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The Results of Studying Diapausing Eggs (Cysts) of Brine Shrimp (*Artemia*) on the Bottom of Hypersaline Lakes of Altai Krai

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Abstract—The results of 2020 studies of bottom cysts of brine shrimp *Artemia* in Lake Kuchukskoe and Lake Bolshoe Yarovoe in Altai krai (Russia) are presented. *Artemia* cysts were observed year round at the bottom of both lakes. The number of cysts depended on the type of bottom sediment. The biomass of bottom cysts in Lake Kuchukskoe was 154.2 t in spring, of which available and visually intact cysts constituted from 11.8 to 25.2 t. Bottom cysts of *Artemia* were not available for 27% of the lake area in Lake Kuchukskoe due to the high salinity of the water and salt precipitation. The viability of cysts from shallow bottom areas was 8.9% in spring. In Lake Kuchukskoe, cysts from accumulations on the coast, washed away by the runoff of melt water, played the main role in the formation of first generation of *Artemia* in spring. The deep water of Lake Bolshoe Yarovoe predetermined the peculiarities of the temperature regime in spring, which prevented brine shrimp cysts from ascending from the bottom if the depth exceeded 7.0 m. Significant silt deposits at the bottom of the lake were an aggressive environment for cysts, as was followed by the presence of different groups of cyst quality: visually intact, with defective chorion, gray or black. The biomass of bottom cysts in Lake Bolshoe Yarovoe was 38 934 t in autumn 2020, which exceeded the reproductive capacity of the *Artemia* population per year. This testified to the long-term period of accumulations of cysts at the bottom and their partly participation in the formation of the *Artemia* first generation in spring. In spring, the maximum hatching rate of nauplii from bottom cysts was 16% from depths exceeding 9.0 m and 26%, from a depth of 8.0 m. The viability of bottom cysts from Lake Bolshoe Yarovoe does not exceed 15% at the average; unhatched floating cysts settle back to the bottom in summer.

Keywords: *Artemia* (brine shrimp), hypersaline lakes, types of sediments, diapausing eggs (cysts), brine shrimp cysts on the bottom, hatching

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INTRODUCTION

The diapausing thick-shelled eggs (cysts) of the crustacean *Artemia* Leach, 1819 are widely used in aquaculture as feeds for the earliest stages. The great economic demand for this bioresource has led to an increased interest in the study of the biota of hypersaline lakes; first and foremost, this is the productivity of populations of brine shrimp in different types of water bodies. The current methodology for determining the total and commercial stocks of brine shrimp (at the cyst stage) includes a number of indicators, one of which is accounting for benthic cysts (*Metodicheskie* ..., 2019). However, cysts of brine shrimp cannot be considered fully benthic organisms adapted to life on the bottom (Zhadin, 1950; Konstantinov, 1979). Therefore, in this work, the term “bottom cysts” is adopted, which means the biomass of cysts of brine shrimp on the sediment surface and in the deeper layers.

Despite the long history of studying hypersaline water bodies and their biota around the world, the peculiarities of the accumulation and storage of cysts of brine shrimp in bottom sediments, as well as their viability and hatching success, remain unexplored. A significant number of the cysts sinking to the lake bottom and a huge variation in this indicator in space and time are noted in a number of publications (Litvinenko et al., 2016, 2018, 2020; Vizer and Rostovtsev, 2016; Semik and Ushakova, 2017). From the beginning of research (2000) to the present, determining the number of cysts in sediment samples and extrapolating the average value to the entire area of the reservoir were the main study foci (*Metodicheskie* ..., 2019; Litvinenko et al., 2018). Following this approach, many features remain unexplored: the dependence of the number of cysts on the type of sediment and the depth of occurrence and the influence of the degree of development of the littoral (including the washed and flooded coast) on the distribution of the cysts (cyst stages) in the reservoir. The viability of the cysts that sunk to the lake bottom in winter and under the influence of unfavorable environmental factors is the most important indicator.

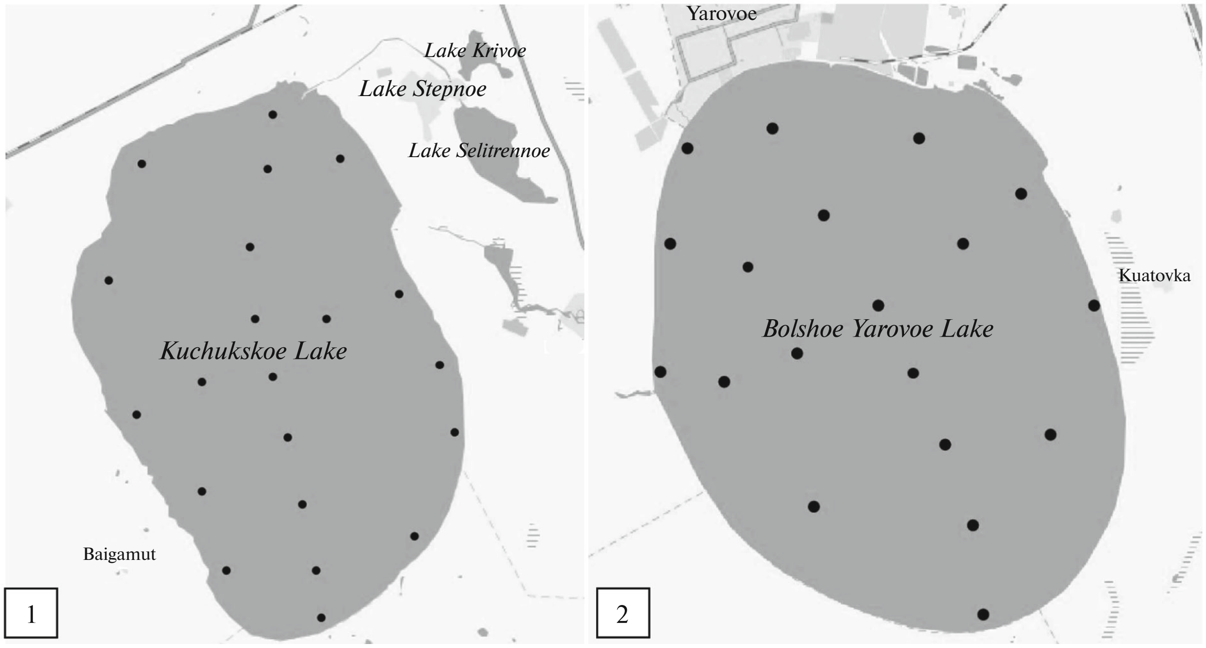

Fig. 1. Scheme of stations for sampling and phenological observations, 2020. (1) Lake Kuchukskoe; (2) Lake Bolshoe Yarovoe.

