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# THE CO-OCCURENCE OF TWO ANOSTRACAN SPECIES *BRANCHINECTELLA MEDIA* (SCHMANKEWITSCH, 1873) AND *PHALLOCRYPTUS SPINOSUS* (MILNE-EDWARDS, 1840) (CRUSTACEA) IN SALINE LAKES FROM THE AURES REGION (NORTH-EAST ALGERIA)

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ANOSTRACANS
COMPETITION
PHENOLOGY
SALINE LAKES
ALGERIA

ABSTRACT. – The co-occurrence of two anostracan species *Branchinectella media* and *Phallocryptus spinosus* have been surveyed monthly in four saline lakes from the Aures region (Chott Tinsilt, Garaa El-Tarf, Sebkha Djendli and Garaa O'Lembarek), in North-East Algeria, between 2013 and 2014. *Branchinectella media* occurred during the winter at low water temperatures ranging between 5 and 16 °C and a salinity between 2.24 and 44 ppt (g/L), while *P. spinosus* preferred spring with a temperature range between 18 and 22 °C and a salinity from 12.08 to 48.6 ppt. The presence of both species overlapped only in March in the four sites. *Phallocryptus spinosus* always occurred with higher monthly densities between $5.2 \pm 0.99$ and $27.7 \pm 3.81$ ind.$L^{-1}$ *versus* $1.65 \pm 0.64$ to $10.55 \pm 0.78$ ind.$L^{-1}$ for *B. media*. *Phallocryptus spinosus* was two times larger than *B. media* ($F = 26.80$, $P < 0.001$) and its females were two times more fertile ($t = 12.89$, $P < 0.0001$) in the four sites. From the present study, we concluded that *B. media*, which is considered as a rare species according to the literature, was sensitive to environmental factors, mainly the temperature, and was dominated by *P. spinosus*, making it vulnerable, especially to the effects of the predicted climate warming.

## INTRODUCTION

Anostracan branchiopods live in general in ephemeral water bodies. The majority of the species live in fresh water and only a few taxa are known either from saline or hypersaline waters, *e.g.* the genera *Branchinecta*, *Phallocryptus*, *Branchinectella*, *Artemia* and *Parartemia* (Rogers 2013). North Africa is rich in salt lakes which are concentrated in semi-arid and arid regions. In the local jargon, they are called Chotts, Sebkhas, Garâas or Dayas. As shallow and very vast environments, they could cover a surface of about hundreds or even thousands of square kilometers for few centimeters deep. Their filling depends on local climatic conditions and more precisely on the balance between precipitation and evaporation, which makes them unstable environments. Indeed, they dry completely for several months or even years, and their salinity varies considerably, ranging from a few grams per liter to more than 360 g/L (Amarouayache *et al*. 2009). Anostracans have adapted to these extreme conditions, thanks to their short life cycle being closely linked to the hydrological budget. They have an efficient osmoregulation system (Bilton *et al*. 2001, Incagnone *et al*. 2015) and their females produce resting eggs (Alonso 1985, Timms 2009). These are dispersed by wind and animals (endozoochory) in new environments (Beladjal *et al*. 2007, Frisch *et al*. 2007, Beladjal & Mertens 2009), where they can develop if conditions are adequate.

Large branchiopod fauna of Algeria has been well studied in the early last century by Gauthier (1928). Samraoui *et al*. (2006) provided a list of nineteen species, mostly from saline lakes where anostracans are dominant. Among them, *Phallocryptus spinosus* (Thamnocephalidae), which was long considered as *Branchinella spinosa* (Milne-Edwards, 1840) until the taxonomic revision of Rogers (2003, 2006), who used the Algerian population of Boughezoul (Medea province) and that of Bechuanaland (Botswana) for its classification in the genus *Phallocryptus* Biraben, 1951. This species has a primarily Palearctic distribution, which extends from the Western Mediterranean in Spain (Alonso 1985, Thiéry 1996) to Ukraine (Keitmaier *et al*. 2008), Central Asia, Pakistan (Brtek & Thiéry 1995), Kazakhstan (Belk & Brtek 1995), Iran (Vakili & Sari 2012) and India (Padhye *et al*. 2017) in the Eastern Palearctic. It has been also recorded beyond the Palearctic, in Oman (Thiéry 1996) and in Botswana (Hulsmans *et al*. 2006). This species is reported in several chotts and sebkhas in Algeria (De Los Rios & Amarouayache 2016) and was the subject of recent studies on the biology and ecology of some populations (Amarouayache 2014). Iranian populations were studied in order to check the possibilities of their use in aquaculture farming (Atashbar 2012, Gharibi *et al*. 2016).

