Observations on *Phallocryptus spinosa* (Branchiopoda, Anostraca) populations from the high plateaus of Northeastern Algeria

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Abstract

Phallocryptus spinosa (Thamnocephalidae) is a small primitive crustacean which lives in temporary saline pools of the Palaearctic region called Chott, Sebkha, Garâa or Daya in North Africa. Data on its biology and life history are very scarce despite its ecological importance in wetlands. Four populations living in the Northeastern High Plateaus of Algeria, Garâas Guellif, El-Tarf, Ank Djemel and Sebkha Ez-Zemoul, have been studied in point of view of their morphmetry (13 parameters) and their reproduction. The population of Sebkha Ez-Zemoul, living in higher salinities and in sympatry with *Artemia salina* was different from the 3 other populations that are close together. Its adults are the largest (21.77 ± 2.34 mm and 23.65 ± 2.36 mm for males and females respectively) and produce more cysts 771.47±8.45 cysts/brood of 293.11±10.41 µm diameter. Associated carcinological fauna and some behavioral traits are also approached.

Key words: *Phallocryptus spinosa*, biometry, fecundity, associated fauna, Algeria.

Introduction

Large Branchiopods are considered as the flagship group of the ecosystems and the temporary pool invertebrates in general (Colbum, 2004). They are used for the assessment of their quality and function (Brendonck et al., 2008). They are threatened because of the degradation and the destruction of their habitats (IUCN France & MNHN, 2012).

Among Anostracan, the Thamnocephalid *Phallocryptus spinosa* (Milne-Edwards 1840) was long considered as *Branchinella spinosa* (Milne-Edwards 1840). Rogers (2003, 2006) gave a new description of the species and classified it in the genus *Phallocryptus* (Biraben 1951), which comprises three other species, of which *P. tserensodnomi* (Alonso & Ventura, 2013) has been recently discovered in Mongolia. *P. spinosa* has a Palaearctic distribution which extends to the Botswana (Hulsmans et al., 2006) and Ukraine (Ketmaier et al., 2008). It lives in shallow (0.2 – 0.8 m) saline (3 - 300 psu) temporal lakes. Their females produce cysts to bridge period of drought ensuring the continuity of the species (Brendonk, 1996).

Studies on biological and behavioural aspects of *P. spinosa* are relatively scarce (Mura, 1992; Alonso, 1996; Brendonck & Riddoch, 1997; Ampe & Thiéry, 1998; Defaye et al., 1998) and generally concern their distribution and seasonality (Garcia et al., 1997; Thiéry & Puff, 1998; Abatzopoulos et al., 1999; Mura, 1993; 2001; Vakili & Sari, 2012). Recently Ketmaier et al., (2008) analyzed patterns of sequence divergence in mitochondrial DNA in 15 populations from the Mediterranean basin, Ukraine, Iran, Uzbekistan and Botswana concluding genetic differentiation among populations. The recent study of Alonso and Ventura (2013) showed that *P. tserensodnomi* from Mongolia was close to *P. spinosa* and might have some genotypes' of African populations.

P. Spinosa is known in Algeria from four large biotopes in the eastern High Plateaus (Samraoui *et al.*, 2006) where it co-occurs with *Artemia salina* in Sebkha Ez-Zemoul (Amarouayache et al., 2012). It has been recorded in Boughzoul, an artificial lake of 1000 ha near the locality of Djelfa in the central High Plateaus by Beladjal et al., (1995). This species with *Artemia* (Artemiidae), govern the feeding and reproduction of the migratory birds, especially the Flamingo Phoenicopterus *Ruber roseus* (Samraoui & Samraoui, 2008). The knowledge about this species is of great importance to envisage protection measures with regard of its ecological role. The aim of this study is to provide some data on biometry and reproduction of four populations among which one lives in sympatry with *A. salina*. Carcinological fauna living with *P. spinosa* was also identified, and other ecological observations are given.