Studies of the state of the bottom cysts of brine shrimp in the hypersaline lakes of Altai krai are just beginning. Our study aims to assess the role of the bottom cysts in the formation of the first generation of brine shrimp, as well as its actual stock. In this regard, the following tasks have been set: study the sediments of various types at different depths in hypersaline reservoirs of different types, determine the number of cysts of brine shrimp in the composition of sediments of different types, and determine the viability of the bottom cysts of brine shrimp in vitro.

MATERIALS AND METHODS

The data were collected during the growing season of 2020 during the monitoring of hypersaline reservoirs in Altai krai by the example of Lakes Kuchukskoe and Bolshoe Yarovoe. Archival materials on the level of water salinity for 2010–2019 accessible in the AltaiNIRO database were also used.

In 2020, hydrobiological surveys were carried out monthly in April and June–September using a small motor vessel. In October, studies at Lake Bolshoe Yarovoe were also performed from a motor boat and at Lake Kuchukskoe from the shore in shallow areas while driving around by car, because salt precipitation was observed over the entire area of the lake. For each lake, a scheme of sampling stations was developed in the laboratory using a Navitel C 500 GPS navigator (Czech Republic) (Fig. 1). The number of stations depended on the lake shape, its area, the development coefficient of the coastline, and the diversity of biotopes (*Metodicheskie* ..., 2019).

The morphometric characteristics of the lakes, including the main coefficients (Vereshchagin, 1930; Ivanov, 1948; Bogoslovskii, 1960), were calculated using original data and remote sensing obtained from satellite maps using the Calculator for Calculating Areas, Lengths and Distances (http://3planeta). The prevailing wind directions (wind rose) were determined according to the data courtesy by the Altai Center for Hydrometeorology and Environmental Monitoring (TsGMS) obtained at the Slavgorod meteorological station.

At each station, the following measurements were performed: depth (using a marked cord on a ratchet winch), water temperature and the content of oxygen dissolved in water (using the dissolved oxygen meter with electronic thermometer Ekspert-001-4.01 (Russia)) at the surface and near the bottom, transparency of water (using a Secchi disk), and water salinity (using the refractometer Atago Master-S 28 M (Japan)) in the surface layer (at a depth of at least 0.2 m) and near the bottom.

Zooplankton and water samples for hydrochemical analyses were taken and processed in accordance with the accepted methods (*Metodicheskie* ..., 2019). In 2020, 8 water samples for hydrochemical parameters and 152 zooplankton samples at Lake Kuchukskoe and 6 water samples and 215 zooplankton samples at Lake Bolshoe Yarovoe were collected.

In order to study the bottom cysts, sediment samples from Lake Kuchukskoe were taken with a Petersen grab (capture area of 0.025 m^2; Russia) and with a tubular rod grab (DTSh, capture area of 0.005 m^2; Russia). It turned out to be impossible to use a stratification bottom grab (DChS-250, Russia) at Lake Kuchukskoe, which is characterized by significant salt deposits, due to the high density of the bottom sediments.

At Lake Bolshoe Yarovoe, bottom sediments were sampled with a Petersen grab (capture area of 0.025 m^2; Russia); in autumn, an additional stratification grab (DChS-250, capture area of 0.025 m^2; Russia) was used. At Lake Bolshoe Yarovoe, where the average depth was 4.5 m, we did not use a DTSh tubular rod grab with a rod length of 3.0 m.

The sediment type was determined and the sample was washed in a mesh bag № 46 (mesh size of 178 μm), which was supplied then with a label indicating the date of sampling, the name of the reservoir, station number, sampling depth, and the part of the sample (for DChS-250 only).

Samples were processed according to the accepted methods (Kiselev, 1969; *Instruktsiya* ..., 2000; *Metodicheskie* ..., 2019; *Manual* ..., 1986), with the following additions developed by AltaiNIRO specialists:

The samples were transported in sieve bags, in which they were washed, which made it possible to avoid the loss of soft, silty sediment, as well as to dry the sample in air.

Under laboratory conditions, the samples were stored in a refrigerator at a temperature of 4–6°C to ensure the viability of the cysts of brine shrimp contained in the sample.

Samples with a high content of sand, salt, clay, and plant detritus were weighed on an electronic balance CAS SWN-06 (South Korea) with an accuracy of 0.01 g. A 1.0-g sub-sample, weighted using an electronic scale Metler Toledo (Germany) with a 0.0001-g accuracy, was diluted with water. The objects in the sample were then counted in the Bogorov's chamber under a trinocular stereoscopic microscope Mikromed MC-2 ZOOM (Russia). Depending on the initial sample weight, from 3 to 10 subsamples were taken.

Samples with a high content of silts were washed in a sieve bag to a minimum volume, placed completely in a beaker, and diluted with water to a certain volume. The sample was thoroughly mixed; then 1 mL aliquot was taken with a pipette in 7–10 replicates and processed in a similar way.

During quantitative processing of samples, all cysts of brine shrimp were counted. Any damage, features of the surface of the chorion (structure and/or color), internal contents under pressure, or the presence of cyst shells were noted. When calculating the abundance, only visually intact cysts were taken into account.

In order to compare the data obtained by using bottom grabs of different designs at Lake Kuchukskoe, the relative abundance of the bottom cysts was used, which was the number of cysts per 1 g of sediment.

When using DChS-250 grab, the abundance of cysts per 1 m^3 was calculated, since the middle and lower sections of the bottom grab had different volumes.

A total of 80 sediment samples were taken and processed at Lake Kuchukskoe and 93 samples at Lake Bolshoe Yarovoe. The biomass of bottom cysts of brine shrimp was calculated according to the equation given in (*Metodicheskie rekomendacii* ..., 2019):

$$W_3 = N_3 S m,$$

where N_3 is the number of the cysts of brine shrimp sunk to the lake bottom, individuals/m^2; S is the lake area, m^2; m is the average cyst mass in wet tons, 1×10^{-11} t.

Calculating the hatching of nauplii from the bottom cysts caused the greatest complexity. Hatching is the complete releasing of an embryo from the cyst shells and the appearance of free-floating *crustacean larvae in plankton community* (Manual ..., 1986; Solovov and Studenikina, 1990; Van Stappen, 1996; *Metodicheskie* ..., 2019). It was necessary to study the survival of embryos in the bottom cysts under certain conditions of particular lake. Most of the developed and generally accepted methods of cyst incubation are aimed at obtaining the maximum result (Spektorova, 1984; *Manual* ..., 1986; Solovov and Studenikina, 1990; Litvinenko et al., 2016), which does not reflect hatching in the natural conditions of the reservoir.

For incubation, brine shrimp cysts were obtained by washing out a sufficient amount of them from the samples, successively separating heavy and light impurities with an aqueous solution of NaCl (220 g/kg) and fresh water.

