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INTRA- AND INTERPOPULATION VARIABILITY OF CYSTS AND ADULTS OF *ARTEMIA* (BRANCHIOPODA: ANOSTRACA) IN SIBERIAN POPULATIONS (MORPHOMETRY)

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The size of *Artemia* cysts is an important indicator of their value as a food resource; to some extent, it allows to identify populations. The data on cysts of *Artemia* parthenogenetic populations (diameter, chorion thickness, and presence of spots on a shell), sampled in hyperhaline lakes of Western Siberia in different years, were analyzed, as well as the data on the morphometry of *Artemia* adults, reared from cysts at the same salinity. Significant intra- and interpopulation variability in the indicators was established. The absolute values of the cyst diameter were in the range of 210–330 µm, the mean values for the samples – 243.5–282.9 µm, the mean values for populations – 257.8–279.6 µm; the absolute values of the decapsulated cyst diameter were in the range of 196–294 µm, the mean values for the samples – 236.5–262.6 µm, the mean values for populations – 239.9–253.2 µm; the absolute values of the cyst chorion thickness were in the range of 3.3–16.9 µm, and the mean values for populations – 6.6–12.4 µm. In the main commercial fishing lakes, which account for about 70 % of the total catch of *Artemia* cysts in Russia, the cysts had similar mean population sizes: 262–268 µm. The absence of intrapopulation anchoring of the cyst diameter and chorion thickness values is concluded; so, they cannot serve as reliable indicators, identifying Siberian populations. Statistically significant correlation ($r = -0.5$) was established between salinity of a mother water body and *Artemia* embryo diameter. Cyst spotting, not exceeding 5 % in almost all the samples, reached 24 % in cysts of Kuchukskoye Lake. Analysis of morphometric indicators of *Artemia* adults, reared from cysts, showed as follows: the mean body length (9.27–11.63 mm), abdomen width (0.53–0.69 mm), and distance between eyes (1.36–1.52 mm) were closely correlated with salinity of a water body (*r* values were of –0.76; –0.62; and –0.67, respectively). Cluster analysis of a set of morphometric indicators of *Artemia* adults showed the unification of populations based on salinity.

Keywords: *Artemia parthenogenetica*, cyst diameter, morphometry of *Artemia* adults, chorion thickness, salinity, population variability, Western Siberia

Out of seven *Artemia* species, living on the planet, three are found in Russia: in Crimean water bodies – *Artemia salina* (Linnaeus, 1758) and *Artemia urmiana* Günther, 1899; in Tuva (Svatikovo Lake) – *Artemia sinica* Cai, 1989 (Litvinenko et al., 2009, 2016 ; Shadrin et al., 2012 ; Shadrin & Anufriieva, 2012). In Russia, most populations belong to the group of those, unspecified to species level and reproducing parthenogenetically – *Artemia parthenogenetica* Bowen & Sterling, 1978. The need to identify commercial samples of *Artemia* cysts was highlighted as early as 2004 (Xiaomei et al., 2004). By that time, various *Artemia* species and geographical races began to enter the world market, both from the American continent and Asia (Western Siberia, Kazakhstan, Turkmenistan, and China). These cysts differed significantly in some characteristics from cysts of *Artemia franciscana* Kellog, 1906 from Great Salt Lake (USA), which are considered "standard" in aquaculture practice (Xiaomei et al., 2004). It concerned the peculiarities of diapause, chorion color, cyst and nauplius size, content of highly unsaturated fatty acids, buoyancy, hatching ratio and rate, *etc.* Later, due to illegal, unreported, and unregulated fishing, it became necessary to identify parthenogenetic populations in Russia. Numerous publications appeared (Egorkina et al., 2008 ; Litvinenko et al., 2018 ; Starovoitova & Burmistrova, 2017 ; etc.), which focused either on significant interpopulation differences in cyst diameter, allowing to identify populations, or on instability of this indicator in the same population in different years. A common drawback of these works was a small number of studied populations in different years.

In this regard, we were faced with the aim to study intra- and interpopulation variability of *Artemia* cysts, using an extended data series, and, on the basis of the results obtained, consider possible ways of identifying *Artemia* population by the cyst diameter, chorion thickness, chorion structure, and size of *Artemia* adults, reared from cysts up to mature stage.

MATERIAL AND METHODS

The cysts of *Artemia* parthenogenetic populations from 13 hyperhaline lakes of Western Siberia were studied. These lakes are as follows: Bolshoye Medvezhye (in the years of sampling, salinity was of 168–293 $g \cdot L^{-1}$), Maloye Medvezhye (164–321), Gashkovo (132–164), Nevidim (112–175), Sorochie (213–263), Aktoban (83–190), Siverga (74–86), Ulzhay (105–235), Ebeyty (135–344), Bolshoye Yarovoye (147–155), Maloye Yarovoye (201), Kuchukskoye (240), and Kulundinskoye (80 $g \cdot L^{-1}$) (Fig. 1). Cyst samples were collected in 2008–2019. The analysis was carried out by cyst diameter (43 samples), chorion thickness (31 samples), and morphometry of *Artemia* adults (7 samples).

Before measuring the diameter, cysts were placed in NaCl solution (10 $g \cdot L^{-1}$), with 1 % Lugol's iodine added, for 2 hours; then, they were filtered and placed in a Petri dish with 1 % Lugol's iodine and left in the dark for 12 hours. The next day, using an Altami SM0870 stereoscope, the diameter of hydrated cysts was measured (300 cysts from each sample).

To determine the chorion thickness, first, the diameter of hydrated cysts was measured; then, sodium hypochlorite (5 % of active chlorine) was added to remove cyst shell; 10–15 minutes later, embryo diameter was measured. The sample was of 100 cysts in each population. The chorion thickness (T_{ch}) was determined by the formula:

$$T_{ch} = (d_c - d_e)/2 \,, \qquad (1)$$

where d_c is cyst diameter;

d_e is embryo diameter.

