

Size-age structure of *Artemia parthenogenetica* and *A. tibetiana* (Crustacea: Anostraca) populations in hypersaline lakes of southern Western Siberia, 2025

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Abstract

Endorheic hypersaline lakes of diverse hydrochemical types in southern Western Siberia, with zooplankton communities dominated by the genus *Artemia* Leach, 1819, are reliable indicators of water availability and are sensitive to climate-driven water-level changes. The abundance and size-age structure of *A. parthenogenetica* and *A. tibetiana* in the salt lakes of Altai Krai, and their environmental correlates, constitute a key research focus. In April–July 2025, ten lakes were surveyed: Bolshoye Shklo, Bolshoye Yarovoye, Krivaya Puchina, Kuchukskoye, Malinovoye, Maloye Shklo, Maloye Yarovoye, Mormyshanskoye, Tanatar III, and Shukyrtuz. Principal component analysis of the collected data distinguished the brine chemistry of habitats occupied by sexual versus parthenogenetic populations. The effects of lake morphometry (area and depth), physicochemical parameters (transparency, temperature, salinity, dissolved oxygen, chlorophyll a), and ionic composition (CO_3^{2-} , HCO_3^- , SO_4^{2-} , Cl^- , Mg^{2+} , $\text{N}^+ + \text{K}^+$) on *Artemia* population characteristics were examined. Temperature and salinity proved to be the primary determinants.

Keywords

Abiotic factors, abundance, age structure, brine shrimp, crustaceans, ecological factors, populations, salt lakes

Introduction

Brine shrimps, also termed as branchiopod crustaceans, are inhabitants of hypersaline waters on different continents (Sorgeloos et al. 1986). Salt lakes are distinguished by extreme conditions, as they are exposed to strong ultraviolet radiation, high salinity, significant temperature fluctuations, cycles of drying and hydration, oxidation and anoxia (Tanguay et al. 2004; MacRae 2016).

Parthenogenetic and bisexual populations of *Artemia* dwell in diverse hypersaline lakes located in the south of Western Siberia. They can survive in these extreme conditions, producing diapausing eggs widely used for aquaculture because of their well-adapted reproductive system (Scelzo and Voglar 1980; Vesnina 2016). In natural populations, the development rate of different-age stages in crustaceans depends on environmental conditions. Different-type water bodies in the region under study are characterized by specific hydrological and physical-chemical conditions determined by long-term fluctuations in the water regime (Anufrieva and Shadrin 2023). With decreasing moisture content, such lakes become very shallow and saline, thereby causing changes in the salinity of brine and its ionic composition. Succession of biocenoses occurs due to interannual and seasonal fluctuations in water levels that lead to the dominance of halobionts in the hypersaline lake systems. Based on ongoing relative stability of the ecosystem during the period of summer stagnation (July), it is possible to identify certain trends in the dependence of the main environmental factors of the aquatic settings on the development and functioning of the examined *Artemia* populations (Shadrin and Anufrieva 2012).

The aim of this work is to study the abundance and size-age structure of *A. parthenogenetica* and *A. tibetiana* populations in different-type lakes located in the south of Western Siberia (Altai Krai), as well as their dependence on environmental factors.

Materials and methods

Ten hypersaline lakes in southern Western Siberia (Altai Krai) were surveyed at monthly intervals from April to July 2025: Bolshoye Shklo (52°63' N, 79°05' E), Bolshoye Yarovoye (52°52' N, 78°36' E), Krivaya Puchina (52°26' N, 79°21' E), Kuchukskoye (52°65' N, 79°75' E), Malinovoye (51°42' N, 79°44' E), Maloye Shklo (52°34' N, 79°02' E), Maloye Yarovoye (53°02' N, 79°07' E), Mormyshanskoye (52°30' N, 81°17' E), Tanatar III (51°39' N, 79°47' E), Shukyrtuz (52°22' N, 79°24' E) (Fig. 1).

We sampled lakes Kuchukskoye, Bolshoye Yarovoye, and Maloye Yarovoye at 5, 10, and 8 stations, respectively, and all other lakes at 3 stations each. Quantitative sampling was conducted in July, with qualitative sampling performed during the remaining months. Table 1 summarizes the number of samples collected and processed.

