Resting eggs bank and their hatching pattern in two co-occuring anostracans Phallocryptus spinosus (Milne-Edwards, 1840) and Branchinectella media (Schmankewitsch, 1873) (Crustacea) from saline lakes of the Aurès region (Northeastern Algeria)

Lynda Rais
Universite Abbes Laghrour Khenchela
Mounia Amarouayache (m.derbal@yahoo.fr)
University of Badji Mokhtar Annaba Faculty of Sciences: Universite Badji Mokhtar Annaba Faculte des Sciences https://orcid.org/0000-0002-1244-0156
André Rochon
UQAR-ISMER: Universite du Quebec a Rimouski Institut des sciences des sciences de la mer de Rimouski

---

Research Article

Keywords: Fairy shrimps, cysts, SEM, Chotts, Algeria

Posted Date: December 4th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3676641/v1

Additional Declarations:
Tables 1 to 4 are available in the Supplementary Files section.

Resting eggs bank and their hatching pattern in two co-occuring anostracans *Phallocryptus spinosus* (Milne-Edwards, 1840) and *Branchinectella media* (Schmankewitsch, 1873) (Crustacea) from saline lakes of the Aurès region (Northeastern Algeria)

Lynda Rais[1] · Mounia Amarouayache*[2] · André Rochon[3]

Department of Molecular and Cellular Biology, Abbes Laghrour University, Khenchela, Algeria[1]
Marine Bioresources Laboratory, Badji Mokhtar University, Annaba, Algeria[2]
ISMER-UQAR, Québec, Canada[3]

✉ Mounia Amarouayache
m.derbal@yahoo.fr

Abstract

Phallocryptus spinosus (Milne-Edwards, 1840) and *Branchinectella media* (Schmankewitsch, 1873) are two anostracan species co-occurring in several saline lakes of the eastern steppic zone of Algeria. Both species produce resting eggs to persist in their temporary and unstable habitats, constituting a resting eggs bank. *B. media* has a restricted distribution worldwide and it is known to prefer cold temperatures (16 °C max). Resting egg banks and hatching patterns of both species have been assessed in seven saline lakes. Results showed that densities of *P. spinosus* resting eggs were always higher than those of *B. media*, whatever the lake ($F = 4.66$, $P = 0.0005$). They range between 93000 ± 75 and 1495000 ± 366.5 resting eggs.m^{-2} for *P. spinosus*, and between 670 ± 30 and 365000 ± 268 resting eggs.m^{-2} for *B. media*. Hatching percentages of eggs incubated at temperatures of 16 °C and 22 °C, and salinities of 0 and 5 psu, showed that 16 °C and 5 psu was the most suitable combination for *B. media,* where 38.88 ± 1.93% of eggs hatched under these conditions. For *P. spinosus*, 81.11 ± 1.92 % of eggs hatched at 22 °C and 5 psu. Resting eggs diameters measurements of both species as well as scanning electron micrographs of *B. media* are provided.

Keywords Fairy shrimps • cysts • SEM • Chotts • Algeria

Acknowledgements

Authors are grateful to Dr Beladjal Lynda (Gent University, Belgium) for her valuable comments and suggestions on the paper.

Statements and declarations

Conflict of interest The authors declare no conflict of interest

Consent for publication All authors have read and approved the final manuscript

Introduction

The Aurès region, situated in the eastern High Plateaus of Algeria, is recognized by its richness in saline lakes, called Chotts, Sebkhas or Garâas, of which most are protected according to RAMSAR convention (Boumezber 2014). In North Africa, these names designate huge shallow areas deprived of vegetation and temporary filled with brackish or saline waters. Such environments are ideal for the presence of large branchiopods, which are currently considered as the flagship of invertebrates in temporary aquatic ecosystems (Brendonck 1996; Rogers 2009). Among this group, anostracans are the most conspicuous to saline lakes (Brendonck and Williams 2000; Rogers and Timms 2014; Mayer-Milne et al. 2021). These crustaceans can resist long periods of drought mainly by the production of resting eggs ensuring the survival and perenniality of the species. These are diapause embryos surrounded by a chitinous shell, which protects them against mechanic shocks and ultraviolet radiations (Beladjal et al. 2008; Mertens et al. 2008; Pinceel et al. 2018). Their diameter and external morphology are characteristic of the species and can be used for identification (Mura 1992; Timms 2004). Resting eggs are in general more or less spherical, measuring between 100 and 500 µm (Timms and Lindsay 2011). The ornaments of the chorion are adapted to the environment and allow protection against predators (Dumont et al. 2002) and snatch from the ground (Jocqué et al. 2007; Timms and Lindsay 2011). Resting eggs increase chance for the perenniality of the species by their dispersion in new habitats by abiotic means such as wind, runoff or via living organisms such as mammals, migratory birds, amphibians, insects and fish (Beladjal et al. 2007; Frisch et al. 2007; Beladjal and Mertens 2009). Following a flood, resting eggs hydrate and hatch, releasing a free larva which develops if environmental conditions are favorable. Indeed, each species has its requirements in relation to temperature and salinity essentially (Pinceel et al. 2013; Atashbar et al. 2014). At each hydroperiod, only a small portion of the eggs hatch during the flood, while the remaining fraction in the sediment forms a persistent egg bank whose lifespan can be over two decades (Rogers 2014). This risk adaptation strategy has been described and assigned as bet-hedging (Beladjal and Mertens 2017; Wang and Rogers 2018).

