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Impacts of Salinity, Temperature, and pH on the Morphology of *Artemia salina* (Branchiopoda: Anostraca) from Tunisia

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Hachem Ben Naceur, Amel Ben Rejeb Jenhani, and Mohamed Salah Romdhane (2012) Impacts of salinity, temperature, and pH on the morphology of *Artemia salina* (Branchiopoda: Anostraca) from Tunisia. *Zoological Studies* **51**(4): 453-462. This study was carried out on natural populations of the brine shrimp *Artemia salina* from 16 salt lakes in Tunisia, with the purpose of determining the impacts of some physicochemical parameters on morphological characters of adult specimens. Males (*n* = 20) and females (*n* = 20) from each site were measured using a binocular microscope equipped with an ocular micrometer. Up to 13 and 12 morphologic characters were considered for males and females, respectively. The results showed that the physicochemical parameters provoked different degrees of variation among the studied populations. Pearson's correlation coefficient showed highly negative significant correlations of salinity and highly positive ones of pH with the width of the 3rd abdominal segment, length of the furca, number of setae inserted on the left branch of the furca, number of setae inserted on the right branch of the furca, width of the head, diameter of the compound eyes, and the maximal distance between them. However, there were no or only a few significant correlation between the temperature and different morphological characters. Furthermore, the principal component analysis divided the different populations studied into 2 groups based on the salinity of the brine.
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Key words: Salt lakes, Physicochemical characteristics, *Artemia salina*, Morphology, Tunisia.

Inland saline lakes are found throughout the arid and semi-arid basins of the world. They include a vast array of lakes of different sizes, salinities, ionic compositions, flora, and fauna (Hammer 1986, Williams 1996). These habitats are strongly dependent on their hydrological budget, and their limnological and ecological characteristics and physicochemical parameters show large interannual variabilities and different behaviours from year to year. Planktonic food webs are complex, with large and variable numbers of species, trophic levels, and dynamic interactions between trophic levels (Wu et al. 2010). Organisms living in hypersaline lakes are thought to evolve relatively fast due to mutagenic effects of these special environments affected by high ultraviolet radiation and salt concentrations (Hebert et al. 2002). The genus *Artemia* belongs to the order Anostraca which includes fairy shrimps. These brine shrimp are the main zooplanktonic organism inhabiting hypersaline environments worldwide (Triantaphyllidis et al. 1998). In fact, *Artemia* species are extremely osmotolerant and are mostly found in salinities ranging 45-200 psu, although they are able to live in brackish water as well as in supersaturated brine at 340 psu. *Artemia* populations are also adapted to widely changeable temperatures of < 6 to 35°C, and to different ionic brine compositions. Moreover, pH values which they can tolerate vary from neutral to highly alkaline (Van Stappen 2002). Therefore, these physicochemical differences

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*To whom correspondence and reprint requests should be addressed. E-mail:hachem_b_naceur@yahoo.fr have important impacts on *Artemia* populations by influencing their reproductive strategies through ovoviviparism versus cyst production (Camargo et al. 2004) and morphological characters of adult specimens (Gilchrist 1960, Hontoria and Amat 1992, Litvinenko et al. 2007, Asem and Rastegar-Pouyani 2008, Ben Naceur et al. 2011a).

In Tunisia, the climate is characterized by long, dry summers and short, rainy winters, which generally imply the lack of a well-developed permanent surface hydrographic network. For this reason, ephemeral and temporary aquatic habitats are the most common and representative types of superficial waters, with saline ecosystems representing 29% of the total wetland surface area in the country (Ben Naceur et al. 2009a b).

In Mediterranean saltmarshes (and/or saltworks) a bisexual *Artemia* species occurs together with 2 parthenogenetic strains. Bowen and Sterling (1978) suggested that bisexual *Artemia* populations in the Mediterranean area be referred as *A. tunisiana* while the binomen *A. salina* should be restricted to the extinct population described by Schlosser (1756) in Lymington (England). Browne (1988) showed that all of the bisexual populations sampled from the Mediterranean region were able to interbreed and should be classified as *A. salina*. Using amplified fragment length polymorphism methods, Triantaphyllidis et al. (1997) confirmed that all bisexual brine shrimp from the Mediterranean basin should be classified as *A. salina*. However, the biodiversity of *Artemia* populations in this area has suffered great changes after the presence of the species, *A. franciscana*, was recorded in France (Thiery and Robert 1992), Italy (Mura et al. 2004), Spain, and Morocco (Amat et al. 2005). In Tunisia, Ben Naceur et al. (2009b) reported 21 *Artemia* sites, but there are few data on the ecology, taxonomy, morphology, physiology and biochemistry of *Artemia* populations living there.

Our objective in this paper was to determine and elucidate the impacts of salinity, temperature, and pH on morphological characters of adult *Artemia* specimens from wild populations found in natural habitats in Tunisia.

MATERIALS AND METHODS

Field study

Our studies were conducted in 16 Tunisian shallow salt lakes distributed in different hydrogeographical zones (Table 1). Adult brine shrimp samples were collected with a 150-μm-mesh plankton net and preserved with a neutralized 5% formalin solution before being studied in the laboratory. During sampling, water temperature, salinity and pH were measured *in situ* using a portable multiprobe instrument (Multi/340i/SET, WTW, Weilheim, Germany).

Table 1. List of localities and types of biotopes where *A. salina* populations were studied, abbreviations, geographical coordinates, and species references

Site	Abbreviation	Geographical coordinates	Surface (km^2)	Species references
Chott Ariana	AR	36°55'38"N 10°15'22"E	39	[1,3]
Sabkhet Korzia	KOR	36°24'47"N 09°47'10"E	12	[3]
Sabkhet Assa Jriba	AJ	36°14'09"N 10°26'20"E	60	[3]
Sahline saltwork	SAH	35°45'58"N 10°46'58"E	12	[2,3]
Sabkhet Moknine	MOK	35°36'20"N 10°55'37"E	40	[1,3]
Sabkhet Sidi El Héni	SH	35°37'43"N 10°22'46"E	350	[3]
Bkalta saltwork	BK	35°34'19"N 11°01'39"E	1.2	[1,3]
Sabkhet El Jam	JAM	35°09'29"N 10°43'48"E	30	[3]
Sabkhet Mchiguigue	MCH	34°57'16"N 10°02'28"E	24	[3]
Sabkhet Noueiel	NOL	34°27'28"N 09°54'51"E	110	[3]
Zarzis saltwork	ZAR	33°24'48"N 11°03'43"E	1.5	[3]
Sabkhet El Melah	MEL	32°21'34"N 10°55'22"E	150	[3]
Mhabeul saltwork	MHB	33°24'35"N 10°51'20"E	3	[3]
Sabkhet El Adhibet	ADH	33°05'42"N 11°24'29"E	125	[2,3]
Chott El Djerid	CJ	33°56'21"N 08°26'50"E	7000	[3]
Chott El Gharsa	CG	34°09'07"N 08°04'07"E	320	[3]

[1] Vanhaecke et al. 1987; [2] Romdhane et al. 2004; [3] Ben Naceur 2010.