As part of this work, the incubation of cysts of brine shrimp, collected in the spring in Lakes Kuchukskoe and Bolshoe Yarovoe, was carried out under optimal conditions described in the literature (Spektorova, 1984; *Manual* ..., 1986; Solovov and Studenikina, 1990; Klepikov, 2012; Litvinenko et al., 2016). We used cone-shaped glass cylinders, in which continuous aeration was maintained from the bottom part, the incubation temperature was 25–29°C, water salinity was 30.0–35.0 g/L, pH was about 8, illumination was 1000 Lux, and the concentration of wet cysts was 4.0 g per 1 L of incubation solution. The brine shrimp cysts, sampled in autumn period from Lake Bolshoe Yarovoe, were incubated in four hatchery solutions: (1) water NaCl solution (20–30 g/kg; hereinafter "artificial solution"), (2) natural brine with total salt concentration of 150–160 g/kg (brine), (3) natural brine diluted with fresh water by total salt concentration of 60–70 g/kg (hereinafter "0.5 brine"), and (4) natural brine diluted with fresh water by total salt concentration of 25–30 g/kg (hereinafter "0.25 brine"). Other incubation conditions were optimal (see above).

Simultaneously, cysts collected from the water column and from its surface and the bottom cysts from the upper sediment layer and from the deep silt depos-

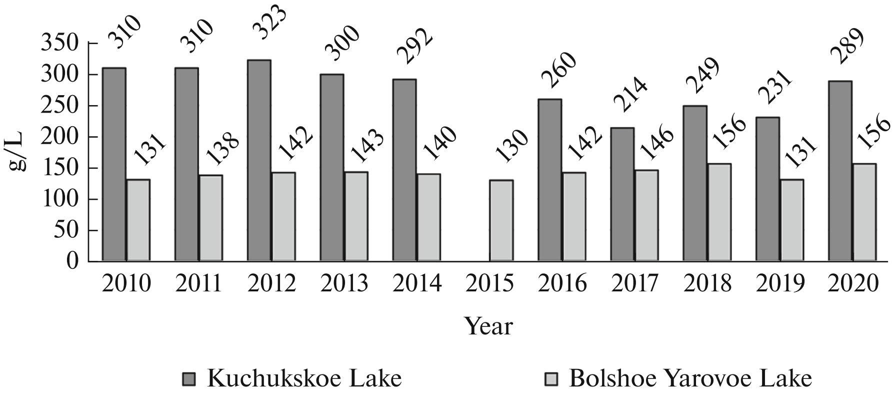

Fig. 2. Annual long-term dynamics of water salinity (g/L) in hyperhaline Lakes Kuchukskoe and Bolshoe Yarovoe, 2010–2020.

its (sediment depth exceeding 20 cm) were also incubated. In total, there were 32 glass cones in the incubator. The exposure time was 24, 48, 72, and 168 h. The 1.0-mL subsample was taken with a mechanical pipette in four replicates from each cone and fixed with an alcoholic solution of iodine. The number of nauplii, embryos, and unhatched cysts was counted under a Micromed MC -2 ZOOM trinocular microscope.

Mathematical analysis was performed in MS Excel software (2016).

RESULTS

Lakes Kuchukskoe and Bolshoe Yarovoe, studied in 2020, are located on the Kulunda Plain (*Vodoemy* …, 1999). The lakes have a round-shape basin, elongated from north to south; the shoreline development index is close to 1.0 for both lakes (Table 1). Lake Kuchukskoe is characterized as a shallow, self-sustaining reservoir with low depth and volume indices; together with the openness index, this indirectly indicates a greater mixing of water masses and the absence of differences in physicochemical parameters in the surface and near-bottom layers. This is confirmed by the water temperature and salinity homogeneity in the lake.

Lake Bolshoe Yarovoe can be classified as deepwater when compared to other hypersaline reservoirs of Russia. Compared to Lake Kuchukskoe, Lake Bolshoe Yarovoe has a significantly lower openness index and a greater depth index, which indicates less mixing of water masses. According to published (Solovov and Studenikina, 1990; Permyakova, 2012; Vesnina and Permyakova, 2013) and original data, temperature stratification is observed in the lake in spring with a thermocline located at a depth of 6.0–7.0 m. The temperature difference between the surface and bottom layers in April 2020 was 8–10°C. In summer, relatively uniform heating of the water column is observed.

In terms of water salinity, Lakes Kuchukskoe and Bolshoe Yarovoe are hypersaline (Alekin, 1970) or ultrahaline water bodies (Oksiyuk and Zhukinskii, 1993). In terms of chemical composition, the lake waters belong to the chloride-sulfate and chloride classes of the sodium group. The average annual water salinity of the solar salt Lake Kuchukskoe in 2010–2020 varied from 214 to 323 g/L; the dry residue fluctuation was 109 g/L (Fig. 2).

In hypersaline Lake Bolshoe Yarovoe, in 2010–2020, the average annual water salinity fluctuated within 26 g/L due to the total water volume, which made it possible to characterize the hydrochemical regime of this lake as more stable.

All mineralized water bodies of Altai Krai are characterized by seasonal changes in water salinity with a minimum value observed in the spring. In Lake Kuchukskoe, a significant increase of salt content in brine is observed during the period of greatest insolation (June–July); the seasonal fluctuation of salinity was 71 g/L in 2010–2020 (Fig. 3). In Lake Bolshoe Yarovoe, the trend is gentler; the water salinity from spring to autumn increases by an average of 18 g/L, generally characterized by greater stability.

The lakes experience a significant anthropogenic load. The brine of Lake Kuchukskoe serves as a raw material for the salt extraction. Once every 2 years, a significant amount of water from the lake is pumped into an evaporator pond (Vesnina and Permyakova, 2011). The city of Yarovoe, with enterprises and a housing and communal complex, is located on the shores of Lake Bolshoe Yarovoe; the city takes water from the lake and discharges wastewater. The lakes are used for recreational purposes with public beaches and undeveloped beaches. The extraction of brine shrimp (at the cyst stage) is also an important factor of the anthropogenic impact.

The stability of the development of populations of brine shrimp and the regularity of industrial fishing in relation to brine shrimp (at the stage of cysts) are predetermined both by abiotic and biotic conditions.