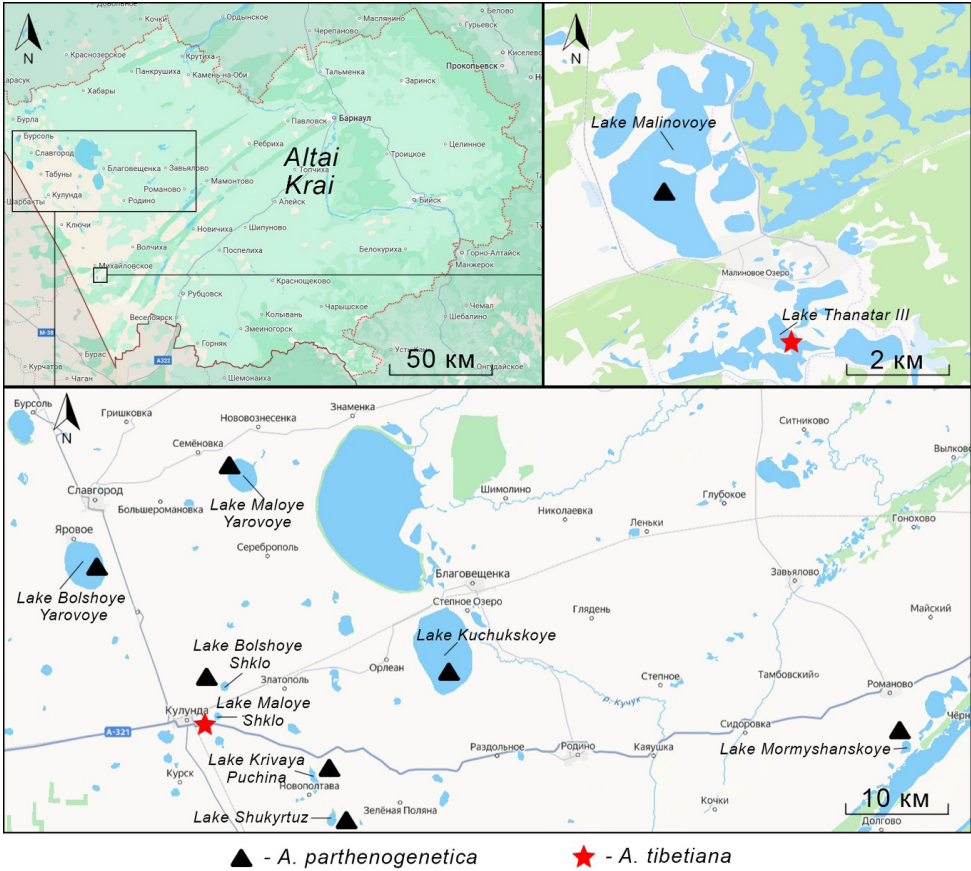


Figure 1. Study lakes and distribution of parthenogenetic and bisexual populations of *Artemia* in the south of Western Siberia.

Table 1. Study lakes and distribution of parthenogenetic and bisexual populations of *Artemia* in the south of Western Siberia

Lake	Qualitative	Quantitative
Bolshoye Shklo	3	3
Bolshoye Yarovoye	3	10
Krivaya Puchina	2	3
Kuchukskoye	3	5
Malinovoye	3	3
Maloye Shklo	3	3
Maloye Yarovoye	2	8
Mormyshanskoye	3	3

Lake	Qualitative	Quantitative
Tanatar III	3	3
Shukyrtuz	3	3
Total	28	44

Hydrobiological samples were taken in line with the standard methods (Methodical guidelines 2002; Vezhnovets 2005) using a small Apstein's plankton net (mesh size: 64 microns). The mouth area of the net was 113.04 cm² with a diameter of 12 cm. The depth of the sampled points in the lakes ranged from 0.3 to 7.3 m. The correction factor for filtration efficiency ranged from 2.66 at a depth of 0.3 m to 64.61 at a depth of 7.3 m. Sampling was performed by hauling the net from the bottom to the water surface.