The resting egg bank has been rarely evaluated in anostracans, such as *Tanymastigites perrieri* (Daday, 1910) in Morocco (Thiéry 1997), *Chirocephalus ruffoi* Cottarelli and Mura 1984 in southern Italy (Mura 2004), *Phallocryptus spinosus* (Milne-Edwards, 1840) and *Branchinecta ornata* (Daday 1910) in Botswana (Hulsmans et al. 2006). Only resting egg banks of the brine shrimp *Artemia* (Leach 1819) have been assessed in Algeria (Amarouayache and Kara 2010; Belakri and Amarouayache 2015). The objective of this study is to estimate the density of the resting eggs of two anostraceans, *P. spinosus* and *Branchinectella media* (Schmankewitsch, 1873) co-occuring in seven saline lakes from northeastern High Plateaus of Algeria. The hatching performance of resting eggs are tested in two salinities and two different temperatures, in order to better understand the co-habitation phenology of the two species and their fate in these lakes. In our knowledge, no scanning electron micrographs of *B. media* are available in literature contrary to *P. spinosus;* we provide here SEM photos of its resting eggs for the first time.

Materials and methods

Study sites

Study sites are part of the Aurès wetland complex, situated in the eastern High Plateaus at an altitude of around 1000 m. It is a steppic zone with semi-arid climate, where summers are hot (up to 50°C) and dry and winters are cold with several days of snow and frost. Precipitations are comprised between 300 and 500 mm with very high evaporation rates. Seven saline lakes have been considered in this study (Fig. 1). Four of them are situated in Oum El Bouaghi district and are classified as RAMSAR sites since 2004. Garâa El-Tarf is the largest one, covering an area of about 33460 ha, followed by Garâa Ank Djemel with an area of 8550 ha, Garâa Guellif and Chott Tinsilt. Sebkha Djendli and Sebkha Ain Yagout are located at Batna district. Sebkha Ouled Lembarek, which is the saltiest, is situated in Khenchela district. The pH is neutral to alkaline (7.5-8.5) in the seven sites and maximum depth never exceeds 1 m. Water temperature is between 2 and over 22°C and salinity is between 2 and 50 psu in general, except for Sebkha Ouled Lembarek where water reaches saturation in salts. Geographical coordinates of the seven sites and other characteristics are summarized in Table 1. Among birds occurring at saline lakes of Aurès, the flamingo *Phoenicopterus roseus* Pallas, 1811 is the most common (Nouri et al. 2016).

Sampling and estimation of resting egg banks

To determine the abundance of resting eggs of *Phallocryptus spinosus* and *Branchinectella media*, the soil of each lake was sampled during summer (June/July) of 2015, when these environments were dry. A total of 10 soil samples were taken at random all along a 100 m transect, using a spatula on a quadrat of 900 cm² of surface over a depth of 10 cm. Sampling was carried out at two opposite sides (S1 and S2) in the seven saline lakes (Fig. 1). Samples were dried at 40 °C in an oven during 72 h and stocked.

In laboratory, resting eggs were isolated from sediment. A sample of 100 g of soil was washed with fresh water through four sieves of decreasing mesh diameter: 500 µm, 400 µm, 200 µm and 100 µm. The two large meshes (500 and 400 µm) allowed to eliminate the large particles from the soil to prevent clogging. Resting eggs of the two species were retained on the 200 µm mesh sieve for *P. spinosus* and 100 µm for *B. media*. These egg diameters of the two species are defined according to Alonso (1990) for *B. media* and Amarouayache et al. (2014) for *P. spinosus*. Very fine mud particles pass through the 100 µm sieve. The sieving operation should not exceed 15 minutes to avoid hydration and metabolism recovering (Levens and Sorgeloos, 1996). The Rochon et al. (1999) protocol was used to separate eggs from impurities of the same size and the debris that remain stuck to the chorion. The eggs are transferred in a beaker containing 10 ml of distilled water. Then the mixture (water + eggs) is exposed to the ultrasonic cleaner for 30 seconds and centrifuged at 3000 rpm. This step separates the small debris that remain stuck to the eggs. Finally, eggs of each species are recovered in a Dollfuss curve and counted under a binocular magnifier.

The number of resting eggs found in 100 g of soil sample is multiplied by 10 to obtain the number per kilogram of soil. One kilogram of soil represents an area of 900 cm^2. The result is converted into number of resting eggs in one square meter.

Resting eggs diameter and morphology

Hydrated resting eggs diameter of the two species was determined after dissection of the ovisacs of females collected in four saline lakes (Garâa El-Tarf, Sebkha Djendli, Sebkha Ouled Lembarek and Chott Tinsilt).

Collected eggs are hydrated in freshwater for one hour, and then measured under an optical microscope equipped with an eye micrometer at x 10 magnification.

The eggs for *Branchinectella media* were removed from the brood pouches of 2 - 3 mature ovigerous females which were preserved in 70% alcohol and then stored in 90% alcohol for several months prior to scanning electron microscopy (SEM).

The following steps have been applied according to a procedure developed by A. Rochon (unpublished):
- Wet sieving at 20 µm on a Nytex membrane to remove fine particles;
- Each specimen of cyst was isolated by hand using a glass pipette and then rinsed in a bath of distilled water;
- The specimens were transferred to a "Cambridge" type aluminum sample holder and were then covered with a layer of a palladium gold alloy using a sputter coater, model SC 7680 (Quorum Technologies).
- The specimens were photographed using a JEOL JSM-6460 L.V. SEM.