Morphological study

Adult males (n = 20) and females (n = 20) were measured using a binocular microscope equipped with an ocular micrometer at each site. Up to 13 and 12 morphologic characters were respectively considered for males and females. The total length (tl), abdominal length (al), width of the 3rd abdominal segment (wts), length of the furca (lf), number of setae inserted on the left (nlf) and right (nrf) branches of the furca, width of the head (wh), diameter of the compound eyes (dy), maximal distance between them (dby), length of the 1st antenna (la), width of the ovisac (wo) (female), width of the 2nd abdominal segment (wss), and width of the frontal knob (fk) (males) were recorded, and the abdomen length to total body length ratio (ra, %) was also calculated for both sexes (Amat et al. 2005).

Morphological differentiation among the diverse samples was established by statistical treatment of the data by an analysis of variance (ANOVA; Tukey's test, $p < 0.05$) using Statistica (vers. 5; StatSoft, Tulsa, OK, USA). For the analysis of the data obtained, the coefficient of variation was calculated, and a principal component analysis (PCA) was used to interpret major patterns of variation in morphological characteristics according to the environmental data by examining the strength of each variable in explaining the principal directions of variation for the different samples.

Statistical analyses were done using XLSTAT-Pro 7.5.2 (©1995-2004 Addinsoft). Morphometric and physicochemical variables were correlated using Pearson's correlation coefficient to find the degree of association among them.

RESULTS

The physicochemical parameters measured for each site are shown in table 2. On the basis of these parameters (especially salinity), the monitored salt lakes were divided into 5 groups: 4 salt lakes with brine salinities ranging 40-60 psu, 2 salt lakes with brine salinities ranging 120-130 psu, 5 salt lakes with brine salinities ranging 220-260 psu, 4 salt lakes with brine salinities ranging 268-290 psu, and 1 salt lake with a brine salinity of 330 psu.

According to tables 3 and 4, statistical comparisons (ANOVA, Tukey's test) of different morphometric characters showed different degrees of variation among populations, and showed no particular similarity between *Artemia* populations collected from sites presenting similar physicochemical conditions.

The correlation analysis (Table 5) between physicochemical parameters and morphological characters revealed the following. There were highly significant negative correlations of salinity

Table 2. Physicochemical parameters of saline ponds where *A. salina* populations were collected in Tunisia

Group	Locality	Temperature (°C)	Salinity (psu)	pH	Sampling month
i)	CG	22.4	40	8.6	Dec. 2006
	JAM	10.8	55.5	8.9	Dec. 2008
	AR	14	60	8.47	Apr. 2006
	NOL	23.1	60	8.32	Apr. 2006
ii)	SAH	23.9	120	8.21	Feb. 2006
	ADH	16.5	130	8.5	Jan. 2007
iii)	KOR	19.2	220	8.16	Mar. 2006
	MEL	16.6	233	7.73	Dec. 2006
	MHB	16	240	7.76	Dec. 2006
	MCH	25.2	260	7.83	Apr. 2006
	ZAR	14.4	260	8.01	Dec. 2006
iv)	MOK	26.1	268	7.8	Dec. 2006
	SH	18.6	270	7.73	Mar. 2006
	AJ	10	280	7.7	Dec. 2008
	BK	22.6	290	7.92	Apr. 2007
v)	CJ	24.8	330	7.25	Dec. 2006

Abbreviations of localities are defined in table 1.

with wts, lf, nlf, nrf, wh, dy, and dby. The only significant positive correlation was recorded between salinity and ra in females. There were highly significant positive correlations of pH with those same morphological characters cited above. The only significant negative correlation with pH was found for ra in males and for al and ra in females. There was an absence or presence of weak correlations ($r < 0.524$) between temperature and the different morphological characters.

Inverse relationships of salinity with wts, lf, nlf, nrf, wh, dy, and dby existed for female samples. The effects of salinity on the morphologic characters of *Artemia*, when a highly significant correlation between salinity and a character was obtained, are shown in figure 1.

Among male samples, the PCA showed that salinity, wts, lf, nlf, nrf, wh, dby, and dy (component 1), and temperature, tl, al, and fk (component 2) were the most important variables for their differentiation, with respective contributions of 83.1% and 73.67%. For female samples, the

Table 3. Mean values (standard deviation) of morphometric characters of males of different *A. salina* populations. Abbreviations of localities are defined in table 1. Total length (tl), abdominal length (al), width of the 2nd abdominal segment (wss), width of the 3rd abdominal segment (wts), length of the furca (lf), number of setae inserted on the left branch of the furca (nlf), number of setae inserted on the right branch of the furca (nrf), width of the head (wh), diameter of the compound eyes (dy), maximal distance between them (dby), length of the 1st antenna (la) and width of the frontal knob (fk), and abdomen length to total body length ratio (ra, %). The same letters indicates non-significant differences among the means in each row of the main column ($p = 0.05$). All values except the ratio are in millimeters

