby*	*dy*	*la*	*fk*
November 2005	8.4^{b} (0.7)	4.1^{ab} (0.4)	0.8^{e} (0.1)	0.6^{c} (0.1)	0.4^{b} (0.1)	10.0^{d} (3.1)	9.7^{c} (3.1)	0.8^{c} (0.1)	1.6^{b} (0.2)	0.4^{b} (0.0)	1.0^{b} (0.2)	0.2^{b} (0.0)
December 2005	8.9^{b} (0.9)	4.5^{bc} (0.5)	0.6^{b} (0.1)	0.5^{b} (0.1)	0.3^{b} (0.1)	8.1^{c} (2.3)	7.6^{b} (2.4)	0.8^{c} (0.1)	1.7^{b} (0.2)	0.4^{b} (0.0)	1.1^{c} (0.1)	0.2^{b} (0.0)
January 2006	9.7^{c} (1.4)	4.7^{c} (0.8)	0.7^{c} (0.1)	0.5^{b} (0.1)	0.8^{c} (0.3)	5.9^{ab} (2.8)	6.7^{b} (3.0)	0.8^{c} (0.1)	1.6^{b} (0.2)	0.4^{b} (0.1)	1.1^{c} (0.2)	0.2^{b} (0.0)
March 2006	9.1^{bc} (1.8)	4.4^{bc} (1.0)	0.7^{c} (0.2)	0.5^{b} (0.1)	0.4^{b} (0.1)	6.3^{b} (2.4)	6.2^{ab} (2.4)	0.7^{b} (0.1)	1.7^{b} (0.4)	0.4^{b} (0.1)	1.1^{c} (0.1)	0.2^{b} (0.0)
April 2006	7.5^{a} (0.6)	3.8^{a} (0.3)	0.4^{a} (0.0)	0.3^{a} (0.2)	0.2^{a} (0.0)	4.6^{a} (2.3)	4.6^{a} (2.3)	0.6^{a} (0.1)	1.2^{a} (0.1)	0.3^{a} (0.0)	0.8^{a} (0.2)	0.2^{a} (0.0)

Same letters show non-significant differences within rows (P=0.05). Characters: *tl* = total length; *al* = abdomen length; *wss* = width of 2nd abdominal segment; *wts* = width of 3rd abdominal segment; *lf* = length of furca; *nlf* = number of setae on left furcal branch; *nrf* = number of setae on right furcal branch; *wh* = width of head; *dy* = diameter of compound eyes; *dby* = maximal distance between compound eyes; *la* = length of 1st antenna; *fk* = width of the frontal knob; *wo* = width of ovisac.

Table 4. Pearson correlation matrix (r_{xy}) between environmental parameters and adult morphological characteristics of *Artemia salina*. Bold numbers are significant at P=0.05