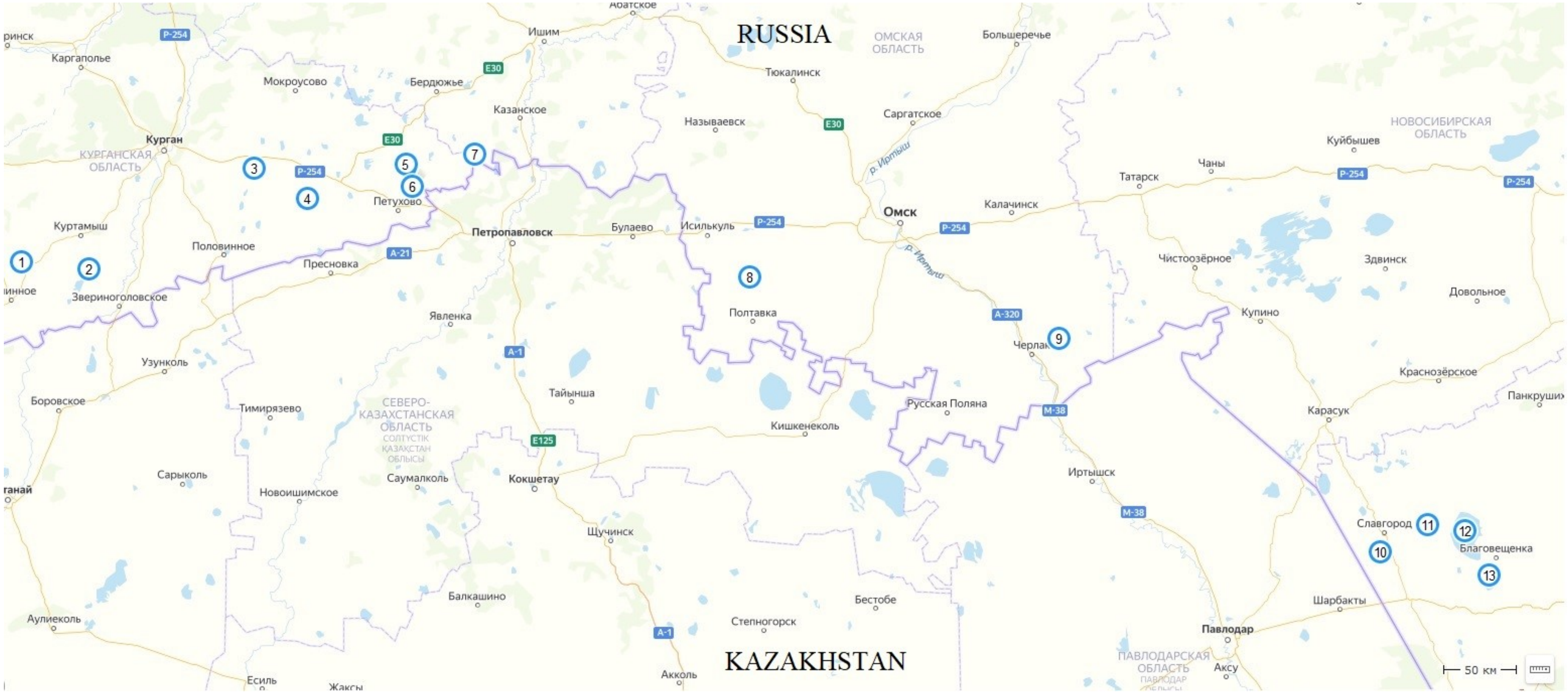


Fig. 1. Location of hyperhaline lakes (sources of *Artemia* cysts studied): 1 – Sorochie; 2 – Gashkovo; 3 – Aktoban; 4 – Nevidim; 5 – Maloye Medvezhye; 6 – Bolshoye Medvezhye; 7 – Siverga; 8 – Ebeyty; 9 – Ulzhay; 10 – Bolshoye Yarovoye; 11 – Maloye Yarovoye; 12 – Kulundinskoye; and 13 – Kuchukskoye

To breed *Artemia* nauplii, cysts were incubated in conical vessels under artificial light (1000 lux), at the temperature of +23…+25 °C, aeration, and in a saline solution (sea salt – 20 g·L^{-1}, $NaHCO_3$ – 2 g·L^{-1}) for 24 hours. Cultivation of *Artemia* adults was carried out in glass 2-L flasks at +23…+25 °C, aeration, and salinity of 100 g·L^{-1} (in salt solution: sea salt – 55 g·L^{-1}, NaCl – 15 g·L^{-1}, $MgSO_4$ – 11.9 g·L^{-1}, $NaHCO_3$ – 10 g·L^{-1}, $CaCl_2$ – 4.5 g·L^{-1}, and KCl – 3.6 g·L^{-1}). *Artemia* adults were fed with rice flour and *Scenedesmus* sp. suspension. Nauplii stocking density was of 100 ind.·L^{-1}; as *Artemia* grew, the density was reduced to 10–20 ind.·L^{-1}.

Artemia adults, that reached maturity, were fixed with Lugol's iodine and measured under an MBS-10 stereoscopic microscope with ocular micrometer. Out of each population, 10–30 *Artemia* adults were measured. In total, nine indicators were analyzed: eight plastic (body length, tl; abdomen length, al; abdomen width, aw; distance between eyes, de; eye diameter, ed; furka length, fl; length of the first antenna, la; and head width, hw) and one meristic (number of setae on furka, sf).

Statistical data processing was carried out by generally accepted methods (Lakin, 1990). Intrapopulation variability (cyst samples from one lake in different years and different dates of the same year) and interpopulation variability (cyst samples from different lakes) were analyzed by the main statistical indicators of quantitative variability: arithmetic mean (*M*), minimum (*min*) and maximum (*max*) values, arithmetic mean error (*m*), coefficient of variation (*CV*, %), standard deviation (*SD*), and sample size (*n*). Correlation coefficient (*r*) was calculated between salinity of a mother water body (source of cysts) and morphometric indicators of *Artemia* cysts and adults. The significance of the differences between the samples was assessed by the Student's *t*-test (t_{st}) at a significance level of $p \leq 0.05$ and $p \leq 0.001$. Cluster analysis was performed by Statistica 13.3 software.

RESULTS

1. Morphometry of cysts.

Diameter. In the samples studied, the absolute values of the cyst diameter were in the range of 210–330 µm, the mean values for the samples – 243.5–282.9 µm, and the mean values for populations – 257.8–279.6 µm (Table 1, Fig. 2). The largest cyst diameter was registered in the populations of Maloye Yarovoye [(279.6 ± 0.8) µm], Kuchukskoye [(278.9 ± 0.8) µm], and Aktoban [(275.9 ± 7.0) µm] lakes. The cysts in the populations of Nevidim and Ulzhay lakes were somewhat smaller: (273.3 ± 1.4) and (272.9 ± 2.1) µm, respectively. The smallest cysts were recorded in the populations of Gashkovo [(257.8 ± 4.5) µm] and Maloye Medvezhye [(258.4 ± 2.0) µm] lakes. Cysts from Bolshoye Yarovoye, Ebeyty, Bolshoye Medvezhye, and Kulundinskoye lakes had similar values: (261.6 ± 9.0), (265.2 ± 2.1), (265.8 ± 1.8), and (267.8 ± 0.8) µm, respectively. Consequently, cysts of the main commercial fishing lakes with an annual mean catch of 783 tons in total (Bolshoye Medvezhye – 102 tons, Ebeyty – 133, Bolshoye Yarovoye – 308, and Kulundinskoye – 240), accounting for about 70 % of the total catch in Russia, had similar sizes: 262–268 µm.

Analysis of the cyst diameter variability (Table 1) in an individual sample (*n* = 300) showed that the standard deviation of the indicator was in the range of 8.8–17.0 (mean SD_1 = 12.6), and the coefficient of variation was 3–6 % (mean CV_1 = 4.7 %).

Analysis of the cyst diameter variability (see Table 1) in an individual population (*n* = 3…7) showed that the standard deviation of the indicator was in the range of 3.5–15.7 (mean SD_2 = 7.3), and the coefficient of variation was 1.3–6.0 % (mean CV_2 = 2.7 %).

Table 1. Estimates of the cyst diameter variability in individual samples and lake populations (μm, unless otherwise stated)

Lake population	Year of sampling	Variability in samples						Intrapopulation variability					
		M_1	*min*	*max*	SD_1	CV_1, %	m_1	M_2	*min*	*max*	SD_2	CV_2, %	m_2
Gashkovo	2015	253.45	221	281	10.1	4	0.58	257,76	247	266	9.0	3.5	5.18
	2017	264.45	234	303	11.7	4	0.68						
	2018	265.95	242	287	8.8	3	0.51						
	2019	247.19	210	314	12.0	5	0.69						
Nevidim	2009	274.6	243	301	12.0	4	0.69	273.27	266	276	3.5	1.3	1.55
	2011	273.3	241	318	14.0	5	0.81						
	2012	275.23	247	303	10.8	4	0.63						
	2014	266.39	234	298	10.6	4	0.61						
	2015 (1)*	275.55	250	309	12.7	5	0.73						
	2015 (2)*	274.53	250	312	12.4	5	0.71						
Aktoban	2017	282.87	235	326	17.0	6	0.98	275.92	234	235	9.8	3.6	9.83
	2019	268.97	234	330	14.0	5	0.81						
Ebeyty	2008	270.87	226	313	13.8	5	0.8	265.16	257	271	5.6	2.1	2.28
	2011	261.84	228	298	11.6	4	0.67						
	2009	269.66	219	297	14.8	5	0.85						
	2013	256.64	223	289	13.1	5	0.76						
	2015	262.69	212	309	15.2	6	0.87						
	2017 (1)*	271.43	240	306	12.6	5	0.73						
	2017 (2)*	262.99	219	300	13.1	5	0.76						
Bolshoye Medvezhye	2008	268.83	226	298	12.7	5	0.73	265.84	260	272	4.8	1.8	1.95
	2009	264.47	228	298	13.8	5	0.79						
	2010	261.48	219	300	12.9	5	0.75						
	2011	263.63	226	294	12.5	5	0.72						
	2012	259.60	216	326	13.9	5	0.8						
	2014	272.01	237	302	12.4	5	0.71						
	2017	270.88	219	295	12.0	4	0.69						
Maloye Medvezhye	2009	257.10	221	291	12.9	5	0.75	258.43	253	265	4.9	1.9	2.19
	2011	252.57	216	292	13.2	5	0.76						
	2013	253.55	212	296	12.3	5	0.71						
	2014	261.49	227	286	11.4	4	0.66						
	2015	260.87	217	289	12.0	5	0.69						
	2017	265.11	215	288	11.4	4	0.66						
Ulzhay	2009	274.96	226	306	12.8	5	0.74	272.88	267	280	4.8	1.7	2.39
	2012	270.98	227	314	12.0	4	0.69						
	2013	267.39	236	295	9.6	4	0.55						
	2015	271.12	233	304	12.1	4	0.70						
	2018	279.95	239	315	13.6	5	0.78						

Continue on the next page…

Lake population	Year of sampling	Variability in samples						Intrapopulation variability					
		M_1	*min*	*max*	SD_1	CV_1, %	m_1	M_2	*min*	*max*	SD_2	CV_2, %	m_2
Bolshoye Yarovoye	2014	269.48	215	302	13.7	5	0.79	261.55	244	272	15.7	6.0	11.0
	2017	243.52	212	289	10.7	4	0.62						
	2018	271.66	231	309	13.7	5	0.79						
Maloye Yarovoye	2017	279.55	240	309	13.0	5	0.75	279.55					
Kuchukskoye	2017	278.89	236	314	13.5	5	0.78	278.89					
Kulundinskoye	2018	267.76	219	297	13.7	5	0.79	267.76					
In total:													
mean		266.02	227.44	301.95	12.58	4.7	0.73	268.82	253.5	267.1	7.3	2.7	4.54
minimum		243.52	210	281	8.8	3.0	0.51	257.76	234	235	3.5	1.3	1.55
maximum		282.87	250	330	17.0	6.0	0.98	279.55	267	280	15.7	6.0	11.0

Note: * – samples were taken in the same year with an interval of one month.

