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The role of salinity as an environmental filtering factor in the determination of the Diptera taxonomic composition in the Crimean waters

Nickolai V. Shadrin[1], Victor P. Belyakov[2], Alexandra I. Bazhora[2] and Elena V. Anufriieva[1,*]

[1] The A. O. Kovalevsky Institute of Marine Biological Research of Russian Academy of Sciences, 2 Nakhimov Av., Sevastopol 299011, Russia
[2] Institute of Limnology of Russian Academy of Sciences, Sevastyanova Str. 9, St.-Petersburg 196105, Russia

Abstract – Salinity is one of the most important factors in aquatic ecosystems, but its filtering role in the Diptera community species composition is still poorly studied. This issue was studied in the diverse Crimean water bodies. A total of 425 samples were collected from freshwater to hypersaline waters. In 73% of the 425 samples examined, representatives of Diptera were found; their larvae and pupae belonged to seven families. Chironomid species composition was analyzed in 47 samples. The most common were Chironomidae, which were in 94% of samples with Diptera, Ceratopoganidae were in 8% of samples, Ephydridae were in 5% of samples, Simuliidae were in 4% of samples, Culicidae and Chaoboridae were in 4% of samples, and Syrphidae was in only one sample. A total of 14 Chironomidae genera and 21 species and forms were found; 9 of them are new for Crimea. Reduction of the dominance variants in the community, as well as the number of chironomid species, with increasing salinity may indicate an increase in the filtering role of salinity in the Diptera community species composition that is still poorly studied.

Keywords: Diptera / Chironomidae / freshwater / hypersaline / species structure

Résumé – **Le rôle de la salinité comme facteur de filtrage environnemental dans la détermination de la composition taxonomique des diptères dans les eaux de Crimée.** La salinité est l'un des facteurs les plus importants dans les écosystèmes aquatiques, mais son rôle de filtre dans la composition des espèces de la communauté des diptères est encore peu étudié. Cette question a été étudiée dans les divers plans d'eau de Crimée. 425 échantillons ont été prélevés en eau douce et en eau hypersaline. Dans 73% des 425 échantillons examinés, des diptères ont été trouvés ; leurs larves et leurs pupes appartenaient à 7 familles. La composition des espèces de chironomides a été analysée dans 47 échantillons. Les plus fréquents étaient les Chironomidae, qui étaient présents dans 94% des échantillons de diptères, les Ceratopoganidae dans 8 % des échantillons, les Ephydridae dans 5% des échantillons, les Simuliidae dans 4 % des échantillons, les Culicidae et Chaoboridae dans 4% des échantillons et les Syrphidae dans un seul échantillon. Au total, 14 genres et 21 espèces et formes de Chironomidae ont été trouvés ; neuf d'entre eux sont nouveaux pour la Crimée. La réduction des variantes de dominance dans la communauté, ainsi que le nombre d'espèces de chironomidés, avec l'augmentation de la salinité peut indiquer une augmentation du rôle filtrant de la salinité dans la composition des espèces de la communauté de diptères est encore peu étudié.

Mots-clés : Diptères / Chironomidae / eau douce / hypersaline / structure des espèces

1 Introduction

Amphibiotic Diptera, larvae of which often constitute a significant share of benthos biomass in diverse water bodies, play an important role in aquatic ecosystem functioning and their connections with terrestrial ecosystems (Armitage *et al.*, 1995; Cummins and Merritt, 1996; Silina, 2016). A number of Diptera species have adapted to habitat in various extreme biotopes (Kohshima, 1984; Armitage *et al.*, 1995; Krivosheina, 2014), including hypersaline waters in different regions (Beadle, 1969; Kokkinn and Williams, 1988; El-Shabrawy and El Sayed, 2005; Przhiboro, 2014; Shadrin *et al.*, 2017). Often species composition of Diptera determines