The second species known from saline lakes, *Branchinectella media* (Schmankewitsch, 1873), as unique representative of the genus, is seen as less common (Alonso 1985). In the Mediterranean basin, it is reported only in Iberian Peninsula (Alonso 1990, Garcia *et al*. 1997, Pérez-Bote 2004) and Tunisia (Marrone *et al*. 2016), where it is rare, and in Algeria where it is relatively widespread in chotts and sebkhas of the Steppic zone, located between the two Atlas, the Tellian and the Saharan (De Los Rios & Amarouayache 2016). It was recorded in Low Lands of Aral, Caspian and Black seas, also between Turkey and Russia (Belk & Brtek 1995), as well as in Ukraine, where it is considered as rare and endangered species (Alexandrov 1994). *Branchinella media* has been recorded in several lakes of Siberia (Van Stappen *et al*. 2009), in Mongolia (Alonso 2010, Marrone *et al*. 2015) and even in the Arctic, at Novaya Zemlaya in the Barents Sea (Vekhoff 1997). However, data about biology and life history of this species are scarce, except descriptive data (Alonso 1985, Dumont & Negrea 2002).

Co-occurrence between two or more large branchiopod species is quite a common phenomenon (Thiéry 1991, Padhye & Dahanukar 2015, Stoch *et al*. 2016, Alfonso 2017). However, when species co-occur, they share the environment in opposite regions and/or develop during different periods of the year (Amat 1983, Mura 1993), characterized by abiotic and biotic conditions to which some species are more adapted than others (Chunco *et al*. 2012). Thus, the problems primarily related to trophic competition are solved (Beladjal *et al*. 1997). The local climatic conditions are a constraint as well, because of the reduction of the hydroperiod and the increase in the temperature and evaporation in the arid regions with saline waterbodies (Ripley *et al*. 2004). The fact that Algeria undergoes high hydric stress resulting from the low rainfall rate and increase in the temperature in last decades (Meddi 2003) is to have dramatic consequences on wetland biodiversity in general in the near future.

The co-occurrence between *P. spinosus* and *B. media* has been recorded in Spain (Garcia *et al*. 1997, Baltános & Alcorlo 2004), in Tunisia (Marrone *et al*. 2016, Stoch *et al*. 2016) and in Algeria since the extensive works of Gauthier, at the beginning of the last century (Gauthier 1928). The aim of this study is to survey, during a humid cycle, the dynamics of these two species co-occurring together in 4 saline lakes of the Aures region, renowned for its

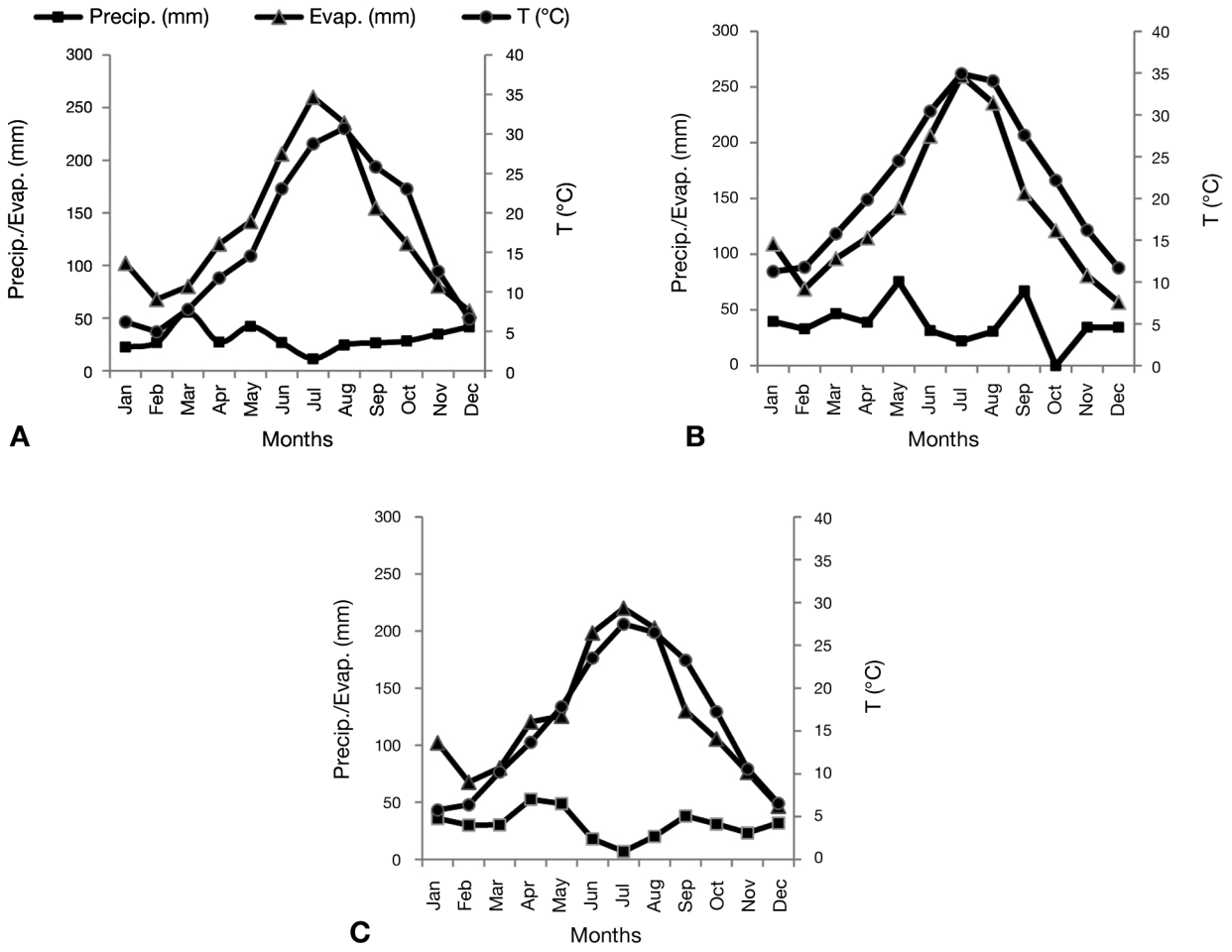


Fig. 1. – Precipitation, evaporation (in mm) and temperature (°C) in 3 provinces of Aures region registered between 2003-2013 (National Meteorological Office). **A**: Oum El-Bouaghi; **B**: Khenchela; **C**: Batna.

richness in chotts and sebkhas of international importance (Anonymous 2004). The objective is to better understand the mechanisms of the co-occurrence between these two species as well as their phenology.

## MATERIALS AND METHODS

*Study sites*: The area of Aures is situated in the North-East of Algeria in the High Plateaus (1000 a.s.l). It includes 3 provinces, Batna, Khenchela and Oum El Bouaghi, covering an area of about 28,771 km$^2$. It is rich in saline waterbodies, especially chotts and sebkhas. The climate of the region is semi-arid with a cold winter and several days of snowfall, as well as a hot and dry summer (Fig. 1A, B, C). High fluctuations of temperature characterize the climate of that region. January is the coldest month with the lowest mean temperature of 0.9 °C recorded over ten years in Batna. The hottest month is July with a maximum mean temperature of 34.9 °C recorded over ten years in Khenchela. The average annual precipitation recorded over 10 years is 366.68, 486.46 and 517.3 mm for Batna, Oum El Bouaghi and Khenchela provinces, respectively. Evaporation rates are very high and reach more than 1600 mm per year. Strong winds blow on the region, sometimes exceeding 70 km/h (ONM, National office of meteorology).

Four saline lakes were considered in this study (Fig. 2).

1) Garâa El-Tarf (35°41'11"N, 07°08'00"E, 834 m a.s.l.), situated in Oum El Bouaghi, is the largest one, covering an area of about 33,460 ha.

2) Chott Tinsilt (35°53'51"N, 6°30'00"E, 792 m a.s.l.), also situated in Oum El Bouaghi, covers an area of 2,154 ha. These two sites have been classified as protected sites in Ramsar convention since February 2004.

3) Sebkha Djendli (35°42'00"N, 6°31'55"E, 833 m a.s.l.), situated in Batna, is of 3,800 ha area.

4) Sebkha O'Lembarek (Tazouguert II) (35°23'57"N, 7°19'52"E, 1069 m a.s.l.), situated in Khenchela, is the smallest with 950 ha area. It is also the saltiest and water can be saturated in salt. The pH there is neutral, but is lightly alkaline in the three other sites (7.5-8).

The maximum depth in the four sites never exceeds 1 m.

*Sampling methods*: Water temperature and salinity have been measured during each sampling using a field multiparameter (Hanna, HI 829). The samples of anostracans *Phallocryptus spinosus* and *Branchinectella media* were collected monthly during the wet period from December 2013 to April 2014 in two different sides for each site. According to water column thickness as well as density of the individuals, a water volume, ranging between 1 L and 10 L, was filtered through a sieve of 400 µm

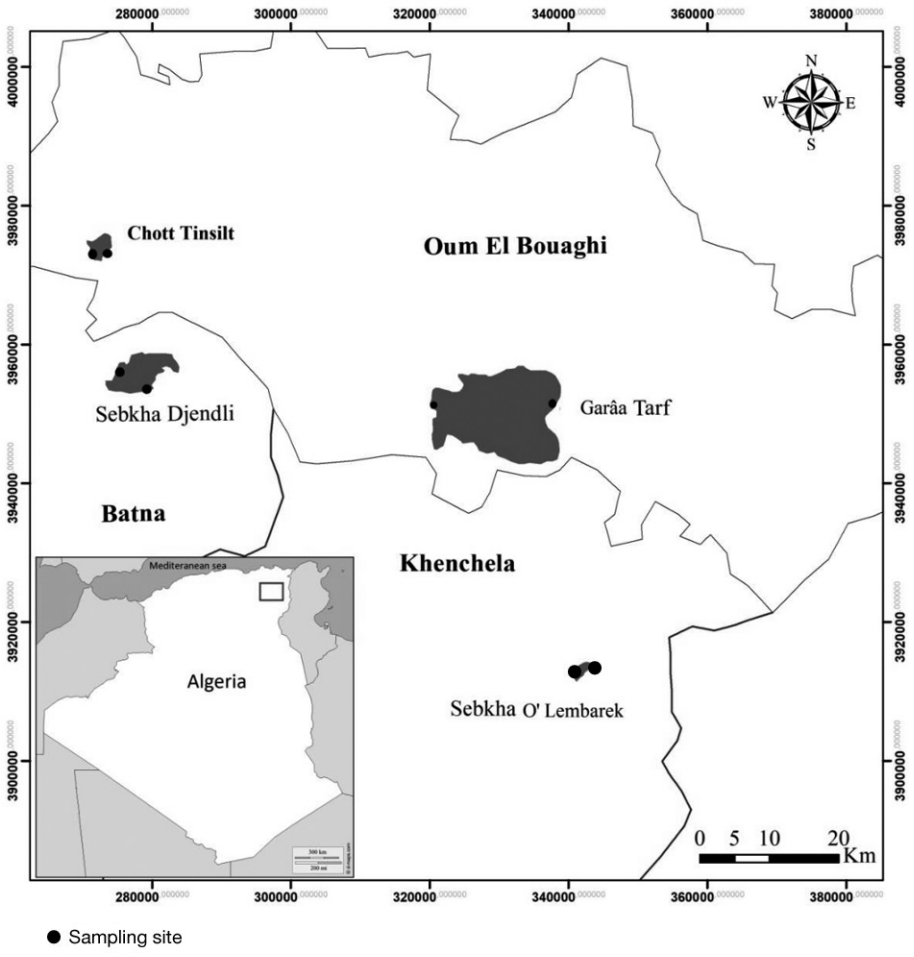


Fig. 2. – Map of the study region Aures (Oum El Bouaghi, Khenchela, Batna).