Males											
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
tl	**-0.47**	**-0.43**	**0.24**	**0.45**	**0.37**	-0.16	**0.39**	**0.41**	**0.35**	0.18	**-0.29**
al	**-0.35**	**-0.35**	0.15	**0.34**	**0.35**	-0.02	**0.35**	**0.33**	**0.31**	0.10	**-0.29**
wss	**-0.61**	**-0.60**	**0.33**	**0.58**	**0.40**	**-0.26**	**0.43**	**0.51**	**0.38**	**0.37**	**-0.33**
wts	**0.96**	**0.73**	**-0.77**	**-0.92**	**-0.29**	**0.27**	**-0.35**	**-0.35**	**-0.25**	**-0.43**	0.07
lf	**-0.55**	**-0.31**	**0.27**	**0.59**	**0.69**	**-0.39**	**0.73**	**0.73**	**0.73**	-0.12	**-0.47**
nlf	**-0.38**	-0.02	**0.56**	**0.39**	-0.18	-0.13	-0.13	**-0.21**	-0.17	-0.02	**0.41**
nrf	**-0.39**	-0.01	**0.52**	**0.41**	-0.06	**-0.20**	-0.01	-0.08	-0.04	-0.07	**0.30**
wh	**-0.68**	**-0.32**	**0.64**	**0.69**	**0.21**	**-0.23**	**0.27**	0.19	**0.21**	0.06	0.06
dy	**-0.55**	**-0.44**	**0.49**	**0.52**	0.06	-0.10	0.09	0.08	0.02	**0.32**	0.05
dby	**-0.55**	**-0.38**	**0.47**	**0.54**	0.17	-0.15	**0.21**	0.18	0.15	0.19	-0.01
la	**-0.48**	**-0.49**	**0.27**	**0.46**	**0.31**	-0.07	**0.32**	**0.33**	**0.27**	**0.27**	**-0.25**
fk	**-0.65**	**-0.47**	**0.51**	**0.64**	**0.27**	**-0.21**	**0.31**	**0.30**	**0.25**	**0.23**	-0.10
Females											
tl	**-0.66**	**-0.45**	**0.38**	**0.68**	**0.62**	**-0.23**	**0.65**	**0.61**	**0.60**	0.00	**-0.40**
al	**-0.50**	**-0.39**	**0.20**	**0.54**	**0.70**	0.07	**0.70**	**0.54**	**0.63**	-0.16	**-0.52**
Wo	-0.01	-0.06	-0.08	0.02	0.19	0.02	0.18	0.17	0.18	-0.04	-0.19
wts	**-0.68**	**-0.41**	**0.46**	**0.70**	**0.54**	**-0.23**	**0.58**	**0.51**	**0.53**	-0.02	**-0.29**
lf	**-0.76**	**-0.55**	**0.51**	**0.74**	**0.46**	**-0.40**	**0.52**	**0.56**	**0.48**	**0.24**	**-0.27**
nlf	**-0.72**	**-0.28**	**0.74**	**0.73**	0.15	**-0.27**	**0.21**	0.13	0.15	0.02	0.17
nrf	**-0.71**	**-0.37**	**0.67**	**0.72**	0.19	**-0.22**	**0.25**	0.18	0.18	0.10	0.08
wh	**-0.68**	**-0.34**	**0.50**	**0.72**	**0.56**	**-0.20**	**0.60**	**0.48**	**0.54**	-0.14	**-0.26**
dy	**-0.72**	**-0.56**	**0.47**	**0.71**	**0.47**	**-0.23**	**0.51**	**0.50**	**0.45**	**0.21**	**-0.30**
dby	**-0.58**	**-0.41**	**0.45**	**0.56**	**0.23**	**-0.23**	**0.27**	**0.28**	**0.22**	**0.22**	-0.08
la	**-0.72**	**-0.49**	**0.54**	**0.72**	**0.4**0	**-0.25**	**0.44**	**0.41**	**0.38**	0.16	-0.18

For abbreviations, see Tables 1 and 3.

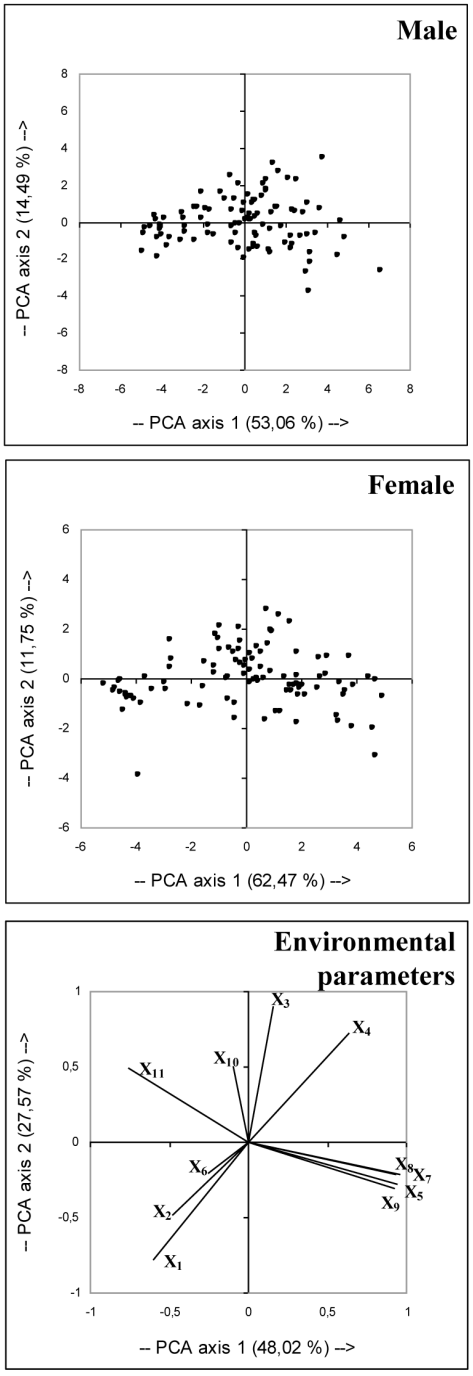

Fig. 2. Principal component analysis (PCA) of morphological characteristics of adult *Artemia salina* and environmental parameters studied. For abbreviations, see Table 1

Table 5. Contributions of principal components F1 and F2 of physicochemical parameters to the variance of adult morphological characters of *Artemia salina*