The obtained samples were processed in a Bogorov chamber under binocular microscope MBS–10 with an eyepiece micrometer in accordance with the standard procedure (Krylov et al., 2024). For *Artemia* spp. populations, the following size-age stages were identified: orthonauplii (0.1–0.5 mm), metanauplii (0.6–1.0 mm; 1,1–1,5 mm; 1,6–2,0 mm; 2,1–2,5 mm; 2,6–3,0 mm), juveniles (3.1–4.0 mm; 4,1–5,0 mm; 5,1–6,0 mm), pre-adults (>6.1 mm), sexually mature females (>6.1 mm, with an ovisac present) and males (>6.1 mm, with enlarged second antennae). In terms of population structures, brine shrimps were represented by all age groups: naupliar and juvenile stages, pre-mature individuals, adult females and males. *Artemia* abundance for different age stages was calculated in triplicate per 1 ml sample. Counting of adult individuals (present in the entire sample) was implemented in a Petri dish (Kuchko et al. 2016; Kononova and Fefilova 2018).

Species identification of the brine shrimp *Artemia* was performed using identification keys for the genus *Artemia* Leach, 1819 (Rogers et al. 2019; Asem et al. 2023) and DNA barcoding. Material for DNA identification was fixed in 96% ethanol. The analysis was conducted at the Department of Biotechnology of the South-Siberian Botanical Garden, Altai State University. Total DNA from several dozen organisms from each lake was extracted using the Diamond DNA Plant Kit (Altaibiotek LLC, Russia) according to the manufacturer's protocol. For species identification, a fragment of the mitochondrial COI gene was amplified using polymerase chain reaction (PCR) with the primer pair LCO-1490 (forward: GGTCAACAAATCATAAAGATATTGG) and HCO-2198 (reverse: TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994). This method is described in detail in our previous article (Bezmaternykh et al. 2026). DNA barcoding was performed on 30 samples.

At each station, in conjunction with hydrobiological sampling during the monthly surveys (April–July), we recorded: (i) surface brine temperature (0.2 m depth) with a thermometer; (ii) surface brine salinity with a portable ATAGO refractometer (Kenco Instruments Co., USA); (iii) dissolved oxygen with a DO9100 portable meter; (iv) pH with an MW101 PRO portable pH meter; (v) Secchi disk transparency; and (vi) water depth with a sounding line.

In July, surface water samples (1.5 L) were collected in plastic containers from the central areas of the lakes for subsequent chemical analysis, following standard protocols (Boeva 2009, 2012). The hydrochemical analysis was performed in the Laboratory of Biogeochemistry of IWEP SB RAS. Water samples from the near-surface water layer were used to determine the content of phytoplankton photosynthetic pigments (1.5 L per plastic bottles). Algae were concentrated by vacuum filtration on membrane filters "Vladipor" brand MFAS-OS-3 with a pore diameter of 0.8 microns. Photosynthetic pigments of phytoplankton were analyzed in acetone extract by means of the spectrophotometric method (GOST 17.1.4.02-90; Guide 1992; Sirenko 1982).

Water surface areas (April–October 2025) of the study hypersaline lakes were estimated based on the analysis of images obtained from the "Resurs-P No. 4" and "Resurs-P No. 5" satellites. Images were taken in cloudless weather (cloud level less than 10%). For analysis, we employed the open-source Roscosmos Geoportal (<https://gptl.ru/>). Water volumes in lakes were calculated as the product of their areas and average depths. Statistical analyses were performed using MS Excel 2013 and PAST 4 software. Multivariate methods included principal component analysis (PCA) and partial least squares (PLS) regression (Halafyan 2007).

Results and discussion

Brine temperature in the studied lakes ranged from 19.1 °C (Tanatar III) to 27.0 °C (Maloye Yarovoye), brine salinity varied from 45.9 g/L (Maloye Shklo) to 210.9 g/L (Kuchukskoye), the pH from 7.6 (Kuchukskoye) to 9.8 (Tanatar III), dissolved oxygen from 7.3 (Shukyrtuz) to 12.9 mg/L (Maloye Shklo), chlorophyll a content from 0.4 (Mormyshanskoye) to 40.0 mg/m³ (Tanatar III), the average lake depth from 0.4 (Kryvaya Puchina) up to 5.0 m (Bolshoye Yarovoye), brine transparency from 0.3 (Kuchukskoye) up to 2.7 m (Bolshoye Yarovoye, and the lake area from 0.9 (Tanatar III) up to 175.2 km² (Kuchukskoye).