Table I. – Salinity (S) and water temperature (T) during the sampling hydroperiod 2013-2014. GET: Garâa El-Tarf, SD: Sebkha Djendli, CT: Chott Tinsilt, SOL: Sebkha O'Lembarek.

| Months | December | | January | | February | | March | | April | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sites | T°C | S (ppt) | T°C | S (ppt) | T°C | S (ppt) | T°C | S (ppt) | T°C | S (ppt) |
| GET | 5.0 | 2.30 | 8.2 | 2.24 | 14.3 | 8.30 | 18 | 12.08 | 22 | 27.2 |
| SD | 12.7 | 2.16 | 8.0 | 4.01 | 13.9 | 11.04 | 19 | 23.10 | 20 | 24.0 |
| CT | 14.0 | 2.33 | 12.7 | 2.54 | 11.7 | 8.30 | 20 | 25.50 | 22 | 16.4 |
| SOL | 5.0 | 8.54 | 8.5 | 23.5 | 16.5 | 42.10 | 20 | 44.00 | 21 | 48.6 |

Table II. – Total length (Lt ± standard deviation, n = 30) of males (♂) and females (♀), sex-ratio (SR) and mean fecundity (F, n = 20)) of *Phallocryptus spinosus* and *Branchinectella media* in the four studied sites. GET: Garâa El-Tarf, SD: Sebkha Djendli, CT: Chott Tinsilt, SOL : Sebkha O'Lembarek.

| Species | Parameter | GET | SD | CT | SOL |
|---|---|---|---|---|---|
| *Phallocryptus spinosus* | L♂ (mm) | 14.55 ± 0.71 | 13.87 ± 0.09 | 16.34 ± 0.64 | 14.26 ± 0.68 |
| | L♀ (mm) | 15.00 ± 1.16 | 14.50 ± 0.74 | 14.75 ± 0.42 | 15.21 ± 0.44 |
| | Lt (♂ + ♀) | 14.74 ± 0.90 | 13.99 ± 0.90 | 15.78 ± 0.95 | 14.66 ± 0.57 |
| | SR | 4.00 | 2.80 | 0.66 | 3.00 |
| | F (offspring/brood) | 27.50 ± 7.50 | 35.5 ± 6.45 | 50.5 ± 8.56 | 30 ± 5.32 |
| *Branchinectella media* | Lt ♂ | 7.02 ± 0.29 | 7.58 ± 0.74 | 7.19 ± 0.37 | 7.17 ± 0.34 |
| | Lt ♀ | 8.17 ± 0.29 | 8.38 ± 0.43 | 8.46 ±0.35 | 8.48 ± 0.19 |
| | Lt (♂ + ♀) | 7.48 ± 0.41 | 7.85 ± 0.46 | 7.73 ± 0.54 | 7.78 ± 0.52 |
| | SR | 0.75 | 1.50 | 1.80 | 0.70 |
| | F (offspring/brood) | 17.00 ± 4.87 | 16.5 ± 6.46 | 19.00 ± 5.87 | 16.50 ± 3.89 |

of mesh size. Collected animals were fixed in 4 % formalin. The identification of both species has been carried out according to Alonso (1996) identification keys. To assess monthly density, expressed by the number of individuals per liter, only identifiable adults and subadults have been taken into account for each species. The counting of the individuals was realized using Dollfus cell placed under stereomicroscope. Sex-ratio (SR) was determined by the number of males per number of females. The body length of males and females (tip of the head to the anus) (Amat 1980) of both species was measured under optical microscope (Optika) equipped with an ocular micrometer. Females fecundity of both species was monthly determined after ovisac dissection and offspring counting.

*Statistical analysis*: Pearson correlation coefficient was determined between the physicochemical factors (salinity and temperature) and individual density of each species and between the densities of the two species. Total lengths of adults were compared between the different sites by analysis of variance (ANOVA). Fecundity of the two species was compared using Student-t test (Minitab 16 software).

## RESULTS

Data about water salinity and temperature throughout the sampling period are listed in table I. The most saline site during the study period was O'Lembarek with salinity between 8.54 and 48.6 ppt (g/L). The 3 other sites have almost identical salinity range (2-27 ppt). Water temperature in the 4 sites varied during sampling between 5 °C and 22 °C.

*Branchinectella media* developed in the 4 sites between December and February-March. The monthly densities of adults and subadults of this species evolved between 1.65 ± 0.64 and 10.55 ± 0.78 ind.$L^{-1}$. *Phallocryptus spinosus* developed between March and April in the 4 sites with monthly densities which varied between 5.2 ± 0.99 and 27.7 ± 3.81 ind.$L^{-1}$ (Fig. 3A-D). Pearson correlation test revealed the existence of a positive correlation either between water temperature and density of individuals (Pearson r = 0.75, $p < 0.01$) or between salinity and density of individuals (Pearson r = 0.45, $p < 0.04$) for *P. spinosus*. In the case of *B. media*, a negative correlation was observed between water temperature and density of individuals (Pearson r = –0.41, $p < 0.05$). No significant association was observed between the densities of the two species (Pearson r = –0.42, p = 0.06).

The total length values of males and females and both sexes, the sex-ratio and fecundity of the 4 populations of both species are listed in Table II. For *P. spinosus*, total lengths of adults were between 13.87 ± 0.09 and 16.34 ± 0.66 mm in Sebkha Djendli and Chott Tinsilt, respectively. For *B. media*, the extreme values of total lengths of adults were between 7.02 ± 0.29 and 8.48 ± 0.19 mm in Garâa El-Tarf and Sebkha O'Lembarek, respectively. According to ANOVA, *P. spinosus* is significantly larger than *B. media* ($F = 26.80$, $p < 0.001$). The sex-ratio is generally in favor of males in both species. For *P. spinosus,* it ranges between 0.66 and 4 in Chott Tinsilt and Garâa El-Tarf, respectively. For *B. media*, sex-ratio values are between 0.7 and 1.8 in Sebkha Djendli and Chott Tinsilt, respectively.

For fecundity (Table II), extreme values were between 27.5 ± 7.50 and 50.5 ± 8.58 offspring/brood in Garâa El-Tarf and Chott Tinsilt, respectively, for *P. spinosus* and between 16.5 ± 3.89 and 19 ± 5.87 offspring/brood in the Sebkha Djendli and Chott Tinsilt, respectively, for *B. media*. Student-t test showed very highly significant difference between fecundity of both species ($t = 12.89$, $p < 0.00001$).