Table 1. Main characteristics of the studied hyperhaline lakes of Altai Krai, 2020

Indicator	Lake/Name of the lake	
	Kuchukskoe Lake	Bolshoe Yarovoe Lake
Area, km^2	175.7	73.7
Length of the lake, km	19.6	11.5
Maximum width of the lake, km	12.5	8.0
Shoreline development coefficient	1.10	1.03
Maximum depth, m	3.3	9.5
Average depth, m	0.9	4.5
The coefficient of volume	0.27	0.47
The coefficient of openness	195.2	16.3
The coefficient of depth	0.16	1.07
Coast	Gentle waterlogged (70%), high steep (30%)	Steep and eroded (83%), gentle and sandy (17%)
Nutrition of the lake	Precipitation nutrition, Kuchuk River and Solonovka stream flow into	Precipitation and ground nutrition
The nature of salt accumulation	Solar salt lake	Brine lake
Limits (max–min) of average annual salinity (2010–2020), g/L Maximum–minimum average annual salinity (2010–2020), g/L	214–323	130–156
Limits (max–min) of seasonal salinity (2010–2020), g/L Maximum–minimum seasonal salinity (2010–2020), g/L	231–302	128–146
pH	7.71	7.89
Category of economic use	High, conditionally commercial	High, commercial
Temperature regime	Temperature stagnation	Spring stratification with thermocline at a 6.0–7.0 m depth, stagnation in summer

Lake Bolshoe Yarovoe is a commercial water body with a regular use of the raw material base; Lake Kuchukskoe is conditionally commercial (*Metodicheskie* ..., 2019). Previously, three categories of economic use of hypersaline lakes were distinguished (Permyakova and Vesnina, 2009); at present, all water bodies in which *Artemia* lives are in the highest category.

Lake Kuchukskoe In April (April 26, 2020), the sediments were composed of medium and fine sand with silt and plant residues (detritus) at depths reaching 1.5 m. Shallow areas were marshy, with a significant layer of silt deposits (black and gray silts). At depths exceeding 1.5 m, the bottom of the reservoir was covered with salt sediment, which did not dissolve even during the spring desalination of the reservoir.

In sediment samples, there were visually intact cysts (65.4% of the total number) and eggs with damaged chorion or missing it (29.8%). We also encountered cysts with a gray chorion (4.8%), which were confined to silty sediments.

In April, the average abundance of the cysts at the lake bottom was 86.7 ± 48.85 thous. ind./m^2 (C_v = 251.9%). The cysts were absent at 65% of the stations (central part of the lake); in shallow waters, the abundance ranged from 22.08 to 936.72 thous. ind./m^2, averaging 102.8 ± 54.50 thous. ind./m^2 or 9.4 ± 3.25 ind./g (Table 2). At the same time, the number of cysts in the water column was 345.4 ± 170.37 thous. ind./m^3 on average for the water area; at the stations on the southern side of the lake, clusters with a density of up to 4210.0 thous. ind./m^3 were observed. Hatching and development of the nauplii of the first generation was more intense in shallow areas in the southwestern part of the water area, where their abundance ranged from 12.3 to 60.5 thous. ind./m^3. In the central part of the lake, the nauplii abundance did not exceed 1.0 thous. ind./m^3. Therefore, bottom cysts covered by a layer of salt sediments remained inaccessible for

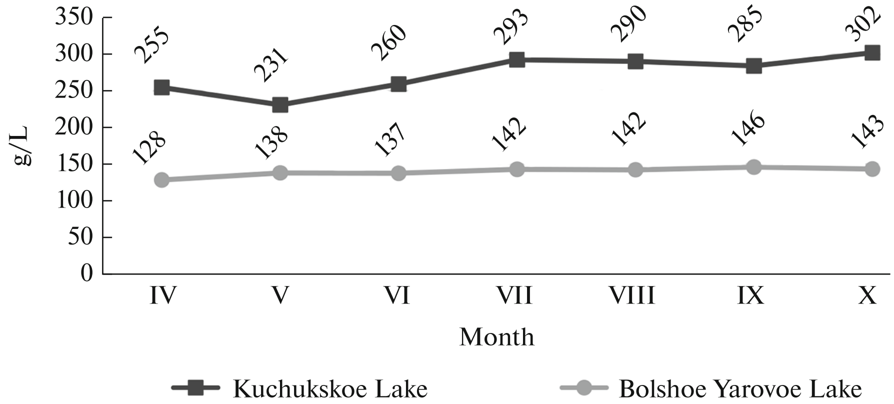

Fig. 3. Average monthly dynamics of water salinity (g/L) in Lakes Kuchukskoe and Bolshoe Yarovoe, 2010–2020.

ascending, so accumulations of cysts on the shore and in shallow areas washed away by melt water into the lake played the main role in the formation of the first generation of these crustaceans. The process of spring melting ensured the desalination and hydration of winter eggs, followed by the hatching of nauplii.

In summer, a 3–5-cm thick silt layer, formed on the surface of the salt sediment, was most likely washed in from shallower, salt-free areas due to wave activity. There were changes in the distribution of cysts of brine shrimp along the bottom compared to April. In June (June 18, 2020), the central part of the bottom remained under the pressure of salt sediment; the average abundance of the bottom cysts in the lake was 232.7 ± 124.15 thous. ind./m^2 (C_v =238.6%) or 23.6 ± 17.34 ind./g. In July and August, this value increased 1794.5 ± 578.30 and 2537.9 ± 552.81 thous. ind./m^2, respectively. The maximum content of cysts in the bottom sediments reached 2075 ind./g in July, and the average content was 344.0 ± 137.58 ind./g during this month. In August, it decreased to 93.4 ± 33.40 ind./g. Regard must be paid to more even distribution; the coefficient of variation in cyst abundance at stations decreased to 81.5% in comparison with the spring–summer period. The biomass of the bottom cysts by August was 4594 t, or 254 kg/ha, of which 92% were located in the central part of the lake.

On the contrary, in the water column, the concentration of cysts decreased through the summer period. The minimum number of cysts in the water column in July (9.9 ± 1.51 thous. ind./m^3) coincided with their highest concentration at the bottom (344.0 ± 137.58 ind./g). In August, there were 37.1 ± 8.27 thous. ind./m^3 of floating diapausing eggs of brine shrimp. In addition, the fishing for the brine shrimp cysts apparently played a certain role. In July–August 2020, 196 t of brine shrimp cysts were caught in Lake Kuchukskoe (data provided by Verkhneobsky Territorial Directorate of the Russian Federal Agency for Fisheries), which accounted for 69% of the total catch for 2020.

According to the results of hydrobiological surveys, the entire area of the lake bottom was covered with a crystalline salt in the autumn period (September 26 and October 16, 2020). There were no cysts of brine shrimp in the salt or on its surface. In September, the abundance of diapausing eggs in the water column was the highest in shallow areas of the southwestern part of the lake (123.1–185.0 thous. ind./m^3); in the center and northern parts of the lake, this indicator was 7.8–144.2 thous. ind./m^3. In October, the floating cyst abundance decreased to 17.2 ± 3.5 thous. ind./m^3.