Morphological character	Males		Females	
	F1	F2	F1	F2
Total length	10.886	9.447	12.487	4.121
Abdomen length	8.344	12.277	8.317	7.286
Width of 2nd abdominal segment	9.110	0.915	-	-
Width of ovisac	-	-	0.463	15.934
Width of 3rd abdominal segment	9.546	1.926	11.624	0.302
Length of furca	5.723	4.470	10.474	0.001
No. of setae on left furcal branch	4.632	31.812	6.322	34.495
No. of setae on right furcal branch	4.343	34.695	6.260	33.795
Width of head	9.563	1.157	10.769	0.075
Maximal distance between compound eyes	12.172	0.072	12.698	0.788
Diameter of compound eyes	11.423	0.602	9.302	3.191
Length of 1st antenna	5.319	1.411	11.285	0.012
Width of frontal knob	8.937	1.219	-	-

eyes (*dby*), width of 3rd abdominal segment (*wts*), length of 1st antenna (*la*), width of the head (*wh*), and length of the furca (*lf*), with a cumulative contribution of 69.33% (Table 5; Fig. 2).

Considering the impact of biotic and abiotic parameters on the *Artemia* morphological structure, it is clear that salinity, temperature, and dissolved oxygen for physicochemical parameters of water, as well as densities of total phytoplankton, diatoms, cryptophytes, and dinophytes, were the major factors affecting *Artemia* morphology (Fig. 2).

DISCUSSION

Sabkhet El Adhibet is an ephemeral site, depending totally on rainfall. Generally, high salinity is the norm in this ecosystem; low salinities coincide with rainfall, as freshwater runs over the flats. In summer, this semi-arid ecosystem is hot and dry, with salt crystals covering the sediment surface. Moreover, the flats are heterogeneous, exhibiting spatial and temporal variability in salinity, temperature, pH, dissolved oxygen, and microalgae density affecting zooplankton biodiversity. Gliwicz et al. (1995)

reported that, in spite of the simplicity of the trophic structure (short food chain) and the limited biodiversity of this ecosystem, it is difficult to assess the consequences of abiotic fluctuations and shifts in phytoplankton concentration/composition on the *Artemia franciscana* population.

In natural environments, temperature, feeding conditions, and salinity are important factors influencing *Artemia* populations (Wear & Haslett 1987; Van Stappen et al. 2001; Torrentera & Dodson 2004). Our study of the correlations between environmental parameters and *Artemia* populations in Sabkhet El Adhibet revealed that physicochemical parameters present significant connections with the reproductive mode and offspring output. These results confirm those reported by Camargo et al. (2004) and Torrentera & Dodson (2004). In fact, induction of diapause in *Artemia* may be under maternal control in response to environmental conditions that determine the metabolic state of the mature embryo (Marcus 1984; Gajardo & Beardmore 1989). Baid (1967), Vanheacke et al. (1984), Triantaphyllidis et al. (1995) and Torrentera & Dodson (2004) reported that the termination of diapause in *Artemia* and the selection of reproductive mode are mainly under environmental control. Camargo et al. (2004) state that the variation in physicochemical conditions of some thalassohaline (marine) sites in the Colombian Caribbean did not influence *Artemia* biomass production. Conversely, Torrentera & Dodson (2004), who studied the ecology of the brine shrimp *Artemia* in the Yucatan (Mexico) salterns, showed that the *Artemia* population dynamics and abundance are highly influenced by environmental factors, principally oxygen, salinity, and temperature. Furthermore, Camargo et al. (2004) reported that the reproductive experiment (mean cyst production per female) does not entirely agree with the estimated cyst production potential but may be due to a combination of certain parameters (i.e. salinity, oxygen concentration, low nitrate, and starvation of the adult *Artemia* population after reaching a high density). In our case, the analysis of physicochemical parameters and *Artemia* densities in Sabkhet El Adhibet did not reveal any correlation between them, confirming the findings of Camargo et al. (2004).

Phytoplankton studies and monitoring are useful for the control of the physicochemical and biological conditions of the water. The dynamics of phytoplankton is influenced many of the environmental processes that affect species diversity. D'Agostino & Provasoli (1968) recognized that food quality and quantity could induce in *Artemia* the oviparity reproduction mode. Newman (2001, cited in Camargo et al. 2004) reported that food density might be the determining factor for *Artemia* to select the oviparous mode of reproduction rather than food quality. Moreover, Camargo et al. (2004) found that chlorophyll *a* was negatively correlated to cyst production, potentially supporting the hypothesis reported by Ballardin & Metalli (1963), D'Agostino & Provasoli (1968), Amat (1985), and Román & Rodríguez (1986), that insufficient food plays an important role in cyst production. On the other hand, Lenz (1987) observed that zooplankton population dynamics are influenced by abiotic factors (salinity, temperature, and nutrient concentration) and by biological interactions (predation, competition, and grazers). Sorgeloos et al. (1986) reported that the best conditions for *Artemia* biomass production are at the lower salinity levels (100 ppt) and under conditions of very regular food availability.