Group	Locality	tl	al	wss	wts	lf	nlf	nrf	wh	dby	dy	la	fk	ra (%)
i)	CG	8.00^{g}	3.73^{efg}	0.57^{j}	0.50^{ij}	0.44^{g}	13.95^{e}	14.35^{d}	0.85^{i}	1.8686^{f}	0.45^{g}	1.15^{h}	0.21^{ef}	46.62^{abc}
		(0.43)	(0.28)	(0.05)	(0.05)	(0.08)	(2.19)	(2.48)	(0.05)	(0.10)	(0.03)	(0.09)	(0.02)	(2.46)
	JAM	5.97^{a}	2.54^{a}	0.48^{efgh}	0.46^{hi}	0.53^{i}	17.8^{f}	17.5^{e}	0.8^{hi}	1.51^{de}	0.38^{ef}	0.88^{def}	0.15^{ab}	42.72^{a}
		(0.45)	(0.25)	(0.05)	(0.06)	(0.07)	(3.4)	(2.98)	(0.06)	(0.11)	(0.03)	(0.13)	(0.02)	(3.04)
	AR	6.89^{cde}	3.23^{bc}	0.51^{ghi}	0.44^{gh}	0.47^{gh}	12.65^{e}	12.80^{d}	0.77^{gh}	1.65^{e}	0.41^{fg}	0.84^{cde}	0.17^{abc}	46.82^{bc}
		(0.56)	(0.36)	(0.06)	(0.03)	(0.07)	(3.87)	(3.65)	(0.07)	(0.16)	(0.04)	(0.11)	(0.02)	(2.92)
	NOL	6.39^{abc}	4.19^{h}	0.50^{fgh}	0.49^{hij}	0.53^{hi}	17.85^{f}	17.95^{e}	0.77^{gh}	1.52^{de}	0.40^{f}	0.85^{cde}	0.17^{bcd}	66.0^{i}
		(0.58)	(0.43)	(0.06)	(0.07)	(0.08)	(3.38)	(3.55)	(0.07)	(0.13)	(0.03)	(0.15)	(0.02)	(8.17)
ii)	SAH	8.13^{gh}	4.19^{h}	0.53^{hij}	0.40^{fg}	0.24^{ef}	6.30^{c}	5.75^{b}	0.74^{fg}	1.53^{de}	0.38^{ef}	0.88^{def}	0.21^{f}	51.49^{defg}
		(0.60)	(0.43)	(0.06)	(0.04)	(0.08)	(2.85)	(2.88)	(0.08)	(0.16)	(0.05)	(0.16)	(0.03)	(2.47)
	ADH	8.32^{gh}	4.40^{h}	0.42^{cd}	0.40^{fg}	0.21^{def}	5.50^{bc}	5.70^{b}	0.69^{ef}	1.30^{bc}	0.29^{ab}	0.88^{def}	0.14^{a}	53.03^{fgh}
		(0.75)	(0.38)	(0.04)	(0.03)	(0.04)	(1.36)	(1.34)	(0.05)	(0.10)	(0.03)	(0.09)	(0.02)	(3.43)
iii)	KOR	8.27^{gh}	4.13^{gh}	0.41^{c}	0.39^{ef}	0.18^{bcd}	3.2^{ab}	3.2^{ab}	0.66^{de}	1.43^{cd}	0.33^{cd}	0.93^{ef}	019^{cde}	49.93^{cdef}
		(0.54)	(0.33)	(0.03)	(0.04)	(0.05)	(1.32)	(1.58)	(0.05)	(0.11)	(0.03)	(0.08)	(0.01)	(2.24)
	MEL	7.27^{ef}	4.00^{fgh}	0.47^{defg}	0.38^{def}	0.19^{cde}	4.35^{abc}	4.35^{ab}	0.66^{de}	1.30^{bc}	0.32^{bcd}	0.81^{bcde}	0.19^{def}	55.05^{gh}
		(0.58)	(0.40)	(0.06)	(0.06)	(0.05)	(2.06)	(2.03)	(0.10)	(0.19)	(0.05)	(0.15)	(0.03)	(2.48)
	MHB	6.32^{abc}	3.16^{bc}	0.39^{bc}	0.29^{ab}	0.09^{a}	1.95^{a}	1.90^{a}	0.52^{a}	1.23^{b}	0.30^{bc}	0.70^{ab}	0.17^{bcd}	49.95^{cdef}
		(0.60)	(0.37)	(0.04)	(0.03)	(0.04)	(1.10)	(1.02)	(0.06)	(0.13)	(0.04)	(0.11)	(0.02)	(2.38)
	MCH	6.83^{cde}	3.70^{def}	0.40^{bc}	0.34^{cde}	0.27^{f}	9.30^{d}	9.20^{c}	0.64^{cde}	1.24^{b}	0.32^{bcd}	0.73^{abc}	0.16^{ab}	54.11^{gh}
		(0.66)	(0.46)	(0.07)	(0.05)	(0.09)	(4.54)	(5.03)	(0.05)	(0.11)	(0.03)	(0.11)	(0.02)	(2.91)
	ZAR	6.18^{ab}	2.95^{ab}	0.39^{bc}	0.30^{abc}	0.12^{ab}	1.90^{a}	1.90^{a}	0.55^{ab}	1.22^{b}	0.30^{bc}	0.77^{bcd}	0.16^{ab}	47.85^{cd}
		(0.44)	(0.54)	(0.03)	(0.03)	(0.03)	(1.12)	(1.07)	(0.04)	(0.09)	(0.03)	(0.12)	(0.02)	(8.33)
iv)	MOK	8.6^{h}	4.81^{i}	0.34^{ab}	0.34^{cde}	0.13^{abc}	3.2^{ab}	3.45^{ab}	0.56^{ab}	1.42^{cd}	0.33^{cd}	0.98^{fg}	0.16^{abc}	55.74^{h}
		(0.48)	(0.33)	(0.03)	(0.03)	(0.04)	(1.58)	(1.54)	(0.04)	(0.1)	(0.02)	(0.11)	(0.02)	(2.62)
	SH	6.76^{bcde}	3.68^{def}	0.33^{a}	0.28^{a}	0.1^{a}	1.95^{a}	2^{a}	0.51^{a}	1.06^{a}	0.26^{a}	0.93^{ef}	0.19^{cde}	54.46^{gh}
		(0.44)	(0.22)	(0.04)	(0.03)	(0.02)	(0.76)	(0.86)	(0.04)	(0.12)	(0.03)	(0.08)	(0.01)	(1.98)
	AJ	7.7^{eg}	3.3^{bcd}	0.56^{ij}	$0.0.52^{j}$	0.27^{f}	12.7^{e}	12.5^{d}	0.69^{ef}	1.6^{e}	0.35^{de}	1.1^{gh}	0.17^{bcd}	42.8^{ab}
		(0.7)	(0.35)	(0.07)	(0.04)	(0.06)	(2.8)	(2.7)	(0.06)	(0.15)	(0.03)	(0.13)	(0.03)	(1.35)
	BK	6.57^{abcd}	3.46^{cde}	0.44^{cdef}	0.32^{abc}	0.20^{cde}	6.00^{c}	5.85^{b}	0.60^{bcd}	1.19^{ab}	0.30^{bc}	0.64^{a}	0.18^{bcd}	52.61^{efgh}
		(0.34)	(0.24)	(0.06)	(0.04)	(0.05)	(2.43)	(2.30)	(0.05)	(0.13)	(0.03)	(0.08)	(0.03)	(1.89)
v)	CJ	7.1717^{def}	3.50^{cde}	0.44^{cde}	0.33^{bcd}	0.23^{def}	3.05^{ab}	3.35^{ab}	0.57^{abc}	1.33^{bc}	0.30^{bc}	0.92^{ef}	0.17^{abc}	48.76^{cde}
		(0.80)	(0.46)	(0.06)	(0.05)	(0.05)	(1.61)	(1.73)	(0.07)	(0.17)	(0.04)	(0.10)	(0.03)	(2.28)

PCA showed that salinity, lf, nlf, nrf, wh, dby, and dy (component 1), and tl, al, and ra (component 2), were the most important variables for their differentiation, with respective contributions of 76% and 66.43% (Table 6).