Therefore, during the growing season of 2020, the bottom sediments were forming with a certain cyclicity. At the same time, bottom cysts of brine shrimp in

Table 2. Relative abundance of brine shrimp cysts in the bottom sediments of Lake Kuchukskoe, 2020

Data	The number of bottom artemia cysts ($M \pm m$, ind. /g) at different depths (m)		
	0–1.0	1.0–2.0	>2.0
26.04.2020	9.4 ± 3.25	0	0
18.06.2020	36.8 ± 20.46	0	0
17.07.2020	6.5 ± 5.05	177.9 ± 82.57	755.0 ± 419.00
18.08.2020	0.5 ± 0.40	114.7 ± 48.93	108.2 ± 37.64

M is mean value; *m* is error of mean.

the central part of the lake remained under the pressure of salt sediment throughout the entire study period and did not participate in the replenishment of the floating cysts and planktonic nauplii of brine shrimp. The cysts from accumulations on the southwestern coast of the lake and from shallow bottom sediments played the greatest role in the development of the first generation of nauplii. It is noteworthy that the highest concentrations of cysts were observed in the same areas both in autumn and in spring; this was due to the prevailing wind direction in the summer–autumn period (Fig. 4).

One of the main objectives of the study was to determine the viability of the bottom cysts of brine shrimp (nauplii hatching rate). In order to do this, we examined samples of cysts of brine shrimp taken on April 26, 2020, i.e., after the winter diapause has finished. The nauplii hatching rate from the floating cysts sampled from water surface was 20.27% after 24 h of exposure. However, in the next 6 h, the hatching rate decreased to 13.12%, and dead nauplii were noted (Table 3). This may be explained by the activation of a part of the embryos (end of diapause), which did not hatch during the subsequent period of transportation due to the lack of favorable conditions, while losing the energy reserves necessary for life. It is known that on the first day nauplii do not feed, but use the yolk reserve (*Manual* ..., 1986), which, apparently, was insufficient under the prevailing conditions. After 48 h of incubation, the nauplii hatching rate was 23.81%. The share of viable embryos in the cysts sinking to the lake bottom was low and did not exceed 20%. There was an increase in hatching nauplii over time from 1.36% to 17.19% (Table 3).

The biomass of cysts of brine shrimp, located at the bottom in April 2020, was calculated as

$$W_3 = 86.72\times10^3\times175.7\times10^6\times10^{-11} = 152.4 \text{ t (method 1)}.$$

Based on our results, it goes without saying that it is inappropriate to consider the entire water area of

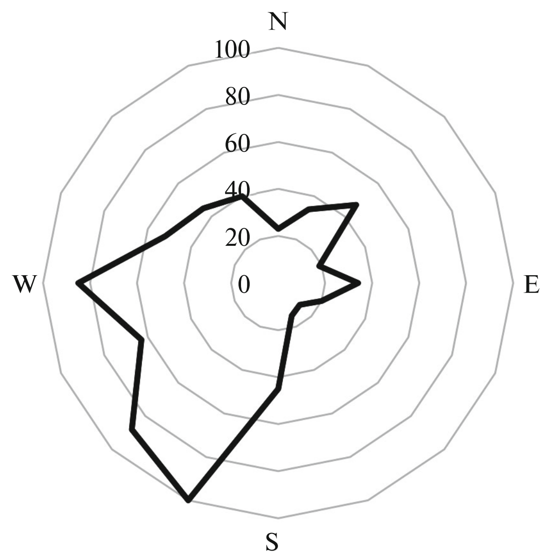

Fig. 4. Prevailing wind directions in the area of Lake Kuchukskoe, August–October 2020.

Lake Kuchukskoe to calculate the biomass of the bottom cysts. In order to determine the area favorable for cysts ascending from the bottom in spring, we used the coordinates of sampling sites, a depth map, and a satellite map. According to the station grid and the corresponding types of sediments obtained in the spring–summer period of 2020, the area available for the bottom cyst ascension was 129 km^2, or 73% of the total area of the lake. In April, the average nauplii hatching rate from the bottom cysts was 8.9%, with a maximum of 19.0%. The biomass of the bottom cysts, taking into account the above indicators, was calculated then as

$$W_3 = 102.78\times10^3\times129\times10^6\times10^{-11} = 132.6 \text{ t};$$
$$132.6\times0.089 = 11.8 \text{ t}$$
$$\text{or } 132.6\times0.19 = 25.2 \text{ t (method 2)}.$$

Therefore, in April 2020, there were 132.6 t of cysts of brine shrimp in the accessible water area of Lake

Table 3. Hatching of brine shrimp cysts from Lake Kuchukskoe, April 26, 2020

Place of sampling, № of sample	Percentage of hatched nauplius (%) during exposure (h)					
	24 h		30 h		48 h	
	H–	H+	H–	H+	H–	H+
Cysts from bottom sediments, no. 1 Bottom Artemia cysts, 1	1.36	5.13	6.58	9.02	8.80	16.82
Cysts from bottom sediments, no. 2 Bottom Artemia cysts, 2	3.94	6.08	3.64	9.52	17.19	19.03
Cysts from the water surface, no. 3 Artemia cysts from the water surface, 3	20.27	28.15	13.12	15.22	23.81	30.41

H– is hatching of nauplii only; H+ is hatching of both nauplii and embryos.

Table 4. Relative abundance of brine shrimp cysts in the bottom sediments of Lake Bolshoe Yarovoe, 2020

Data	Abundance, thous. ind./m^2	
	$M \pm m$	*Lim*
27.04.2020	1501.7 ± 594.66	0–4744
21.06.2020	34812.0 ± 10410.31	20–65861
14.07.2020	30120.2 ± 8813.11	15–101676
16.08.2020	25704.3 ± 5950.60	567–94340
20.09.2020	52828.3 ± 33437.53	94–660800

M is the mean value, *m* is the error of the mean, and *Lim* is the limits of variation (min–max mean).

Kuchukskoe, of which 11.8–25.2 t were viable. The difference between the indicators calculated in two ways was 84–92%.

Lake Bolshoe Yarovoe. According to the results of studies in 2020, the sediments in Lake Bolshoe Yarovoe in shallow areas are represented by coarse and medium-dispersed sand, mobile in the surf zone. At a depth of 0.8–1.0 m, the sediment is dense, sandy with an admixture of clay. At the depths exceeding 2.5–3.0 m, the lake bottom is covered with silts of intense black color smelling hydrogen sulfide, which have a finely dispersed (colloidal) structure. The silt thickness varies in different parts of the deep-water part of the reservoir. In the central part of the lake, the silt layer exceeds 30 cm; in shallower areas (depth 4.0–6.0 m) it ranges 10–15 cm. In the thickness of the silt (15–20 cm depth) there are coal combustion products.