*Corresponding author: lena_anufriieva@mail.ru

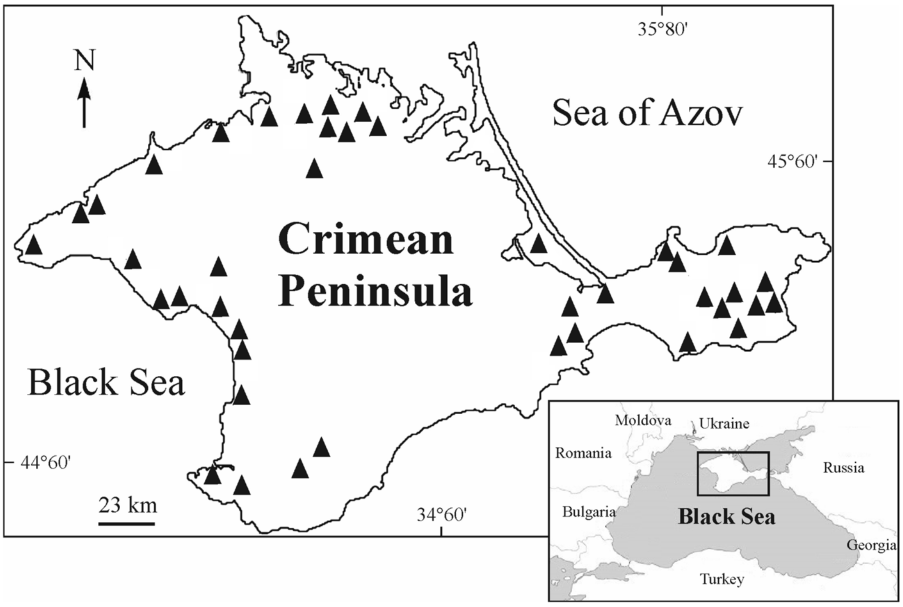

Fig. 1. The Crimean peninsula and distribution of studied water bodies.

functioning and dynamics of an entire aquatic ecosystem, so understanding the regularities of its formation is an important task of aquatic ecology. Both the deterministic causes (*e.g.* the environmental filtering, the biotic interactions) and the stochastic factors (*e.g.* accidental drift, the effects of extreme weather events, irregular visits to a water body by migrating birds or insects) are involved in the formation of community species composition (Williams, 1998; El-Shabrawy *et al.*, 2015; Kraft *et al.*, 2015; Shadrin *et al.*, 2016; Tolonen *et al.*, 2018). Salinity is one of the most important factors in aquatic ecosystems (Williams, 1998), but its filtering role in the Diptera community species composition is still poorly studied and understood, despite some studies (*e.g.* Topping, 1971; Williams, 1998; Piscart *et al.*, 2005; Shadrin *et al.*, 2017).

Crimea is the largest peninsula in the Black Sea (area 27,000 km^2). Crimean fauna, which has many endemic species (Polishchuk, 1998; Apostolov, 1999) and is a mixture of different faunisitic components, belongs to two ecoregions of the Palearctic ecozone. The northern Crimean portion extends to the Euro–Siberian region, while the southern part extends to the Mediterranean Basin. Crimea is characterized by high species diversity in many groups of animals. Saline and hypersaline water bodies constitute the characteristic aquatic habitat type in the region (Shadrin *et al.*, 2017). Natural freshwater bodies such as springs and adjacent pools, small mountain and steppe rivers and cave waters are not numerous in Crimea. Artificial water bodies however are more abundant, for example the North Crimean Canal, which is one of the largest canal systems in Europe (currently closed), ponds, decorative pools, rice fields and other small water bodies (Oliferov and Timchenko, 2005; Anufriieva *et al.*, 2014). The species composition of Diptera in the various Crimean water habitats has not been well studied; only the species composition of Culicidae (Razumeiko *et al.*, 2014) and Ephydridae (Przhiboro and Shadrin, 2012; Krivosheina, 2014) has been relatively well studied. At present, 26 species of Ephedridae (Przhiboro and Shadrin, 2012; Krivosheina, 2014) are known in Crimea, which is more than 1.44% of the 1800 species in the world fauna (Kubátová-Hiršová, 2009). The species composition of chironomids has been previously studied in few types of fresh and saline water bodies (Baranov, 2011a,b, 2013; Baranov and Ferrington, 2013; Belyakov *et al.*, 2017; Shadrin *et al.*, 2017), and endemic chironomid species were found in Crimea (Baranov, 2011a). Previously, chironomid species identification was made by authors in 28 samples of all collected and only from hypersaline waters; the results of this was published (Shadrin *et al.*, 2017; Belyakov *et al.*, 2017). All 425 collected samples were not analyzed before this study. These few studies do not give possibility to evaluate how a salinity filters community species composition.