Hatching test

Resting eggs of both species *Phallocryptus spinosus* and *Branchinectella media* were isolated from the soil collected in two saline lakes: El-Tarf and Ain Yagout. The choice of these sites is justified by the abundance of eggs in the first site and low density in the second. The resting eggs of the two species were collected in situ from the ground after sieving as explained previously. For each experience, 30 resting eggs were immediately incubated in 10 ml graduated tubes under different temperature and salinity conditions. The temperature/salinity combinations chosen are as follows: (16°C/0 psu, 16°C/5 psu, 22°C/0 psu and 22°C/ 5 psu.). The choice of these salinity levels is inspired from the natural conditions, at the beginning of the hydroperiod after first water flows. We evaluated the hatching performances of resting eggs based on those advocated by Lavens and Sorgeloos (1996). Incubation time represents time at the appearance of the first nauplius (T_0). Time of synchronization of hatching (Ts) is deduced from the difference in time at the appearance of 10 % and 90 % of nauplii. Hatching percentage (H%) allows appreciation of hatching quality of full resting eggs.

Statistical analysis

The densities of resting eggs of *Phallocryptus spinosus* and *Branchinectella media* contained in the soil samples from the seven sites were compared by three-way ANOVA using SAS software 9.1.3. One-way ANOVA-test was used to compare results of hatching quality of two species (Minitab software). The *t*-test of comparison was used for eggs diameter and hatching percentage of two species. $P < 0.05$ was chosen as the significance level.

Results

The total number of resting eggs of the two species *Phallocryptus spinosus* and *Branchinectella media* estimated in the sediments of the seven studied sites are illustrated in figure 2. The minimum density of *P. spinosa* resting eggs was observed in Sebkha Ain Yagout with 93000 ± 75 eggs.m^{-2} against a maximum number observed in Garâa El-Tarf with 1495000 ± 366.5 eggs.m^{-2}. For *B. media*, the minimum number was also observed in Sebkha Ain Yagout with 670± 30 eggs.m^{-2} and the maximum number was observed in Sebkha Djendli with 365000 ± 268 eggs.m^{-2}.

According to the ANOVA statistical test, there is a very highly significant difference (F = 4.66, P = 0.0005) between the resting egg densities of the seven sites. In each site, a significant difference is recorded between the densities of the eggs of the two species; the density of the resting eggs of *P. spinosus* is higher in the seven sites than that of *B. media* (F = 25.73, P < 0.005). Regardless of the site or the sampling station, the number of *P. spinosus* eggs is always higher than that of *B. media.*

Among the 7 salt lakes, we noticed a very highly significant difference between the 2 sampling stations in Garâa El-Tarf (F = 14.22, P < 0.0005), Garâa Ank Djemel (F = 10.17, P < 0.0005), Garâa Guellif (F = 5.30, P < 0.0005), Sebkha Djendli (F = 5.17, P < 0.0005), Chott Tinsilt (F = 5.38, P < 0.0005), Sebkha Ouled Lembarek (F = 5.69, P < 0.0005). For Sebkha Ain Yagout, there is no significant difference between the eggs number in both sampling stations (F = 65.95, P = 0.66).

Resting eggs diameter and morphology

Resting egg diameters of the two species *Phallocryptus spinosus* and *Branchinectella media* are presented in table 2. *P. spinosus* resting eggs are larger than of *B. media* for all the studied sites. The diameter of *P. spinosus* hydrated resting eggs varies between 280.93 ± 20.30 µm and 315.86 ± 44.15 µm for the populations of Chott Tinsilt and Garâa El-Tarf respectively. For *B. media*, the diameter of hydrated resting eggs varies between 152.00 ± 12.97 µm and 174.40 ± 17.37 µm for the populations of Garâa El-Tarf and Sebkha Ouled Lembarek respectively. The *t*-test revealed a very highly significant difference between the size of resting eggs of the two species (F = 39.27, P < 0.001).

The shape of *B. media* resting eggs is irregular. It is more or less spherical with five to six invaginations (Fig. 3a), with a slightly pointed side (Fig. 3b) and a slightly flattened opposite side (Fig. 172 3c). The outer membrane is smooth with some ridges on the surface (Fig. 3d).

Hatching quality of resting eggs

Tables 3 and 4 summarize hatching characteristics of resting eggs for both species. The first larvae of *Phallocryptus spinosus* appeared after 12 hours in the two incubation combinations (22 °C/0 psu, 22 °C/5 psu). Synchronization time (T_{90}-T_{10}) was of 72 hours for eggs incubated at 22 °C/5 psu. For *B. media*, the first larvae appeared after 24 h of incubation of the resting eggs at 16°C/0 psu and synchronization time was of 94 h for the cysts incubated at 16 °C/0 psu.

The highest percentage of hatching (81.11 ± 1.92 %) was obtained for *P. spinosus* resting eggs from Garâa El-Tarf incubated at 22 °C/5 psu. The lowest rate (12.22 ± 1.92 %) was recorded at 16 °C/5 psu for resting eggs from Ain Yagout.

For *B. media,* hatching percentage of 38.88 ± 1.93 % was significantly higher for resting eggs collected from Garâa El-Tarf and incubated at 16 °C/5 psu. The lowest hatching percentage of 17.73 ± 1.93 was obtained from Sebkha Ain Yagout resting eggs incubated at 22 °C/5 psu.

The statistical test revealed that salinity had no effect on the hatching of resting eggs of the two species (P = 0.120 and P = 0.668 for *P. spinosus* and *B. media* respectively). Regarding the temperature, a very highly significant difference exists, 16 °C is favorable for *B. media* (F = 2.64, P < 0.001) while 22 °C is more favorable for the hatching of *P. spinosus* resting eggs (F = 4. 35, P < 0.001).