In April, the concentration of cysts at the bottom of this lake averaged 1501.7 ± 594.71 thous. ind./m^2 and, in the water column, 39.3 ± 25.8 thous. ind./m^3. Only nauplii of brine shrimp were present in the zooplankton composition; their abundance in some shallow coastal areas (1.5 m in depth) reached 273.4 thous. ind./m^3 and, on average for the lake, it was 5.9 ± 0.96 thous. ind./m^3. Accumulations of brine shrimp cysts were observed on the water surface and along the shoreline. In some samples, nauplii hatching was observed during transportation, so it was not possible to study the cysts in the laboratory. Despite the active hatching of nauplii, their abundance in the water column was very low, which was explained by low water temperature.

In June, the average number of brine shrimp of the first generation was 28.6 ± 2.48 thous. ind./m^3, of which 96% were represented by nauplii. Sexually mature females were not numerous; gametes of early stages were observed in the egg sacs. We conclude that there were both long-lasting hatching of nauplii from overwintered cysts and the absence of cyst-bearing in females. This was reflected in the cyst abundance in the water column, when no accumulations were observed, and their average abundance in the lake was 17.4 ± 3.38 thous. ind./m^3. At the same time, the content of cysts at the bottom in June increased by 23 times compared to April **(Table 4)**. Apparently, a significant number of diapausing eggs of brine shrimp sank to the bottom during the warming of the water column and convection processes in the spring period.

In June–August, the average abundance of the bottom cysts changed slightly (28610.4 ± 2629.55 thous. ind./m^2; C_v=15.9%), but varied strongly from station to station (C_v=0–94%). In the water column, the number of cysts increased as their accumulations were formed in certain parts of the lake in the direction of the prevailing winds. In August, their abundance was 100.8 ± 24.94 thous. ind./m^3, considering brine shrimp fishing (at the cyst stage). According to previous studies of the chorological structure of brine shrimp cysts in the water column, the process of their settling was most intensive in August–October (Permyakova, 2012; Vesnina, Permyakova, 2013). In September 2020, the number of the cysts sinking to the lake bottom increased to 52828.2 ± 33437.53 thous. ind./m^2 with significant variation across stations (C_v = 283.1%).

Taking into account the average abundance of the bottom cysts in September (52828.2 thous. ind./m^2) and the area of Lake Bolshoe Yarovoe (Table 1), the biomass of the bottom cysts was 38934 t, or 5283 kg/ha. Such indicators of biomass exceed the reproductive capacity of the population of brine shrimp in one growing season. The average long-term (2000–2019) total stock of brine shrimp (at the cyst stage) in Lake Bolshoe Yarovoe, excluding the bottom cysts, was assessed in 880 t (Vesnina et al., 2019). Consequently, the accumulation of cysts of brine shrimp in bottom sediments occurred over a number of years.

The range of cyst abundance was significant at some stations, from 0 to 660.8 million ind./m^2 (Table 4). In this case, the average values did not give a reliable idea of the biomass of brine shrimp cysts located on the bottom, so there was a need for a more detailed consideration of their spatial distribution.

In the sediments characterized by a significant content of medium and coarse sand with an admixture of clay (0.5–1.0-m depth), the cyst abundance was 0–12506.36 thous. ind./m^2 during the growing season. Their maximum number was noted at a shallow station

Table 5. Abundance of brine shrimp cysts in the silty bottom sediments of Lake Bolshoe Yarovoe, autumn 2020

Data	Plot (depth, m)	Group of Artemia cysts	Abundance of brine shrimp cysts (million ind./m^3) in the silt layer				
			The density of Artemia cysts (million ind./m^3) in the silt layer				
			0–5 cm	5–10 cm	10–15 cm	15–20 cm	>20 cm
20.09.2020	Center (9.4 m)	Externally-whole	26.25	208.40	128.00	249.47	252.27
		Defective	14.00	93.20	78.20	82.20	282.53
		Gray or black color	0.00	7.00	0.20	27.53	0.27
	South (6.8 m)	Externally-whole	76.60	115.44	75.20	44.00	–
		Defective	92.60	157.04	108.80	52.80	–
		Gray or black color	1.33	3.73	6.40	28.00	–
16.10.2020	North (7.0 m)	Externally-whole	103.60	131.68	66.08	19.25	–
		Defective	88.00	131.84	42.24	130.75	–
		Gray or black color	1.60	59.20	53.76	5.45	–
	Center (9.4 m)	Externally-whole	30.40	126.24	163.84	252.48	115.60
		Defective	12.00	70.24	81.12	62.72	225.60
		Gray or black color	0.00	1.12	5.60	4.48	3.20

in the surf zone following the wind direction, where accumulations of brine shrimp cysts formed. In April (April 27, 2020), when sexually mature females were absent and cyst biomass increased naturally, the average abundance of diapausing eggs at shallow water stations was 261.9 thous. ind./m^2, but in August is was 6028.1 thous. ind./m^2. Presumably, the presence of cysts of brine shrimp in the loose, mobile sandy bottom of Lake Bolshoe Yarovoe was due to their accumulation in the water column and the formation of such accumulations in the coastal zone. The relative abundance of the cysts sinking to the lake bottom was 1.0–24.0% at depths not exceeding 6.0 m.

The highest content of bottom cysts was registered in silt deposits at depths of 6.1 to 9.5 m. Their relative abundance ranged from 76 to 90% during the growing season, with an average of 86.1% of the total abundance during this period.

Therefore, there is a correlation between the content of the bottom cysts and the type of sediment, which will be the task of further studies considering the data obtained from various types of hypersaline reservoirs.

A qualitative analysis of samples revealed the presence of outwardly different eggs of brine shrimp in the sediments of Lake Bolshoe Yarovoe; these eggs might be conditionally divided into three groups: (1) visually intact cysts (without visible damage), (2) eggs with or without a damaged chorion (there were cracks or uncolored areas on the shell; the complete absence of the chorion was possible), and (3) cysts with an unnatural color of the chorion (from gray to black; the contents are orange or gray, unstructured). Visually intact cysts accounted for 40.4–69.5% of the total number. Unnaturally colored eggs were confined to silty sediments and accounted for 1.1 to 3.2% of the total number.

In addition to the spatial distribution of the bottom cysts, their content in different layers of silty sediments was analyzed (Table 5). The highest content of visually intact cysts was observed in the central, deepest part of the lake, where their abundance was 776.47 million ind./m^3 in the 0–20-cm sediment layer. In the northern and southern parts of the lake, the silt layer was thinner; the cyst concentration in the entire silt layer was comparable (320.61 and 311.24 million ind./m^3, respectively). The content of damaged cysts corresponded to that of intact cysts: the highest abundance was in the lake center (500.91 million ind./m^3), a bit lower (392.83–411.24 million ind./m^3) in the northern and southern part. However, the content of cysts with unnaturally colored chorion (gray and black) was maximum in the northern part of the lake (120.01 million ind./m^3); in the central and southern parts of the lake, their abundance was 24.70–29.47 million ind./m^3. This could be explained by the location of the Yarovoe thermal power plant on the northern shore, which discharged wastewater into the lake here, so the products of coal combustion in the form of smoke spread directly over the lake and, apparently, partially settled along with precipitation.