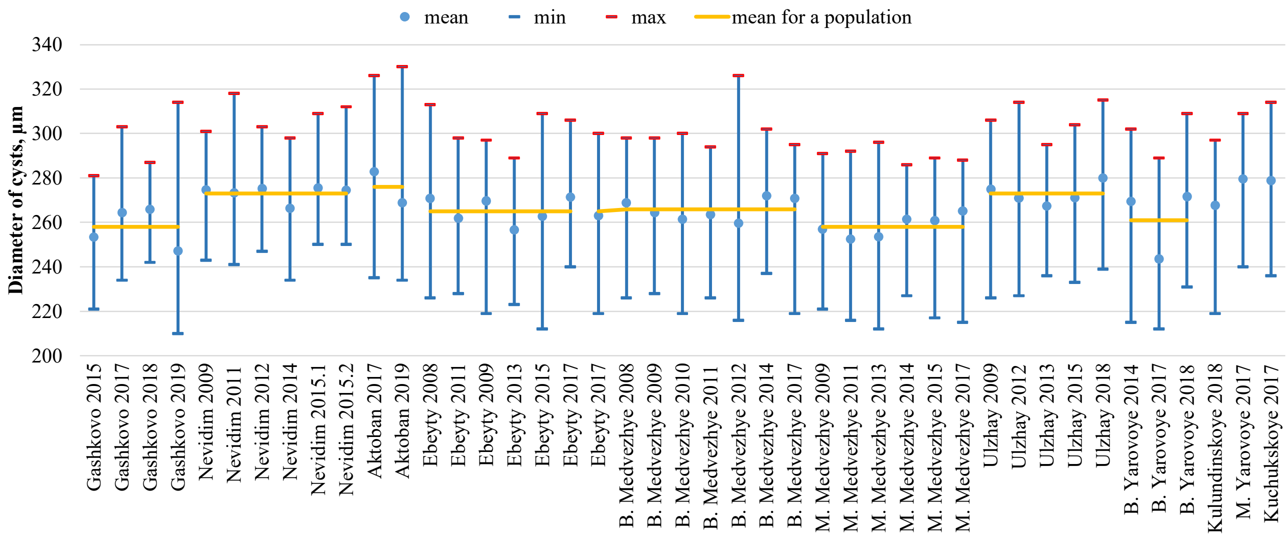


Fig. 2. Diameter of *Artemia* cysts from 11 lakes of Western Siberia

Comparative analysis of the data on interpopulation variability of the Siberian populations studied (n = 11) (M_3 within 258–280 μm; $M_3 \pm m_3$ = 268.82 ± 2.47; SD_3 = 7.8; CV_3 = 2.9 %) with the data of intrapopulation variability, *inter alia* variability of individual samples, showed that the variability in the indicators is ranked as follows: $SD_1 > SD_3 > SD_2$ and $CV_1 > CV_3 > CV_2$.

The results of our study show that the variability in the mean values of the cyst diameter between populations is somewhat higher than in an individual population in different years and much lower than in an individual sample.

The analysis of the significance of the differences between all the samples (43 cyst samples) showed as follows: 78 % of the compared pairs have differences at a significance level $p \leq 0.001$, and 86 % of the compared pairs – at a significance level $p \leq 0.05$.

The analysis of the significance of intrapopulation differences showed that cysts from Nevidim Lake differ the least of all (only the sample of 2014 stood out). In other populations, the ratio of significantly different samples of different years was, at a high significance level, within 62–100 % (on average 75 %), which is close to the comparison estimates for the entire dataset.

Cluster analysis of the data similarity by the cyst diameter (Fig. 3) showed that the same populations in different years could be included in different clusters. There were no differences on the territorial basis either. The populations of Altai lakes, the most distant from other ones, were present in all the clusters, except for D. Cysts from Nevidim (2015) and Ebeyty (2017) lakes, sampled with an interval of one month (see Table 1), got into different clusters on the dendrogram.

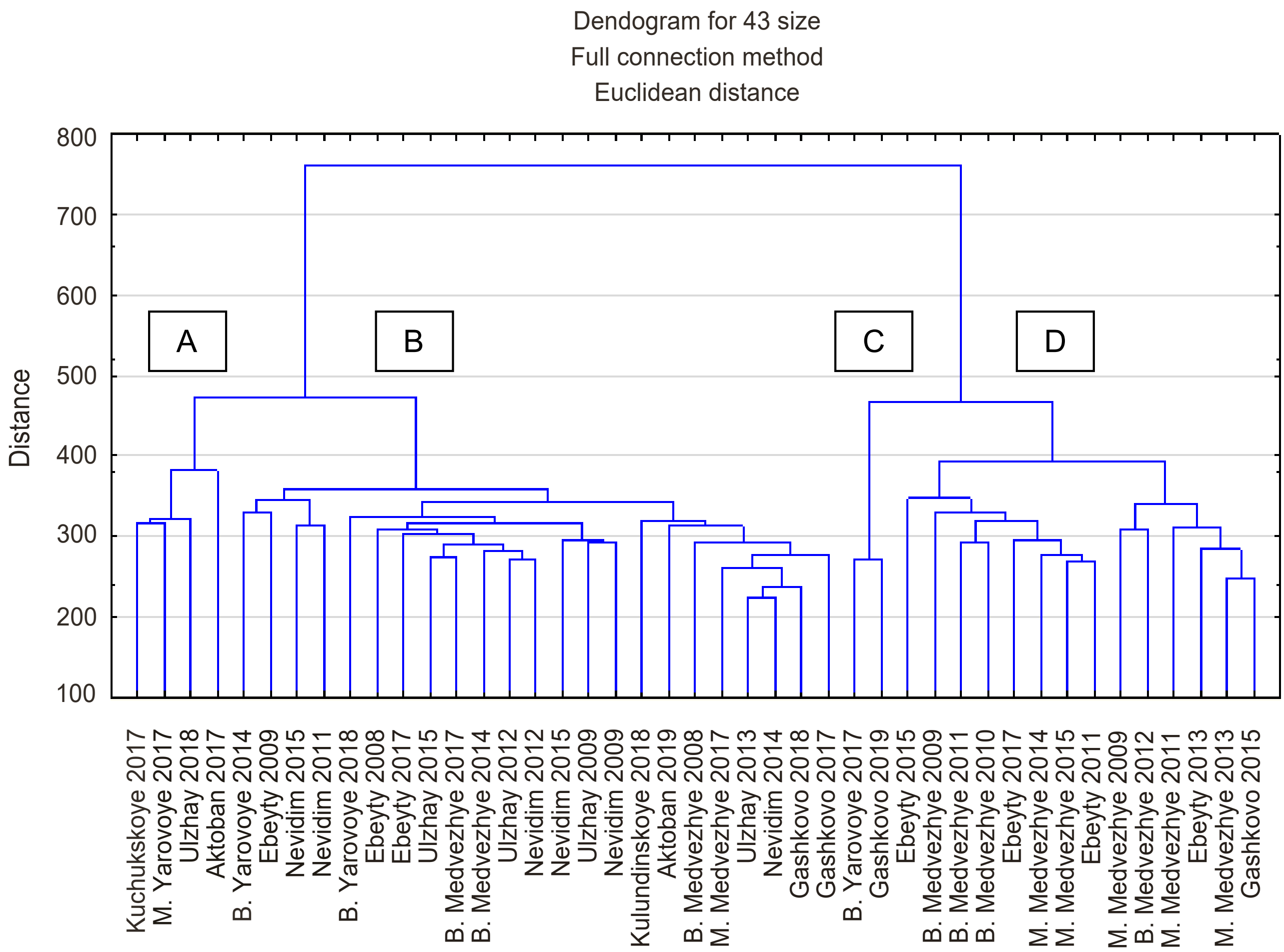


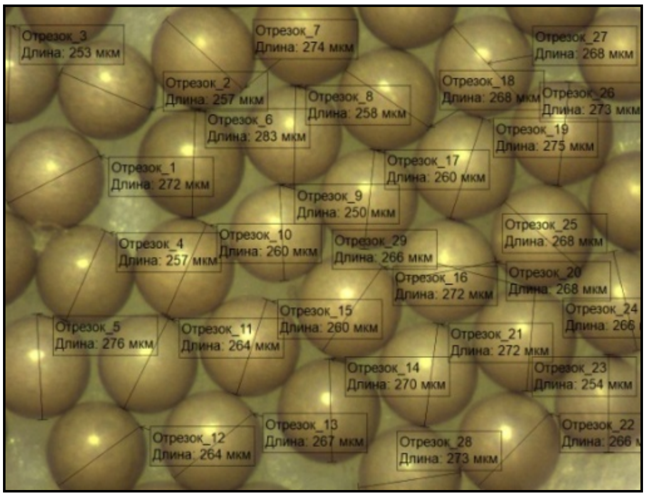
Fig. 3. Dendrogram of cluster analysis by the diameter of *Artemia* cysts of the populations studied

From the photographs (Fig. 4), a conclusion can be drawn about a morphological non-uniformity of cysts in size, both in different *Artemia* populations and in the same population in different years.

Bolshoye Yarovoye, 2017, (243.52 ± 0.62) µm

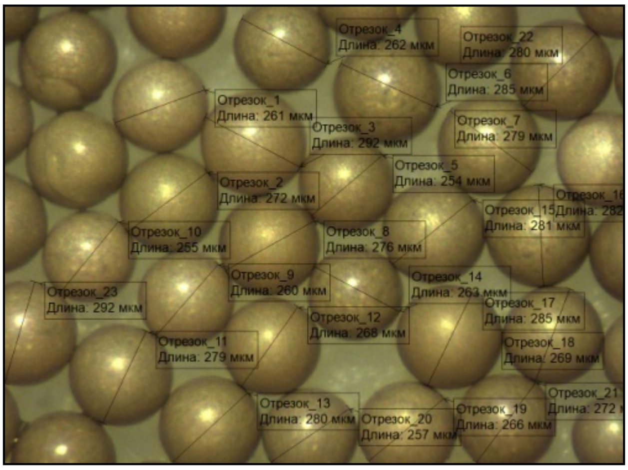
Bolshoye Yarovoye, 2018, (271.66 ± 0.79) µm

Gashkovo, 2019, (247.19 ± 0.69) µm



Gashkovo, 2017, (264.45 ± 0.68) µm

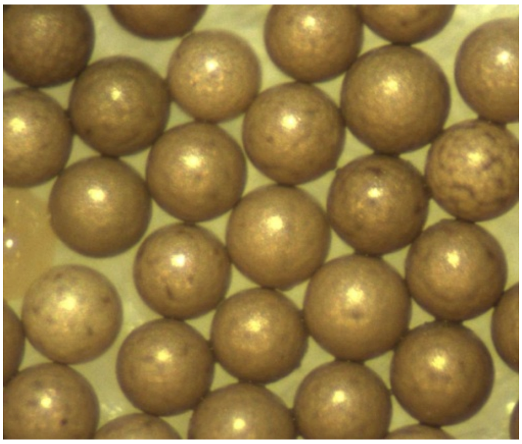
Bolshoye Medvezhye, 2012, (259.60 ± 0.80) µm



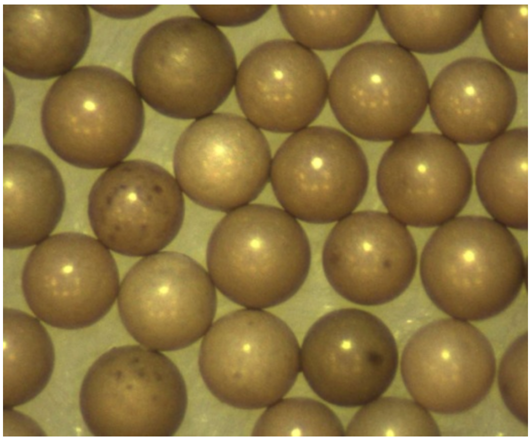
Bolshoye Medvezhye, 2014, (272.01 ± 0.71) µm

Fig. 4. Photos of cysts of the populations, studied in different years, with the greatest size variability (отрезок is segment; длина is length, µm)

Cyst spotting. Cysts with dark spots (Fig. 5) were rare in most populations; their ratio generally did not exceed 5 % and reached 24 % in Kuchukskoye Lake only (Fig. 6). This indicator can probably be used as one of the identifying for *Artemia* population of Kuchukskoye Lake.



Kuchukskoye, 2017, (278.89 ± 0.78) μm, 24 % of cysts with spots



Maloye Yarovoye, 2017, (279.55 ± 0.75) μm, 4 % of cysts with spots

Fig. 5. Cysts with spots on a shell

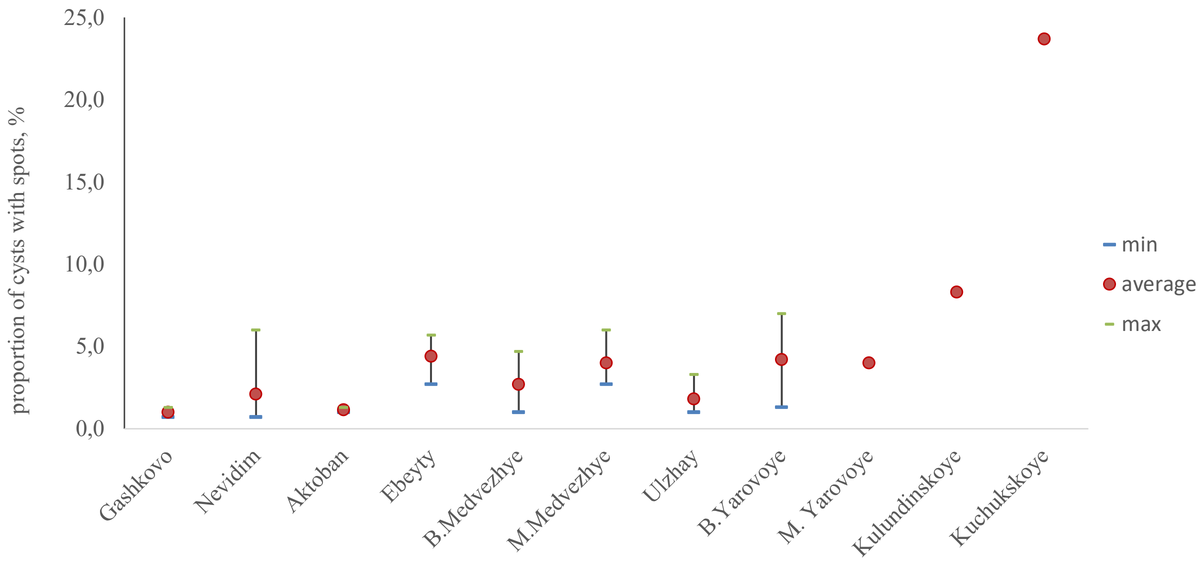


Fig. 6. Ratio of cysts with spots in *Artemia* populations, studied in different years

Embryo (decapsulated cyst) diameter. The absolute values of the embryo diameter were in the range of 196–294 μm (Table 2). The largest embryos were registered in the populations of Ulzhay (253 μm) and Nevidim (249 μm) lakes; medium-sized ones were recorded in Ebeyty, Bolshoye Medvezhye, and Maloye Medvezhye (244–245 μm) lakes; and the smallest ones were found in Lake Gashkovo (240 μm).

The analysis of the embryo diameter variability in an individual sample (n = 100) showed that the standard deviation of the indicator was in the range of 9.3–19.1 (mean SD_1 = 13.3), and the coefficient of variation was 3.9–7.9 % (mean CV_1 = 5.4 %).