The chemical composition of brine saw the following variations in concentrations of basic anions and cations: CO₃²⁻ – from 0.003 (Kuchukskoye) to 26.0 g/L (Tanatar III); HCO₃⁻ – from 0.329 (Kryvaya Puchina) to 59.2 g/L (Tanatar III); SO₄²⁻ – from 2.0 (Maloye Yarovoye) to 56.3 g/L (Mormyshanskoye); Cl⁻ – from 5.9 (Mormyshanskoye) to 125.4 g/L (Kuchukskoye); Ca²⁺ – from 0.016 (Tanatar III) to 4.7 g/L (Maloye Yarovoye); Mg²⁺ – from 0.064 (Tanatar III) to 7.5 g/L (Bolshoye Yarovoye); Na⁺+K⁺ – from 16.4 (Maloye Shklo) to 76.2 g/L (Kuchukskoye).

When studying different-type lakes, we detected parthenogenetic and bisexual populations referred to two species: *A. parthenogenetica* Barigozzi, 1974 and *A. tibetiana* (Abatzopoulos et al., 1998) (Bezmaternykh et al. 2026).

A. parthenogenetica was found in lakes of the chloride (Bolshoye Shklo, Bolshoye Yarovoye, Krivaya Puchina, Kuchukskoye, Malinovoye, Maloye Yarovoye, Shukyrtuz) and sulfate type (Mormyshanskoye), while *A. tibetiana* – in the chloride (Maloye Shklo) and bicarbonate (Tanatar III) lakes.

Statistical analysis (PCA) of chemical characteristics of the study objects suggests that the chemical composition of lakes, inhabited by *A. parthenogenetica*, significantly differs from that of the reservoirs with the detected *A. tibetiana*, as evidenced from the 95% probability ellipse (Fig. 2).

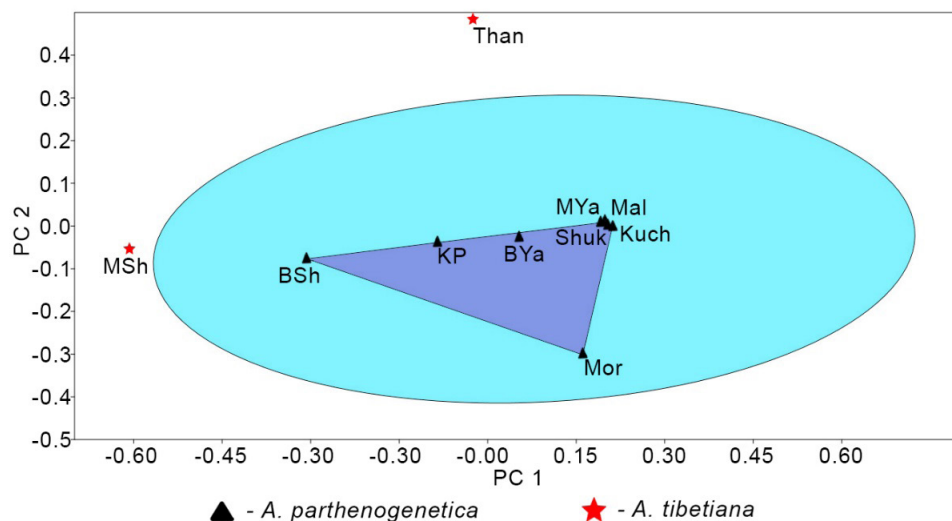


Figure 2. PCA-based results of hydrochemical characteristics of the studied lakes: BSh – Bolshoye Shklo, BYa – Bolshoye Yarovoye, KP – Krivaya Puchina, Kuch – Kuchukskoye, Mal – Malinovoye, MSh – Maloye Shklo, MYa – Maloye Yarovoye, Mor – Mormyshanskoye, Than – Thanatar III, Shuk – Shukyr্তুz.