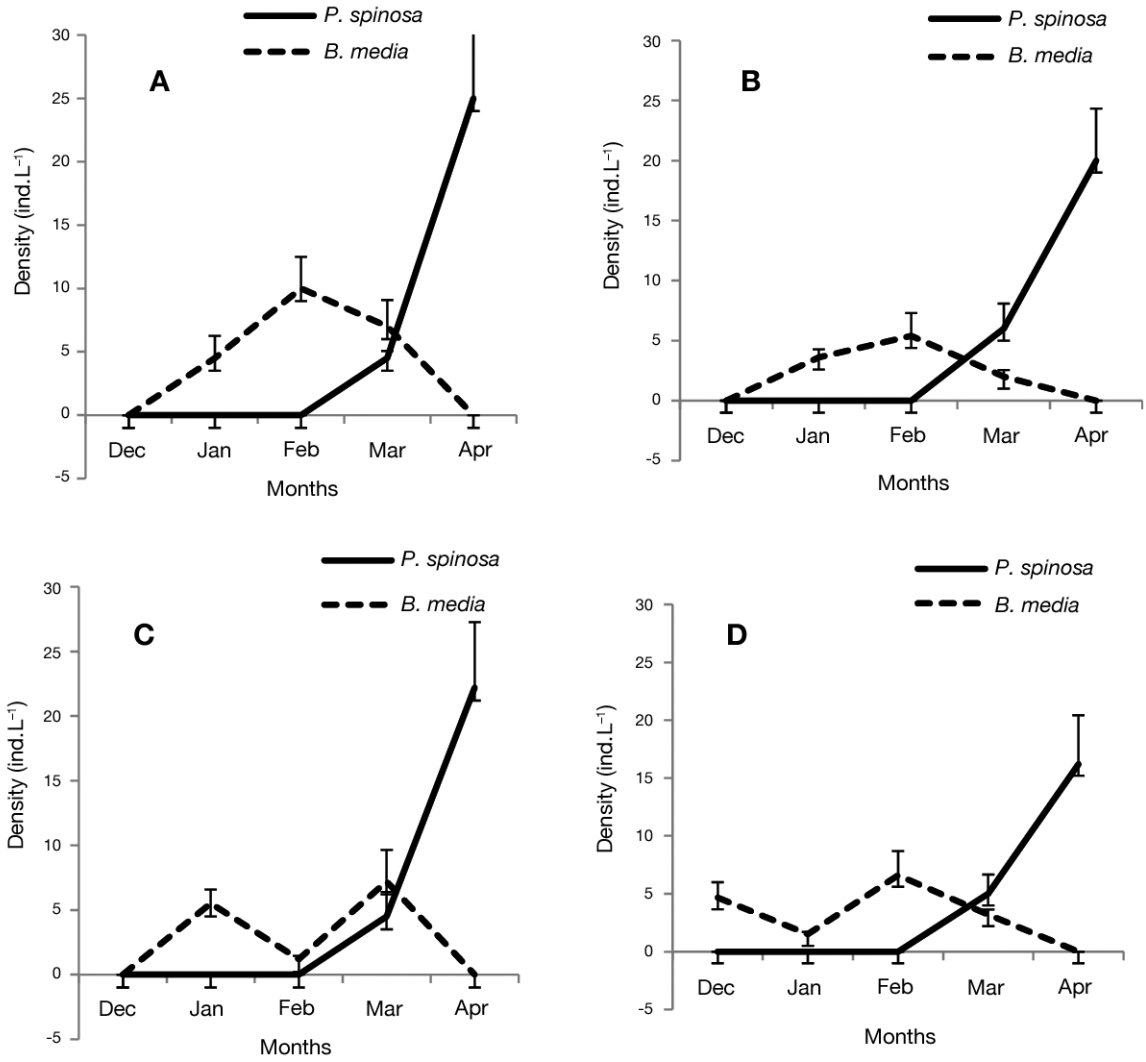


Fig 3. – Density (ind.L$^{-1}$) of *Phallocryptus spinosus* and *Branchinectella media* in the four sampled sites, (**A**) Garâa El Tarf, (**B**) Sebkha Djendli, (**C**) Chott Tinsilt, (**D**) Sebkha O'Lembarek during the wet period 2013-2014.

## DISCUSSION

Chotts and sebkhas of North Africa are known for their richness in large branchiopods (Samraoui *et al*. 2006). They are considered as the flagship of invertebrates (Belk 1998), meanwhile, they constitute a food web for migratory water birds. They are often used to evaluate the quality of temporary freshwater ecosystems (Brendonck *et al*. 2008). In the studied sites, *Phallocryptus spinosus* and *Branchinectella media* co-occur together and develop simultaneously in winter/spring. These 2 species are typical of environments with moderate salinity, which dry up in summer and autumn in the Mediterranean region (Alonso 1990, Garcia *et al*. 1997, Baltanãs & Alcorlo 2004, Pérez-Bote 2004). Great variations of the abiotic factors, especially temperature and salinity, are observed there and depend on the hydrological budget and local climatic conditions. In Algeria, these two species were found to often co-occur in at least 7 sites (De Los Rios & Amarouayache 2016), but *P. spinosus* is more frequent and recorded alone in several sites (R.L. pers obs). Among studied sites, Sebkha O'Lembarek is the saltiest; salinity can reach saturation (more than 340 ppt), but no cysts or active stages of the brine shrimp *Artemia* Leach, 1819 was found there with the two considered species (R.L. pers obs). The monthly survey of the co-occurrence of both species revealed that *B. media* prefers to live at low temperatures (5 to 16 °C), whereas *P. spinosus* develops at relatively high temperatures (18 to 22 °C). The co-occurrence of these two species overlapped during March in the 4 sites, with close densities. At this period, the sites were at their maximum of filling. Similar studies pointed out that the co-occurrence of different species of branchiopods in the same ecological niche in temporary ponds seems to be accomplished thanks to various strategies which make it possible for the species to split resources simultaneously in space and time (Amat 1983, Thiéry 1987, Moscatello *et al*. 2002, Wang *et al*. 2012). Beside