The PCA revealed 2 main directions of variation, with axis 1 explaining 45.44% and 48.04% and axis 2 explaining an additional 17.56% and 20.77% for males and females, respectively (Table 6, Fig. 2). Examination of the inter-set correlations of *Artemia* morphological characters with physicochemical variables revealed that the strongest direction of variation was primarily led by salinity, which showed high positive correlations with dby, dy, wss, wts, wh, lf, nlf, and nrf, while pH showed negative correlations with those characters according to the PCA axis 1. The 2nd gradient (axis 2) was characterized by strong inter-set correlations of fk, tl, and al with temperature. All of these variables were positively correlated to the PCA axis 2.

Table 4. Mean values (standard deviation) of morphometric characters of females of different *A. salina* populations. Abbreviations of localities are defined in table 1. Total length (tl), abdominal length (al), width of the 3rd abdominal segment (wts), width of the ovisac (wo), length of the furca (lf), number of setae inserted on the left branch of the furca (nlf), number of setae inserted on the right branch of the furca (nrf), width of the head (wh), diameter of the compound eyes (dy), maximal distance between them (dby), length of the 1st antenna (la), and abdomen length to total body length ratio (ra, %). The same letters among means in each row of the main column indicate a non-significant differences ($p = 0.05$). All values except the ratio are in millimeters

Group	Locality	tl	al	wts	wo	lf	nlf	nrf	wh	dby	dy	la	ra (%)
i)	CG	9.43^{hi}	4.76^{efgh}	0.64^{g}	2.06^{c}	0.42^{fg}	11.90^{f}	11.90^{f}	0.94^{i}	1.57^{g}	0.31^{f}	0.83^{f}	50.35^{b}
		(0.98)	(0.62)	(0.06)	(0.26)	(0.04)	(1.45)	(1.29)	(0.11)	(0.13)	(0.03)	(0.09)	(2.04)
	JAM	6.57^{a}	3.04^{a}	0.47^{ef}	1.25	0.52^{h}	18.35^{g}	18.2^{g}	0.78^{gh}	1.38^{ef}	0.28^{f}	0.66^{de}	46.24^{a}
		(0.3)	(0.2)	(0.05)	(0.14)	(0.06)	(2.83)	(2.66)	(0.06)	(0.16)	(0.02)	(0.05)	(2.27)
	AR	8.26^{cde}	4.24^{cde}	0.97^{h}	1.87^{bc}	0.39^{f}	9.65^{e}	9.60^{e}	0.86^{h}	1.52^{fg}	0.28^{f}	0.59^{bcd}	51.15^{bcd}
		(0.89)	(0.63)	(0.11)	(0.21)	(0.07)	(2.18)	(2.26)	(0.06)	(0.12)	(0.03)	(0.12)	(2.56)
	NOL	7.37^{ab}	3.42^{ab}	0.60^{g}	1.56^{abc}	0.47^{gh}	16.85^{g}	17.30^{g}	0.81^{gh}	1.47^{fg}	0.28^{f}	0.59^{bcd}	46.34^{a}
		(0.90)	(0.50)	(0.09)	(0.34)	(0.05)	(3.20)	(3.31)	(0.09)	(0.19)	(0.03)	(0.07)	(2.3)
ii)	SAH	9.14^{fghi}	4.89^{gh}	0.49^{f}	1.47^{abc}	0.24^{e}	4.20^{bcd}	4.20^{bcd}	0.76^{fg}	1.26^{de}	0.25^{e}	0.68^{e}	53.29^{cdef}
		(1.22)	(0.80)	(0.06)	(0.40)	(0.08)	(2.21)	(2.09)	(0.07)	(0.19)	(0.03)	(0.10)	(2.66)
	ADH	8.98^{efgh}	4.82^{fgh}	0.43^{cdef}	1.30^{abc}	0.22^{de}	4.95^{bcd}	4.95^{cd}	0.70^{ef}	1.13^{bcd}	0.23^{bcde}	0.62^{cde}	53.55^{defg}
		(1.00)	(0.63)	(0.04)	(0.31)	(0.05)	(2.09)	(2.06)	(0.05)	(0.12)	(0.02)	(0.08)	(1.73)
iii)	KOR	9.28^{ghi}	4.96^{h}	0.46^{ef}	1.47^{abc}	0.17^{bcd}	2.1^{a}	2.2^{ab}	0.65^{cde}	1.19^{cd}	0.22^{bcd}	0.63^{de}	53.42^{cdef}
		(0.63)	(0.41)	(0.04)	(0.18)	(0.05)	(1.33)	(1.36)	(0.05)	(0.1)	(0.01)	(0.05)	(2.07)
	MEL	8.36^{cdef}	4.38^{defg}	0.44^{def}	1.63^{abc}	0.16^{bc}	3.25^{abc}	2.90^{ab}	0.64^{bcde}	1.15^{cd}	0.23^{de}	0.53^{ab}	52.45^{bcde}
		(0.83)	(0.48)	(0.07)	(0.33)	(0.05)	(1.33)	(1.25)	(0.08)	(0.13)	(0.02)	(0.07)	(1.7)
	MHB	7.74^{bcd}	3.95^{cd}	0.36^{ab}	1.42^{abc}	0.11^{ab}	2.15^{a}	2.25^{ab}	0.59^{abc}	1.06^{abc}	0.22^{bcd}	0.51^{ab}	51.0^{bc}
		(0.77)	(0.49)	(0.05)	(0.27)	(0.06)	(1.31)	(1.41)	(0.07)	(0.13)	(0.02)	(0.10)	(2.31)
	MCH	7.61^{ab}	4.25^{cd}	0.38^{bc}	1.26^{ab}	0.19^{cde}	5.25^{d}	5.25^{d}	0.63^{abc}	1.06^{a}	0.20^{ab}	0.54^{ab}	55.63^{fgh}
		(0.35)	(0.30)	(0.05)	(0.17)	(0.06)	(2.49)	(2.49)	(0.05)	(0.06)	(0.02)	(0.06)	(2.59)
	ZAR	7.34^{ab}	3.76^{bc}	0.36^{ab}	1.82^{bc}	0.10^{a}	1.35^{a}	1.35^{a}	0.57^{ab}	0.96^{a}	0.22^{abcd}	0.48^{a}	51.23^{bcd}
		(0.50)	(0.33)	(0.04)	(2.75)	(0.03)	(0.59)	(0.59)	(0.06)	(0.12)	(0.03)	(0.07)	(2.77)
iv)	MOK	9.82^{i}	5.67^{i}	0.42^{bcde}	1.34^{abc}	0.11^{ab}	1.95^{a}	1.85^{a}	0.65^{cde}	1.2^{d}	0.22^{bcde}	0.53^{ab}	57.73^{h}
		(0.6)	(0.4)	(0.05)	(0.22)	(0.03)	(1.35)	(1.13)	(0.05)	(0.07)	(0.02)	(0.07)	(2.1)
	SH	7.94^{bc}	4.25^{cd}	0.6^{a}	1.47^{a}	0.1^{a}	2.45^{a}	2.2^{a}	0.59^{a}	1.13^{ab}	0.19^{a}	0.45^{a}	54.31^{efg}
		(0.49)	(0.34)	(0.04)	(0.18)	(0.03)	(1.32)	(1.2)	(0.04)	(0.13)	(0.01)	(0.06)	(2.71)
	AJ	8.22^{cde}	4.31^{def}	0.42^{cdef}	1.4^{abc}	0.17^{bcd}	5.45^{d}	5.6^{d}	0.67^{de}	1.19^{cd}	0.25^{e}	0.62^{cde}	52.39^{bcde}
		(0.7)	(0.43)	(0.05)	(0.16)	(0.05)	(1.93)	(1.81)	(0.05)	(0.06)	(0.01)	(0.04)	(1.4)
	BK	7.13^{bc}	3.97^{cde}	0.37^{bcd}	1.10^{ab}	0.19^{cde}	5.30^{cd}	5.45^{d}	0.59^{abcd}	0.94^{abc}	0.20^{abc}	0.52^{abc}	55.93^{gh}
		(0.46)	(0.33)	(0.04)	(0.21)	(0.02)	(1.56)	(1.67)	(0.05)	(0.08)	(0.04)	(0.07)	(2.87)
v)	CJ	8.49^{defg}	4.33^{def}	0.46^{ef}	1.61^{abc}	0.21^{cde}	3.05^{ab}	3.10^{abc}	0.67^{de}	1.25^{d}	0.23^{cde}	0.62^{de}	50.97^{bc}
		(0.84)	(0.52)	(0.06)	(0.25)	(0.05)	(1.15)	(1.25)	(0.07)	(0.11)	(0.02)	(0.08)	(2.9)

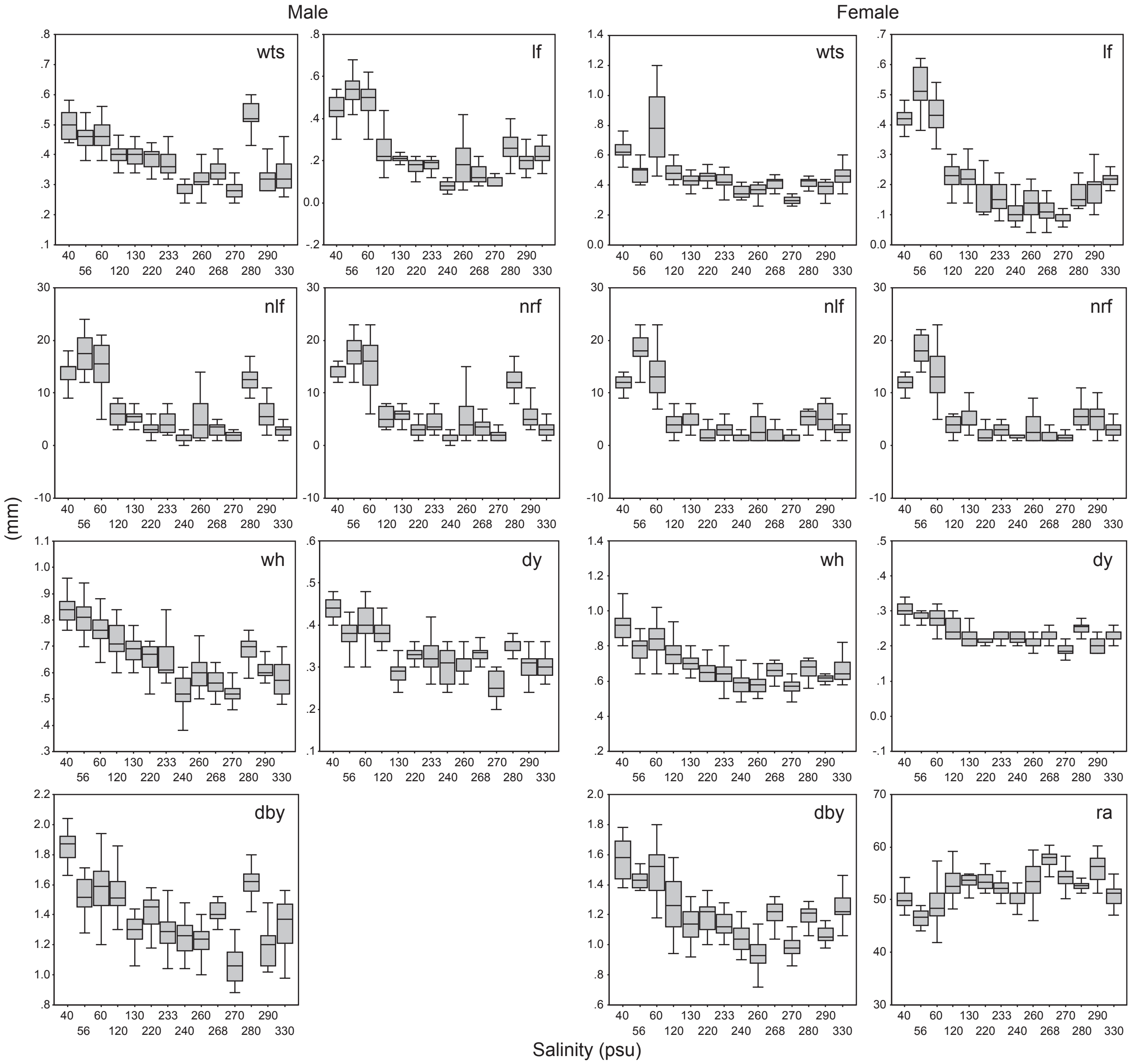

Fig. 1. Effects of salinity on morphological characters of *A. salina*. See abbreviations of morphometric characters in table 2 and 3.