Materials and methods

Sites and sampling: *Phallocryptus spinosa* and its associated fauna were sampled in the temporary pools (locally Sebkhas and Garâas) of the Northeastern High Plateaus (800-1000 m altitude), situated in a semi-arid region (350-450 mm) formed at the Pleistocene (Fig. 1). They are shallow (max 0.5 m), alkaline (pH = 8.5) and generally dried in summer. The thin water layer is led away by winds that generally blow from the Northwest. Sebkha Ez-Zemoul (35°53'N - 06°30'E) is the

most saline (30-255 psu) with an area of 6,100 ha and is exploited for the salt by ENASEL farm. Garâa El-Tarf (35°41'N-07°08') is the largest with 33,460 ha, then Garâa Ank-Djemel (35°N47'-06°51') with 6,700 ha and Garâa Guellif (35°47'N-06°51') of 5,500 ha. These 3 Garâas have RAMSAR site statute. Their salinity at the sampling period was between 15 and 20 psu.

P. spinosa have been sampled in Sebkha Ez-Zemoul during an exceptional rainy year in January 2003 with 250 mm for this month, and 767.6 mm.year^{-1}, a record rate during the 3 last decades. Water salinity has decreased at this period to 30 psu and water column was 70 cm at the shore near the crystallizing ponds of the salt farm. In the 3 Garâas, individuals were sampled in May 2005 at the shore when water depth was around 2 cm. A sieve of 125 µm mesh vacuum was used to filtration and crustaceans were fixed in 4 % formalin.

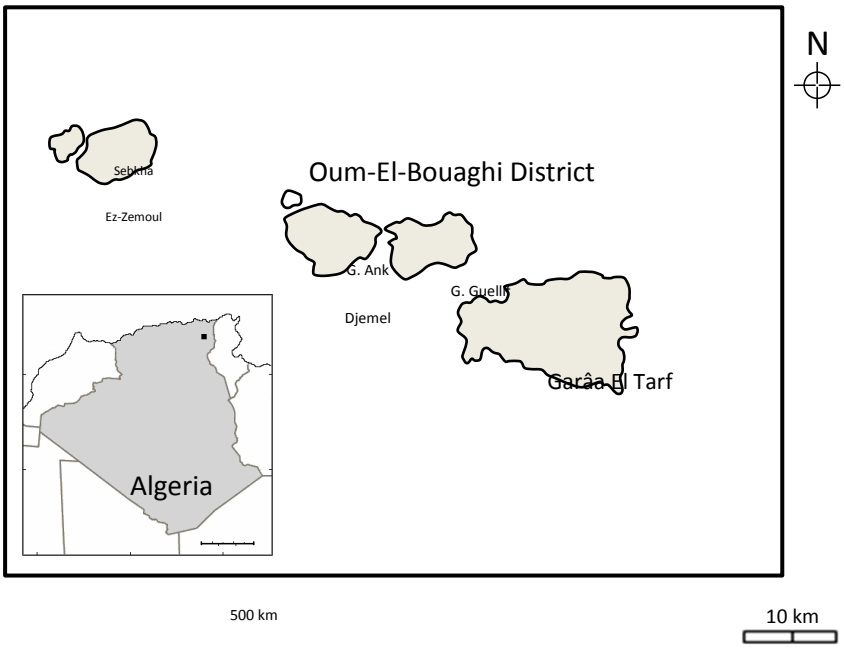

Figure 1. Geographic situation of sampling sites of *Phallocryptus spinosa*.

Biological and ecological observations: for each population, 20 males and 20 females have been measured using a compound microscope equipped with an ocular micrometer. One numeric (setae number) and 13 metric parameters have been considered for morphometric study according to Amat (1980, modified): total length, abdominal length, furca length, length of first antenna, length of ovisac, width of ovisac, width of the third abdominal segment, width of head, distance between complex eyes, maximal diameter of complex eye. The length and the width of the first abdominal spin were also considered.

The size at first sexual maturity was determined as corresponding to the length of which 50% of the females presented signs of reproduction such as the presence of embryos or cysts inside the ovisacs. Those were dissected and the cysts were counted to determine fecundity. Cysts from each population were hydrated during 1h and their mean diameter was measured. Other observations as the population structure and associated carcinological fauna were also approached.