There are differences in the relative abundance of cysts in sediment layers by stations (Fig. 5). The surface sediment layer (0–10 cm) in the northern and southern part of the lake contains 62–73% visually intact cysts. The relative abundance of damaged and unnaturally colored eggs increases in the layers deeper than 5 cm. The lake depths at these stations are comparable (6.8 and 7.0 m), the thermocline in the spring–summer is deeper, and cyst accumulation on

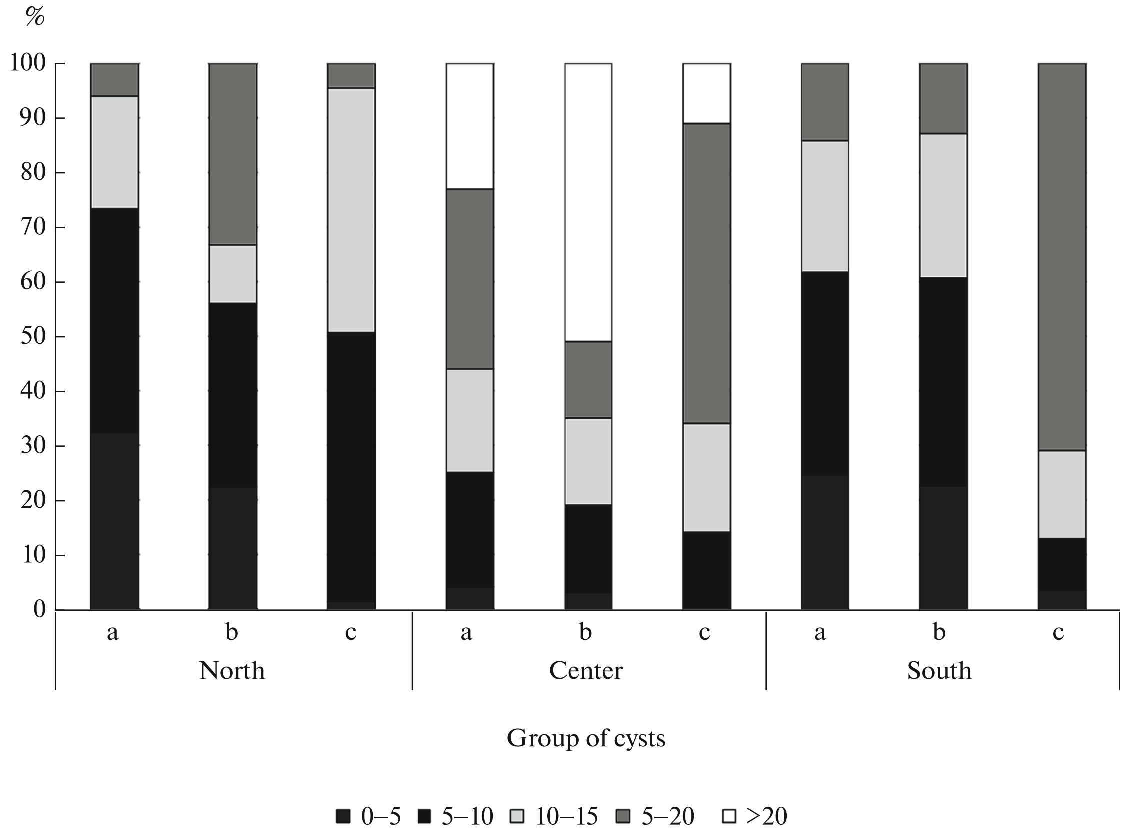

Fig. 5. Relative abundance of brine shrimp cysts of differing quality in the layers of the silty bottom sediments (cm) of Lake Bolshoe Yarovoe, 2020. (a) Visually intact cysts, (b) cysts with defective shells, and (c) cysts of gray or black color.

the bottom at such depths is more pronounced (which explains the high content of visually intact cysts in the upper layers of the sediment).

In the central, deepest part of the lake, in the upper layers of silt (0–10 cm), there is a minimum relative abundance of visually intact cysts (25% of their total number); the same trend may be traced for damaged (19%) and unnaturally colored eggs (14%).

The highest content of all identified groups of cysts is noted in the sediment layers deeper than 15 cm. This may conditionally indicate a longer presence of cysts in bottom silts and slower processes of descending and ascending. The cysts probably sink to a certain depth and keep between two water masses of different densities during the process of water column cooling and when a layer of fresh water forms on the lake surface due to precipitation in the autumn. Some cysts sink to the bottom; apparently, their bulk hibernate in the water column. Such a process is also described for the Great Salt Lake (Utah, United States), where the survival of cysts in the winter period in coastal discharges and the water column was studied (Belovsky et al., 2019). The same process was observed by colleagues from the Altai Branch of Russian Federal Institute of Fisheries and Oceanography in laboratory experiments when adding fresh water to lake brine with cysts without active mixing. Most of cysts descended, but did not sink to the bottom, keeping in between the surface and the bottom on the margin of two water densities. The salinity of the water in the upper layer was 40 g/kg and, in the lower layer, 120 g/kg.

In the spring, the viability of the bottom cysts (nauplii hatching rate) washed out of the silt from a depth exceeding 9.0 m was 4.46–15.93% and, from a depth of 8.0 m, 17.28–26.12% (Table 6). Therefore, the viability of the bottom cysts, which underwent diapause under the conditions of a reservoir, was quite low even under optimal conditions and varied depending on the sampling depth.

Samples of cysts of brine shrimp collected in September were incubated using different brine solutions. Nauplii hatching in natural brine was absent; the cysts dehydrated and floated to the surface. Also, the exposure time and the composition of the incubation solution had no effect for cysts sampled from the bottom sediments deeper than 20 cm (Table 7). Signs of viability of cysts sampled from the water column were recorded after 72 h of incubation in natural brine with a salinity of 20–30 g/kg. Diapausing eggs from the upper layers of the bottom sediments (0–10 cm) also had hatching potential; their hatching rate was 1.5–8.1%.

Table 6. Parameters of nauplii hatching from bottom brine shrimp cysts from Lake Bolshoe Yarovoe, 2020

Sampling depth, m	Date	Exposure, h	H	Hatching, %
More than 9.0 m	April 27, 2020	24	H–	4.46
			H+	7.27
		30	H–	9.49
			H+	15.93
Before 8.0 m	April 27, 2020	24	H–	23.53
			H+	26.12
		30	H–	17.28
			H+	21.99

H– is nauplii hatching only; H+ is hatching of both nauplii and embryos.