environmental variables, the competition mechanism between species for the same ecological niche requires differences in body size and shape (Timms & Sanders 2002). Thus, according to their size, their feeding mode differs from each other. The majority of anostracan species are non-selective filter feeders; the size of the filtered particles differs according to the species (Beladjal *et al*. 1997). Only large specimens of *P. spinosus* feed on cladocerans, copepods or even other anostracan larvae (Alonso 1985, Defaye *et al*. 1998, Sarma & Nandini 2002).

*Phallocryptus spinosus* is two to three times more fertile than *B. media* in the 4 sites, with values ranging between 27 to 50 offspring/brood and 16 to 19 offspring/brood, respectively. Moreover, *B. media* is endowed of a globulous ovisac, while *P. spinosus* has an elongated one, which confers to its females a better introduction into the ground, and thus, the spawning of resting-eggs is carried out with a few millimeters below the sediment surface (A.M. pers obs). Differences in term of fecundity and ovisac shape have direct impact on the final densities in the ground (banks of resting-eggs). Indeed, several authors reported that the persistence of the population of a given species depends on its resting-egg bank (Timms & Sanders 2002, Ripley *et al*. 2004, Rogers 2014). On the other hand, the strong winds blowing on the region favor the dispersion of the new laid restring-eggs of *B. media* in new environments, but also limit their stock in the site of origin.

Variability of sex-ratio is marked especially in *P. spinosus* populations, where one can count 4 males for one female, in comparison with *B. media* where the sex ratio varies between 0.70 and 1.80. Several studies showed genetic and environmental effect on sex-ratio variation in anostracans (O'Neill *et al*. 1997, Browne & Wanigasekera 2000, Beladjal *et al*. 2002). A high survival rate of males in comparison with females was observed in high water temperature and salinity in some anostracan species (Beladjal *et al*. 2003, Dumont & Negrea 2002). The male dominance favors the mixing of genes, thus limiting consanguinity in new generations; consequently, this fact would contribute to species persistence (Lievens *et al*. 2016).

Although developing time of *B. media* is longer (3 to 4 months) than that of *P. spinosus* (2 months), the comparison between the two species in terms of density, size and fecundity revealed a clear dominance of *P. spinosus* in the 4 sites. This species is larger, more fertile and more abundant than *B. media*. Positive correlation between water temperature and density of *P. spinosus* suggests that this environmental factor favors the multiplication of the individuals, probably by the increase of resting egg hatching rate. Indeed, Atashbar *et al*. (2012) obtained high hatching rates at temperatures ranging between 19 and 25 °C for the same species and concluded its preferences for high water temperature (Atashbar *et al*. 2014, Gharibi *et al*. 2016). The development of *B. media* during winter months shows its preference for low temperatures. According to Vekhoff (1997), this species is regarded as the most tolerant to temperature variations and can stand cold waters of the Arctic, since it was recorded in Novaya Zemlya (Coulson *et al*. 2014). Since the rhythm and the extent of the climate change quicken, the effect on the species distribution becomes alarming. As examples, Alexandrov (1994) reported that *B. media* was rare and in danger of extinction in Ukraine, and no more recorded in Turkey (Mura *et al*. 2005). Additionally to its presence in some Siberian and Arctic waterbodies, where the genetic identity of the species should be checked, the distribution of *B. media* is limited to few sites in the Mediterranean basin either in Spain, Tunisia, or Algeria.

According to our results *B. media* is a species which prefers low temperatures of chotts and sebkhas from Eastern High Plateaus. Because of climate warming, which is particularly marked in these semi-arid areas (Nichane & Khelil 2015), and the dominance by *P. spinosus*, the existence of *B. media* in Algeria, as well as in other localities, is threatened. Conservation measures should be taken to protect this species. In addition, due to the possible existence of two or more closely related taxa among Palearctic populations lumped under the same binomen of *B. media*, both morphological and molecular taxonomic studies are needed in order to detect cryptic species within the genus *Branchinectella*.

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