Table 5. Pearson's correlation matrix between physicochemical parameters and morphological characters of adult specimen for 16 *A. salina* populations. Numbers in boldface indicate significant correlations at $p < 0.05$. Abbreviations are defined in tables 1, 3, and 4

Males	tl	al	wss	wts	lf	nlf	nrf	wh	dy	dby	la	fk	ra
T (°C)	**0.261**	**0.524**	**-0.184**	**-0.233**	**-0.113**	**-0.193**	**-0.184**	**-0.125**	-0.063	-0.009	-0.044	**0.198**	**0.488**
S (psu)	0.008	0.017	**-0.452**	**-0.575**	**-0.746**	**-0.657**	**-0.659**	**-0.746**	**-0.581**	**-0.642**	**-0.171**	-0.071	0.004
pH	-0.005	-0.083	**0.309**	**0.473**	**0.619**	**0.566**	**0.564**	**0.662**	**0.443**	**0.492**	0.104	-0.050	**-0.125**
Females			wo										
T (°C)	**0.278**	**0.340**	-0.025	-0.096	-0.103	**-0.156**	**-0.148**	-0.004	-0.023	**-0.149**	0.040	-	**0.325**
S (psu)	-0.019	**0.159**	**-0.148**	**-0.641**	**-0.799**	**-0.742**	**-0.740**	**-0.736**	**-0.653**	**-0.678**	**-0.470**	-	**0.485**
pH	-0.031	**-0.150**	0.083	**0.451**	**0.693**	**0.663**	**0.658**	**0.588**	**0.471**	**0.518**	**0.407**	-	**-0.365**

T, temperature; S, salinity.

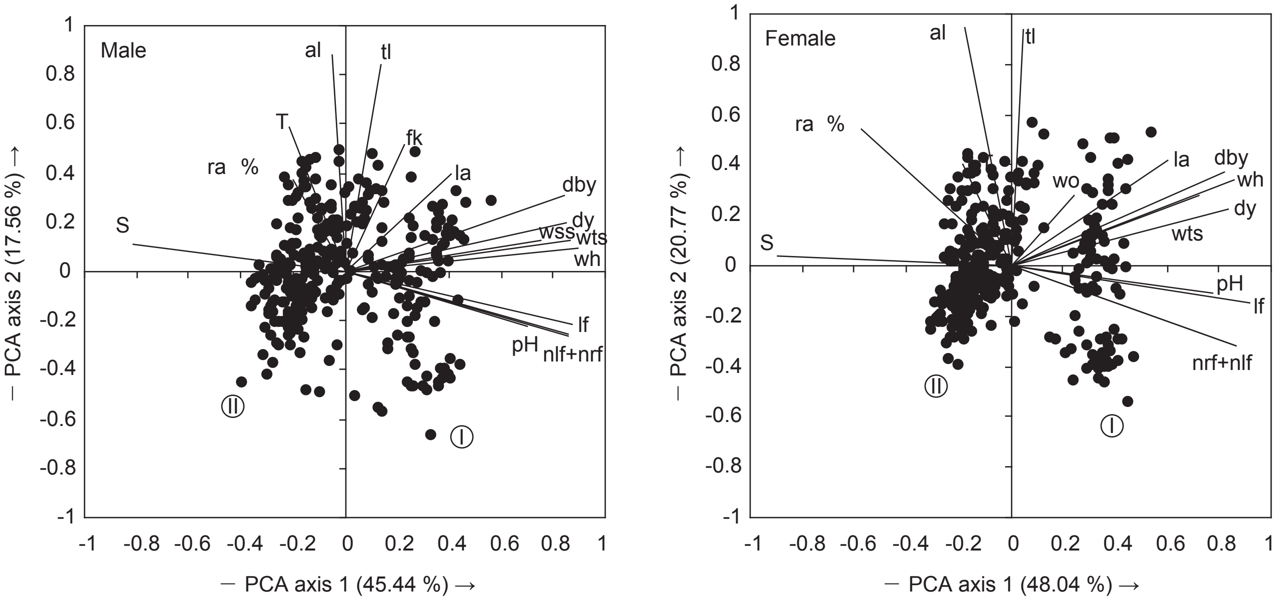

Fig. 2. Results obtained from application of a principal component analysis to correlate the morphological traits of adult *A. salina* specimens with physicochemical parameters recorded in the different hypersaline habitats studied. (I) AR, JAM, NOL, CG; (II) KOR, AJ, SAH, MOK, SH, BK, MCH, ZAR, MEL, MHB, ADH, CJ. Abbreviations of sampling localities are defined in table 1.

Table 6. Contribution of each morphological trait to the distribution of the results of the 1st 2 components of the principal component analysis applied to physicochemical parameters and morphological data analyzed. Abbreviations are defined in table 3 and 4

	Males				Females			
	Contribution of a variable (%)		Component		Contribution of a variable (%)		Component	
Variables	1	2	1	2	1	2	1	2
T (°C)	0.621	12.371	-0.212	0.590	0.486	5.180	-0.187	0.402
S (psu)	9.013	0.452	-0.810	0.113	11.179	0.050	-0.897	0.039
pH	6.739	1.793	0.700	-0.224	8.282	0.383	0.773	-0.109
tl	0.265	25.030	0.139	0.839	0.029	28.308	0.046	0.939
al	0.031	27.626	-0.048	0.881	0.415	28.825	-0.173	0.948
wss	7.756	0.599	0.751	0.130	-	-	-	-
wo	-	-	-	-	0.808	2.561	0.241	0.282
wts	10.348	0.544	0.867	0.124	7.216	2.559	0.721	0.282
lf	10.471	1.686	0.873	-0.218	11.707	0.719	0.918	-0.150
nlf	10.183	2.266	0.860	-0.252	10.409	3.237	0.866	-0.318
nrf	10.112	2.368	0.857	-0.258	10.411	3.241	0.866	-0.318
wh	11.046	0.344	0.896	0.098	10.286	3.742	0.861	0.341
dby	9.839	3.339	0.846	0.306	9.403	4.483	0.823	0.374
dy	10.022	1.448	0.854	0.202	9.737	1.573	0.838	0.221
la	2.301	5.710	0.409	0.401	5.087	5.702	0.605	0.421
fk	0.695	9.385	0.225	0.514	-	-	-	-
ra	0.557	5.039	-0.201	0.376	4.545	9.437	-0.572	0.542
Total variance explained								
Percent of variance	45.442	17.562			48.035	20.768		
Cumulative percent	45.442	63.004			48.035	68.803		

T, temperature; S, salinity.

