Aquaculture Studies, 21(1), 41-46
http://doi.org/10.4194/2618-6381-v21_1_05

RESEARCH PAPER

Study of Cysts Biometry and Hatching Percentage of the Brine Shrimp *Artemia salina* (Linnaeus, 1758) from the Sebkha of Sidi El Hani (Tunisia) According to Successive Generations

Imene Sellami[1,*], Hachem Ben Naceur[1], Adnane Kacem[1]

[1]University of Monastir, Higher Institute of Biotechnology of Monastir, Research Laboratory: LR14ES06 Bioresources, Integrative Biology and Valorization, Avenue Tahar Hadded, BP 74, 5000 Monastir, Tunisia.

Article History
Received 20 October 2020
Accepted 16 December 2020
First Online 17 December 2020

Corresponding Author
Tel.: +0021699375365
E-mail: imen.sellami2012@gmail.com

Keywords
Artemia salina
Cyst diameter
Chorion thickness
Hatching percentage
Subsequent generations

Abstract

Artemia salina cysts harvested from the Sebkha of Sidi El Hani were hatched and cultivated in the laboratory. The obtained nauplii were acclimated at 90 ppt and fed with *Dunaliella salina* microalgae until the adult stage called generation 1 (G1) was reached. Ten couples were isolated and acclimated in the same conditions. Observations of the offspring lasted for a maximum of 60 days. Cysts obtained from G1 were collected and counted. Cyst diameter, chorion thickness and hatching percentage were established. Nauplii obtained from G1 were labelled G2. The third (G3) and the fourth (G4) generations were studied similarly. A significant variation of untreated cyst diameter between G1 (234.7±14.1 µm) and the subsequent generations, notably G3 (210.9±14 µm) were registered. Decapsulated cyst diameter delayed significantly from 218.5±12.8 (G1) to 190.8±10.1 (G3). The chorion thickness ranged between 8.1 (G1) and 10.5 (G2). Therefore, an interesting boost of untreated cysts hatching percentage was observed from 69±2.3% (G1) to 76±3.7% (G4). These results highlighted the variability of cyst diameter across successive generations, even cultivated under laboratory scale. An improvement of hatching quality was detected when environmental conditions became favorable.

Introduction

The brine shrimp *Artemia* is a cosmopolitan crustacean that colonizes hypertonic media, considered lethal for the majority of aquatic species, varying from 9 ppt (Brisset *et al.,* 1982) to 340 ppt (Post & Youssef, 1977; Gajardo & Beardmore, 2012). A recent detailed study of osmoregulatory performance in *Artemia salina* adults conducted by Sellami *et al.*, (2020) highlighted their powerful hypo-osmoregulatory capacity, as they keep their hemolymph osmolality strongly not up to even the foremost concentrated media. Using Na^+/K^+-ATPase immunolocalization, they confirmed the involvement of three main organs in active ion transport, the metepipodites, the maxillary glands and the anterior part of the digestive tract.

Depending on environmental conditions, this small crustacean is able to breed either ovoviviparously, producing free-swimming larvae "nauplii" or oviparously, producing encysted embryos "diapause cysts" enclosed within a chitinous shell (Dai *et al.,* 2011; Ma *et al.,* 2013; Tan & Macrae, 2018; Iryani *et al.*, 2020). Diapause is a reversible physiological state during which metabolism is considerably reduced and enhanced stress resistance facing extreme environmental conditions (Podrabsky & Hand, 2015; Tan & Macrae, 2018). Exposure to favorable environmental conditions such as desiccation and/or cold promotes resumption of cyst development, but if conditions are not conducive to growth, they undergo quiescence, remaining dormant and stress tolerant until adequate moisture, oxygen and temperature triumph (Robbins *et al.*, 2010; Tan & Macrae, 2018)

Artemia cysts present a basic component of aquaculture diets as they contribute to the expansion of the aquaculture industry. Cultivation of fish and shellfish evolve considerable growth advantages after the introduction of *Artemia* nauplii in larviculture. *Artemia*

nauplii present an excellent live food item in larviculture (Ben Naceur *et al.*, 2013; Van Stappen *et al.,* 2020) due especially to its small size that matches perfectly with mouth gape sizes of first larval life stages of cultured species. They also contain basic nutritional requirements, enzymes and other crucial dietary elements for early-stage fish and crustacean larvae (Newmark, 1992; Sorgeloos *et al.,* 2001).

World production of *Artemia* cysts has oscillated around 4000 tonnes per year (Camara, 2020). The Great Salt Lake (Utah, USA) and saline biotopes in Russia, Kazakhstan and China are the major suppliers of *Artemia* cysts to the world aquaculture industry (Litvinenko *et al.*, 2015). The global demand for *Artemia* cysts increases constantly in aquaculture. However, cyst production has decreased due to the worldwide decline of saline lakes that constitute an important ecosystem for such species (Wurtsbaugh *et al.,* 2017). This continuously boost of cysts demand has escalated the search for new *Artemia* strains with good quality cysts and adequate sized nauplii to justify commercial exploitation (Dhont & Sorgeloos, 2002; Camara *et al.,* 2004). Several parameters, such as biometrics of cysts and nauplii, hatching and nutritional qualities, were involved in the characterization of *Artemia* strain and, therefore, the marketing price of its cysts.

The present study aims to investigate the variability of cyst diameter, chorion thickness, and hatching percentage of the branchiopod *Artemia salina* isolated from the wild natural site Sebkha of Sidi El Hani and cultivated as a closed population in the laboratory across four generations.

Material and Methods

Geographical location of the Sebkha of Sidi El Hani Heading

The Sebkha of Sidi El Hani (SH) is a NW-SE lengthened depression in the Sahel area (eastern Tunisia). The area of the Sebkha of Sidi El Hani is approximately 370 km^2. The average water depth is 0.4 m, but it can reach 0.8 m at some locations. The seasonal temperature fluctuates between 2 and 13°C in winter and between 33 and 39°C in summer (Tagorti *et al.,* 2013). The seasonal average of salinity oscillates between a minimum of 180 ppt in winter registered after rainfall and up to 320 ppt in summer (Mathlouthi, 2010). The presence of *Artemia* in SH was first reported by Gauthier (1928) and identified as *Artemia salina* (Ben Naceur *et al.*, 2012a).

Cyst Samples

Artemia salina cysts were collected in summer 2015 from the banks of the Sebkha of Sidi El Hani. Then, they were mixed with salt collected on site for conservation. Once in the laboratory, they were cleaned, separated and stored according to the protocol described by Sorgeloos *et al.* (1986) which consists in a separation by density difference in saturated brine to discard heavy debris (sand, stone, pieces of glass...) followed by separation in fresh water using a decantation funnel to distinguish the full “living” cysts that settled at the bottom of the funnel, empty cysts “dead” and light debris that floated on the surface. This step should not exceed 15 min to avoid the risk of triggering embryonic metabolism and hatching of the cysts. The recovered cysts were spread and dried in an oven at a temperature of 35 °C for 48 h then stored in plastic bottles and kept away from humidity.

Culture Experiments

Cysts harvested from the wild population were incubated during 48 h in 1 L of filtered seawater at a salinity of 35 ppt and a temperature of 25°C, under constant illumination (2000 Lux) and continuous aeration (2 mg/L) to keep the cysts in suspension (Lavens & Sorgeloos, 1996). The obtained nauplii represented the first generation labelled G1. These larvae were cultivated into three containers of 5 L each, filled with water at 90 ppt. These media had been prepared from a mixture of seawater sterilized in an autoclave and raw salt harvested at the Sebkha of Sidi El Hani. In each container, water salinity was checked using a salinometer (Lovibond SensoDirect con110). All containers were placed at a temperature of 25 °C and a photoperiod of 16 h light / 8 h dark. *Artemia* nauplii density was adjusted to 50 individuals per L. These larvae were fed twice a week by adding 100 ml of Chlorophyceae *Dunaliella salina* culture at an approximate density of 100,000 cells. mL^{-1}. The salinity of the microalga culture was the same as that used for the culture of nauplii. The medium was renewed twice a week to ensure the elimination of *Artemia* waste and agglomerated microalgae. Virgin females were separated from mass cultures as soon as signs of ovarian development appeared. Males were also isolated from stock cultures as soon as they had clasped females. A male–female pair of G1 (n = 10 pairs) was placed in each 20 ml plastic tube, and the first crossing was performed, at the same culture conditions as described for parental stocks. The culture medium was changed once a week to ensure waste disposal. Upon the death of a partner, males and females were again paired with a survivor from stock cultures. Observations of the offspring were recorded daily for a maximum period of 60 days. Oviparous offspring (cysts) was filtered and counted under magnifying glass. Emerged nauplii, called G2 (second generation), were transferred into separate culture media under the same conditions and maintained until maturity. The second crossing yielded the third generation G3 from which G4 was further generated. The recovered cysts, for each generation, were dried in an oven at a temperature of 35 °C for 48 h then stored in an Eppendorf tube kept away from humidity.

Untreated and Decapsulated Cysts Diameter

Dehydrated cysts from each generation were placed in Petri dishes with fresh water for 1 hour until the cysts were fully hydrated (became spherical); cysts were photographed with an Olympus optical microscope equipped with a digital camera. Moreover, decapsulation of hydrated cysts was performed according to Sorgeloos *et al.* (1986) method, and then photographed; the photographs of untreated and decapsulated cysts were analyzed using the public domain ImageJ software (version 1.49, v), to measure the diameter of one hundred cysts for each generation studied.

Measurement of Chorion Thickness

Chorion thicknesses were determined according to the following formula (Vanhaecke & Sorgeloos, 1980):

$$Ch = (U - D) / 2$$

Where: Ch = Chorion thickness, U = Untreated cysts, D = Decapsulated cysts

Determination of Hatching Percentage (H %)

The hatching percentage of untreated cysts were determined according to the method described by (Sorgeloos *et al.*, 1986). A known number of cysts from each generation were incubated over 48 hrs in 50 ml cylindroconical cones filled with filtered seawater at a salinity of 35 ppt and a temperature of 25°C, under constant illumination (2000 Lux) and continuous aeration to keep the cysts in suspension (Lavens & Sorgeloos, 1996). Six subsamples of 250 µl each were taken from each cone with a micropipette and placed in a small vial after 24 h and 48 h of hatching. The obtained nauplii were fixed with Lugol solution and counted under a magnifying glass.

The hatching percentage was calculated according to the following formula (Sorgeloos & Kulasekarapandian, 1984). :

$$H\% = [N / (N + C)] \times 100$$

Where: N = number of nauplii, C = number of cysts.

Statistical Analysis

Statistical analyses were performed using Graphpad Prism (version 6, GraphPad Software Incorporated, La Jolla, CA 268, USA). Normality and homogeneity of variance were respectively checked using the D'Agostino-Pearson test and Barlett's test. For data fitting homogeneity of variance requirement, a one-way ANOVA considering generations as the main factor was performed; critical differences between groups were appraised using the Tukey's multiple comparisons test. For data not fitting homogeneity of variance and data due to the small sample size, a non-parametric Kruskal-Wallis test followed by a Dunn's multiple comparisons test was used. Data are presented as mean±SD, and the level of statistical significance was set at $P<0.05$.

Results

Cysts Diameter and Chorion Thickness

Data on cysts diameter, chorion thickness, and results of statistical analysis (one-way ANOVA, Tukey's test, $P<0.05$) for four successive generations of *Artemia salina* from the Sebkha of Sidi El Hani, are summarized in Table 1. A significant variation of untreated cysts diameter was observed between G1 (234.7±14.1 µm) and the following generations, notably *G3* (210.9±14 µm) as shown in Table 1 (one-way ANOVA, $P<0.05$). Decapsulated cysts diameter delayed significantly from 218.5±12.8 to 190.8±10.1 for G1 and G3, respectively (Table 1; one-way ANOVA, $P<0.05$). The chorion thickness ranged between 8.1 (G1) and 10.5 (G2) as represented in Table 1.

Hatching Percentage (H%)

After 24 hours of incubation, hatching percentage showed a significant increase from 52.3±2.2% to 63.6±3% between the first and the latest generation as described in Table 2 (one-way ANOVA, $P<0.05$).

An interesting improvement in untreated cysts hatching percentage was also observed from 69±2.3% (G1) to 76±3.7% (G4), following 48 hours of incubation respectively (Table 2; one-way ANOVA, $P<0.05$).

Table 1. Biometric determination (mean±SD) of cysts across four generations of *A. salina* population cultivated from the Sebkha of Sidi El Hani (units in µm). Notes: Different letters indicate significant differences between data (Tukey's test. $P<0.05$)

Generation	Untreated cysts diameter	Decapsulated cysts diameter	Chorion thickness
G1	234.7±14.1 ^{c}	218.5±12.8 ^{c}	8.1
G2	228.5±15.4 ^{c}	207.4±12.7 ^{b}	10.5
G3	210.9± 14.0 ^{a}	190.8±10.1 ^{a}	10.1
G4	220.1±11.2 ^{b}	201.3±11.7 ^{b}	9.4

Discussion

Characterization of *Artemia* species for aquaculture use is crucial to judge the economic value of cysts. Among the most important criteria implicated in the characterization of *Artemia* species are the determination of cysts diameter as well as their hatching percentage.

Cyst biometry is commonly used as an indicator of the number of cysts.g^{-1}. This last one increases with cysts of small diameter. Such improvement in the amount of cysts.g^{-1} may consequently lead to produce more nauplii.g^{-1} (Camargo *et al.,* 2005). In a comparative study of the biometrical variation of *Artemia* strains from different geographical locations, Vanhaecke and Sorgeloos (1980) have categorized cysts into three groups: 1) The smallest cysts belonging to the Adelaide strain (Australia) and *Artemia* from the San Francisco Bay with 225.5 and 235.6 µm, respectively; 2) the largest parthenogenetic cysts with diameters varying from 267.0 to 284.9 µm; And 3) strains with cysts of intermediate size and very thin chorion, which is characteristic for *A. franciscana* from Chaplin Lake and the Great Salt Lake with 240.0 µm and 244.2 - 252.2 µm, respectively. Based on cysts classification, *Artemia salina* isolated from the wild natural site Sebkha of Sidi El Hani and cultivated as a closed population in the laboratory across four generations revealed similarity with the first group of the smallest cysts diameter. Otherwise, in comparison with the commercial *A. franciscana* populations usually used as a reference in scientific works: the Great Salt Lake (GSL- Utah, USA) cyst diameter (244.2 - 252.5 µm) and the San Francisco Bay (SFB -California, USA) (223.9 -228.7 µm) (Vanhaecke & Sorgeloos, 1980), our results afford a comparative diameter with SFB.

Several extensive studies affirmed the variability of cysts diameter between different species and populations of *Artemia*. A detailed survey of biometric and ecologic comparison between *Artemia* from Mexico and Chile confirmed that the cyst diameter of *Artemia franciscana* varied from 220.5 to 241 µm for fully cyst and 5.4 to 7.9 µm for chorion thickness from Chile and also 200.4 to 292.3 µm for untreated cyst and 2.11 to 10.78 µm for chorion thickness from Mexico (Castro *et al.,* 2006). In the same context, the biometric determination of *Artemia franciscana* cysts samples from six locations in the Colombian Caribbean and San Francisco Bay showed a wide variation among both untreated (201 - 252.9 µm) and decapsulated cysts (183.4 - 234.2 µm) with a chorion thickness ranging between 3.1 and 13.2 µm (Camargo *et al.*, 2005). Studying 14 *A. franciscana* populations, (Hontoria, 1990) perceived that cyst diameter ranged between 217 and 230 µm, except Great Salt Lake (Utah, USA) and Galera Zamba (Colombia) cysts, with 242 and 245 µm diameter. Abatzopoulos *et al.* (2006) have attributed this variability to seasonal fluctuations in physicochemical parameters and food availability in different regions of Lake Urmia and the differences can be observed at different times in a single site.

Based on cysts classification, Ben Naceur *et al.* (2012b) examined the biometry of *Artemia* cysts harvested from different salt lakes in Tunisia and classified *A. salina* of Sidi El Hani as a population with an intermediate cyst diameter (260.2 µm hydrated cysts and 236.1 µm for decapsulated cysts) with a chorion thickness about 12.1 µm. The present study showed a variation of cyst diameter from the first to the following generations oscillating between 234.9 µm (G1) and 210.9 µm (G3) for untreated cysts and between 190.7 µm (G1) and 218.5 µm (G3) for decapsulated cysts with a chorion thickness varying from 10.5 µm (G2) to 8.1 µm (G1).

Abatzopoulos *et al.* (2006) mentioned that despite the previous use of cyst diameter as a characterization parameter for different strains of *Artemia*, this criterion does not allow identification of cysts of unknown origin. This is due to the wide difference in cyst diameter that may exist between different samples of the same population. However, other studies suggested that cyst diameter from the same strain or inoculum in different countries appear to have a constant diameter that seems to be genetically controlled (D'Agostino, 1965; Claus *et al.*, 1977; Vanhaecke & Sorgeloos, 1980). In our case, in spite of the stability of culture conditions, a significant difference of cyst diameter between the studied generations was observed, confirming the results obtained by Abatzopoulos *et al.* (2006).

Several factors could control hatching performance like the degree of diapause termination, cysts energy content and impurities (empty shells, salt, sand), storage conditions, hydration/dehydration (H/D) cycles, hatching period as well as production conditions affecting the parental generation (Sorgeloos *et al.,* 1986; Van Stappen, 1996).

Our results showed a significant improvement in the % of hatching across generations, ranging between 69% (G1) and 76% (G4). These findings revealed an honest hatching percentage as compared only with Tunisian populations (14.3% - 63.7%, (Ben Naceur *et al.,* 2012) but also with cysts harvested from Urmia Lake (35%, (Abatzopoulos *et al.*, 2006), Colombian Caribbean sites (46.7% - 53.1%, (Camargo *et al.*, 2005), and GSL

Table 2. Hatching percentage (mean ± SD) of cysts of four generations of *A. salina* population cultivated from the Sebkha of Sidi El Hani after 24 h and 48 h hatching incubation. Notes: Different letters indicate significant differences between data. (Tukey's test. P<0.05)

		G1	G2	G3	G4	*F-value*	*P-value*
H%	After 24 h	52.3±2.2^{a}	63.0±0.6^{b}	59.3±3.5^{b}	63.6±3.0^{b}	12.18	0.002
	After 48 h	68.5±2.3^{a}	71.2±3.3^{ab}	72.1±2.1^{ab}	76.0±3.7^{b}	5.063	0.015

'non-commercial cysts' (43.9%, (Sorgeloos *et al.*, 1986). Evaluating the quality of *Artemia salina* from Chott Marouane (Northeast Algeria), Kara *et al.* (2004) showed also a wide variability of hatching percentage ranging between 0.9%, 55%, and 24.7% for cysts collected in 1994, 2000, and 2001 respectively.

The poor hatchability of *Artemia* cysts could be attributed to improper processing of cysts after sampling, and its long-term storage (Vanhaecke & Sorgeloos, 1982). El-magsodi *et al.* (2014) confirmed that cyst metabolism, as initiated after hydration, is to a certain degree reversible and that cysts are often converted from a hydrated, metabolically active mass of cells into a dehydrated, ametabolic state. They also revealed that repeated H/D cycles cause a decreased hatching output and inferior quality of those nauplii hatching. In our case, grandmothers (G1) obtained from cysts that had been subjected to repeated H/D cycles (collected directly from the Sebkha) release cysts with lower hatching output in comparison with those obtained from adults cultivated under laboratory conditions and hadn't been exposed to repeated H/D cycles. Statistical analysis showed significant dissimilarities of the hatching percentage between grandmothers (G1) and the subsequent generations. However, no significant differences between G2, G3, and G4 were detected.

Conclusion

This study asserts the variability of cyst diameter among successive generations, even cultivated under stable conditions (laboratory scale). Whereas, an improvement of the hatching quality was observed when environmental conditions became favorable.

References

Abatzopoulos, T. J., Baxevanis, A. D., Triantaphyllidis, G. V, Criel, G., Pador, E. L., Van Stappen, G., & Sorgeloos, P. (2006). Quality evaluation of *Artemia urmiana* Günther (Urmia Lake, Iran) with special emphasis on its particular cyst characteristics (International Study on Artemia LXIX). *Aquaculture*, 254(1–4), 442–454.

Ben Naceur, H., Ben Rejeb Jenhani, A., & Romdhane, M. (2012a). Review of the biogeography of *Artemia* Leach, 1819 (Crustacea: Anostraca) in Tunisia. *International Journal of Artemia Biology*, 2(1), 24–39.

Ben Naceur, H., Ben Rejeb Jenhani, A., & Romdhane, M. S. (2012b). Biometrical study of *Artemia spp.* (Anostraca: Artemiidae) cysts harvested from different salt lakes in Tunisia. *North-Western Journal of Zoology*, 8 (2): 308-314.

Ben Naceur, H., Ben Rejeb Jenhani, A., & Romdhane, M. S. (2012). Quality characterization of cysts of the brine shrimp *Artemia salina* from Tunisia focusing on their potential use in aquaculture. *Journal of Biological Research-Thessaloniki*, 17, 16–25.

Ben Naceur, H., Ghazali, N., Ben Rejeb Jenhani, A., & Romdhane, M. S. (2013). Study of the fatty acid composition of *Artemia salina* cysts from Tunisia. *Journal of the Marine Biological Association of the United Kingdom*, 93(7), 1795–1803. https://doi.org/10.1017/S0025315413000623

Brisset, P., Versichele, D., Bossuyt, E., De Ruyck, L., & Sorgeloos, P. (1982). High density flow-through culturing of brine shrimp *Artemia* on inert feeds—preliminary results with a modified culture system. *Aquacultural Engineering*, 1(2), 115–119. https://doi.org/10.1016/0144-8609(82)90003-6

Camara, M. R., Monteiro, P. A., Reis, L. G., & Costa, M. F. (2004). Farming *Artemia* in a multi-cycle culture system in Northeastern Brazil. *World Aquaculture*. 35(2): 40-42.

Camara, M. R. (2020). After the gold rush: A review of *Artemia* cyst production in northeastern Brazil. *Aquaculture Reports*, 17:100359. https://doi.org/10.1016/j.aqrep.2020.100359

Camargo, W. N., Durán, G. C., Rada, O. C., Hernández, L. C., Linero, J. G., Muelle, I. M., & Sorgeloos, P. (2005). Determination of biological and physicochemical parameters of *Artemia franciscana* strains in hypersaline environments for aquaculture in the Colombian Caribbean. *Saline Systems*, 4, 1–11. https://doi.org/10.1186/1746-1448-1-9

Castro, T. B., Gajardo, G., Castro, J. M., & Castro, G. M. (2006). A biometric and ecologic comparison between *Artemia* from Mexico and Chile. *Saline Systems*, 2(13), 1–10. https://doi.org/10.1186/1746-1448-2-13

Claus, C., Benijts, F., & Sorgeloos, P. (1977). Comparative study of different geographical strains of the brine shrimp *Artemia salina*. In E. Jaspers, (Ed.), Fundamental and Applied Research on the Brine Shrimp *Artemia salina* (L.) in Belgium. *European Mariculture Society, Special Publication*, (2), pp. 91-105.

D'Agostino, A. S. (1965). Comparative studies of *Artemia salina* (Development and Physiology). New York University, Ph.D. thesis, University Microfilm 66-5653. University Microfilms, Inc., Ann Arbor, Michigan.Dai, Z., Li, R., Dai, L., Yang, J., Chen, S., Zeng, Q., Yang, F., & Yang, W. (2011). Determination in oocytes of the reproductive modes for the brine shrimp *Artemia parthenogenetica*. *Bioscience Reports*, 31, 17–30. https://doi.org/10.1042/BSR20090141

Dhont, J., & Sorgeloos, P. (2002). Applications of *Artemia*. In *Artemia*: Basic and applied biology (pp. 251–277). Springer.

El-magsodi, M. O., Bossier, P., Sorgeloos, P., & Van Stappen, G. (2014). Hatching and nutritional quality of *Artemia* cysts progressively deteriorates as a function of increased exposure to hydration / dehydration cycles. https://doi.org/10.1007/s10499-014-9763-9

Gajardo, G. M., & Beardmore, J. A. (2012). The brine shrimp *Artemia*: adapted to critical life conditions. *Frontiers in Physiology*, 3, 1–8. https://doi.org/10.3389/fphys.2012.00185

Gauthier, H. (1928). Recherches sur la faune des eaux continentales de l'Algérie et de la Tunisie. In Thesis and Dissertations. Minerva, Alger, Algerie.

Hontoria, F. (1990). Caracterizacion de tres poblaciones originarias del area levantina espanola del crustaceo branquiopodo *Artemia*. Aplicacion en acuicultura. Universidad Autónoma de Barcelona, Bellaterra, Spain.

Iryani, M. T. M., Sorgeloos, P., Danish-daniel, M., Tan, M. P., Wong, L. L., Mok, W. J., Satyantini, W. H., Mahasri, G., & Sung, Y. Y. (2020). Cyst viability and stress tolerance upon heat shock protein 70 knockdown in the brine

shrimp *Artemia franciscana*. *Cell Stress and Chaperones*, Clegg 2011, 1–5.

Kara, M. H., Bengraine, K. A., & Derbal, F. (2004). Quality evaluation of a new strain of Artemia from Chott Marouane (Northeast Algeria). *Aquaculture*, 235(1-4), 361-369. https://doi.org/10.1016/j.aquaculture.2004.02.016

Lavens, P., & Sorgeloos, P. (1996). Manual on the production and use of live food for aquaculture. In P. Lavens & P. Sorgeloos (Eds.), *FAO Fisheries Technical Paper. Food and Agriculture Organization (FAO)*. https://ftp.fao.org/.../w3732e00.pdf

Litvinenko, L. I., Litvinenko, A. I., Boiko, E. G., & Kutsanov, K. (2015). *Artemia* cyst production in Russia. *Chinese Journal of Oceanology and Limnology*, 33(6), 1436–1450. https://doi.org/10.1007/s00343-015-4381-6

Ma, W.-M., Li, H.-W., Dai, Z.-M., Yang, J.-S., Yang, F., & Yang, W.-J. (2013). Chitin-binding proteins of *Artemia* diapause cysts participate in formation of the embryonic cuticle layer of cyst shells. *Biochemical Journal*, 449(1), 285–294. https://doi.org/10.1042/BJ20121259

Mathlouthi, W. (2010). Etude des ressources zooplanctoniques de la Saline SAÎDA et de Sebkha Sidi El Hani. Mastère : Aquaculture et Biotechnologie Marine, Institut Supérieur de Biotechnologie de Monastir, université de Monastir, Tunisie.

Newmark, F. (1992). Comparación experimental de las cepas de Colombia de *Artemia* (Galera Zamba, Manaurey Pozos Colorados). En la larvicultura de postlarvas, cultivo y evaluación de microorganismos como alimento. Volumen 1, Programa Iberoamericano de Ciencias y Tecnología Para El Desarrollo. Subprograma II Acuicultura. 1992, 231-239.

Podrabsky, J. E., & Hand, S. C. (2015). Physiological strategies during animal diapause: lessons from brine shrimp and annual killifish. *The Journal of Experimental Biology*, 218, 1897–1906. https://doi.org/10.1242/jeb.116194

Post, F. J., & Youssef, N. N. (1977). A procaryotic intracellular symbiont of the Great Salt Lake brine shrimp *Artemia salina* (L.). *Canadian Journal of Microbiology*, 23(9), 1232–1236. https://doi.org/10.1139/m77-184

Robbins, H. M., Stappen, G. Van, Sorgeloos, P., Sung, Y. Y., Macrae, T. H., & Bossier, P. (2010). Diapause termination and development of encysted *Artemia* embryos: roles for nitric oxide and hydrogen peroxide. *The Journal of Experimental Biology*, 213(9), 1464–1470. https://doi.org/10.1242/jeb.041772

Sellami, I., Charmantier, G., Naceur, H. B., Kacem, A., & Lorin-Nebel, C. (2020). Osmoregulatory performance and immunolocalization of Na+/K+-ATPase in the branchiopod *Artemia salina* from the Sebkha of Sidi El Hani (Tunisia). *Tissue and Cell*, 63. https://doi.org/10.1016/j.tice.2020.101340

Sorgeloos, P., Dhert, P., & Candreva, P. (2001). Use of the brine shrimp, *Artemia sp.*, in marine fish larviculture. *Aquaculture*, 200, 147–159.

Sorgeloos, P., & Kulasekarapandian, S. (1984). Production and use of *Artemia* in aquaculture. *CMFRI special publication*, 15, 1-73.

Sorgeloos, P., Lavens, P., Léger, P., Tackaert, W., & Versichele, D. (1986). Manual for the culture and use of brine shrimp *Artemia* in aquaculture. Belgium, Artemia Reference Center, Faculty of Agriculture, State University Ghent, pp. 319.

Tagorti, M. A., Essefi, E., Touir, J., Guellala, R., & Yaich, C. (2013). Geochemical controls of groundwaters upwelling in saline environments: Case study the discharge playa of Sidi El Hani (Sahel, Tunisia). *Journal of African Earth Sciences*, 86, 1–9. https://doi.org/10.1016/j.jafrearsci.2013.05.004

Tan, J., & Macrae, T. H. (2018). Stress tolerance in diapausing embryos of *Artemia franciscana* is dependent on heat shock factor 1 (Hsf1). *PLoS ONE*, 13(7), e0200153. https://doi.org/10.1371/journal.pone.0200153

Van Stappen, G. (1996). Use of cysts. In P. Lavens & P. Sorgeloos (Eds.), Manual on the production and use of live food for aquaculture (Vol. 361, pp. 107–136). FAO.

Van Stappen, G., Sui, L., Hoa, V. N., Tamtin, M., Nyonje, B., de Medeiros Rocha, R., Sorgeloos, P., & Gajardo, G. (2020). Review on integrated production of the brine shrimp *Artemia* in solar salt ponds. *Reviews in Aquaculture*, 12(2), 1054–1071. https://doi.org/10.1111/raq.12371

Vanhaecke, P., & Sorgeloos, P. (1980). International Study on *Artemia* *. XIV. Growth and Survival of *Artemia* Larvae of Different Geographical Origin in a Standard Culture Test. 3, 303–307.

Vanhaecke, P., & Sorgeloos, P. (1980). International Study on *Artemia* IV. The biometrics of *Artemia* strains from different geographical origins. *The brine shrimp Artemia*, 3, 393–405.Vanhaecke, P., & Sorgeloos, P. (1982). International study on *Artemia*. XVIII. The hatching rate of *Artemia* cysts—a comparative study. *Aquacultural Engineering*, 1(4), 263–273.

Wurtsbaugh, W. A., Miller, C., Null, S. E., Justin De Rose, R., Wilcock, P., Hahnenberger, M., Howe, F., & Moore, J. (2017). Decline of the world's saline lakes. *Nature Geoscience*, 10(11), 816–821. https://doi.org/10.1038/NGEO3052