Discussion

Few studies focussed on the resting egg banks of large branchiopods. In this study, number of resting eggs were close to those reported in other studies, as examples between 833 and 31449 eggs.m^{-2} for *Phallocryptus spinosus* from saline lake of Makgadikgadi in Botswana (Hulsmans et al. 2006), more than 1700 eggs in 100 cm^2 for *Chirocephalus diaphanus* Prevost, 1803 in ditches at Rocheford du Gard in France (Thiéry 1997), and between 6200 and 250000 eggs.m^{-2} for *Branchipodopsis* spp. carried out in temporary rock pools at the summit of the Korannaberg Mountain (Free State Province, South Africa) (Vanschoenwinkel et al. 2010). Thiéry (1997) reported a close relationship between the distribution and abundance of the resting egg banks and the behavior of the female during oviposition and environmental factors, such as plant distribution and wind direction. Thus, the saline lakes of the Aurès region are characterized by strong winds and the absence of vegetation, which help the dispersion of the resting eggs during the dry phase. The density of *P. spinosus* resting eggs is much higher than that of *B. media* in all considered sites. This is probably due to a higher fecundity and may be high density of free-living individuals, and/or larger body size after maturation of *P. spinosus* (Rais and Amarouayache 2018) and to the fact that females introduce their tubular ovisac into the ground to lay their eggs (Kraus et al. 2004). At the opposite, the density of *B. media* resting eggs is low, probably because of a reduced fecundity and the globular shape of females' ovisacs, which release their eggs in the water column, thus facilitating their dispersion. Kraus et al. (2004) were the first authors to demonstrate how females of *Streptocephalus torvicornis* (Waga, 1842) introduce their tubular ovisac into the ground to release their eggs. Vanschoenwinkel et al. (2010) have shown the role of plant cover in protecting the resting egg bank, which acts as a real nursery. Thus, aquatic plants reduce the erosion of the resting egg bank by the wind and form a protective layer in dry periods. When ornaments exist at the surface of resting eggs may be a deterrent for egg predators such as planarians (Dumont et al. 2002), which is not the case for *B. media* resting eggs that have smooth surface. Recent investigations sustain the idea that eggs ornamentation combined with size determine their dispersion capacity, but the mode of laying and ovisac' shape have not been taken into account (Meyer-Milne et al. 2021).

Branchinectella media resting eggs are easily recognizable and distinguished from other anostracans; their morphology is simple, resembling that of the brine shrimp *Artemia* (Leach 1819) by the smooth surface, but presents differences in terms of invaginations. These invaginations on a smooth surface are reminiscent of those of the genus *Parartemia* (Sayce 1903) from Australia (Timms et al. 2004). The two close genera live in saline to hypersaline environments, generally without predators, which may explain the absence of anti-predatory ornaments on the surface of resting eggs, such as spines and/or transverse ridges. *B. media* eggs are small, with about 150 µm of diameter. Apart from the record of Alonso (1985) (200 µm) for a Spanish population, no data are available on resting eggs diameter for this species. No great differences were found between resting eggs diameters of *P. spinosus* compared with the previous study of Amarouayache (2014). According to Bengtson et al. (1991), the resting eggs diameter of a population should be stable; it is genetically determined, and large differences should not be found between samples taken at different times. On the other hand, Timms and Lindsay (2014) reported that a positive relationship between female length and the eggs size was found and variations in the egg's diameters are related to the levels of food and salinity in the environment.

Hatching characteristics depend on the incubation conditions and differ according to the requirements of each species towards physicochemical parameters, mainly temperature and salinity (Sorgeloos et al. 1983). The hatching performances of the resting eggs of the two species *P. spinosus* and *B. media* from Garâa El-Tarf are always the best compared to Sebkha Ain Yagout, which is different from other sites by its small area and its situation near to a national road. It should be noted that we never observed adults form of *B. media* in this site and individuals of *P. spinosus* rarely reach sexual maturity. This data shows that Sebkha Ain Yagout is not very favorable for the development of these crustaceans. According to a study by Wanschoenwinkel et al. (2010) on hatching and the resting egg banks of several populations of *Branchipodopsis* spp. from South Africa, individuals living in biotopes with a very short hydrological cycle do not reach maturity because of the early desiccation of the environment, which is in agreement with our observations. On the other hand, the low hatching rate (generally < 50%) is explained by the strategy of risk minimization or "bet-hedging" (Wang and Rogers 2018) where only part of the resting eggs hatch while the rest constitute the resting eggs bank, which ensures the continuity of the species in the case where the medium dries quickly before the larvae reach reproduction.

The hatching performance of large branchiopods in different combinations of temperature and salinity has been tested in some species of anostracans, and most were for aquaculture purposes, such as *Artemia* (Browne and Wanigasekera 2000), and less frequently *P. spinosus* (Atashbar et al. 2014). The results of the present study showed that, contrary to temperature, change in low degrees of salinity (0-5 psu) had no effect on the hatching performances of *P. spinosus* and *B. media* eggs. Indeed, *B. media* eggs have a higher hatching percentage at 16°C, unlike *P. spinosus* eggs, which prefer a higher temperature (22 °C). This is in agreement with the results concerning the life histories of the two species. In fact, *B. media* develops in winter at low temperatures (5 to 16 °C) and *P. spinosus* develops in spring at higher temperatures (18 to 22 °C) (Rais and Amarouayache 2018). The appearance time of the first larvae and the time of synchronization are shorter for *P. spinosus* eggs at 22 °C and for *B. media* eggs at 16 °C. The other combinations of temperature and salinity extend the synchronization time and the incubation time. The temperature seems to be an important factor controlling the presence of the two co-occuring species in several sites in the region. 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 and the clear dominance by *P. spinosus*, the existence of *B. media* in Algeria is threatened and this species deserves IUCN statut of protection.