The influence of physicochemical factors on structural and functional parameters of *A. parthenogenetica* and *A. tibetiana* during summer stagnation was studied in detail. On 10–15 July 2025, the size-age structure of *Artemia* populations corresponded to the development of its second generation. The *Artemia* populations were represented by various age stages, including nauplii, juveniles, pre-adults, and adults. The monthly proportions of these stages varied among the lakes. In Lake Bolshoye Shklo, the parthenogenetic lineage was dominated by nauplii (23.8%), while adult females were the least abundant (5.1%). Juvenile stages were not recorded. In Lake Bolshoye Yarovoye, the parthenogenetic lineage was dominated by juveniles (73.3%), while pre-adults were the least abundant (0.1%). In Lake Krivaya Puchina, the parthenogenetic lineage was dominated by pre-adults (46.5%), while adult females were the least abundant (8.7%). In Lake Kuchukskoye, the parthenogenetic lineage was dominated by nauplii (71.3%), while pre-adults were the least abundant (1.5%). In Lake Malinovoye, the parthenogenetic lineage was dominated by adult females (53.6%), while pre-adults were the least abundant (14.3%). Juvenile stages were not recorded. In Lake Maloye Yarovoye, pre-adults accounted for 54.7% of the zooplankton in July, while adult females were the least abundant (17.4%). Juvenile stages were absent from the zooplankton (Fig. 3).

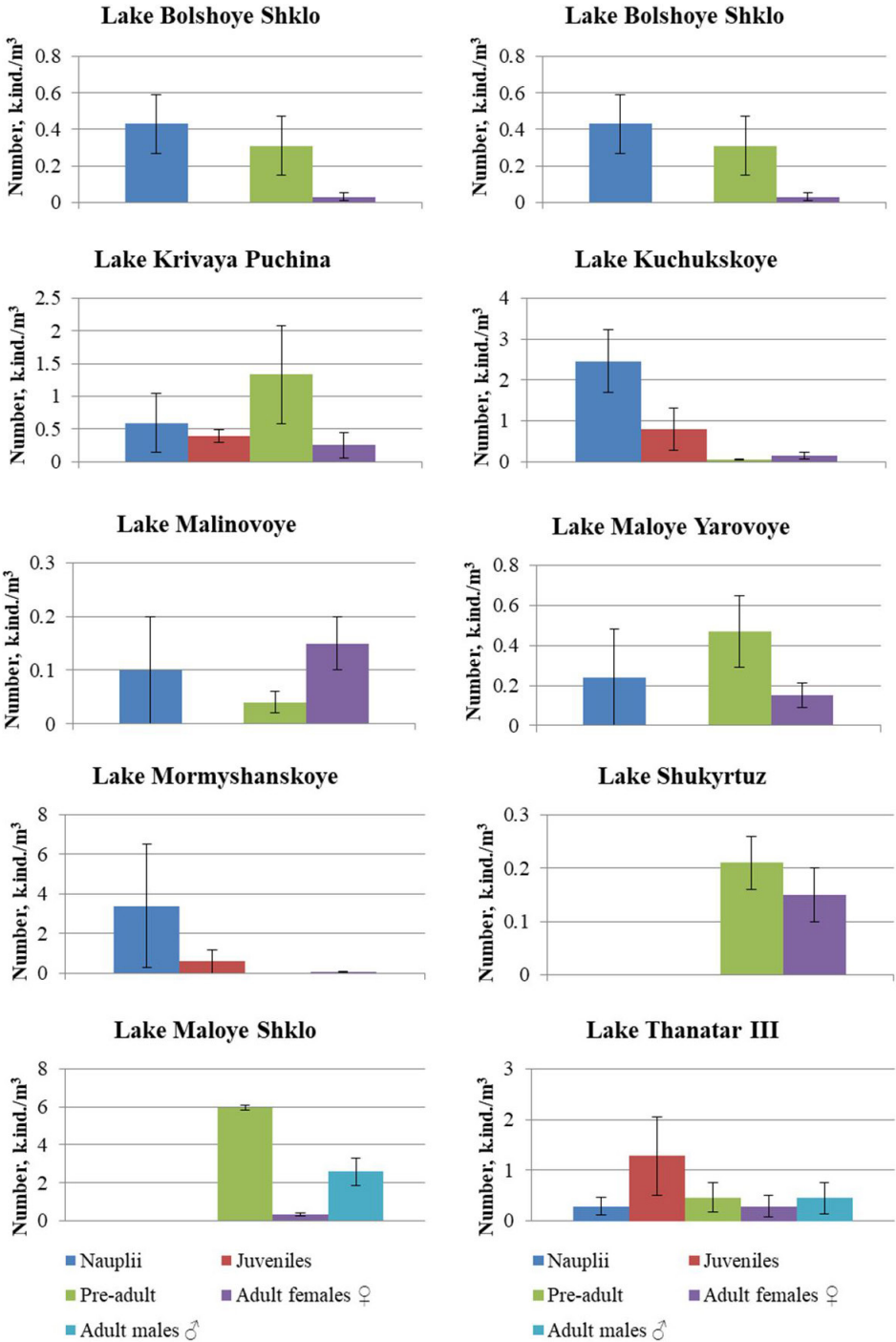


Figure 3. Number of different-age stages in *A. parthenogenetica* and *A. tibetiana* (±SE) during the period of summer stagnation (July 2025).

In terms of size-age characteristics, *A. tibetiana* populations generally showed a dominant position in the abundance of juvenile (on average 1.92 ± 0.74 thousand ind./m³), pre-mature (3.22 ± 1.24 thousand ind./m³) individuals and adult males (1.51 ± 0.59 thousand ind./m³) during summer stagnation. On the contrary, nauplius (0.44 ± 0.15 thousand ind./m³) and adult females (0.31 ± 0.12 thousand ind./m³) were the least abundant.

A two-block Partial Least Squares (PLS) analysis was performed using PAST 4.0 to explore the covariance between the age-structure matrix (4 stages: number of adult females, pre-adult, juveniles, nauplii) and the hydrochemical matrix (9 variables: water salinity, electrical conductivity, Ca²⁺, Cl⁻, CO₃²⁻, HCO₃⁻, Mg²⁺, Na⁺+K⁺, SO₄²⁺). Significance of each latent axis was assessed via permutation tests (99 runs). Variables with loading values > |0.5| on significant axes were considered biologically meaningful. Using two-block PLS, we found that 91.7% of the variance in the age structure of *Artemia* populations is associated with hydrochemical variables, but only the 2nd and 3rd latent axes were statistically significant ($p < 0.05$). Nauplii showed positive correlations with total salinity and electrical conductivity, while pre-adults were positively associated with SO₄²⁺ and negatively with Cl⁻ and Na⁺+K⁺. These results suggest a stage-specific response of *Artemia* to ionic water composition, likely related to ontogenetic shifts in physiological tolerance (Fig. 4). Previously, a similar alteration in the age structure of the *A. parthenogenetica* population influenced by environmental factors was recorded in Lake Kulundinskoye (Vesnina and Bezmaternykh 2023).

In most lakes (Bolshoye Shklo, Bolshoye Yarovoye, Kuchukskoye, Maloye Yarovoye, Mormyshanskoye), a trend towards an increase in the number of age stages with increasing brine temperature was observed during the period from April to June 2025 (Fig. 5). This trend was not observed in three lakes (Krivaya Puchina, Malinovoye, Shukyrtyuz).

A comparison of the main environmental factors and abundance characteristics of Lake Maloye Shklo and Lake Tanatar III is presented in Table 2. Unfortunately, the small sample size of numerical characteristics does not allow for precise conclusions regarding the impact of abiotic factors on the size-age structure of *A. tibetiana* populations. Nauplii and juveniles were not detected in Lake Maloye Shklo.

Thus, a comparison of two populations of *A. tibetiana* revealed a decrease in the abundance of pre-mature individuals and adult males along with an increase in juvenile individuals and Nauplius at a rise of brine mineralization, pH, CO₃²⁻ concentration, HCO₃⁻, Na⁺+K⁺, as well as a decrease in brine temperature, depth, transparency, area, dissolved oxygen concentration, SO₄²⁻, Ca²⁺ and Mg²⁺.

Other studies repeatedly show the effect of brine temperature and salinity on size, age, and numerical parameters of hydrobionts, including halophiles (Hammer 1986; Vizer 2015; Ermolaeva and Fetter 2021). For instance, the dynamics of size-age characteristics of *Artemia* populations depend on the duration of the dry season, brine temperature, and precipitation frequency responsible for the variability of salinity and ionic composition of brine. Fluctuations in microalgae number and other factors also determine this dynamics (Van Stappen et al. 2020).