The object of this study is to use the multi-year collections from the Crimean water bodies to determine the taxonomic diversity of Diptera, in particular the species composition of chironomids, in different types of water bodies with salinity from 0 to 340 g/L. This study will improve knowledge of the chironomid fauna in the Crimean Peninsula, and assess the role of salinity as a natural filtering factor in the determination of Diptera taxonomic composition in the Crimean waters.

2 Materials and methods

In different seasons, a total of 425 samples of zooplankton, benthos, and floating filamentous algae mats were collected from 41 water bodies of various types between 2005 and 2018 (Fig. 1). Plankton samples were also used because in hypersaline waters, most benthic animals, including chironomid larvae, are present in the water column rather than in the benthos (Shadrin *et al.*, 2017). There are three reasons why benthic animals shift to live in the water column or floating green algae mats: (1) increased water density under high salinity; (2) the common occurrence of bottom anoxic conditions in hypersaline waters; (3) forming of bottom salt crust if salinity is higher than 150–160 g/L. Description of used

Table 1. Diptera families in the Crimean water bodies found in this study.

Family	Frequency of occurrence in samples with Diptera, %	Salinity range, g/L
Chironomidae	94.0	0–320
Ceratopoganidae	8.0	5–270
Ephydridae	6.0	10–340
Simuliidae	5.0	0–5
Culicidae	4.0	5–15
Chaoboridae	4.0	0–10
Syrphidae	0.3	122

standard sampling methods was given (Balushkina *et al.*, 2009; Belmonte *et al.*, 2012; Shadrin *et al.*, 2017). For this study, the identification of Diptera families and chironomid species was carried out in new 47 occasionally selected samples with chironomids from waters with a salinity of 0–300 g/L, in addition to 28 samples analyzed before (Shadrin *et al.*, 2017; Belyakov *et al.*, 2017). The entire range of salinity was conditionally divided into several intervals: In salinity range from 0 to 2 g/L (ponds, two spring pools, the Bodrak river), 10 samples were analyzed on chironomid species composition, in range of 5–10 g/L (ponds, pools, lakes) – 7 samples, in range of 15–45 g/L (ponds, pools, lakes) – 12 samples, in range of 50–80 g/L (ponds, lakes) – 9 samples, in range of 100–190 g/L (lakes) – 6 samples, and in range of 200–340 g/L (lakes) – 3 samples.

During sampling, salinity, temperature and pH were measured by a refractometer (Kelilong WZ212) and a pH meter (PHH-830). Samples were fixed with 4% formalin and analyzed using an Olympus SZ-ST and LOMO MBS-9 stereo microscope. Chironomidae larvae species were identified using different keys (Pankratova, 1970, 1983; Hirvenoja, 1973; Wiederholm, 1983; Makarchenko and Makarchenko, 1999). The average values of larvae abundance, standard deviations, the coefficients of variability and correlations between different parameters as well as the regression equations were calculated in the standard program MS Excel 2007. The confidence level of the correlation coefficients was determined (Müller *et al.*, 1979). Student's *t*-test was used to compare averages.

3 Results

Diptera larvae and pupae, belonging to seven families, were found in 73% of the 425 samples collected during 2005–2018 (Tab. 1). Only one Diptera family larvae was found in about 90% of all samples with Diptera. In the case of joint occurrence of species of several families in a sample, there were different variants of dominance. In the salinity range of 0–5 g/L, in 67% of all cases, Chironomidae dominated in number with the presence of Ephydridae (33% of all such cases) or Simuliidae (33% of all such cases) and Ceratopoganidae, Culicidae and Chaoboridae (16% of all such cases). Ephydridae (35% of total Diptera larvae abundance) and Culicidae (35% of total abundance) dominated in the hydrogen sulfide spring with a strong H_2S smell (salinity of 10 g/L); Chironomidae (24% of total abundance) and Chaoboridae (6%) were also present here. