



DIMENSIONS OF ARTEMIA CYSTS IN THE ARAL SEA POPULATION, UZBEKISTAN

Utemuratova F. Zh.
Tashkent State Agrarian University

Summary

With the salinization of the Aral Sea and the transformation of its remnant into a hyperhaline reservoir, *Artemia* (*Artemia parthenogenetica*) appeared in the plankton and has now become dominant. There are practically no data on *Artemia* morphometry of this new population. The diameter of resting and decapsulated cysts and the thickness of the chorion collected in November 2021 on the southern coast of the Aral Sea remnant (Uzbekistan) were determined. The sizes of the resting cysts were 198 - 330 (265.3 + 4.09) μm , decapsulated - 198 - 297 (242.8 + 3.37) μm , the calculated size of the chorion was 11.1 μm .

Keywords: brine shrimp, *Artemia*, *Artemia* cysts, Aral Sea, Uzbekistan.

Introduction

Various populations of *Artemia* have been identified in temperate and tropical zones on all continents except Antarctica. The modern distribution was also influenced by the artificial introduction of *Artemia* into different regions. (Liu and Zheng, 1990; Abatzopoulos et al., 1998; Triantaphyllidis et al., 1998; Hontoria and Amat, 1992; Gajardo et al., 2002). *Artemia* are adapted to different temperature conditions and a very wide range of water salinity (from 7.0 g/l to more than 300 g/l) (Abreu-Grobois and Beardmore, 1982; Browne and Bowen, 1991; Hontoria and Amat, 1992).

In the early 1990s, *Artemia* (*Artemia parthenogenetica*) was discovered in the Aral Sea, and since the 2000s it has become a permanent and dominant component of the reservoir, accounting for more than 99% of the total zooplankton biomass (Mirabdullaev et al., 2010). However, many questions of the biology of the emerging population of this reservoir in Central Asia have not been studied.

Specialists conduct research on the phylogeny of *Artemia* using a number of methodological approaches, incl. comparison of biometric and morphological characteristics of cysts (Asem et al., 2007; Asem and Sun, 2014). In this study, we analyzed the diameter of hydrated and decapsulated *Artemia* cysts and the thickness of their chorion in individuals of the Aral Sea population collected on the southern shore of the reservoir.





Getting cysts

Cysts were collected in November 2021 off the coast of the southern part of the remnant of the Aral Sea on the territory of Karakalpakstan (Uzbekistan).

Artemia cysts harvested in the water or on the shore and containing various impurities (sand, algae, shells, bird feathers, egg shells, etc., which accounted for up to 60% of the collection mass) were cleaned by conventional methods by employees of the Aral Sea LLC Aquaculture. Cleaning with water was carried out in a special device, where eggs are separated by specific gravity. Clean samples of cysts were brought to the laboratory and stored in a refrigerator at a temperature not exceeding -5°C .

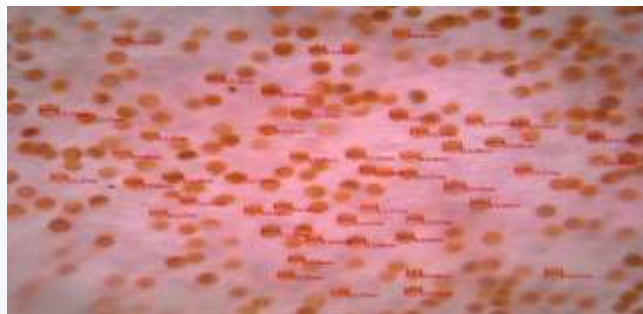
Resting cyst diameter

To determine the size, a small number of randomly selected cysts (about 1000 pieces) were placed in a Petri dish with a minimum amount of fresh water.

The diameter of the resting cysts was determined in micrometers with an accuracy of $0.1\ \mu\text{m}$ (Fig. 2) immediately after being placed in water.

Part of the sample was photographed using a usb microscope, incl. took a photo of the micrometer object (to determine the magnification).

The maximum diameter of at least 100 random cysts was determined. Based on the results of measurements of 100 cysts, the average and other statistical data were calculated. indicators of one variable. The samples were compared using the t-test ($p < 0.05$).



Rice. 2. Photos of measurements of cysts in the usb-microscope online

Diameter of decapsulated cysts

Cysts were decapsulated according to the generally accepted method (Bruggeman et al., 1980). Resting cysts, after their diameter was measured, were poured from a Petri dish into a 150 ml beaker with a minimum amount of fresh water and 100 ml of liquid bleach (with NaOCl content $\sim 15\%$) was added, after which for 5-10 min everything was mixed for uniform passage of the decapsulation process.



The start of the decapsulation process and its end could be observed with the naked eye.

After 5-10 minutes note removal of cyst membranes. Upon completion of decapsulation, the sample was washed through a 125 μm sieve to remove the chemicals used for decapsulation. It is known that with prolonged exposure to chemicals (high concentration of NaOCl), embryos gradually degrade, which will introduce errors in measurements.

In our study, it was not the goal to determine the yield of embryos, so the applied method is sufficient. A minimum of 100 embryos were selected for diameter measurement. The diameter of decapsulated cysts was also determined in micrometers with an accuracy of 0.1 μm . To do this, they were photographed using a usb microscope (including a photo of a micrometer object to determine the magnification).

A minimum of 100 embryos were measured, while not fully hydrated cysts, embryos with an excretory membrane, not completely filled with yolk mass, and with other abnormalities were not measured. The standard statistics of the measured sample were determined.

Chorionic Thickness

It's calculated indicator, which was determined using the arithmetic mean of the diameters of hydrated and decapsulated cysts. The indicator was calculated using the following formula (Vanhaecke, Sorgeloos, 1980):

Chorionic thickness = (average diameter of resting cysts - average diameter of decapsulated cysts) / 2.

Results

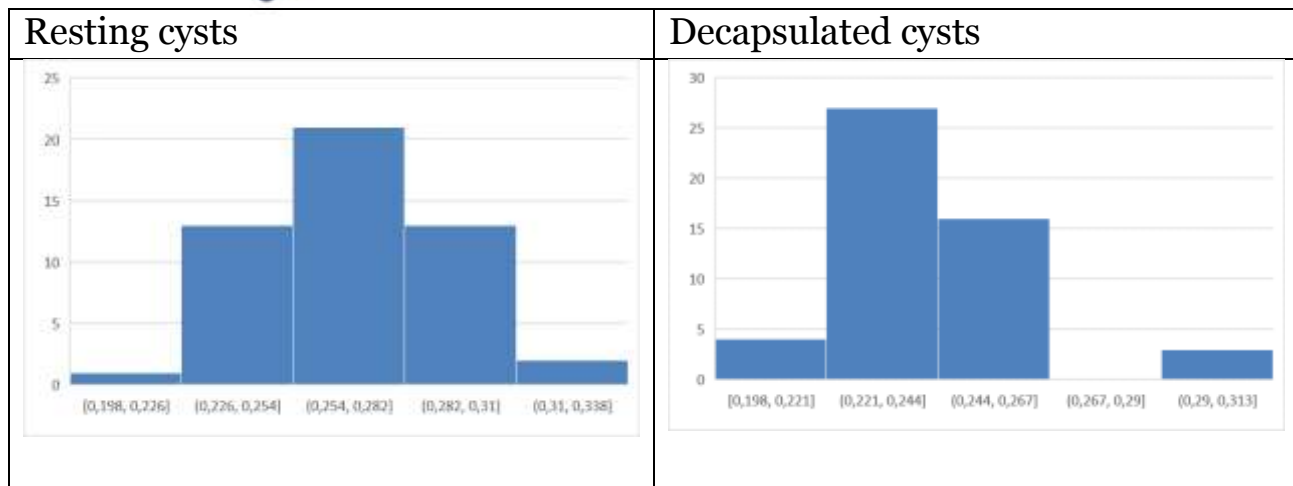
Diameter of cysts collected near the southern coast of the Aral Sea remnant

The distribution of the diameters of the resting cysts is shown in Figure 3. The minimum value of the resting cysts in diameter was 198 μm , the maximum was 330 μm , the arithmetic mean of the sample was 265.3 + 4.09 μm . The variability of cyst sizes was rather low, the coefficient of variation was 10.09%.

Minimum diameter value decapsulated cysts was 198.0, the maximum was 297.0, the average was 242.88 + 3.37 μm , the coefficient of variation of the index for cysts was 9.8%.

Comparison by Student's t-test, which was 4.2, showed that the difference between the average sizes of cysts resting and decapsulated is reliable.





Rice. Fig. 3. Size distribution of the diameter of hydrated (left) and decapsulated (right) *Artemia* cysts in the sample (along the “x” axis - the diameter of cysts, microns, along the “y” axis - occurrence, pieces.)

Chorionic Thickness

The chorion thickness of the cysts collected on the south bank was calculated as 11.2 μm .

Discussion

Various populations of *Artemia* are scattered throughout the temperate and tropical zones of the world, about 600 reservoirs are known where they live. A high adaptability of brine shrimp to a number of environmental factors, primarily salinity, was revealed, and their analysis is carried out for systematic, taxonomic and evolutionary studies. Various methodological approaches are used to study the evolution and phylogeny of *Artemia*, including comparison of biometric characteristics (Hontoria and Amat, 1992; Gajardo et al., 2002; Van Stappen, 2002; Asem et al., 2007).

Experts, studying *Artemia* cysts of various geographical origin, identified 3 groups according to the size of cysts (Vanhaecke, Sorgeloos, 1980):

1. The smallest cysts of the *Adelaid* and *Artemia* strains are from the San Francisco Bay Area, including the SFB grafted strain from Macau and Barotac Nuevo.
2. Parthenogenetic strains from China, France, Italy and India, characterized by large cysts.
3. Strains with medium-sized cysts.

In 14 *A. Franciscana* populations, mean population cyst diameters were 217 - 230 μm (Asem et al., 2007). The diameter of untreated *Artemia* cysts from Namibia and



Madagascar was determined to be 247.7 and 285.9 μm on average (Triantaphyllidis et al., 1996). In *A. tibetiana*, the largest cysts have been identified so far, 323 μm on average (Abatzopoulos et al., 1998). Cohen et al. (1999) showed a mean population diameter in 4 Argentine *Artemia* populations of 230.3 - 246.1 μm . Very large *Artemia* cysts of the parthenogenetic population of Lake Akkikkol (China) (315.8 μm) were found (Ma, Wang, 2003).

In the laboratory of *Artemia* Reference Center, Ghent University, Belgium, using the standard methods developed in this laboratory, some parameters of *Artemia* cysts of the Aral Sea were studied in the first years of their detection in the saline Aral Sea. The measured length of Aral *Artemia* nauplii at the Instar I stage after 24 hours was 0.490 mm on average (the result of measurements of 500 individuals from various collections), and the diameter of hydrated decapsulated cysts averaged 0.247 mm (1000 cysts of various collections were measured) (Abdurakhimova, Mustafayeva, 2009).

Our data are somewhat different from the data in previous years in the Aral Sea, for example, cysts in collections near the southern coast have 198 - 297 (average 242.9) microns.

Comparing the sizes of dormant and decapsulated *Artemia* cysts from different regions of the planet, we can assume that the parthenogenetic *Artemia* of the Aral Sea population belongs to the group with medium-sized cysts.

With regard to the processed material, we note that the main quality parameters of cysts (size, hatchability, fatty acid composition) meet the requirements for primary feed for shrimp and fish in aquaculture, and are close to commercially optimal (cysts with a diameter of less than 250 microns are in greatest demand) (Musaev et al., 2012)

List of Used Works

1. Abatzopoulos, TJ, Zhang, B. and Sorgeloos, P. 1998. International study on *Artemia*: 59. *Artemia tibetiana*: preliminary characterization of a new *Artemia* species found in Tibet (People's Republic of China). *International Journal of Salt Lake Research*, 7:41-44.
2. Abdurakhimova, AN, Mustafaeva, ZA Eksperimentalnie dannie po razvitiyu galofilnogo rachka *Artemia parthenogenetica*. (Experimental data on the development of the halophilic crustacean *Artemia parthenogenetica*).- In: *Materiali nauchnoi konferencii "Aktualnie problem zoologicheskoy nauki"* (conference compilation "Actual problems of zoological science"), Tashkent, Institute of zoology of Uzbekistan Academy of Sciences, 2009 .-pp. 21 - 22 (in Russian).





3. Abreu-Grobois, FA, Beardmore, JA 1982. Genetic differentiation and speciation in the brine shrimp *Artemia*. In: Mechanisms of speciation (ed. C. Barigozzi.), Alan R. Liss Inc., New York, USA, pp. 345-376
4. Asem, A., Rastegar-pouyani, N., Agh, N. Biometrical Study of *Artemia urmiana* (Anostraca: Artemiidae) Cysts Harvested from Lake Urmia (West Azerbaijan, Iran). - Turkish Journal of Zoology, 2007, 31. – p. 171-180.
5. Asem, A., Sun, S.-C. Biometric characterization of Chinese parthenogenetic *Artemia* (Crustacea: Anostraca) cysts, focusing on its relationship with ploidy and habitat altitude. - North-western journal of zoology, 2014, 10(1). – p. 149-157.
6. Browne, RA, Bowen, ST 1991. Taxonomy and population genetics of *Artemia*. In: *Artemia Biology* (eds. RA Browne, P. Sorgeloos, CNA Trotman), CRC Press, Boca Raton, Florida, pp. 221-253.
7. Bruggeman, E., Sorgeloos, P., Vanhaecke, P. 1980. Improvements in the decapsulation technique of *Artemia* cysts: In: *The Brine Shrimp Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. (eds. G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers), Universa Press, Wetteren, Belgium, pp. 261-269.
8. Cohen, R. G., Amat, F., Hontoria, F., Navarro, J. C. Preliminary characterization of some Argentinean *Artemia* population from La Pampa and Buenos Aires provinces. - International Journal of Salt Lake Research, 1999, 8. - p. 329-340.
9. Gajardo, G., Abatzopoulos, TJ, Kappas. I., Beardmore, JA 2002. Evolution and speciation. In: *Artemia Basic and Applied Biology*. (eds. TJ Abatzopoulos, JA Beardmore, JS Clegg, P. Sorgeloos), Kluwer Academic Publishers, Dordrecht, pp. 225-250.
10. Hontoria, F., Amat, F. 1992. Morphological characterization of adult *Artemia* (Crustacea, Branchiopoda) from different geographical origins. American populations, *Journal of Plankton Research*. 14:1461-1471.
11. Liu, JY, Zheng, MP Geographic distribution of *Artemia* in Chinese interior salt lakes. - *Larviculture and Artemia Newsletter*, 1990, 17. – p. 22-24.
12. Ma, L., Wang, W. Comparison of the biological characteristics of two *Artemia* populations from Aqikekule Lake and Ga Hai Lake. - *Marine Sciences*, 2003, 27. - p. 34-37. (in Chinese with English summary).
13. Mirabdullaev, IM, Musaev, AK, Mustafaeva, ZA, Joldasova, IM, Abdaullaeva, LN, Temirbekov, RO, Marden, B., Van Stappen, G. Sukcessiya bioti Aralskogo moray pri perehode ot oligo- k poligalinnomu sostoyaniyu (Succession of the biota of the Aral Sea during the transition from oligo- to polyhaline state). – *Uzbek biological journal*, 2010, special issue to the 75th anniversary of Academician Tashmukhamedov, BA – pp. 66-69. (in Russian).



14. Musaev, AK, Abdurakhimova AR, Mirabdullaev, IM Kachestvo cist artemii Aralskogo moray (Quality of Artemia cysts in the Aral Sea). - Vestnik Karakalpakskogo otdeleniya AN RUz, Nukus, 2012, 1. - pp. 62-64 (in Russian).
15. Triantaphyllidis, GV, Abatzopoulos, TJ, Miasa, E., Sorgeloos, P. International study on Artemia population from Namibia and Madagascar; cytogenetics, biometry, hatching characteristics and fatty acid profiles. – Hydrobiologia, 1996, 335. – p. 97-106.
16. Triantaphyllidis, GV, Abatzopoulos, TJ, Sorgeloos, P. Review of the biogeography of the genus Artemia (Crustacea, Anostraca). - Journal of Biogeography, 1998, 25. - p. 213-226.
17. Van Stappen, G. Zoogeography. In: Artemia Basic and Applied Biology. (eds. TJ Abatzopoulos, JA Beardmore, JS Clegg, P. Sorgeloos), Kluwer Academic Publishers, Dordrecht, 2002, pp. 171-224.
18. Vanhaecke, P., Steyaert, H., Sorgeloos, P. International Study on Artemia. III. The use of Coulter equipment for the biometrical analysis of Artemia cysts. Methodology and mathematics. In: The Brine Shrimp Artemia. Vol. 1. Morphology, genetics, radiobiology, toxicology. (eds. G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers), Universa Press, Wetteren, Belgium, 1980, pp. 107-115.
19. Vanhaecke, P., Sorgeloos, P. 1980. International study on Artemia IV, The Biometrics of Artemia strains from different geographical origin. In: The Brine Shrimp Artemia. Vol. 3, (eds. eds. G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers), Universa press, Wetteren, Belgium, pp. 393-405.
20. Van Stappen, G. Zoogeography. In: Artemia Basic and Applied Biology. (eds. TJ Abatzopoulos, JA Beardmore, JS Clegg, P. Sorgeloos), Kluwer Academic Publishers, Dordrecht, 2002, pp. 171-224.