Results

Reproduction and morphometry: all the females reproduce by oviparity and were gravid at a total length ranged between 11.5 and 23.65 mm according to the origin. Data on fecundity and mean diameter of hydrated cysts for each population are presented in table 1. Females of Sebkha Ez-Zemoul were the most fertile with a mean fecundity of 771.4 ± 78.45 cysts/brood and a maximal individual brood size of 888 cysts. In the 3 Garâas the fecundity was relatively low with a minimal brood size of 15.6 ± 8.45 cysts/brood observed in the Guellif's females. The mean diameter of hydrated cysts was comprised between 276.29 ± 18.79 µm and 293.11 ± 10.41 µm for Guellif and Ez-Zemoul respectively. Morphometric parameters are given in table 2. Populations of the three Garâas are close while individuals from Sebkha Ez-Zemoul clearly present larger lengths for all the parameters, except the setae number. Males' size was between 16.26 ± 0.92 and 21.77 ± 2.34 mm for El-Tarf and Ez-Zemoul's populations respectively and female's size was between 16.14 ± 1.10 and 23.65 ± 2.36 mm and 21.77 ± 2.34 mm for the same populations. Number of setae's was between a minimum of 96.50 ± 24.28 observed in males of Garâa El-Tarf and a maximum of 125 ± 14.26 at females of Garâa Ank Djemel.

Table 1. Reproduction patterns for the Guellif, El-Tarf, Ank Djemel and Ez-Zemoul *P. spinosa* populations. L: Length at first sexual maturity, F: mean fecundity (SD), Cd: mean cyst diameter (SD), Min: minimal cyst diameter and Max: maximal cyst diameter.

Parameter	Guellif	El-Tarf	An Djemel	Az-Zemoul
L mm	14.00	13.00	11.50	23.65
F cysts/brood	23±15.42	15.6±8.45	27.65±14.49	771.47±8.45
Cd µm	276.29±18.79	292.18±21.81	282.84±20.56	293.11±10.41
Min µm	239.20	208.00	239.20	266.00
Max µm	312.00	343.20	322.41	312.00

Population structure and associated fauna: in the three Garâas, *P. spinosa* was present at high densities during the sampling period with an approximate density of 30,000 ind.m^{-2} near the banks. Adults dominated on juveniles and pre-adults at more than 90 %, and nauplii and metanauplii were totally absent. Sex-ratio was generally in favour of males with 1.02, 3.86, 2.09 and 1.87 in Garâas Guellif, El-Tarf, Ank-Djemel and Sebkha Ez-Zemoul respectively. In the Sebkha Ez-Zemoul *P. spinosa* was found with *Artemia salina*, the Harpacticoid *Cletocamptus retrogressus* (Schmankevitch, 1875), the Cladoceran *Moina salina* (Daday, 1888) and the Cypridid Ostracod *Heterocypris* sp. However, the majority of its individuals were dead, floating on the surface of the water and were brought from the other side of the Sebkha (North-East) by the waves. Some individuals carried almost entire Harpacticoids inside their abdomen, probably *C. retrogressus* (Fig. 2). *P. spinosa* of Garâa Guellif was found to live with *M. salina* witch was the most frequent, and rare individuals of the Ostracod *Eucypris lilljeborgi* and the Calanoid *Arctodiaptomus salinus*. In Garâa El-Tarf, *A. salinus* was the most frequent, then *C. retrogressus*, *M. salina* and a non-identified Ostracod. In Garâa Ank Djemel, *M. salina* was the most frequent with few non identified Ostracods.

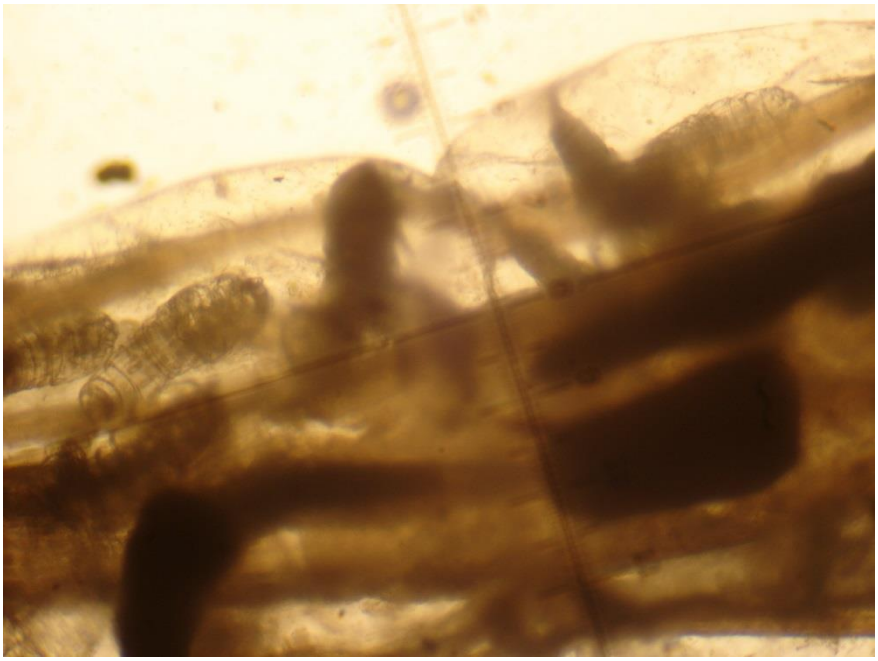

Figure 2. Photograph showing Harpacticoid Copepods inside the body of *Phallocryptus spinosa* from Sebkha Ez-Zemoul.

Table 2. Mean (S.D.) of morphometric parameters of males and females in mm for the Guellif, El-Tarf, Ank Djemel and Ez-Zemoul *P. spinosa* populations. (A) total length, (B) abdominal length, (C) furca length, (D) length of first antenna, I length of ovisac, (F) width of ovisac, (G) width of the third abdominal segment, (H) width of head, (I) distance between complex eyes, (J) maximal diameter of complex eye, (K) length of the first abdominal spine, (L) width of the first abdominal spine, (M) number of setae.

Parameters	Guellif	El-Tarf	Ank Djemel	Ez-Zemoul
A	16.35 (1.45)	16.26 (0.92)	16.92 (2.67)	21.77 (2.34)
	16.25 (1.64)	16.14 (1.10)	17.84 (2.65)	23.65 (2.36)
B	8.87 (0.78)	9.31 (0.52)	9.35 (1.49)	11.82 (1.24)
	9.63 (1.23)	9.84 (0.94)	10.85 (1.83)	12.71 (2.33)
C	1.26 (0.18)	1.16 (0.15)	1.45 (0.29)	1.92 (0.22)
	1.06 (0.27)	0.88 (0.13)	1.28 (0.31)	2.02 (0.19)
D	1.80 (0.17)	1.63 (0.22)	1.60 (0.35)	2.08 (0.35)
	0.94 (0.22)	0.86 (0.07)	0.97 (0.20)	1.37 (0.21)
E	3.82 (0.34)	3.83 (0.37)	4.13 (0.57)	6.11 (0.88)
F	1.31 (0.32)	1.16 (0.20)	1.39 (0.46)	3.4 (3.6)
G	0.99 (0.12)	0.97 (0.22)	1.18 (0.49)	1.41 (0.14)
	0.95 (0.15)	0.86 (0.14)	1.17 (0.24)	1.79 (0.24)
H	1.11 (0.33)	0.90 (0,09)	1.04 (0.18)	1.35 (0.18)
	1.09 (0.14)	1.07 (0.07)	1.09 (0.19)	1.48 (0.18)
I	2.35 (0.44)	2.31 (0.17)	2.45 (0.34)	2.92 (0.33)
	2.07 (0.32)	2.05 (0.19)	2.17 (0.34)	3.00 (0.32)
J	0.65 (0.09)	0.65 (0.08)	0.64 (0.11)	0.89 (0.09)
	0.50 (0.05)	0.048 (0.06)	0.50 (0.10)	0.82 (0.08)
K	0.67 (0.19)	0.72 (0.17)	0.63 (0.15)	0.85 (0.28)
L	0.41 (0.13)	0.43 (0.11)	0.42 (0.10)	0.54 (0.12)
M	107.55 (14.76)	96.5 (24.28)	112.1 (13.49)	99.19 (28.76)
	109.9 (20.22)	98.5 (18.12)	125.2 (14.26)	120.00 (13.53)

