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METHODS OF INVESTIGATIONS

Analysis of Relations between Communities of Hydrobionts in Saline Rivers by Multidimensional Block Ordination

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Abstract—We present the results of a comprehensive study of five saline rivers in the arid region of Lake Elton, including hydrobiological surveys of plankton and benthic communities. The DIABLO method was used for a statistical analysis of the associative links between the species structures of the macrozoobenthos, meiobenthos, and zooplankton communities. This method combines multidimensional ordinations based on *n* blocks of observations and uses special algorithms of canonical correlation. It is shown that a significant portion of the cumulative variation in the data can be explained by a consensus configuration based on information common for all the three groups of observations. The diagrams, constructed and analyzed with the use of the cluster and Generalized Procrustes analysis, enable us to isolate stable associations of taxa typical for particular biotopes with homogeneous environmental conditions. We have constructed graphs of correlation pleiades and calculated indicator significance for particular species of macrozoobenthos, meiobenthos, and zooplankton. It is shown that highly mineralized systems of arid regions are not always characterized by a pronounced specification of life forms of plankton and benthic communities. The interpenetration between benthic and plankton animals results in a high portion of mixed ecological groups. Plankton and benthic communities correlate well enough with each other, which testifies to a close relationship between them due to both biotic interactions and a mutually agreed response to changes in aquatic environment factors. These data enable us to consider the plankton and benthic communities of highly mineralized rivers a nonequilibrium dynamically changing consortia of species.

Keywords: saline rivers, zooplankton, macrozoobenthos, meiobenthos, community structure, canonical correlation, multidimensional ordination, Procrustes analysis, indicator significance

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INTRODUCTION

The systems of running waters in arid regions are characterized by unstable status related to global and regional climatic fluctuations, in particular, as a result of anthropogenic impact [23]. Specific life conditions of hydrobionts are formed in different parts of watercourses with low flow rate, small depth, and highly trophic waters. Mineralization is one of the main factors determining to a great rate the taxonomic composition and trophic relations in aquatic communities. There is usually no pronounced isolation of plankton or benthic communities in shallow saline rivers and lakes: their mass species occur both at the bottom and in the water column [2, 3, 5–8]. Therefore, the study of the combined spatial distribution pattern of these communities in similar biotopes under changing abiotic factors is of great interest for a analysis of the structural organization of aquatic ecosystems [1].

The basis of quantitative ecosystem studies was formed by R. Whittaker upon the use of a direct gradient analysis [21], which simulates the distribution of particular cenopopulations relatively to the axes of two or three complex medium-forming gradients. The modern approach to the study of ecosystems is based on multivariate statistical analysis of tens and hundreds of different variables (population, phenotypic, genetic, ecological, chemical, landscape-geographical, etc.), which are characterized by significant time and spatial variability. The main aim of this analysis is to identify the significance and mechanisms of the effect of all factors in combination on the structural and functional features of the studied communities, as well as the subsequent forecast of their development under different scenarios of environmental activities.

Methods of multidimensional ordination have occupied an important place in ecology from the beginning of the 21th century. They enable us to synthesize the optimal information structure of communities, consisting of axes of new latent variables, which determine the total variation of the analyzed data. The main axes of the ordination concentrate the largest part of the dispersion and are orthogonal, which

Table 1. Rivers of the Lake Elton basin, location of stations, and the main hydrochemical parameters

Rivers	Stations	River reaches	Distance from river mouth, km	Total mineralization, g/dm^3	Oxygen, % of saturation	Mineral phosphorus, mg/dm^3	Ammonium (NH_4^+), mg/dm^3
Solyanka	Sol_1	Upper	5.0	25.7	96	0.011	64.86
	Sol_2	Middle	3.0	26.7	226	0.182	31.48
	Sol_3	Mouth	0.5	25.2	325	0.031	29.05
Lantsug	Lan_1	Middle	3.0	5.95	22	0.836	0.416
	Lan_3	Mouth	0.8	13.6	558	0.005	9.207
Khara	Khar_4	Middle	20.0	6.55	75	0.832	0.356
	Khar_5	Middle	12.0	8.74	64	0.971	0.178
	Khar_5a	Middle	11.0	7.54	52	1.011	0.594
	Khar_6	Lower	5.0	11.77	64	0.005	13.306
	Khar_7	Mouth	0.8	13.27	417	0.005	9.563
Chernavka	Cher_0	Upper	5.0	28.1	67	0.032	45.38
	Cher_1	Middle	3.0	27.5	178	0.032	45.92
	Cher_2	Mouth	0.3	31.7	282	0.015	41.99
Bol'shaya Samoroda	B.S_2	Middle	5.0	16.3	96	0.513	1.782
	B.S_3	Mouth	0.8	10.33	129	0.597	0.178

enables us to visualize a multidimensional cloud of points, projecting them on a plane with a minimal distortion. Various methods of indirect ordination are widely used: principal component analysis, correspondence analysis, and nonmetric multidimensional scaling [4, 13]. The approach based on the reduction of data became the basis for the algorithms of direct ordination: the redundancy analysis and canonical correspondence analysis, which relate the internal variability of species structure of the studied community with the matrix of factors of external impact [15, 20].

At the present time, algorithms of the multidimensional analysis are widely used to solve more and more complicated problems of a quantitative evaluation of correlations in large data sets. The quick development of researches related to the genomics and associated areas requires powerful algorithms to determine biomarkers using latent structures [16]. This results in the appearance of a group of new methods based on block symmetric covariance analysis, Procrustean analysis, canonical correlations, etc. [12].

The aim of this work is to analyze the intrasystem correlations of the taxonomic structure of benthic and plankton communities of small rivers with different mineralization in the Lake Elton basin based on the method of multidimensional ordination with the subsequent integration of individual data blocks (the DIABLO method).

MATERIALS AND METHODS OF THE RESEARCH

The initial data were obtained at hydrobiological surveys of macrozoobenthos, meiobenthos, and zooplankton in saline rivers (Table 1) of the basin of hyperhaline Lake Elton (49°07′30″ N, 46°30′40″ E). The rivers are characterized by a significant mineralization gradient (from 6 to ≥41.1 g/L). A detailed description and a scheme of the studied area, as well as the methods of sampling of hydrobionts and laboratory analysis of the material are given in [3, 5, 7, 23]. The multidimensional statistical analysis was based on the data of simultaneous hydrobiological survey at 15 stations in five saline rivers in August 2013. Three matrixes of numbers T (animals/m^2) of 88 taxonomic groups were formed. The groups included species and genera of zooplankton (28), macrozoobenthos (24), and meiobenthos (36). For the correct combined data processing, the values of the matrixes were transformed into a unified scale of points from 0 to 6, using the algorithm of finding the optimal boundaries [5]. Hydrochemical monitoring was simultaneously performed at the same stations. Its data were used as a basis for grouping the stations according to water mineralization: (1) >25 g/L, (2) 10–25 g/L, and (3) <10 g/L.

A statistical data analysis was performed using the integrated method of multidimensional ordination and the combined classification of *n* groups of observations DIABLO: Data Integration Analysis for Biomarker discovery using a Latent component for Omics studies [18], which included several basic algorithms. The initial variables were decomposed with respect to the axes of the main components using the method of Partial Least Squares (PLS) [22], and then sparse discriminant analysis (sparse PLS-DA) [14]), which does not require assumptions on data distribution, was performed. The use of the PLS-DA enabled us to isolate the portion of variation in taxonomic structures of zooplankton, macrozoobenthos, and meiobenthos caused by the main factor—water mineralization—and

Table 2. Correlation between the blocks of data and the decomposition of the intrablock explained variation, % with respect to main components (MC)

Blocks (communities of hydrobionts)	Correlation between blocks				Portion of explained intrablock variation, %	
	With respect to the 1st MC		With respect to the 2nd MC			
	Macrozoobenthos	Zooplankton	Macrozoobenthos	Zooplankton	1st MC	Sum of 1st+2nd MC
Macrozoobenthos	–	–	–	–	21.94	33.45
Zooplankton	0.901	–	0.89	–	19.45	32.18
Meiobenthos	0.925	0.937	0.929	0.944	15.99	33.90

evaluate the internal correlations within and between the blocks of communities.

The three tables of data of hydrobiological survey were integrated by the generalized canonical correlation analysis with regularization [19], on the basis of which the maximum of the total explained interblock dispersion of projections of latent variables was revealed. The generalized Procrustean analysis [11] was used to construct a consensus configuration, i.e., a mean of particular ordinations, corresponding to each block of the initial data.

Indexes of indicator significance were used to identify the species of hydrobionts, which may be considered statistically significant indicators of the mineralization of a group of water bodies [15]. The d_{jk} indicator index for samples of the k group of biotopes ($k = \{1, 2, 3\}$) was calculated as the product of the relative frequency by the relative mean number of the j species. The species was considered an indicator for the group for which d_{jk} was the maximal: $IndVal_j = \max[d_{jk}]$. $IndVal_j = 1$, if representatives of the j species occur in all samples of only one k group.

The associative links between sets of observations were visualized on the basis of the recommendations [10]. The calculations were performed using the mixOmics software of the R statistical medium (version 3.02) [17].

RESULTS OF THE RESEARCH

The main results of the block multidimensional ordination of plankton and benthic communities related to the decomposition of the total variability of data into intra- and intergroup variations are given in Table 2. A close correlation between the main components of each species complex has been revealed; i.e., the axes of the maximum variation of particular ordinations are characterized by the similar direction in the general multidimensional space. The high statistical significance ($p < 0.001$) of the portion of explained interblock dispersion was confirmed by the randomization test.

The consensus configuration based on linear combinations of the two main components explains 53.7% of the dispersion in all the data sets. A generalized ordination of the total analyzed community of hydrobionts and Procrustes distance from the centroids of the consensus configuration to particular blocks, corresponding to macrozoobenthos, meiobenthos, and zooplankton, is given in Fig. 1.

The first two main components of the partial ordinations (Table 2) explain only ~33% of the intrablock dispersion received after the deduction of the total interblock variation, which is related to high specificity of each species and to the absence of strong correlations between them. The relatively uniform distribution of the residual variation with respect to the groups testifies that the generalization is rather effective for all particular ordinations.

Because it has been revealed that synchronous variation in the species composition of the groups of hydrobionts is statistically significant, we used a number of methods for the specification of stable associations of taxa typical for particular types of biotopes with homogenic environment conditions. The heatmap or the map of cluster relations (Fig. 2) is represented by a matrix, the rows and columns in which are ordered according to the hierarchical classification of observation stations and the species structure related to it. The dendrograms on the left and above the matrix reflect the composition of the objects of the formed clusters, and the color intensity corresponds to the points of species abundance. The diagram shows the areas with specific species composition of hydrobionts, which directly depends on mineralization.

The correlation matrix between the taxa of the blocks, corresponding to macrozoobenthos, meiobenthos, and zooplankton, and the correlation graph based on it (Fig. 3) enable us to specify the composition of groups of species which may be considered related by interspecies interactions.

Indicator indexes *IndVal* were calculated for all 88 species of the analyzed community of hydrobionts with account for the specification of all studied biotopes into three groups according to the total water mineralization. The level of statistical significance p was evaluated with the use of randomization for each species used as an indicator of water salinity. The most typical species indicators of each class are given in Table 3.

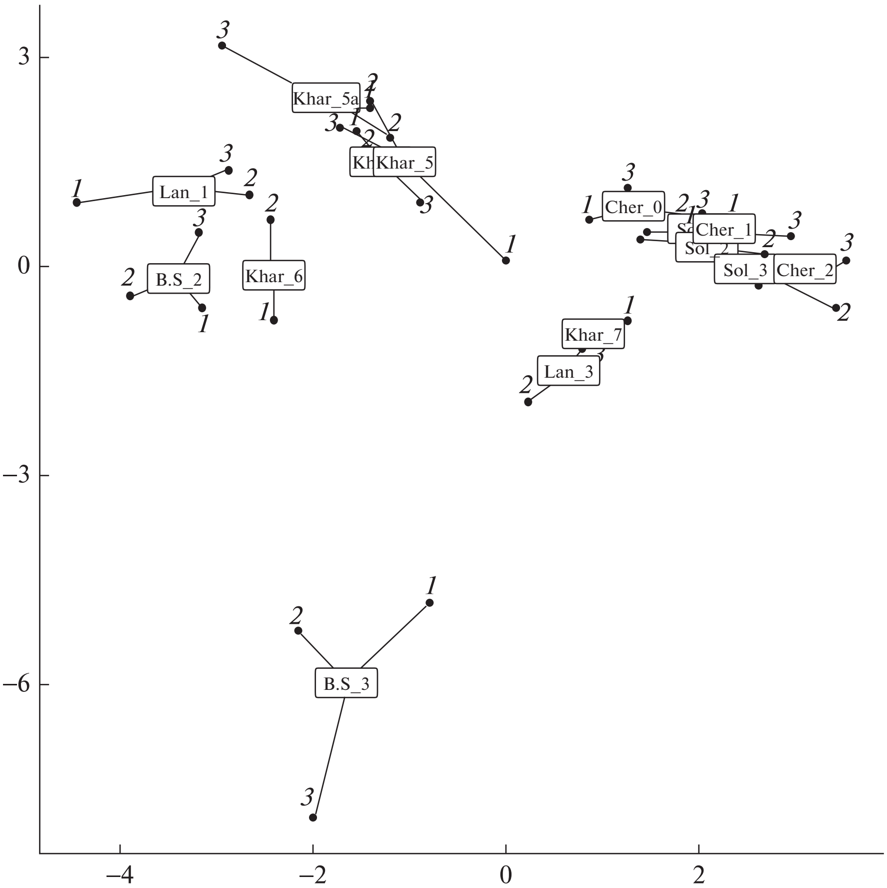

Fig. 1. Ordination of the reaches of rivers in the Lake Elton basin on the basis of generalization of three blocks of data of hydrobiological survey ((*1*) macrozoobenthos, (*2*) zooplankton, and (*3*) meiobenthos); rectangles mark coordinates of consensus configuration; designation of the reaches are given in Table 1.

DISCUSSION OF RESULTS

An analysis of allocation of hydrobionts to particular biotopes has shown that most plankton species are actually assigned to benthoplankton and are characterized by trophic relations with substrate [2, 6–8]. Euplankton species in the studied rivers comprise only 20% of zooplankton and are represented by copepods and rotifers [7]. Benthoplankton include 45% of taxa (mainly copepods of the orders Cyclopoida and Harpacticoida), and euritopic forms (Cladocera and rotifer *Brachionus plicatilis*) and meroplankton (larvae of chironomids and waterbugs of the family Corixidae) comprise 15% each. It should be mentioned that the orders Harpacticoida and Ostracoda dominate in the samples of meiobenthos in all rivers and make it possible to assign this community to eumeiobenthos or benthoplankton.

With respect to abundance, zooplankton is dominated by euritopic species (>50% of the total number) in the polyhaline Solyanka and Chernavka rivers and by benthoplankton and euplankton in the mesohaline Khara, Lantsug, and Bol'shaya Samoroda rivers [7]. A general regularity for all the rivers consists in a decrease in the taxonomic diversity of zooplankton, meiobenthos, and macrozoobenthos under conditions of high nutrient status and productivity in the bottom water layer with the preservation of their high number [5]. Halophilic species of macrozoobenthos such as larvae of Diptera *Cricotopus salinophilus*, *Chironomus aprilinus*, *Ch. salinarius*, *Microchironomus deribae*, *Palpomyia schmidti*, and others are typical for communities of both zooplankton and meiobenthos. Families Ceratopogonidae and Chironomidae are the most resistant to critical environmental factors in saline rivers.

The high interseasonal variability of environmental parameters typical for saline rivers of arid ecosystems has determined the specificity of the processes of formation and transformation of communities, among which the adaptive capabilities of individual species dominate. Our earlier studies of saline rivers did not reveal the most significant factors or their complexes, which determine the structure of plankton and benthic communities [5, 23] in a varying spacetime interval. Changes in the structure of biotic communities of different taxonomic groups are obviously different and depend not only on mineralization variations, but also on the biotope parameters and the specificity of adaptation of particular species.

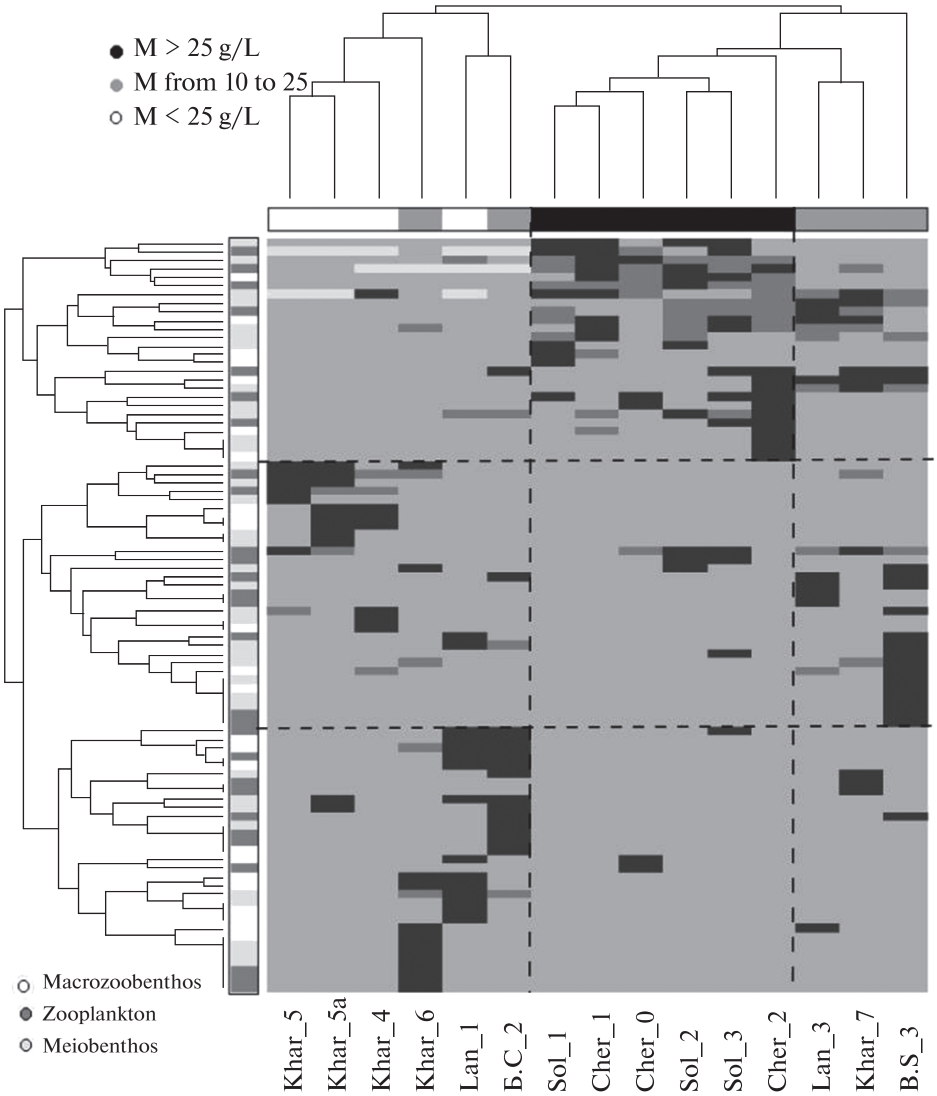

Fig. 2. Map of cluster relations between the reaches of the rivers in the Lake Elton basin with different mineralization *M* (in columns) and taxonomic structure of macrozoobenthos, zooplankton, and meiobenthos (in rows); the color intensity of matrix elements corresponds to the relative abundance of the corresponding species.

The spatial pattern of communities of zooplankton, macrobenthos, and meiobenthos shown in Fig. 2 is pronouncedly mosaic: the boundaries and the area occupied by the interpenetrating taxocenes of different ecological groups within one biotope are overlapped and characterized by fluctuating equilibrium.

Stability and equilibrium are often associated with the *balance of nature* metaphor [9], based on the concept that natural components tend to compensate various external effects, which results in regular fluctuations of populations and the entire species composition. This concept plays a fundamental role in understanding the dynamic equilibrium of communities in strong-mineralized waters of arid regions. The capability of most taxocenes to form temporary associations characterized by consortium relations is probably the main factor of the formation of the structure of communities in highly mineralized rivers.

CONCLUSIONS

Few works devoted to different aspects of the organization of communities in salt rivers have been performed in areas whose geography and functioning are not similar to the region of Lake Elton. The taxonomic spectrum of organisms living in saline rivers of arid regions all over the world significantly differs, which does not allow a direct comparison of the organization of plankton and benthic communities. We have constructed and analyzed stable associations of taxa using cluster and generalized Procrustean analysis. We have assessed their indicator significance for particular communities of saline rivers typical for biotopes with homogeneous environmental conditions. Three

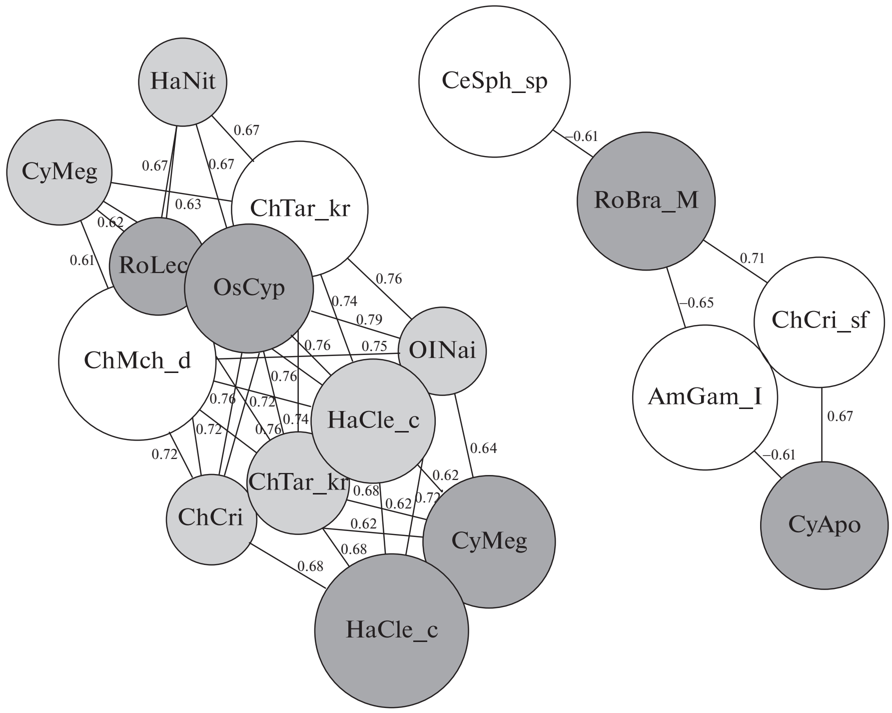

Fig. 3. Graph of correlation between the numbers of taxa of different groups (only the relationships with the correlation coefficient module >0.6 are shown); circles contain the codes of zooplankton (dark gray circles), meiobenthos (light gray circles), and macrozoobenthos (white circles) species given in Table 3.

Table 3. Species of zooplankton (ZP), meiobenthos (MB), and macrozoobenthos (ZB) typical for different groups of stations of saline rivers in the Lake Elton basin specified with respect to mineralization rate

Communities	Species	Species code	Occurrence, %	*IndVal*	*p*
I, mineralization >25 g/L (*n* = 33)					
MB	*Cricotopus salinophilus*	ChCri_sf	**100**	**0.666**	**0.008**
ZP	*Brachionus plicatilis*	RoBra_M	**100**	**0.663**	**0.003**
ZP	*Apocyclops dengizicus*	CyApo	**100**	**1.0**	**0.001**
ZP, ZB	*Palpomyia* sp.	CePal_sp	**83**	**0.794**	**0.007**
ZB	*Chironomus salinarius*	ChChi	**100**	**0.730**	**0.002**
ZB	*Palpomyia schmidti*	CeCer	**100**	**0.842**	**0.001**
II, mineralization 10–25 g/L (*n* = 59)					
MB	*Nais elinguis*	OlNai	**60**	**0.6**	**0.031**
ZP	*Megacyclops viridis* (juv.)	CyMeg	**60**	**0.6**	**0.033**
MB, ZB	*Cletocamptus confluens?*	HaCle_c	40	0.4	0.167
MB	*Megacyclops viridis*	CyMeg	40	0.4	0.145
ZB	*Microchironomus deribae*	ChMch_d	40	0.339	0.289
ZP	*Cyprinotus salinus*	OsCyp	20	0.2	0.596
ZP	*Lecane luna*	RoLec	20	0.2	0.633
MB	*Chironomus aprilinus*	ChChi_g	20	0.2	0.603
III, mineralization <10 g/L (*n* = 35)					
ZP, MB	*Brachionus calyciflorus*	RoBra_P	**75**	**0.75**	**0.007**
ZB	*Glyptotendipes salinus*	ChGly	**50**	**0.5**	**0.049**
ZP	*Acanthocyclops americanus*	CyAca	**75**	**0.568**	**0.027**
ZB	*Cricotopus* gr. *sylvestris*	ChCri	50	0.5	0.052
MB, ZB	*Glyptotendipes salinus*	ChGly_sl	50	0.05	0.056
ZB, MB	*Chironomus plumosus*	ChChi_p	50	0.5	0.064
MB	*Candona* spp.	OsCan	25	0.178	0.715
ZB	*Limnodrilus udekemianus*	OlLim_u	25	0.253	0.251
MB	*Chironomus salinarius*	ChChi	50	0.5	0.058
MB	*Microchironomus deribae*	ChMic	50	0.326	0.277

IndVal, Legendre–Dufrene indicator index [15]; *p*, statistical significance; and *n*, number of revealed species. Statistically significant indicator species at $\alpha = 0.05$ are given in bold type.

groups of species related to different water mineralization have been identified. Statistically significant indicators of hyperhaline conditions (>25 g/L) have been determined. They include chironomids *Cricotopus salinophilus* and *Chironomus salinarius*, ceratopogonids *Palpomyia schmidti*, copepods *Apocyclops dengizicus*, rotifers *Brachionus plicatilis*, and others. The interaction between plankton and benthic communities in saline rivers of the arid region of Lake Elton results in the domination of mixed ecological groups. An analysis of the structure of communities on the basis of different methods of multidimensional analysis evidences that, under conditions of a fluctuating medium of high-mineralized rivers, plankton, macrozoobenthos, and meiobenthos may be considered a specific dynamic association of the consortium type.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. This article does not contain any studies involving animals performed by any of the authors.

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