DISCUSSION

In natural environments, temperature, feeding conditions, and salinity are important factors influencing *Artemia* population dynamics (Browne 1982, Wear and Haslett 1987), while salinity is the most important factor to characterizing salt lakes. In fact, Herbst (2001) classified saline systems into 4 categories based on conditions of habitat stability and salinity stress: stable lakes of low salinity, stable lakes of moderate to high salinity, temporary lakes of low to moderate salinity, and ephemeral lakes with extremely hypersaline brine. In *Artemia*, morphological traits are the basis for describing species and strains, although controversy exists on the method of selecting and using suitable morphological traits and on the degree of their genetic or environmental determination (Gajardo et al. 1998). The impact of the environment is relevant to understanding the extent of morphological differentiation in *Artemia* populations. Often, geographical and ecological separation along with climatic and hydrobiological changes, as a whole, offers a mosaic of environmental conditions that tend to favor local forms (Gajardo et al. 1998). The ability of *Artemia* to change its appearance under the influence of salinity was established in earlier work by Schmankewitsch in 1875 (in Litvinenko et al. 2007), Gaevskaya (1916), Gilchrist (1960), and Amat (1982). Gaevskaya (1916), Amat (1980), Amat et al. (1991), Rodríguez-Almaraz et al. (2006), and Ben Naceur et al. (2011b) showed that a higher salinity leads to a reduced adult body size. Litvinenko et al. (2007) reported that only the number of setae on each furcal branch changed as a function of salinity for *Artemia* populations in Siberian lakes. A morphometric analysis of 125 Siberian populations of *Artemia* by Litvinenko and Boyko (2008) demonstrated that furcal morphological traits (length and width of the furca, ratio of furcal length to abdomen length, and number of setae on the furca) were the most labile, while body length and abdomen length to body length ratio were the most stable. Our study results allowed us to conclude that wts, lf, nlf, nrf, wh, dy, and dby were the most changeable characters as a function of salinity. As to other physicochemical parameters and their impacts on *Artemia* morphological characteristics, few data are available and are often restricted to salinity. Impacts of pH and temperature on *Artemia* populations were mostly studied in terms of growth (Vos 1979), cyst hatching efficiency (Sato 1967), survival, and reproduction (Browne and Wanigasekera 2000). In our study, the impact of pH on *Artemia* morphology showed significant negative correlations with ra in males and, al and ra in females. On the other hand, results obtained herein did not reveal an important impact of temperature on *Artemia* morphological characters, as the most labile character influenced by temperature was abdomen length (al) at $r = 0.524$.

Based on the statistical comparison (ANOVA, Tukey's test), impacts of physicochemical parameters on the different morphological characters varied from 1 population to another. Gilchrist (1960) stated that differences among populations (of the same species) and between males and females (of the same population) may exist, even when animals were cultured under the same conditions, thus demonstrating intrinsic and environmental influences.

On the basis of their morphological traits, the 16 *Artemia* populations sampled from Tunisian salt lakes were clearly separated into 2 groups along the 1st PCA gradient, with populations obtained from Ariana, El Jam, Noueiel, and El Gharsa on the left side of the ordination (Fig. 2), which indicated that these sites were characterized by lower salinities (40-60 psu) and an alkaline pH (8.32-8.9). The other populations, plotted on the right side of the ordination (Fig. 2), were associated with salinities ranging 120-330 psu, and pH values of 7.25-8.5. The contribution of salinity to the segregation of *Artemia* populations in the 2 groups was clear (Table 5). The study of the contribution of each morphological variable showed that wts, lf, nlf, nrf, wh, dby, and dy were the major characters driving this separation. Furthermore, contrary to Pilla and Beardmore (1994), Camargo et al. (2003) and Asem and Rastegar-Pouyani (2008), who showed that male morphometric characters can be at least as informative as those of females, it is evident in our case that females of these populations were more clearly segregated than males.

The data derived from the study of the impacts of physicochemical parameters on the 16 brine shrimp morphological characteristics from Tunisia provide evidence that the width of the 3rd abdominal segment (wts), length of the furca (lf), number of setae inserted on the left branch of the furca (nlf), number of setae inserted on the right branch of the furca (nrf), width of the head (wh), diameter of the compound eyes (dy), and the maximal distance between them (dby) were the variables most influenced by salinity (significant negative correlations). Moreover, pH showed a positive significant correlation with these characters and a significant negative significant correlation with the ratio of abdomen length to total body length (ra) in males and abdomen length (al) and ra in females. On the other hand, except for al and ra, temperature presented only weak correlations with the other morphological characteristics. Furthermore, based on the PCA, the populations studied herein were clearly separated into 2 groups based on the salinity level.

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REFERENCES

Amat F. 1980. Differentiation in *Artemia* strains from Spain. *In* G Persoone, P Sorgeloos, O Roels, E Jaspers, eds. The brine shrimp *Artemia*. Vol. 1. Morphology, genetics, radiobiology, toxicology. Wetteren, Belgium: Universa Press, pp. 19-39.

Amat F. 1982. Diferenciación y distribución de las poblaciones de *Artemia* de España. III. Oviparismo y ovoviviparismo. Estudio cualitativo y cuantitativo. Invest. Pesq. **46:** 3-13.

Amat F, F Hontoria, JC Navarro, EA Gonzalbo, VI Varo. 1991. Bioecología de *Artemia* (Crustacea, Branchiopoda) en la Laguna de La Mata, Torre Vieja, Alicante. Torre de la Sal (CSIC), Spain: Instituto de Acuicultura.

Amat F, F Hontoria, O Ruiz, AJ Green, MI Sanchez, J Figuerola, F Hortas. 2005. The American brine shrimp as an exotic invasive species in the western Mediterranean. Biol. Invasions **7:** 37-47.

Asem A, N Rastegar-Pouyani. 2008. Morphological differentiation of *Artemia urmiana* Günther 1899 (Crustacea: Anostraca) in different geographical stations from the Urmia Lake, Iran. Res. J. Biol. Sci. **3:** 222-228.

Ben Naceur H. 2010. Cratérisation taxonomique, biochimique et éco-biologique pour la valorisation aquacole de l'*Artemia* en Tunisie: cas de Sabkhet El Adhibet (sud-est Tunisien). Thèse de Doctorat en Sciences Agronomiques, Institut National Agronomique de Tunisie, 271 pp.

Ben Naceur H, A Ben Rejeb Jenhani, MS Romdhane. 2009a. Ecobiological survey of the brine shrimp *Artemia salina* from Sabkhet El Adhibet (south-east Tunisia). J. Mar. Biol. Assoc. UK **89:** 1109-1116.

Ben Naceur H, A Ben Rejeb Jenhani, MS Romdhane. 2009b. New distribution record of the brine shrimp *Artemia* (Crustacea, Branchiopoda, Anostraca) in Tunisia. Check List **5:** 281-288.

Ben Naceur H, A Ben Rejeb Jenhani, MS Romdhane. 2011a. *In situ* study of adult *Artemia salina* morphometry and its relationships to the physicochemical water parameters in Sahline saltwork (Tunisia). Oceanol. Hydrobiol. St. **40:** 44-51.

Ben Naceur H, A Ben Rejeb Jenhani, MS Romdhane. 2011b. Influence of some environmental factors on *Artemia salina* life cycle and morphology in Sabkhet El Adhibet (southeast Tunisia). Biological Lett. **48:** 67-83.

Bowen ST, G Sterling. 1978. Esterase and malate dehydrogenase isozyme polymorphisms in 15 *Artemia* populations. Compar. Biochem. Physiol. **61:** 593-595.

Browne RA. 1982. The costs of reproduction in brine shrimp. Ecology **63:** 43-47.

Browne RA. 1988. Ecological and genetic divergence of sexual and asexual brine shrimp *Artemia* populations from the Mediterranean basin. Nat. Geogr. Res. **4:** 548-554.

Browne RA, G Wanigasekera. 2000. Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. J. Exp. Mar. Biol. Ecol. **244:** 29-44.

Camargo WN, JS Ely, GC Duran, P Sorgeloos. 2004. Influence of some physicochemical parameters on *Artemia* biomass and cyst from the Colombian Caribbean. J. World Aquacult. Soc. **35:** 296-305.

Camargo WN, JS Ely, P Sorgeloos. 2003. Morphometric characterization of thalassohaline *Artemia franciscana* populations from the Colombian Caribbean. J. Biogeogr. **30:** 697-702.

Gaevskaya NS. 1916. Variability of *Artemia salina* (L). Russ. Pub. Special Zool. Lab. Acad. Sci. **2:** 1-37.

Gajardo G, N Colihueque, M Parraguez, P Sorgeloos. 1998. International study on *Artemia* LVIII. Morphologic differentiation and reproductive isolation of *Artemia* population from South America. Int. J. Salt Lake Res. **7:** 133-151.

Gilchrist BM. 1960. Growth and form of the brine shrimp *Artemia salina* (L.). Proc. Zool. Soc. Lond. **134:** 221-235.

Hammer UT. 1986. Saline lake ecosystems of the world. Dordrecht, the Netherlands: Dr. W Junk Publishers.

Hebert PD, EA Remigio, JK Colbourne, DJ Taylor, CC Wilson. 2002. Accelerated molecular evolution in halophilic crustaceans. Evolution **56:** 909-926.

Herbst DB. 2001. Gradient of salinity stress, environmental stability and water chemistry as a template for defining habitat types and physiological strategies in inland salt waters. Hydrobiologia **466:** 209-219.

Hontoria F, F Amat. 1992. Morphological characterization of adult *Artemia* (Crustacea, Branchiopoda) from different geographical origin. Mediterranean populations. J. Plankton Res. **14:** 949-959.

Litvinenko LI, EG Boyko. 2008. The morphological characteristics of *Artemia* shrimps from Siberian populations. Inland Water Biol. **1:** 37-45.

Litvinenko LI, AV Kozlov, AI Kovalenko, DS Bauer. 2007. Salinity of water as a factor to determine the development of the brine shrimp *Artemia* population in Siberian lakes. Hydrobiologia **576:** 95-101.

Mura G, F Amat, TJ Abatzopoulos, S Moscatello. 2004. First record of *Artemia franciscana* in an Italian saltwork. Book of abstracts. Fifth International Large Branchiopod Symposium. Toodyay, Western Australia, pp. 35-36.

Pilla EJS, JA Beardmore. 1994. Genetic and morphometric differentiation in Old Word bisexual species of the brine shrimp (*Artemia*). Heredity **72:** 47-56.

Rodríguez-Almaraz GA, C Zavala, R Mendoza, AM Maeda-Martínez. 2006. Ecological and biological notes on the brine shrimp *Artemia* (Crustacea: Branchiopoda: Anostraca) from Carmen Island, Baja California Sur, México. Hydrobiologia **560:** 417-423.

Romdhane MS, H Ben Naceur, S Hamrouni, A Ben Rejeb Jenhani, M El Cafsi. 2004. Biological and biochemical characterisation of *Artemia* from Tunisian wetlands. *In* N Agh, P Sorgeloos, eds. International workshop on *Artemia*. INCO-DEV Project on *Artemia* biodiversity, Urmia, Iran, pp. 89-91.

Sato NL. 1967. Enzymatic contribution to the encystment of *Artemia salina*. Sci. Rep. Tohoku Univ. **33:** 319-327.

Schlösser D. 1756. Extrait d'une lettre de M. le Docteur Schlösser concernant un insecte peu connu. Observation périodique sur la physique, l'histoire naturelle et les beaux arts, de Gautier, pp. 58-60.

Thiery A, F Robert. 1992. Bisexual populations of the brine shrimp *Artemia* in Sète-Villeroy and Villeneuve saltworks (Languedoc, France). Int. J. Salt Lake Res. **1:** 47-63.

Triantaphyllidis GV, TJ Abatzopoulos, P Sorgeloos. 1998. Review of the biogeography of the genus *Artemia* (Crustacea, Anostraca). J. Biogeogr. **25:** 213-226.

Triantaphyllidis GV, GRJ Criel, TJ Abatzopoulos, KM Thomas, J Peleman, JA Beardmore, P Sorgeloos. 1997. International study on *Artemia* LVII. Morphology and molecular characters suggest conspecificity of all bisexual European and North African *Artemia* populations. Mar. Biol. **129:** 477-487.

Vanhaecke P, W Tackaert, P Sorgeloos. 1987. The biogeography of *Artemia*: an updated review. *In* P Sorgeloos, DA Bengtson, W Decleir, E Jaspers, eds. *Artemia* research and its applications. Vol. 1. Wetteren, Belgium: Universa Press, pp. 129-155.

Van Stappen G. 2002. Zoogeography. *In* TJ Abatzopoulos, JA Beardmore, JS Cleeg, P Sorgeloos, eds. *Artemia* basic and applied biology. Dordrecht, the Netherlands: Kluwer Academic Publishing, pp. 171-215.

Vos J. 1979. Brine shrimp *Artemia salina* inoculation in tropical salt ponds; a preliminary guide for use in Thailand. Rome, Italy: Food and Agricultural Organization report THA/008/75/79/WP/4.

Wear RG, SJ Haslett. 1987. Studies on the biology and ecology of *Artemia* from Lake Grassmere, New Zealand. *In* P Sorgeloos, DA Bengtson, W Decleir, E Jaspers, eds. *Artemia* research and applications. Vol. 3. Ecology, culturing, use in Aquaculture. Wetteren, Belgium: Universa Press, pp. 101-126.

Williams WD. 1996. The largest, highest and lowest lakes of the world: saline lakes. Verh. Int. Verein. Limnol. **26:** 61-79.

Wu CH, HU Dahms, EJ Buskey, J Rudi Strickler, JS Hwang. 2010. Behavioral interactions of the copepod *Temora turbinata* with potential ciliate Prey. Zool. Stud. **49:** 157-168.