Table 7. Nauplii hatching rate (%) from brine shrimp cysts from Lake Bolshoe Yarovoe, September 20, 2020

Incubation solution (salinity, g/kg)	Place of sampling of Artemia cysts			
	from the water surface	bottom samples, sediment layer		
		0–5 cm bottom, layer 0–5 cm	0–10 cm bottom, layer 0–10 cm	>20 cm bottom, layer more than 20 cm
Artificial solution (20–30)	0–1.0	1.5–3.8	0–7.1	0
Brine (150–160)	0	0	0	0
0.5 brine (60–70)	0–0.6	0–3.2	0.5–3.6	0
0.25 brine (25–30)	0.3–2.0	1.3–6.0	1.3–8.1	0

DISCUSSION

The formation of diapausing eggs (cysts) is an important ecological adaptation for maintaining the viability of the brine shrimp population. Diapause occurs in the life cycle of animals as an adaptation for experiencing adverse conditions (Khmeleva, 1988). Cysts of brine shrimp overwinter in a pond at the bottom, in the water column, and in coastal discharges. The viability of cysts is a main indicator for the development of these crustaceans in the spring. A combination of certain conditions is necessary to launch the embryo metabolism, as was widely proved in international and Russian literature (Vanhaecke et al., 1981, 1984; Spektorova, 1984; Khmeleva, 1988; Solovov and Studenikina, 1990; Drinkwater and Crowe, 1991; *Instruktsiya* ..., 2000). Such an optimal combination is possible under artificial conditions, which contributes to an increase in nauplii hatching rate, but it is merely impossible to influence the hatching rate in a reservoir.

In many publications, the issue of cyst mortality in winter in a reservoir remains poorly understood. According to the years of research in the Great Salt Lake (Utah, United States), most cysts, giving rise to the first generation of brine shrimp in spring, winter in coastal discharges and in the water column. Their mortality is quite high (34–91%), preconditioned by the availability of food resources for females and the temperature regime in winter and spring (Belovsky et al., 2019). The authors believe that the winter survival of cysts is very important for the population of brine shrimp in the Great Salt Lake and should be taken into account in the commercial fishing of this bioresource (Belovsky and Pershon, 2019). Our results, obtained in 2020 for hypersaline Lake Kuchukskoe, confirm that cysts overwintered in coastal discharges and in shallow waters play the main role for the population replenishment of this species in spring. In the relatively deepwater Lake Bolshoe Yarovoe, characterized by an undeveloped littoral zone, cysts overwinter mainly in the reservoir, i.e., in the water column and at the bottom. The presence of cysts of different quality in sediments and a long period of their accumulation, as well as their uneven horizontal and vertical distribution, have been proven.

A number of works are devoted to the study of the stocks of brine shrimp cysts in the bottom sediments in various types of hypersaline water bodies of Russia (Litvinenko et al., 2016; Vizer and Rostovtsev, 2016; Semik and Ushakova, 2017; Litvinenko et al., 2020). All authors note a significant number of the cysts sinking to the lake bottom, as well as their presence throughout the year. At the same time, the given population fluctuations vary from zero to several dozens of millions, which is consistent with our data and confirms the uneven distribution of the bottom cysts in space and time.

Studies in lakes Kuchukskoe and Bolshoe Yarovoe evidenced a higher concentration of cysts in silts compared to other types of sediments. In the bottom silt deposits of the mineralized lakes of the Kulunda Steppe (Kulunda Plain), a community of anaerobic bacteria with a significant biomass is formed (Sorokin et al., 2006). The pronounced smell of silt in the surveyed lakes indicates the development of sulfate-reducing anaerobic prokaryotes, which cause decomposition processes. Experimentally, it was proven that the storage of cysts with a high content of organic impurities leads to a decrease in their viability and death, even when other conditions were favorable for their diapause (Klepikov, 2012). At the bottom, there are both the absence of conditions to interrupt the diapause and the presence of an aggressive environment, which together lead to the damage to the outer shell of cysts and the death of the embryo, as we have found.

The viability of the cysts sinking to the lake bottom has not been well studied. In Russia, the survival rate of benthic cysts is reported in the range of 1.3–57.5% (Litvinenko et al., 2016). No single trend is found for these indicators in different lakes; in particular, the hatching rate may either increase or decrease in the spring, or it may keep during the entire year at the same level. Thus, there are bottom cysts with varying degrees of viability; the latter is determined by the conditions in each particular lake. Our findings are preliminary, but consistent with those reported earlier.

The fishing of brine shrimp cysts is an important factor. In the reservoirs of Altai krai, the catch of raw material is carried out from the water surface (Lake Bolshoe Yarovoe), from coastal discharges, and from water along the shoreline using artificial concentrators. According to research conducted in the Great Salt Lake (Utah, United States), both the buoyancy of cysts in the water column and their sinking have a genetic and an ecological basis. The selective collection of floating cysts leads to a decrease in their quality and an increase in the mortality of nauplii in the reservoir (Sura and Belovsky, 2016).

CONCLUSIONS

A low diversity of sediment types is observed in the hypersaline lakes of Altai krai studied in 2020; the sediments may be divided into several groups according to the dominant particles. In Lake Kuchukskoe, bottom salt deposits play an important role; they are formed with a pronounced cyclicity. This type of sedimentation affects the brine shrimp cyst biomass accumulation on the lake bottom, so these cysts are unavailable for ascending in the spring. According to our results, we conclude that the bottom cysts play a significant role in the development of the brine shrimp population and contribute greatly to accumulations on the shore and shallow silted areas of the reservoir. The average hatching rate of nauplii from the bottom cysts is 8.9%.

In Lake Bolshoe Yarovoe, silt deposits contain a significant biomass of cysts of brine shrimp, estimated at 38934 t in September 2020. The hydrothermal and hydrochemical regimes, as well as the depth of the reservoir, play a decisive role during their accumulation at the bottom and further ascend. In the lake areas, where the depths do not exceed 7.0 m, the surface sediment layer contains up to 73% of visually intact cysts with nauplii hatching rate of 22% in the spring. In the central, deepest part of the reservoir, the content of cysts at the bottom is maximum, but they are the least accessible. The content of intact cysts in the upper layers of the sediment here is the lowest, and their viability in the spring does not exceed 10%.

Therefore, in the heterogeneous hypersaline water bodies of Altai krai, the role of the cysts sinking to the lake bottom is different in the lake biota, which makes it necessary to take into account indicators such as the area available for cyst ascending, depth, sediment type, and bottom cyst viability when calculating their biomass.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The author declares that she has no conflicts of interest.

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