Discussion

Phallocryptus spinosa lives in Algeria in biotopes much wider than those known from literature comprised between 5 and 135 ha (Garcia et al., 1997; Mura, 1999; Abatzopoulos et al., 1999, Moscatello et al., 2002; Mura & Takami, 2002, Thiery & Puente, 2002), except Tunisian Sebkhas of

area's reaching 32,000ha (Ben Naceur et al., 2009). Adults of *P. spinosa* were dominant in the three Garâas while young stages were totally absent in May. It was certainly the last generation before the complete dry of the sites in summer. In Sebkha Ez-Zemoul, it was observed in January and co-occurred with *Artemia salina* but likely in the North-eastern side, from where dead individuals were brought by the waves. This co-existence, and also with other Anostracan, had been documented by several authors, in Greece (Mura & Hadjistephanou, 1987), Italy (Mura, 1993, Moscatello et al., 2002), France (Defaye et al., 1998; Thiery & Puente, 2002), Iran (Mura & Takami, 2000), Botswana (Hulsmans et al., 2006) and Tunisia (Ben Naceur et al., 2009). It is considered as a complex phenomenon (Maeda-Martinez et al., 1997) complicating the adaptive strategies of these Anostraca life histories and leading to differences in the temporal or spatial distribution (Mura, 1993, Wang et al., 2012). The developing season for *P. spinosa* varies from a site to another. It was found in winter/spring in Tunisia (Ben Naceur et al., 2009), in May in the saline of the Southwest of Sardinia (Mura, 1993), between December and March In the temporary saline lake of Fuente de Piedra in Spain (Garcia et al., 1997) and between January and April in the French Camargue (Thiery & Puente, 2002). Sex-ratio was in favour of males for all the studied populations as reported by Alonso (1996) for Spanish ones, while in Camargue, it was in favour of females (1:3) (Thiery & Puente, 2002).

Total lengths between sexes are not different for Garâas populations, as it is the case for the populations of the Spanish Iberian Peninsula (Alonso, 1996). However, females from Sebkha Ez-Zemoul which is more saline than the 3 others were larger than males, probably because the former should be stronger to carry males during mating when water density is high. Few biological data are available about biometry and reproduction. Alonso (1996) reported a maximal adults size of 40 mm for Spanish populations against 30 mm in the French Camargue (Defaye et al., 1998). Thiéry & Puente (2002) found in the same population lengths which vary between 6 and 30 mm. Mura (1993) found lower values of 6 to 11 mm in a Sardinian population. In point of view of biometrics, the three populations of Ank Djemel, Guellif and El-Tarf are close together but different from that of Ez-Zemoul. Ketmaier et al. (2008) recently studied the phylogeography of the Paleartic species and found no differences between African populations of Algeria (Boughzoul) and that of Botswana. However, Alonso (1996) reported that *P. spinosa* could have two phenotypes according to its diet. Carnivorous individuals reach big sizes, but this predatory behavior is rarely observed in this species (Rogers, 2006). Entire Copepod's bodies were observed inside the abdomen of some *P. spinosa* individuals from Sebkha Ez-Zemoul, which could explain the gigantism in this population.

Females start to reproduce in general at a length of 11.5 mm, which is close to that of the South-western Sardinian population (Mura, 1993). Oviparity was the unique mode of reproduction used as it is common in the species, and the females of Sebkha Ez-Zemoul were the most fertile among the studied populations (771 cysts/brood *vs* < 30 cysts/brood). Alonso (1996) brought back a maximal fecundity of a thousand offspring in Spanish populations. Cyst diameter of the Northeast

Algerian populations (276.29 – 292.18 µm according to the origin) is close to that of Italian populations (Mura, 1992), and intermediate between that of the Camargue (220-265 µm) (Defaye et al., 1998) and the Spanish populations (300 µm) (Alonso, 1996).

Except the co-existence with *Artemia*, the carcinological fauna associated with *P. spinosa* has been rarely reported. This species lives in Spanish marshes, according to salinity and periods, with *Moina salina*, *Cletocamptus retrogressus*, *Arctodiaptomus salinus*, as it was found in this study, and also with *Branchinectella media*, *Cyclops* sp, *Sigara* sp, *Macrothrix* sp and *Daphnia mediterranea* Alonso 1985 (Garcia et al., 1997; Alonso & Ventura, 2013). In Southern artificial saltmarshes of France, it lives with *M. salina*, *C. retrogressus* and *Eurythemora velox* (Thiery & Puente, 2002).

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