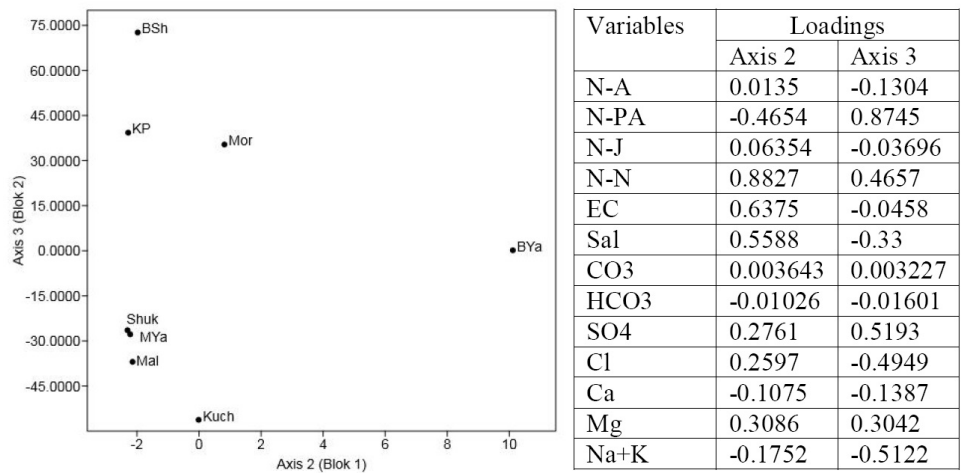


Figure 4. Results of the two-block PLS analysis for the studied lakes. Lakes: BSh – Bolshoye Shklo, BYa – Bolshoye Yarovoye, KP – Krivaya Puchina, Kuch – Kuchukskoye, Mal – Malinovoye, MYa – Maloye Yarovoye, Mor – Mormyshanskoye, Shuk – Shukyrtuz. Age stages: N-A – number of adult females, N-PA – number of pre-adult individuals, N-J – number of juveniles, N-N – number of nauplii. Environmental factors: Ca – calcium ions, Cl – chloride ions, CO₃ – carbonate ions, EC – electrical conductivity, HCO₃ – hydrocarbonate ions, Mg – magnesium ions, Na+K – sodium and potassium ions, Sal – water salinity, SO₄ – sulfate ions.

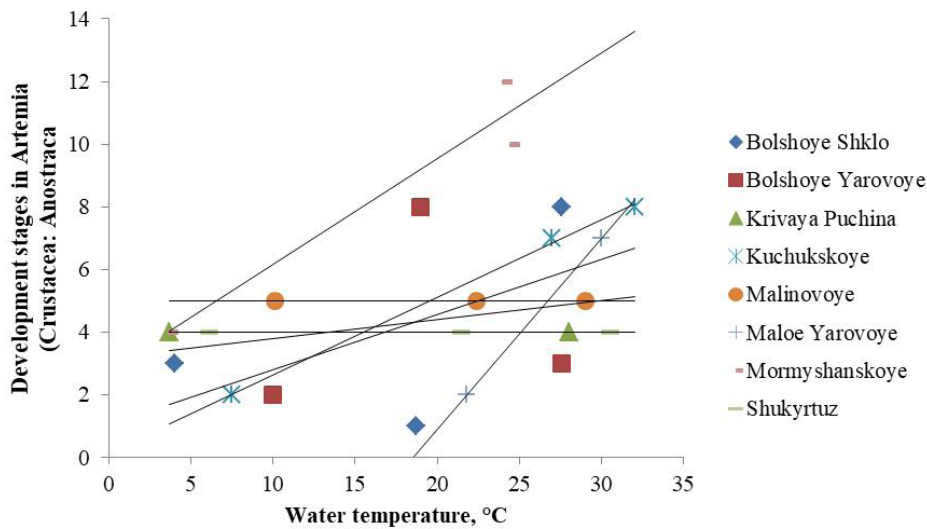


Figure 5. Trends in the increase in the number of age stages depending on the increase in brine temperature in different-type lakes in the south of Western Siberia (April–June 2025)

Table 2. Main environmental and populations characteristics of lakes inhabited by *A. tibetiana*