References

Alonso M (1985) A survey of the Spanish Euphyllopoda. Misc (Zoo.) 9:179-280

Alonso M (1990) Anostraca, Cladocera and Copepoda of Spanish saline lakes. Hydrobiologia 197:221-231

Amarouayache M, Derbal F, Kara MH (2010) Caractéristiques écologiques et biologiques d'*Artemia salina* (Crustacé, Anostracé) de la Sebkha Ez-Zemoul, Algérie nord-est. Rev Écol (Terre Vie) 65:13-22

Amarouayache M (2014) Observations on *Phallocryptus spinosa* (Branchiopoda, Anostraca) population from the high plateaus of Northeastern Algeria. Sustain Agri Food Environ Res 2(4): 22-31. https://doi.org/7770/safer-V2N4-art844

Atashbar B, Agh N, Van Stappen G, Martens L, Beladjal L (2014) Combined effect of temperature and salinity on hatching characteristics of three fairy shrimp species (Branchiopoda: Anostraca). J Limnol 73(3):574-583. https://doi.org/10.4081/jlimnol.2014.954

Beladjal L, Mertens J, Clegg JS (2008) Biochemical and biophysical aspects of the tolerance of anhydrobiotic crustacean embryos to very high temperatures. J Therm Biol 33:117-127. https://doi.org/10.1016/j.jtherbio.2007.11.003

Beladjal L, Mertens J (2009) Diaspore dispersal of Anostraca by flying insects. J Crust Biol 29:266-268. https://doi.org/10.1651/08-3059R.1

Beladjal L, Mertens J (2017) Risk reduction strategies in *Branchipus schaefferi* (Crustacea: Anostraca: Branchiopoda) as adaptation to a variable environment. Hydrobiologia 801(1):153-163. https://doi.org/10.1007/s10750-017-3156-9

Bengtson DA, Leger P, Sorgeloos P (1991) Use of *Artemia* as a food source for aquaculture. In: Browne RA, Sorgeloos P, Trotman CNA (eds) Artemia biology. CRC Press, Inc., Boca Raton, pp 255-285.

Boumezbeur A (2004) Atlas des zones humides algériennes. DGF

Brendonck L (1996) Diapause, quiescence, hatching requirements: what we can learn from large freshwater branchiopods (Crustacea: Branchiopoda: Anostraca, Notostraca, Conchostraca). Hydrobiologia 320:85-97. https://doi.org/10.1007/BF00016809

Brendonck L, Williams WD (2000) Biodiversity in wetlands of dry regions (drylands). In: Biodiversity in Wetlands: Assessment, Function and Conservation, Vol. 1, Gopal B, Junk WJ, Davis JA (eds). Backhuys Publishers: Leiden, The Netherlands, pp 181-194.

Brown R, Wanigasekera G (2000) Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. J Exp Mar Biol Ecol 244:29-44. https://doi.org/10.1016/S0022-0981(99)00125-2

Dumont HJ, Nandini S, Sarma SSS (2002) Cyst ornamentation in aquatic invertebrates: a defence against egg-predation. Hydrobiologia 486:161-167. https://doi.org/10.1023/A:1021346601235

Hulsmans A, Bracke S, Moreau K, Riddoch BJ, De Meester L, Brendonck L (2006) Dormant egg bank characteristics and hatching patterns of the *Phallocryptus spinosa* (Anostraca) population in the Makgadikgadi Pans (Botswana). Hydrobiologia 271:123-132. https://doi.org/10.1007/s10750-006-0233-x

Jocque M, Riddoch BJ, Brendonck L (2007) Successional phases and species replacements in freshwater rock pools with a biological definition of ephemeral water bodies. Freshwat *Biol* 52:1734-1744. https://doi.org/10.1111/j.1365-2427.2007.01802.x

Kraus H, Eder E, Møller Werding B (2004) Cyst deposition bahaviour and the functional morphology of the brood pouch in *Streptocephalus torvicornis* (Branchiopoda: Anostraca). J Crust Biol 21(3): 93-397. https://doi.org/10.1651/C-2470

Lavens P, Sorgeloos P (1996) Manual of the production and use of live food for aquaculture. FAO Fisheries Technical Paper

Mertens J, Beladjal L, Alcantara A, Fougnies L, Van Der Straeten D, Clegg JS (2008) Survival of dried eukaryotes (anhydrobiotes) after exposure to extremely high temperatures. Biol J Linn Soc Lond 93:15-22. https://doi.org/10.1111/j.1095-8312.2007.00902x

Meyer-Milne E, Brendonck L, Pinceel T (2021) Egg morphology may underpin the successful distribution of large branchiopods in temporary waters. Aquat Ecol 55 (1):237-257. https://doi.org/10.1007/s10452-020-09826-1

Mura G (1992) Preliminary testing of Anostraca from Italy for use in freshwater fish culture. Hydrobiologia 242:185-194

Mura G (2004) Structure and Functioning of the "Egg Bank" of a Fairy Shrimp in a Temporary Pool: *Chirocephalus ruffoi* from Pollino National Park (Southern Italy) as a Case Study. Internat Rev Hydrobiol 80(1):35-50. https://doi.org/10.1002/iroh.200310643

Nouri N, Merabet O, Houhamdi M, Bouslama Z (2016) Biodiversity and phenology of aquatic birds in Highlands's wetlands of Oum El Bouaghi (North-eastern Algeria). Adv Environ Biol 10 (5):39+