Table 2. Limit and mean values of the decapsulated cyst (embryo) diameters in the populations studied and their variability

Lake	Years of sampling	Variability in the decapsulated cyst diameter (μm)		
		in the sample, absolute values	intrapopulation, mean values by years	interpopulation, mean values for populations
Ulzhay	2009, 2012, 2013, 2015, 2017	196–294	245.1–262.6	253.2
Ebeyty	2008, 2009, 2011, 2013, 2015	210–280	238.7–251.0	243.9
Bolshoye Medvezhye	2008, 2009, 2010, 2011, 2012, 2014	210–294	240.8–246.1	243.7
Maloye Medvezhye	2009, 2011, 2013, 2014, 2015 (1)*, 2015 (2)*, 2017	210–280	237.0–250.0	245.2
Nevidim	2009, 2011, 2012, 2014, 2015	210–280	244.4–255.6	249.0
Gashkovo	2015, 2017, 2018	196–266	236.5–242.3	239.9

Note: * – see explanation to Table 1.

The analysis of intrapopulation variability in the embryo diameter in an individual population (n = 3…7) showed that the standard deviation of the indicator was in the range of 2.3–6.3 (mean SD_2 = 4.4), and the coefficient of variation was 0.9–2.5 % (mean CV_2 = 1.8 %).

The analysis of interpopulation variability in the embryo diameter of the populations studied (n = 6) showed that SD_3 = 4.7 and CV_3 = 1.9 %.

Comparative analysis of the above data showed that the variability in the indicators is ranked as follows: $SD_1 > SD_3 > SD_2$ and $CV_1 > CV_3 > CV_2$.

According to the results of the study, the variability in the embryo sizes in individual samples is maximum, and the interpopulation variability in the mean values exceeds the intrapopulation one.

Statistically significant differences were recorded for 87 % of the compared pairs of the mean values for populations; differences were insignificant for the pairs Ebeyty – Bolshoye Medvezhye and Ebeyty – Maloye Medvezhye only (at $p \leq 0.05$).

Thus, to identify the populations, it is possible to use the embryo diameters, the interpopulation variability in which, as in the case with the cyst diameter, exceeds the intrapopulation one. It should be borne in mind that the main commercial fishing lakes (Ebeyty, Bolshoye Medvezhye, and Maloye Medvezhye) are characterized by the presence of embryos of similar sizes.

Cyst chorion thickness. In *Artemia* populations studied, the absolute values of the cyst chorion thickness ranged from 3.3 μm (Ulzhay Lake, 2017) to 16.9 μm (Ebeyty Lake, 2009). When comparing the mean chorion thickness for all the populations studied, the thinnest chorion was found in the populations from Gashkovo and Ulzhay lakes [(6.56 ± 0.29) and (7.01 ± 1.22) μm, respectively], and the thickest chorion – in the cysts of Lake Ebeyty [(12.43 ± 1.13) μm]. Cysts from Bolshoye Medvezhye, Maloye Medvezhye, and Nevidim lakes had medium thickness values [(8.08 ± 0.74), (8.54 ± 0.75), and (9.56 ± 1.38) μm, respectively] (Fig. 7).

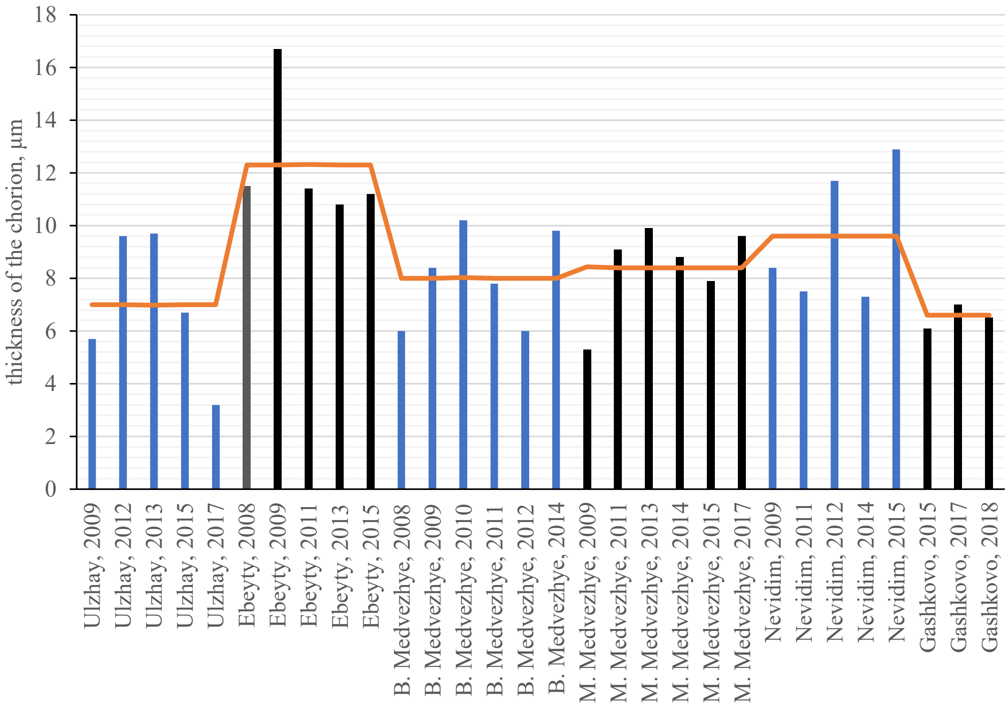


Fig. 7. Chorion thickness of *Artemia* populations studied

The intrapopulation range of the chorion thickness variability, expressed through the ratio of the maximum and minimum values in the lakes in different years, was insignificant in the population of Gashkovo Lake (1.2), average in the populations of Nevidim, Maloye Medvezhye, and Bolshoye Medvezhye lakes (1.8–2.0), and significant in the populations of Ulzhay and Ebeyty lakes (2.9–3.0).

Significant differences in the chorion thickness were established for 33 % of pairs: for Gashkovo – Maloye Medvezhye lakes and for Ebeyty with all the lakes, except for Nevidim.

Thus, of the cysts studied, the most detached in terms of several indicators are the cysts of Gashkovo Lake, as the smallest ones, with a thin chorion and small spotting, as well as the cysts of Kuchukskoye Lake, with a pronounced spotting.

Effect of salinity. It is known that salinity strongly affects *Artemia* rearing and breeding in the natural environment, and it was important to establish the degree of salinity effect of the mother water body brine on morphometry of cyst. On Fig. 8, the graphs are given of the dependence of morphometric indicators of cysts on salinity. Correlation analysis showed a weak negative correlation ($r = -0.21$) between salinity and cyst diameter and a weak positive correlation ($r = 0.25$) between salinity and chorion thickness. A statistically significant negative correlation ($r = -0.5$) was established between salinity and embryo diameter at a significance level of $p \leq 0.05$.

2. Morphometry of *Artemia* adults.

Salinity of the environment is known to be the main morpho-forming factor for *Artemia* adults. To eliminate its effect, we compared the indicators of *Artemia* adults, reared at the same salinity. In Table 3 and Fig. 9, the data are given on the morphometry of *Artemia* adults of Siberian parthenogenetic populations, reared at a salinity of 100 $g \cdot L^{-1}$. Correlation analysis of the morphometric indicators and salinity of the mother water bodies showed as follows: body length of *Artemia* adults was in a significant strong negative correlation with salinity; for abdomen width and distance between eyes, a mean negative correlation with salinity was recorded.

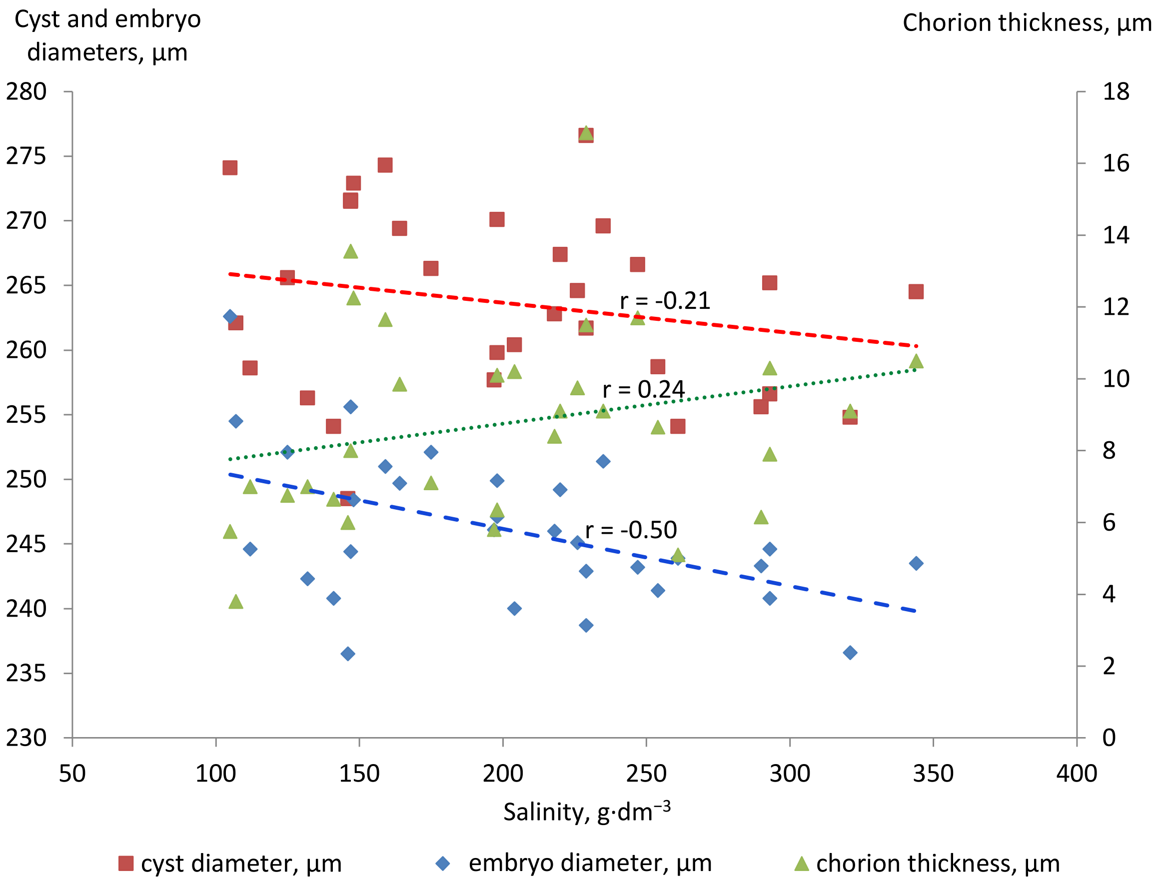


Fig. 8. Diagram of the dependence of morphometric indicators of cysts on brine salinity in a lake (source of cysts)

Table 3. Morphometric indicators of *Artemia* adults of the populations studied (mm, unless otherwise stated)

Lake, year	tl	al	aw	de	ed	sf, pcs	fl	la	hw	Σu, g·L^{-1}
Sorochie, 2009	9.46 ± 0.09	5.38 ± 0.07	0.53 ± 0.01	1.37 ± 0.02	0.24 ± 0.01	7.31 ± 0.28	0.30 ± 0.02	0.91 ± 0.02	0.64 ± 0.01	213
Sorochie, 2015	9.28 ± 0.12	5.24 ± 0.07	0.54 ± 0.01	1.36 ± 0.02	0.24 ± 0.01	7.19 ± 0.51	0.27 ± 0.01	0.96 ± 0.02	0.59 ± 0.01	263
Bolshoye Medvezhye, 2017	9.27 ± 0.64	4.43 ± 0.35	0.63 ± 0.03	1.48 ± 0.04	0.23 ± 0.02	9.0 ± 0.00	0.20 ± 0.01	0.92 ± 0.07	0.62 ± 0.02	168
Ebeyty, 2017	9.50 ± 0.31	4.96 ± 0.41	0.58 ± 0.04	1.48 ± 0.01	0.23 ± 0.01	10.6 ± 0.4	0.26 ± 0.01	0.86 ± 0.03	0.63 ± 0.03	135
Ulzhay, 2015	9.55 ± 0.26	5.02 ± 0.14	0.56 ± 0.03	1.45 ± 0.03	0.24 ± 0.01	10.7 ± 0.45	0.30 ± 0.01	0.91 ± 0.02	0.64 ± 0.03	125
Siverga, 2012	11.63 ± 0.17	5.73 ± 0.13	0.69 ± 0.02	1.52 ± 0.02	0.26 ± 0.01	7.08 ± 0.4	0.24 ± 0.03	0.87 ± 0.03	0.69 ± 0.01	86
Siverga, 2013	10.4 ± 0.14	5.46 ± 0.10	0.59 ± 0.02	1.41 ± 0.02	0.25 ± 0.01	5.33 ± 0.58	0.25 ± 0.01	0.95 ± 0.03	0.57 ± 0.01	74
Correlation with salinity, *r*	−0.76*	−0.25	−0.62	−0.67	−0.48	0.04	0.25	0.40	−0.22	

Note: * – statistically significant correlation. Abbreviations are explained in “Material and Methods” section.

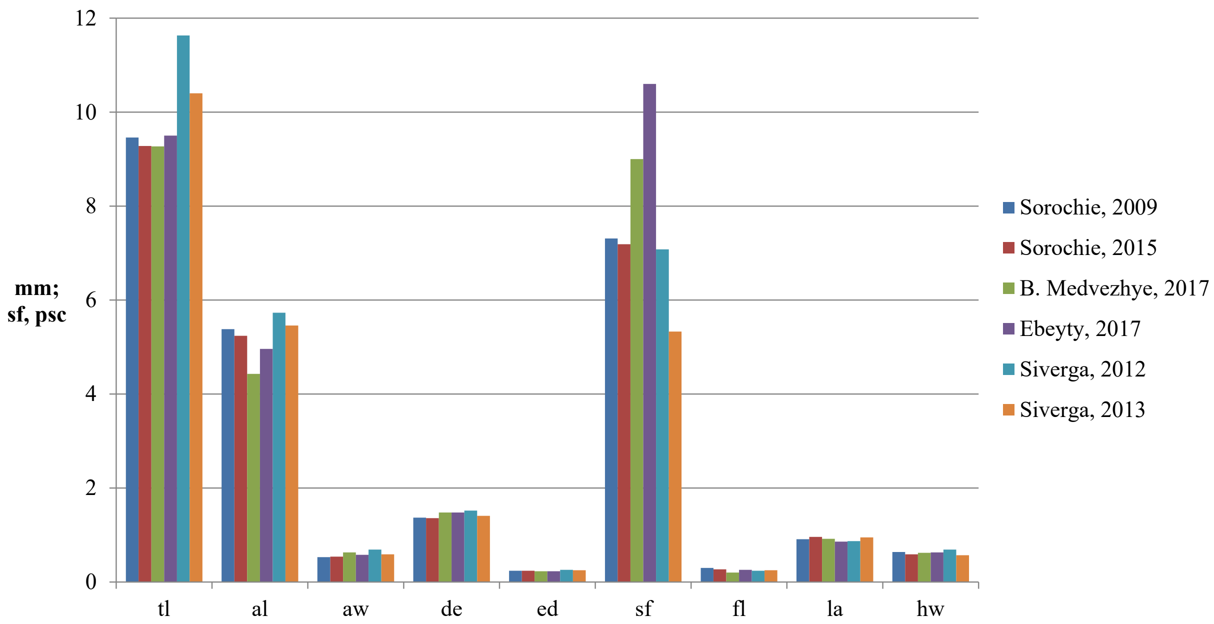


Fig. 9. Morphometric indicators of *Artemia* adults, reared from cysts of different populations

To assess the set of morphometric indicators, cluster analysis was carried out (Fig. 10). It showed the presence of a separate cluster A, formed by the population of Siverga Lake (2012 and 2013, with the lowest salinity of the natural water body: 74–86 g·L^{-1}), and cluster B, which in turn was divided into subclusters B_1 (Ulzhay Lake, 2015 and 2017, 125–135 g·L^{-1}), B_2 (Sorochie Lake, 2009 and 2015, 213–263 g·L^{-1}), and B_3 (Bolshoye Medvezhye, 2017, 168 g·L^{-1}).

Thus, *Artemia* adults, reared from cysts at the same salinity, carry information about the habitat conditions, under which they were formed.

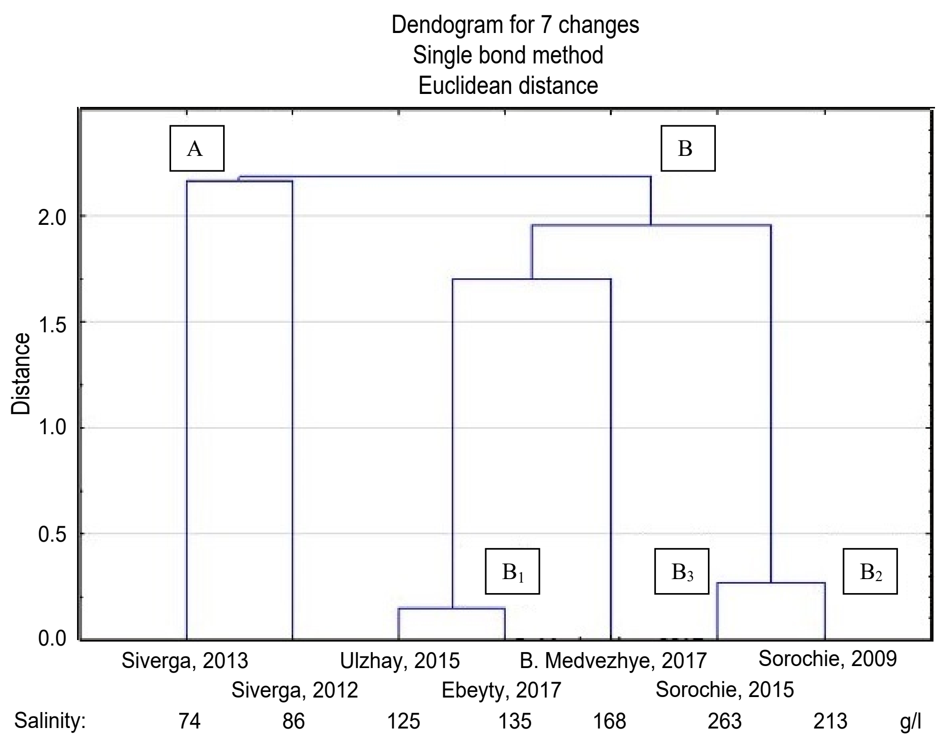


Fig. 10. Cluster analysis of morphometric data on *Artemia* adults, reared from cysts of different West Siberian populations

DISCUSSION

According to several researchers (Vanhaecke & Sorgeloos, 1980), the size of cysts and embryos, as well as chorion thickness, are the indicators, which determine the species and are basically unchanged under new environmental conditions. Practice has shown that there are difficulties both in *Artemia* species identification and in cyst population differentiation. As known (Amat, 1980 ; Asem et al., 2007 ; Camargo et al., 2005 ; Eskandari & Saygi, 2019 ; Litvinenko et al., 2016 ; Pilla & Beardmore, 1994 ; etc.), *Artemia tibetiana* Abatzopoulos, Zhang & Sorgeloos, 1998 is morphologically isolated, with the largest cyst size (up to 330 μm). The smallest cysts (on average 220–250 μm) are found in three species: *Artemia salina*, *Artemia persimilis* Piccinelli & Prosdocimi, 1968, and *Artemia franciscana* Kellog, 1906. The cysts of other species (*A. urmiana* and *A. sinica*) and parthenogenetic populations are mostly in the range of 250–280 μm. In the water bodies, studied by us, the cyst diameter varied in the range of 210–330 μm, with the mean value of 244–283 μm.

Considering *Artemia* bisexual species and parthenogenetic populations, living in Russia, there are difficulties in cyst differentiation by size due to the lack of clear divisions. According to literature sources (Anufriieva, 2014 ; Klepikov, 2012 ; Litvinenko et al., 2018, 2009 ; Solovov & Studenikina, 1990 ; Starovoitova & Burmistrova, 2017 ; Litvinenko et al., 2016), the size range of the cyst diameter absolute values fits into the gradation 220 to 290 μm, and mean for a population – 240 to 280 μm. Larger sizes for *A. parthenogenetica* populations were recorded in Bolshoye Yashaltinskoye and Dzhama water bodies (Kalmykiya): 225 to 370 μm, on average (300 ± 30) μm (Ivanova et al., 2012). For bottom sediments of Crimean water bodies (Anufriieva, 2014), two size groups of the cysts, belonging to *A. parthenogenetica*, are given: 243 μm for diploid races and 279 μm for polyploid ones. All this indicates a genetic non-uniformity of parthenogenetic races. Analysis of the cyst diameter of 25 *Artemia* parthenogenetic populations of Siberia (Litvinenko et al., 2018) showed as follows: more than 50 % of the populations have close (on average 253–261 μm) cyst sizes to that of the main commercial fishing lakes and cannot be identified by this indicator (the differences are insignificant); 33 % of populations have smaller cysts (240–245 μm); and 14 % have larger cysts (265–278 μm). As shown in our study, in the main commercial fishing lakes (Bolshoye Medvezhye, Ebeyty, Bolshoye Yarovoye, and Kulundinskoye), which account for about 70 % of the total catch of *Artemia* cysts in Russia, cysts have similar mean population sizes: 262–268 μm.

Our study has revealed significant intrapopulation variability of cysts in different years. When compared to previously published data, the difference turned out to be even greater than in our study. Thus, according to (Starovoitova & Burmistrova, 2017), in Kulundinskoye Lake over the period 1998–2005, the mean annual cyst diameter was in the range of 233–245 μm, in Bolshoye Yarovoye Lake in 1997–2012 – 235–249 μm, and in Maloye Yarovoye Lake in 1998–2016 – 226–256 μm, that is on average 1.1 times (in some years, even 1.2 times) lower than recorded by us. Such a difference might be related not only to the variability in the cyst size in the same population in different years, but also to the choice of measurement technique. In our research, a more accurate microscopic technique was used, and a large sample (300 pcs) was studied. All this illustrates the difficulty of using the cyst diameter as an identifying indicator.

Identity of cysts of Nevidim Lake, sampled with an interval of one month in 2015, and a significant difference in the sizes of cyst of Ebeyty Lake, sampled in 2017, also raise doubts about the possibility of identifying populations, even when having a cyst bank for each water body during all the periods of commercial fishing.

Cyst spotting, not exceeding 5 % in almost all the samples, reached 24 % in cysts of Kuchukskoye Lake. This fact may be of great importance in identifying a population. This is also evidenced by the data on structural differences in the surface of cysts of Kuchukskoye Lake, revealed by scanning electron microscopy (Egorkina et al., 2008).

As known (Vanhaecke & Sorgeloos, 1980), the chorion thickness does not always depend on the cyst diameter: there are populations with a small diameter and a thick chorion. Basically, the chorion thickness is in the range of 4.7–11.2 μm (Vanhaecke & Sorgeloos, 1980), but there are populations with a smaller and greater mean thickness: 1.31–9.37 μm – in Urmia Lake (Asem et al., 2007); 3.1–13.2 μm – in lakes of Columbia (Camargo et al., 2005); 0.6–8.6 μm – in water bodies of Turkey (Eskandari & Saygi, 2019). The absolute (3.3–16.9 μm) and mean (6.56–12.43 μm) values, obtained by us, expand the range of known values of the chorion thickness upward, which is likely to be due to more severe living conditions of Siberian populations. Comparison of our data with those published earlier (Litvinenko et al., 2000) has shown that the thickness of the cyst shell in several lakes can vary significantly. Thus, the intrapopulation variability in the chorion thickness in Ebeyty Lake had a range of fluctuations from 3.9 μm in 2007 (Litvinenko et al., 2009) to 12.4 μm in 2009; in Ulzhay Lake – from 11.4 μm in 1999 (Litvinenko et al., 2000) to 3.3 μm in 2017. In other populations, fluctuations were not so significant: in Bolshoye Medvezhye and Maloye Medvezhye lakes in 1999 – 9.3 μm, in 2007 – 7.6 μm, and in 2008–2015 – in the range of 5.1–10.3 μm (on average 8.3 μm); in Nevidim Lake in 1999 – 11.6 μm, in 2007 – 8.6 μm, and in 2009–2015 – 7.0–13.5 μm (on average 9.6 μm). The data obtained show a weak fixation of this morphometric indicator for the population, probably resulting from the effect of a set of natural factors, *inter alia* salinity, on a shell thickness. Thus, for *Artemia* populations of lakes of North Kazakhstan Region, where the mean chorion thickness ranged 4.0 to 8.4 μm, with a cyst diameter of 279–307 μm, it was established as follows: with an increase in salinity, the chorion thickness decreases (Volf, 2010). In our study, a weak positive correlation between these indicators was revealed.