Characteristics	Maloye Shklo (M ± SE)	Thanatar III (M ± SE)
Number of nauplii	n/a	0.30 ± 0.17
Number of juveniles	n/a	1.28 ± 0.77
Number of pre-adult individuals	5.97 ± 0.13	5.97 ± 0.13
Number of adult females	0.32 ± 0.08	0.29 ± 0.21
Number of adult males	2.58 ± 0.71	0.45 ± 0.31
Brine salinity, g/L*	45.9 ± 13.8	161.6 ± 48.5
Brine temperature, °C	21.1 ± 1.2	16.0 ± 1.1
Maximum lake depth, m*	0.6 ± 0.0	0.5 ± 0.0
Water transparency, m	0.6 ± 0.0	0.5 ± 0.0
Lake area, km ²	2.2 ± 0.1	0.88 ± 0.0
Dissolved oxygen, mg/L	12.9 ± 0.6	8.6 ± 0.1
pH*	8.8 ± 0.0	9.8 ± 0.0
CO ₃ ²⁻ , g/L*	1.1 ± 0.0	26.0 ± 0.1
HCO ₃ ⁻ , g/L*	3.4 ± 0.0	59.2 ± 0.3
SO ₄ ²⁻ , g/L*	8.9 ± 1.3	4.5 ± 0.7
Ca ²⁺ , g/L*	0.04 ± 0.00	0.02 ± 0.00
Mg ²⁺ , g/L*	0.21 ± 0.02	0.07 ± 0.01
Na ⁺ +K ⁺ , g/L*	16.4 ± 3.3	55.2 ± 11.0
Chlorophyll a, mg/m ³	3.8 ± 0.6	40.0 ± 35.7

Note: * – error of the analysis method.

Differences in life cycles and reproductive characteristics of *A. parthenogenetica* and *A. tibetiana* are manifested in the duration of the pre- and post-reproductive period, the total life expectancy, the number of offspring per brood, the number of broods per female, and the time between broods (Vesnina 2020; Vesnina et al. 2024). In the first breeding season, age is a key factor determining the population growth rate dependent on physical and chemical factors, especially on temperature and salinity of brine (Vesnina and Bezmaternykh 2023). Species richness of *Artemia* in the hypersaline lakes of the study region provides its adaptability to such environmental variations as climate change or anthropogenic impact (Vesnina et al. 2025; Vesnina and Permyakova 2011).

The studied populations of *A. parthenogenetica* and *A. tibetiana* have two reproductive modes influencing the formation of size-age characteristics of parthenogenetic and bisexual populations. The first pattern of reproduction includes diapause dependent on environmental factors, mainly brine mineralization and temperature. To a lesser extent, it depends on photoperiod, iron concentration, and diet (Ver-

sichele and Sorgeloos 1980; Wang et al. 2017, 2019). As a consequence, the formation of cysts containing an embryo (gastrula) covered with a tough chitinous shell occurs (Jackson and Clegg 1996; Liang and MacRae 1999; MacRae 2003). This structure serves as a chorion that protects an embryo from mechanical damage and physical-chemical impacts (Clegg and Conte 1980; Tanguay et al. 2004; MacRae 2016). Such a reproductive regime allows branchiopods to produce offspring under unfavorable environmental conditions, thereby increasing the probability of populations survival in the future (Browne 1980). The second regime is associated with reduced salinity of brine, which leads to the formation of predominantly nauplii in the egg sacs of females with the a survival rate of about 30% (Solovov and Studenikina 1990) that entails changes in the number of size-age groups in populations.

The breeding regime determines the dates of appearance of the *Artemia* first generation. In the studied lakes, it begins unevenly, i.e. in March–April with the warming of shallow areas when brine temperature reaches +3 – +5 °C (Vesnina et al. 2021). This is confirmed by our data on the number of size-age groups in *Artemia* populations from the lakes studied in 2025, as well as by our previous investigations (Vesnina and Vasilyeva 2019).

Conclusion

Parthenogenetic and sexual populations of *Artemia* were detected in lakes of varying hydrochemical types in southern Western Siberia, which differed markedly in their physicochemical properties. In chloride-type lakes (Bolshoye Shklo, Bolshoye Yarovoye, Krivaya Puchina, Kuchukskoye, Malinovoye, Maloye Yarovoye, Shukyr-tuz, and Maloye Shklo), both *A. parthenogenetica* and *A. tibetiana* were recorded. In contrast, only *A. parthenogenetica* was found in the sulfate-type lake (Mormyshanskoye), whereas only *A. tibetiana* occurred in the bicarbonate-type lake (Tanatar III). Environmental conditions influenced the size-age characteristics of the studied populations over the survey period. Statistical analysis revealed that the size-age structure and abundance of *A. parthenogenetica* populations were correlated with several environmental factors, with temperature and brine salinity emerging as particularly influential.

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