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Invertebrates of hyperhaline reservoirs of the Orenburg Region (Russia)

Беспозвоночные животные гипергалинных водоемов Оренбургской области (Россия)

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KEY WORDS: hyperhaline reservoir, resort area, macrozoobenthos, Branchiopoda, Insecta, dominant species, blood-suckers, reservoirs of bacteria, mass breeding.

КЛЮЧЕВЫЕ СЛОВА: гипергалинный водоем, курортная зона, макрозообентос, Branchiopoda, Insecta, доминантные виды, кровососы, переносчики бактерий, массовое размножение.

ABSTRACT. A study of the composition and structure of macrozoobenthos communities of 4 hypergaline reservoirs of resort areas and places of mass recreation in the Orenburg Region: artificial reservoirs on the Tuzlukkol river, a reservoir near the spring of the Gora Boevaya Nature Monument, lakes Maloe Gorodskoe and Tuzluchnoe (Sol-Iletsky lakes) was fulfilled. The taxonomic composition of macrozoobenthos includes 10 species of Branchiopoda and Insecta. It is shown that an increase in the level of mineralization to 159‰ leads to a decrease in species diversity of biota and halobiont *Artemia salina* and halophilic *Cricotopus salinophilus, Culicoides* (*Monoculicoides*) *riethi* and *Ephydra pseudomurina* become dominant species in hyperhaline reservoirs. The development of the shore fly *Ephydra attica* was confirmed in hyperhaline water bodies at a mineralization level of 51–67‰; this species, previously known from the territory of Crimea and the Astrakhan region, is recorded on the territory of the Orenburg region for the first time.

РЕЗЮМЕ. Проведено исследование состава и структуры сообществ макрозообентоса 4 гипергалинных водоемов курортных зон и мест массового отдыха Оренбургской области: искусственных водоемов на реке Тузлукколь, водоема вблизи родника на территории памятника природы Гора Боевая, Малого Городского и Тузлучного озер (Соль-Илецкие озера). Таксономический состав макрозообентоса включает 10 видов Branchiopoda и Insecta. Показано, что увеличение уровня минерализации до 159‰ приводит к снижению видового разнообразия, и доминантными видами в гипергалинных водоемах становятся галобионтный *Artemia salina* и галофильные *Cricotopus salinophilus, Culicoides* (*Monoculicoides*) *riethi* и *Ephydra pseudomurina.* Подтверждено развитие мухи-береговушки *Ephydra attica* в гипергалинных водоемах при минерализации 51–67‰; этот вид, известный ранее в России с территории Крыма и Астраханской области, впервые регистрируется в Оренбургской области.

Introduction

High salinity inland waters are essential for maintaining natural processes in arid zones. Salt water bodies are an important source of halophilic fauna formation, which differs sharply from that of freshwater communities and often contains rare highly specialized forms. Of particular interest to researchers is the study of changes in species diversity in the salinity gradient in water bodies with different salt concentrations [Vare-

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schi, 1987; Zinchenko, Golovatyuk, 2013; Shayhutdinova, 2019a,b]. With an increase in mineralization in such reservoirs, the number of species living in them decreases; in some cases, a single species remains in the pond. For example, *Artemia salina* is the only inhabitant of the reservoir in the western part of Lake Sivash [Chaban, 2012]. It is the only of the gill-footed crustaceans adapted to live in salt waters at salinity up to 300‰ and survive in chloride, sulfate and carbonate waters, in acidic so in alkaline environments. In the arid zone of Uzbekistan, such a unique species is the fly *Ephydra pseudomurina* Krivosheina, 1983, the larvae of which develop in lakes in sulfate waters at a salinity of 50‰ in an alkaline environment [Krivosheina, 1983, 1986]. Despite the fairly extensive information on the composition, the structure of macrozoobenthos communities of mineralized water bodies and their dynamics at the gradient of mineralization [Williams, 1998; Balushkina *et al*., 2007; Zinchenko *et al*., 2011], the new data continue to be relevant due to the fact that many salt water bodies are used to improve the health of people in resort areas [Gogoleva *et al*., 2019]. In these cases, it is necessary to have the most complete data on the species composition of invertebrates of specific water bodies in order to assess their possible impact on humans. The objects of our study were selected hypergaline reservoirs of the Orenburg region, used as resorts and places of mass recreation.

Description of reservoirs

The material was collected in the spring, summer, and autumn seasons of 2019 on four hyperhaline ponds of the Orenburg Region: artificial microreservoirs on the Tuzlukkol river, a reservoir near the spring of the Gora Boevaya Nature Monument, lakes Maloe Gorodskoe and Tuzluchnoe (Sol-Iletsky District).

The Tuzlukkol river crosses the Tuzlukkol salt dome 9.5 km from the source, where spring water outlets are observed and two self-flowing wells from the time of A.S. Khomentovsky [Chibilev *et al*., 2009] are drilled. As a result, a boggy salt marsh area was formed in the river valley. The solonchak is of geological origin and is associated with a close occurrence of Permian salts and gypsum in the core of the salt dome [Petrishchev, 2011]. There are several types of plots on the Tuzlukkol River: fresh, saline and with intermediate salinity indicators. The latter forms a specific adaptive zone inhabited by invertebrates with a high capacity for osmoregulation. We selected hyperhaline plots with salinity from 40 to 159‰ for this research, which may be lower, about 30‰, during short period in spring as a result of snow melting. They are artificially created microreservoirs (stations 1–3) with a varying level of mineralization during the year from 29.5 to 159.0‰, with a depth of 0.7–0.8 m, with a total area of 21.5 m^2 on the territory. The flow velocity in the river does not exceed 0.1 m/s. Bottom sediments of the river are silty with a large amount of plant debris, sometimes muddy with inclusions of mineral particles (mainly sand). More than 5000 tourists may attend the place.

The spring on the territory of the Gora Boevaya Nature Monument has the following morphometric characteristics: a point source, a downward source, and a permanent one. Expiration pattern — pressureless bottom feeding. This is a unique natural object associated with the release of salt formations to the surface. Mineralization was within 51–67‰. Spring water outlet velocity does not exceed 0.01 m/s. The spring is located on the slope of a steep deep ravine in the open air and is a cascade of bowl-shaped natural cavities with a depth of 0.2 m and a width of 0.7 m. Bottom sediments are represented by a mixture of gray silt and clay. This place is a little less popular since the spring can dry out in summer.

There is a unique Iletsk rock salt deposit, which is a salt dome in a salt core brought to the surface on the territory of the city of Sol-Iletsk, Orenburg Region. A complex of lakes was formed as a result of open-pit mining of salt, when large karst funnels, excavations of used open pits and cavities of old mine workings formed in saline sediment were filled with water [Petrishchev, 2011]. These lakes are very popular among tourists and more than 1.5 million people attend them. We have investigated 2 of them — Maloe Gorodskoe and Tuzluchnoe.

Lake Maloe Gorodskoe has a karst origin, the maximum depth reaches 15 m. The area of the lake is 21 030 m^2. The total salt content in this reservoir is from 31.4‰ in the spring to 86.5‰ in the autumn. At the bottom lies a layer of therapeutic mud, the total reserve of which is about 3 thousand m^3. Bottom sediments of the lake are sandy-silty.

Lake Tuzluchnoye also has karst origin. Its area is 23 750 m^2 and a depth of approximately 3–4 m. Mineralization fluctuates throughout the year and amounts to 30.3‰ in spring and 159‰ in autumn. The bottom sediments of Lake Tuzluchnoye are represented by a thick layer (2 m or more) of black silt — a homogeneous fine silt with the remains of decomposed vegetation with the smell of hydrogen sulfide.

Methods

To determine the species diversity of macrozoobenthos of the studied water bodies, samples were taken from silty soils using a DAK-100 automatic box-type scoop on a steel cable with a grip area of 1/100 m^2, and from a sand and gravel substrate using a hydrobiological scraper (knife length 16 cm, scraper pulling 1m). Samples were taken at depth 0.7–0.8 m at Tuzzlukkol reservoirs, at depth 0.2 at Gora Boevaya station, at depth of 0.7–1 m at Maloe Gorodskoe and Tuzluchnoe lakes with 3 replicates each season. The soil was washed through a sieve cloth with a mesh size of 300 μm. Bottom invertebrates were fixed with 4% formaldehyde solution. Sampling and processing of macrozoobenthos samples was carried out according to the methods generally accepted in hydrobiology [Abakumov, 1983]. To determine the constant weight of the larvae, they were kept in a 4% formaldehyde solution for three months, dried on filter paper until the wet spot disappeared, measured and weighed on an HR-100AZG analytical balance. The species composition, abundance and biomass of bottom invertebrates were determined, followed by a conversion to 1 m^2. The classification of V.Ya. Levanidov [1977] was applied to determine the species structure of benthocenoses. The dominance of macrozoobenthos groups in the community was estimated by the percentage of the total number and biomass. Water sampling for chemical analysis, storage, transportation and preparation for research were carried out in accordance with GOST 31861-2012. Hydrochemical analysis of water samples was carried out according to standard methods on the basis of an accredited laboratory in Orenburg Federal State Budgetary Institution State Center for Agrochemical Service "Orenburgsky".

The species composition was determined by fixed larvae using keys and species descriptions [Tsalolikhin 2000, 2001; Krivosheina, 2003; Zinchenko *et al*., 2009; Zorina *et al*., 2014], using light microscopes of the MBS-2 and Standard-25 brands (Carl Zeiss). We did not collect adults specifically except for the case of mass reproduction of *E. pseudomurina* on Lake Tuzluchnoye to confirm the species affiliation of the object. A total of 56 samples were collected and processed.

Results and discussion

The waters of the investigated water bodies are of the sodium chloride type with a varying level of mineralization.

As part of the macrozoobenthos of the artificial microreservoirs on the Tuzlukkol river, 4 taxa were registered within the place. Insects are represented by larvae of 2 species of dipterans (*Cricotopus salinophilus* Zinchenko, Makarchenko et Makarchenko, 2009, *Ephydra pseudomurina* Krivosheina, 1983), 1 species of beetles (*Berosus* (*Enoplurus*) *spinosus* (Steven, 1878)). Of the other invertebrate groups, 1 species of crustaceans (*Artemia salina* (Linnaeus, 1758)) was noted (Table 1). Among the species, there are species that live in salt bodies of water.

In artificial reservoires 1 and 3 on the Tuzlukkol river, the mineralization level reaches 159‰, which provides a stable habitat for certain species: *Ephydra pseudomurina*, *Cricotopus salinophilus*, *Artemia salina*. The number of representatives of the bottom communities of individual sections of the river varies from 51 to 6362 ind./m^2, and the biomass — from 0.40 to 7.35 g/m^2. Benthos stations 1 and 3 are characterized by the dominance of larvae of shore-flies. The rest of the fauna 1 is represented mainly by eurybiont species. At station 2, larvae of *Ephydra pseudomurina* flies massively develop in spring as well as in autumn (abundance 75 ind./m^2 and 775 ind./m^2, biomass 0.34 g/m^2 and 6.17 g/m^2, respectively).

As a part of macrozoobenthos, 5 taxa were registered in the mineral spring on the territory of the Gora Boevaya Nature Monument: Branchiopoda — *Artemia salina*; Insecta: Diptera — *Culicoides* (*Monoculicoides*) *riethi* Kieffer, 1914, *Ephydra glauca* Meigen, 1830, *E. attica* Becker, 1896, *E. pseudomurina* (Table 1). In spring, the most common species in the Gora Boevaya spring are the larvae of *Ephydra pseudomurina*, numbering 1075 ind./m^2. These flies are known to form layers of larvae and pupae up to 2–3 cm thick [Krivosheina, 1983, 1986]. Our samples showed a large number of puparia and flies in the spring. In the autumn period, the communities of ceratopogonids *Culicoides* (*Monoculicoides*) *riethi* are formed in the natural environment of the spring with a dominance of 99.6%.

As a part of macrozoobenthos in Salt-Iletsky lakes, 8 taxa were registered: Branchiopoda — *Artemia salina*; Insecta: Heteroptera — *Sigara assimilis* (Fieber, 1848); Coleoptera — *Hygrotus* (*Coelambus*) *enneagrammus* (Ahrens, 1833); Diptera — *Culex* sp., *Culicoides* (*Monoculicoides*) *riethi*, *Cricotopus salinophilus*, *Ephydra glauca*, *E. pseudomurina* (Table 1).

In the pelagic zone of Lake Maloe Gorodskoye, the gill-footed crustacean *Artemia salina*, along with the larvae of beetles (*Hygrotus* (*Coelambus*) *enneagrammus*), mosquitoes (*Culex* sp.), ceratopogonids (*Culicoides* (*Monoculicoides*) *riethi*), chironomids (*Cricotopus salinophilus*) and shore-flies *E. pseudomurina* lived in large numbers.

In Lake Tuzluchnoe, the larvae of *Ephydra pseudomurina* developed in spring in such quantities that the surface of the water seemed to be covered with a continuous layer of imago. The entire water line of the anthropogenic reservoir was dotted with their empty pupae. The number of larvae of *E. pseudomurina* reached 910 ind./m^2, and the biomass was 4.75 g/m^2. *Cricotopus salinophilus*, *Artemia salina*, *Sigara assimilis*, *Ephydra glauca* were also found in large numbers there. Among the species, there are species that live only in salt bodies of water (*Cricotopus salinophilus*, *Ephydra* spp., *Artemia salina*), and species with wide ecological plasticity. For example, *Sigara assimilis* is able to colonize nearly all aquatic habitats: rivers, streams, lakes, ponds, temporary waters, pools and brackish coastal waters [Boda, Csabai, 2009; Polhemus, Polhemus, 2008]. They occur in waters with salinity up to 100‰ [Strauss, Niederinghaus, 2014].

In general, it should be noted that, compared to mixohaline reservoirs [Shayhutdinova, 2019 a, b], an increase in salinity leads to a decrease in species diversity, and species such as *Artemia salina*, *Ephydra pseudomurina* and *Cricotopus salinophilus* dominate in hyperhaline reservoirs. The main structural indicators of macrozoobenthos communities are presented in Table 2. The benthos abundance during the study period ranged from 923 ind./m^2 (microreservoirs on the Tuzlukkol river) to 8487 ind./m^2 (Maloe Gorodskoe

Table 1. Distribution of species composition and ecological groups (ER — euryhaline, GF — halophilic, GB — halobiont) of macrozoobenthos in the hyperhaline reservoirs of the Orenburg Region.
Таблица 1.Распределение видового состава и экологические группы (ER — эвригалинные, GF — галофильные, GB — галобионтные) макрозообентоса в гипергалинных водоемах Оренбургской области.

Species and taxa	Name of water body						Environmental group
	artificial microreservoirs on the Tuzlukkol river			Gora Boevaya spring	Sol-Iletsky lakes		
	1	2	3		Maloe Gorod-skoe	Tuzluch-noe	
BRANCHIOPODA							
Artemiidae							
Artemia salina (Linnaeus, 1758)	+		+	+	+	+	GB
INSECTA							
Heteroptera Corixidae							
Sigara assimilis (Fieber, 1848)						+	ER, GF
Coleoptera Dytiscidae							
Hygrotus (*Coelambus*) *enneagrammus* (Ahrens, 1833)					+		ER, GF
Hydrophilidae							
Berosus (*Enoplurus*) *spinosus* (Steven, 1878)	+						ER, GF
Diptera Culicidae							
Culex sp.					+		ER, GF
Ceratopogonidae							
Culicoides (*Monoculicoides*) *riethi* Kieffer, 1914				+	+		ER, GF
Chironomidae							
Cricotopus salinophilus Zinchenko, Makarchenko et Makarchenko, 2009	+		+		+	+	ER, GF
Ephydridae							
Ephydra glauca Meigen, 1830				+		+	ER, GF
Ephydra pseudomurina Krivosheina, 1983	+	+	+	+	+	+	ER, GF
Ephydra attica Becker, 1896				+			ER, GF

Table 2. Structural indicators of bottom communities of hyperhaline reservoirs of the Orenburg region.
Таблица 2. Структурные показатели донных сообществ гипергалинных водоемов Оренбургской области.

Index	Sampling point			
	artificial microreservoirs on the Tuzlukkol river	Gora Boevaya spring	Sol-Iletsky lakes	
			Maloe Gorodskoe Lake	Tuzluchnoe Lake
Abundance, ind./m^2	923	2613	8487	1224
Biomass, g/m^2	4.85	7.20	4.31	7.59
Species: d — dominants, ds —subdominants calculated by abundance	*Ephydra pseudomurina* (d = 54.2) *Cricotopus salinophilus* (d = 36.6) *Artemia salina* (ds = 9.2)	*Ephydra pseudomurina* (d = 41.1) *Artemia salina* (d = 30.3) *Culicoides* (*Monoculicoides*) *riethi* (d = 23.5)	*Artemia salina* (d = 83.5) *Cricotopus salinophilus* (ds = 14.7)	*Ephydra pseudomurina* (d = 63.7) *Cricotopus salinophilus* (d = 27.7)
Species: d — dominants, ds —subdominants calculated on biomass	*Ephydra pseudomurina* (d = 74.1) *Cricotopus salinophilus* (d = 18.1) *Artemia salina* (ds = 7.8)	*Artemia salina* (d = 47.8) *Ephydra pseudomurina* (d = 40.8) *Ephydra attica* (ds = 5.3)	*Artemia salina* (d = 69.6) *Cricotopus salinophilus* (d = 17.7) *Ephydra pseudomurina* (ds = 9.1)	*Ephydra pseudomurina* (d = 88.0)

Lake), biomass from 4.31 g/m^2 (Maloe Gorodskoe Lake) to 7.59 g/m^2 (Tuzluchnoe Lake).

Conclusions

As part of the macrozoobenthos of the studied hypergaline reservoirs, 10 taxa were identified. Under the conditions of the mineralization gradient, the benthic fauna variability has pronounced structural and functional features. An increase in mineralization to 159‰ leads to a decrease in species diversity of biota, reflecting the high concentration of dominance of halobiont species *Artemia salina* and halophilic species *Cricotopus salinophilus*, *Culicoides (Monoculicoides) riethi* and *Ephydra pseudomurina*. The development of the shore fly *Ephydra attica* was confirmed in hyperhaline water bodies at a mineralization level of 51–67‰; this species, previously known from the territory of Crimea and the Astrakhan region, is recorded on the territory of the Orenburg region for the first time. The mass reproduction of halophilic species can lead to clogging of therapeutic mud. In addition, some objects, for example, larvae of *Ephydra pseudomurina* are known to be reservoirs for the preservation of opportunistic bacteria of the *Escherichia coli* group in water bodies with high salinity [Gogoleva *et al*., 2019]. Chironomidae larvae may have connections with other dangerous bacterial agents [Meir *et al*., 2008; Halpern, Senderovich, 2015; Laviad, Halpern, 2016]. Two bloodsuckers were identified among dipterans: *Culicoides* (*Monoculicoides*) *riethi* and *Culex* sp., capable of attacking people. In addition, the mass emergence of some Diptera species, such as chironomids *Cricotopus salinophilus*, shore-flies *Ephydra pseudomurina*, *E. glauca* as well as Corixidae bugs *Sigara assimilis* in the daytime and in the evening (attracted by light sources) may be nuisant for people.

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