Dolapsakis et al. (2005) showed that the density of the microalga *Dunaliella salina* (Chlorophyta) reached its minimum in summer, when grazing by *Artemia parthenogenetica* and *Fabrea salina* was intense. During our survey, the statistical analyses did not show any correlation between phytoplankton density and the *Artemia* life cycle (density, population structure, and reproduction). The absence of connections between *Artemia* and phytoplankton density could be explained by the fact that in Sabkhet El Adhibet and during a certain period (especially when salinity did not exceed 70 g L^{-1}), *Artemia* coexisted with some other zooplankton (e.g. Branchiopoda, Cladocera, Rotifera) but studied herein as a monoculture, and then the impact of the other species on phytoplankton density was not considered.

Hypersaline habitats are abundant worldwide and provide important models for studying adaptation of aquatic organisms to extreme environments. Biotopes inhabited by *Artemia* are often characterized by extreme ecological conditions. The formation of body proportions in the brine shrimp is affected by many abiotic factors, such as temperature, ion ratio, oxygen content, acidity, and general mineralization (Litvinenko & Boyko 2008). According to previous studies (e.g. Gaevskaya 1916; Gilchrist 1960; Amat 1980; Litvinenko et al. 2007), salinity and salt composition are the most important ecological characteristics affecting morphological and biometrical *Artemia* parameters. However, there are few published data about the impact of the other water physicochemical parameters on *Artemia* morphology.

In the present study, significant differences among adult specimen samples have been demonstrated for several morphological parameters. Specimens harvested in April (corresponding to the highest salinity) show the smallest sizes for the different morphological characteristics. Correlation analysis between different *Artemia* morphological characteristics and the different environmental parameters revealed that physicochemical water characteristics were correlated with all morphological parameters except width of the ovisac. For phytoplankton density, the results show a positive connection between total phytoplankton density and some morphological characteristics. Our data indicate that salinity and temperature were negatively connected with morphological characteristics, whereas pH, oxygen concentration, and total phytoplankton density were positively connected with these parameters. The impact of salinity on *Artemia* morphology was observed by several researchers (Amat 1982; Amat et al. 1991; Naegel & Rodriguez 2002). Naegel & Rodriguez (2002) mentioned that the main reason for the decrease in size of adults is because at high salinity levels (200–250 g L^{-1}), food becomes a limiting factor and *Artemia* needs more energy for osmoregulation. Bertalanffy & Krywienczyk (1953), studying the oxygen consumption of *Artemia* at one salinity level, revealed that respiration is proportional to the square of body length, and thus to surface area.

PCA revealed that salinity, temperature and oxygen are the physicochemical parameters that affect the largest number of morphological characteristics. In fact, Fig. 2 shows that for samples harvested at lower salinity, adult *Artemia* differ more strongly in their morphological characteristics than those harvested at high salinity (in April at salinity level 285 g L^{-1}). These differences were expressed by a dispersed distribution of specimens collected at lower salinity, whereas the distribution of specimens collected at high salinity is more clumped. The ability of *Artemia* to change its

appearance under the influence of salinity has been established by several authors, Gaevskaya (1916), rearing *Artemia* at different salinities, concluded that increasing salinity results in a reduction of adult brine shrimp body size and of the last abdominal segment (furca). Amat (1980) observed that *Artemia* living in natural environments is usually smaller than when cultured in the laboratory. Further, Litvinenko et al. (2007) revealed that only the number of setae on each furcal branch was changed as a function of salinity. In our morphological follow-up, the correlation analysis showed that salinity has an impact on all morphological structures.

In conclusion, this study allowed us to show relationships between water physicochemical parameters and *Artemia* reproduction characteristics. In contrast, no significant relationship was found between any physicochemical variable and *Artemia* population structure and density. Further, there was no correlation between phytoplankton density and the *Artemia* life cycle (density, population structure, and reproduction). One of the most interesting findings in this survey concerns the follow-up of the adult *Artemia* morphology. In fact, we can observe a relationship between physicochemical parameters and all morphological characteristics except the width of the ovisac. Besides, we showed a connection between phytoplankton density and some adult morphological structures.

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