Pinceel T, Vanschoenwinkel B, Uten J, Brendonck L (2013) Mechanistic and evolutionary aspects of light-induced dormancy termination in a temporary pond crustacean. Freshw Sci 32 (2):517-524. https://doi.org/10.1899/12.157.1

Pinceel T, Buschke F, Weckx M, Brendonck L, Vanschoenwinkel B (2018) Climate change jeopardizes the persistence of freshwater zooplankton by reducing both habitat suitability and demographic resilience. BMC Ecol 18(2):1-10. https://doi.org/10.1186/s12898-018-0158-z

Rais L, Amarouayache M (2018) The co-occurrence 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). Vie et Milieu 68:167-174

Rochon A, De vernal A, Turon JL (1999) Distribution of recent dinoflagellate cysts of the North Atlantic Ocean and adjacent seas in relation to sea-surface parameters. Amer Assoc Strat Palyn Contr Ser 35:1-152.

Rogers C (2009) Branchiopoda (Anostraca, Notostraca, Laevicaudata, Spinicaudata, Cyclestherida). In: Likens GF (ed.), Encyclopedia of Inland Waters 2:242-249. https://doi.org/10.1116/B978-012370626-3.00157-5

Rogers C (2014) Larger Hatching Fractions in Avian Dispersed Anostracan Eggs (Branchiopoda). J Crust Biol 34 (2):135-143. https://doi.org/10.1163/1937240X-00002220

Rogers C, Timms B (2014) Anostracan (Crustacea: Branchiopoda) zoogeography III. Australian bioregions. Zootaxa 3881(5):453-487. https://doi.org/10.11646/zootaxa.3881.5.3

Sorgeloos P, Bossuyt E, Lavens P, Leger P, Vanhaecke P, Versichele D (1983) The use of brine shrimp *Artemia* in crustacean hatcheries and nurseries. Crust Aquac 1:71-95

Sorgeloos P, Lavens P, Leger P, Tackaert W, Versichele D (1986) Manual for the culture and use of brine shrimp *Artemia* in aquaculture. Artemia Reference Center, State of University of Ghent, Belgium.

Thiéry A (1997) Horizontal distribution and abundance of cysts of several large branchiopods in temporary pool and ditch sediments. Hydrobiologia 359:177-189. https://doi.org/10.1023/A:1003124617897

Timms B (2004) Cyst-shell morphology of the fairy shrimps (Crustacea: Anostraca) of Australia. Proc. Linn Soc NSW 125:73-96

Timms B, Lindsay S (2011) Morphometrics of the resting eggs of the Australian species of the fairy shrimp *Branchinella* (Anostraca: Thamnocephalidae). Proc Linn Soc NSW 133:53-70

Vanschoenwinkel B, Seaman M, Brendonck L (2010) Hatching phenology, life history and egg bank size of fairy shrimp *Branchipodopsis* spp. (Branchiopoda, Crustacea) in relation to the ephemerality of their rock pool habitat. Aquat Ecol 44:771-780. https://doi.org/10.1007/s10452-010-9315-y

Wang CC, Rogers C (2018) Bet hedging in stochastic habitats: an approach through large branchiopods in a temporary wetland. Oecologia 188:1081-1093. https://doi.org/10.1007/s00442-018-4272-6

FIGURES CAPTIONS

Fig. 1 Study sites and sampling points at opposite sides (S1 and S2)

Fig. 2 Resting eggs density of *Phallocryptus spinosus* (**black bars**: *P. s*) and *Branchinectella media* (**gray bars**: *B. m*) collected in the seven saline lakes of the Aurès region in two different stations (1 and 2). GET: Garâa El-Tarf, SD: Sebkha Djendli, CT: Chott Tinsilt, GG: Garâa Guellif, GAD: Garâa Ank Djemel, SOL: Sebkha Ouled Lembarek and SAY: Sebkha Ain Yagout

Fig. 3 Photographs of ultrastructure of *Branchinectella media* resting eggs. a, details of invaginations; b, details of pointed side; c, details of egg shell with ridges; d, details of flattened side. Scale bars: 50 µm for (A, B, D), 20 µm for (C)

Figures

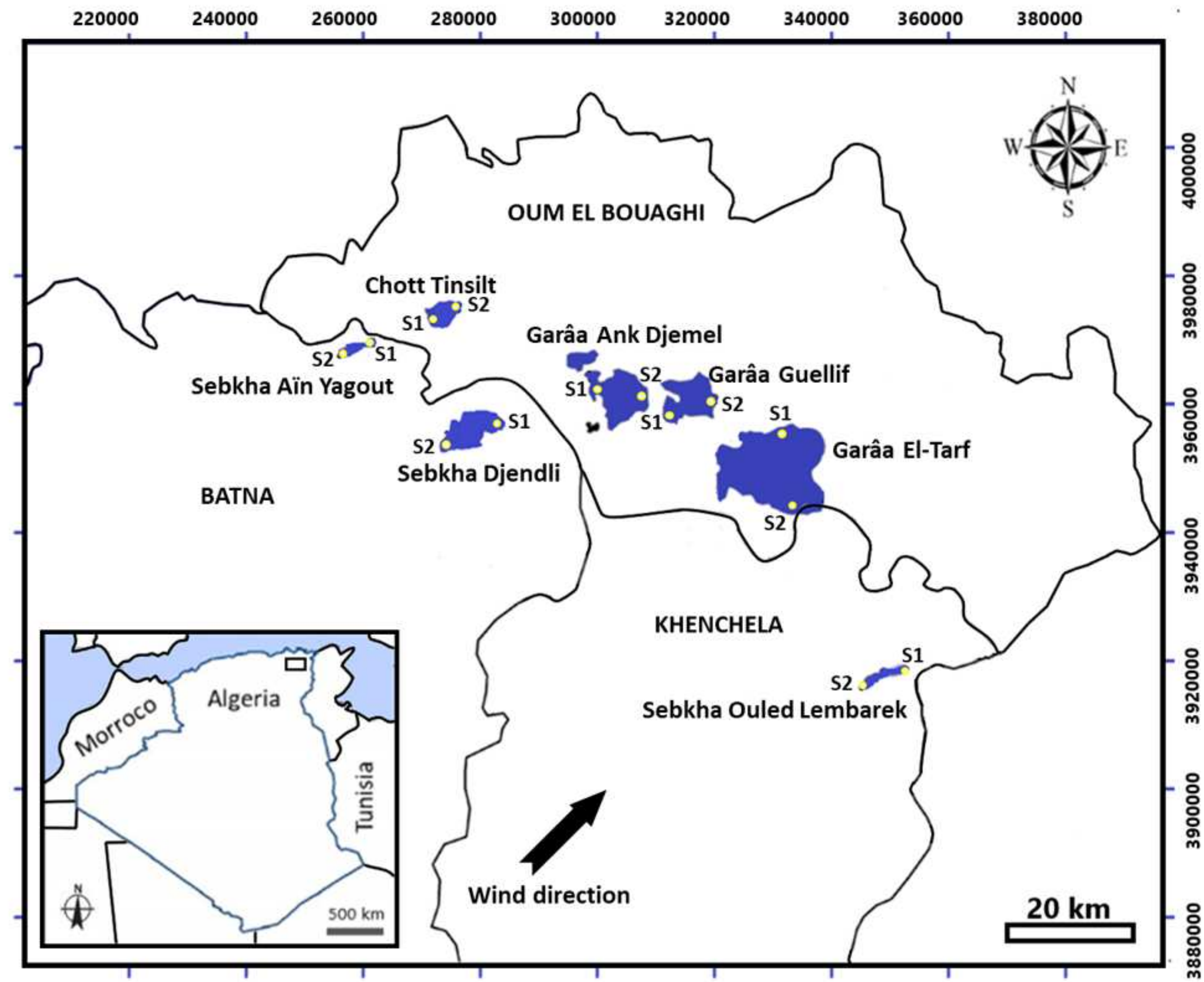

Fig. 1

Figure 1

Study sites and sampling points at opposite sides (S1 and S2)

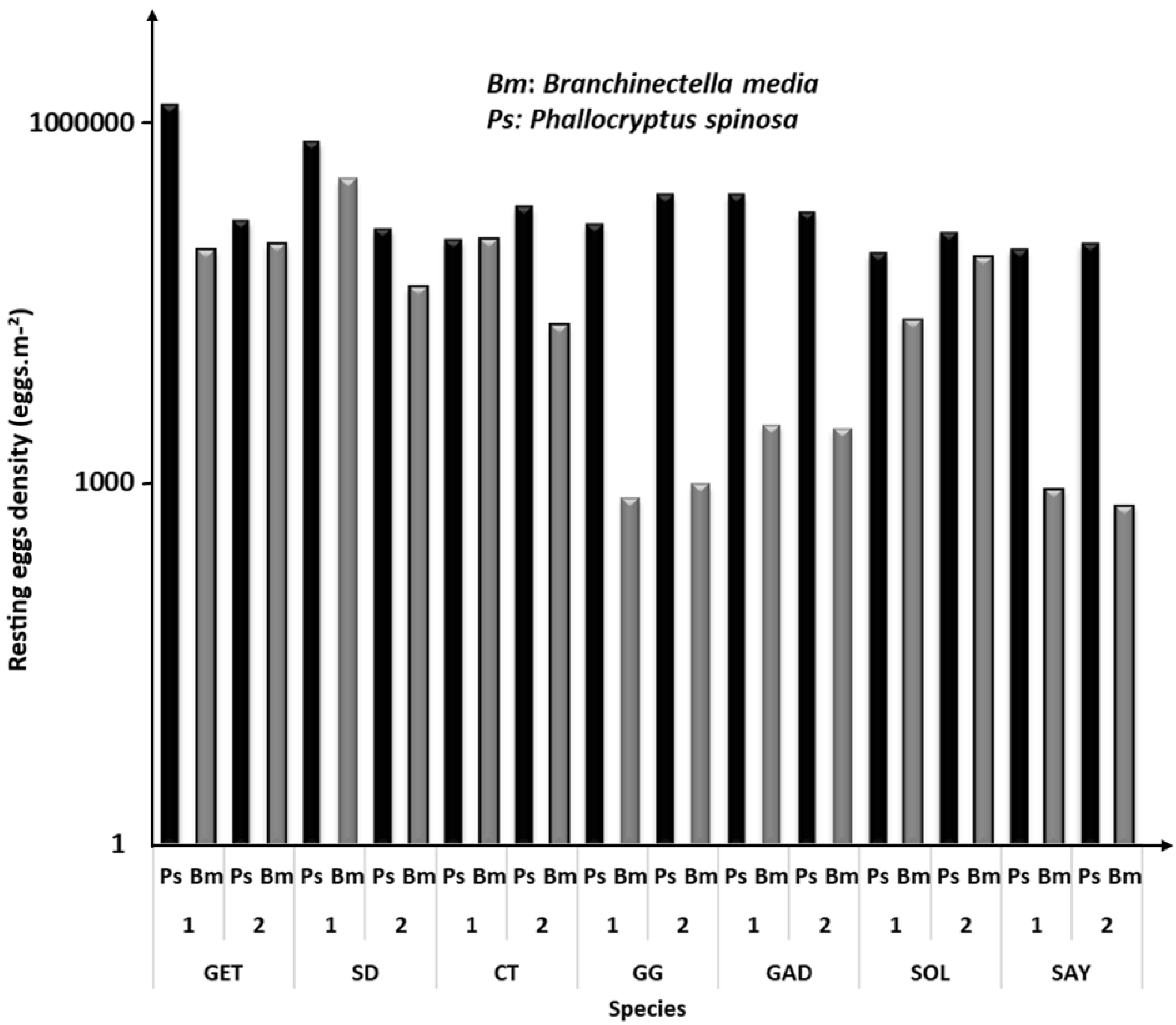

Fig. 2

Figure 2

Resting eggs density of Phallocryptus spinosus (black bars: P. s) and Branchinectella media (gray bars: B. m) collected in the seven saline lakes of the Aurès region in two different stations (1 and 2). GET: Garâa El-Tarf, SD: Sebkha Djendli, CT: Chott Tinsilt, GG: Garâa Guellif, GAD: Garâa Ank Djemel, SOL: Sebkha Ouled Lembarek and SAY: Sebkha Ain Yagout

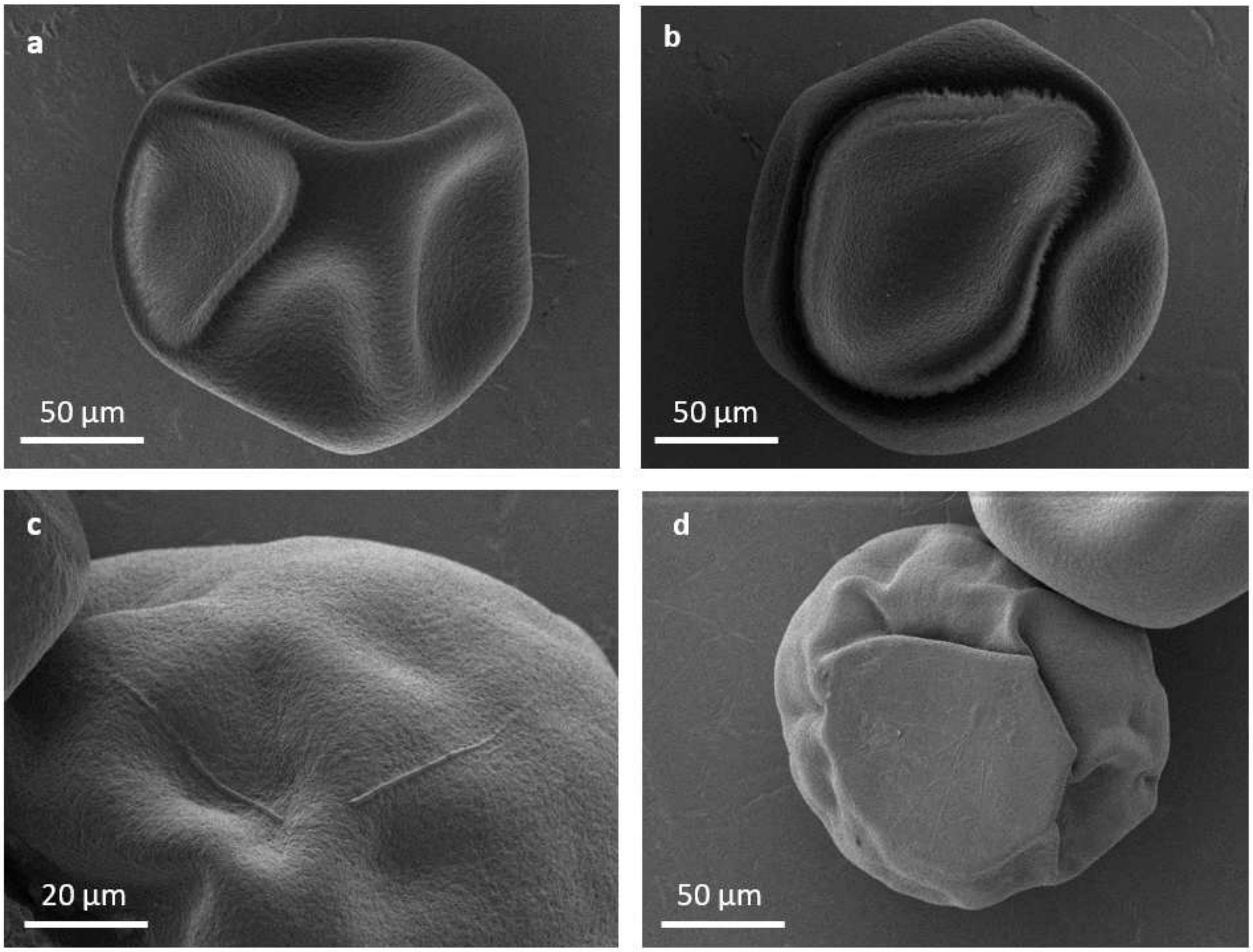

Fig. 3

Figure 3

Photographs of ultrastructure of Branchinectella media resting eggs. a, details of invaginations; b, details of pointed side; c, details of egg shell with ridges; d, details of flattened side. Scale bars: 50 µm for (A, B, D), 20 µm for (C)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Amarouayachecystsbanktab23.docx