Morphometric polymorphism of *Artemia* adults under the effect of environmental factors, primarily salinity, was noted at the turn of the XIX–XX centuries (Gajewskaya, 1916 ; Schmankewitsch, 1875). As shown in numerous studies (Boiko et al., 2016 ; Voronov, 1979 ; Ronzhina, 2009 ; Solovov & Studenikina, 1990 ; Boyko et al., 2012 ; Litvinenko & Boiko, 2008 ; Litvinenko et al., 2016 ; etc.), the sizes of mature *Artemia* adults differ within the same species and, to a large extent, depend on salinity of natural water bodies. An analysis of the morphometry of *Artemia* adults from different water bodies of Asia, Europe, Africa, and America, reared under laboratory conditions at the same salinity (Litvinenko & Boiko, 2008), has shown as follows: the formation of the phenotype of bisexual and parthenogenetic populations depends on the genotype as well, as evidenced by the established differentiation between American and all other populations of the Old World. In our experiments with *Artemia* adults, reared at the same salinity, a clear division into clusters by populations could be explained by the influence both of the genotype and salinity of a mother water body, in which the cysts were formed.

The dendrogram of similarity (Fig. 11), based on literature data on the morphometry of *Artemia* adults of the first generation (Razova, 2019), showed as follows: the American population allocated into a separate cluster, and the B_3 subcluster (Bolshoye Medvezhye Lake, 2017) (see Fig. 10) was joined by the populations of Bolshoye Yarovoye (2016) and Bolshoye Medvezhye (2016) lakes with a similar salinity of natural brine of 150–160 $g \cdot L^{-1}$. Since the sizes of *Artemia* adults of subsequent generations tended to decrease (Razova, 2019), it can be assumed that *Artemia* phenotype, which carries information about the environment, in which cysts were formed, is manifested in the first generation and partially in the second.

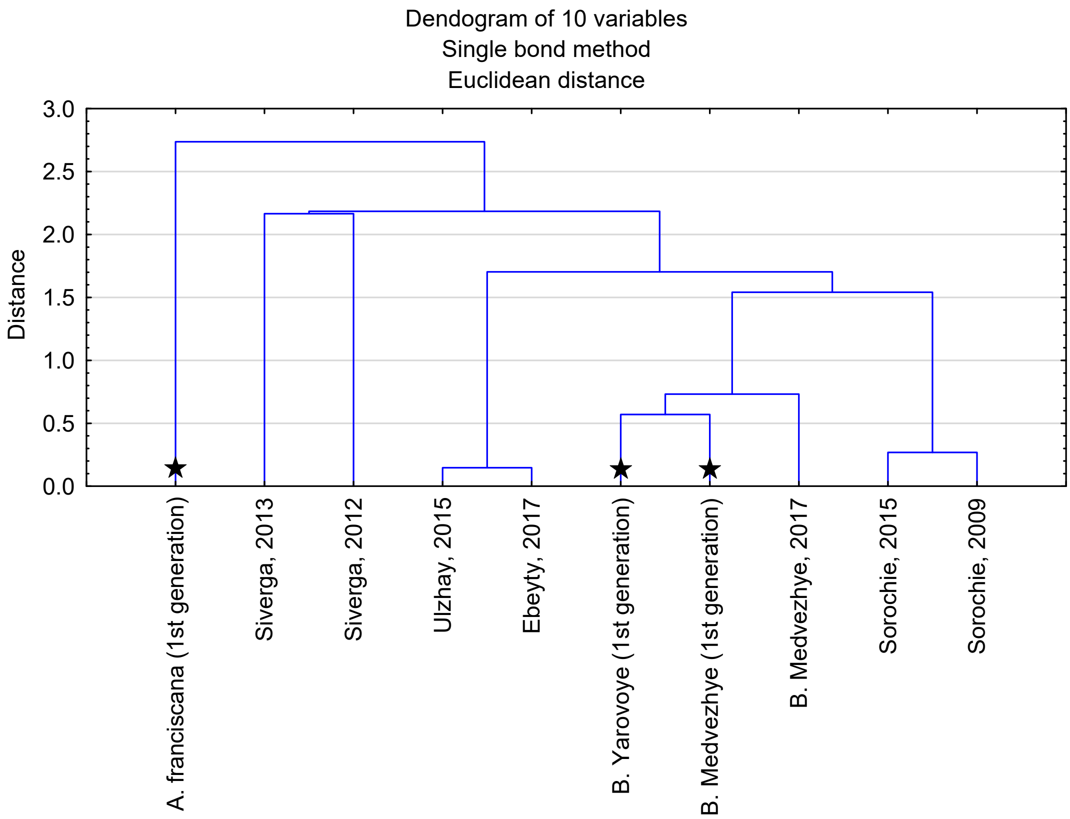


Fig. 11. Dendrogram of morphometric indicators of reared *Artemia* adults (★ – referenced literature data)

Conclusions:

1. *Artemia* cysts of Siberian populations are characterized by significant intra- and interpopulation variability in the diameter of the cysts and embryos and the chorion thickness.
2. Morphometric indicators of cysts, sampled from a water body at different dates of the same year, demonstrate seasonal variability.
3. In the main commercial fishing lakes, which account for about 70 % of the total catch of *Artemia* cysts in Russia, cysts have similar mean population sizes (262–268 μm).
4. Negative significant medium-strength correlation is established between salinity of a mother water body and the embryo diameter.
5. Significant interannual variability in the indicators of *Artemia* cysts in the same population shows that morphological characteristics of cysts cannot serve as reliable indicators, identifying a population.
6. Cyst spotting, possibly resulting from sample preparation, may further be used in differentiation of several *Artemia* populations.
7. Morphometric parameters of *Artemia* adults of the first generation, reared from cysts at the same salinity, carry information about salinity of a mother water body, which should be taken into account when identifying a population.

The work has been carried out within the framework of the state assignment of the Tyumen branch of Russian Federal Research Institute of Fisheries and Oceanography ("Gosrybtsentr") on the applied topic "Improving the fishing regulation system and increasing the efficiency of using the resources of commercial invertebrates of hyperhaline water bodies of the Russian Federation" (No. 076-00005-20-ПР).

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ВНУТРИ- И МЕЖПОПУЛЯЦИОННАЯ ИЗМЕНЧИВОСТЬ ЦИСТ И ВЗРОСЛЫХ СТАДИЙ АРТЕМИИ (BRANCHIOPODA: ANOSTRACA) В СИБИРСКИХ ПОПУЛЯЦИЯХ (МОРФОМЕТРИЯ)

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Размеры цист артемии являются важным показателем ценности их как кормового ресурса и в некоторой степени позволяют идентифицировать популяции. В статье проанализированы показатели цист артемии партеногенетических популяций (диаметр, толщина хориона, наличие пятен на оболочке), отобранных в гипергалинных озёрах Западной Сибири в разные годы, и морфометрические показатели рачков, выращенных из цист при одинаковой солёности. Установлена значительная внутри- и межпопуляционная изменчивость рассмотренных показателей. Абсолютные значения диаметра цист находились в пределах 210–330 мкм, средние значения по пробам — 243,5–282,9 мкм, средние по популяциям — 257,8–279,6 мкм; абсолютные значения диаметра декапсулированных цист — в пределах 196–294 мкм, средние значения по пробам — 236,5–262,6 мкм, средние по популяциям — 239,9–253,2 мкм; абсолютные значения толщины хориона цист — 3,3–16,9 мкм, средние значения по популяциям — 6,6–12,4 мкм. В основных промысловых озёрах, на которые приходится около 70 % от всего вылова цист артемии в России, цисты имели близкие среднепопуляционные размеры (262–268 мкм). Дано заключение об отсутствии внутрипопуляционной закреплённости таких признаков, как диаметр цист и толщина хориона, то есть они не могут служить надёжными показателями, идентифицирующими сибирские популяции. Установлена статистически значимая связь ($r = -0{,}5$) между солёностью материнского водоёма и диаметром эмбрионов артемии. Пятнистость цист, не превышающая 5 % почти во всех их пробах, у цист озера Кучукское составила 24 %. Анализ морфометрических показателей рачков, выращенных из цист, показал, что средняя длина рачков (9,27–11,63 мм), ширина абдомена (0,53–0,69 мм) и расстояние между глазами (1,36–1,52 мм) тесно коррелировали с солёностью материнского водоёма (значения r составили –0,76; –0,62; –0,67 соответственно). Кластерный анализ совокупности морфометрических признаков рачков указывает на объединение популяций по признаку солёности.

Ключевые слова: *Artemia parthenogenetica*, диаметр цист, морфометрия рачков, толщина хориона, солёность, популяционная изменчивость, Западная Сибирь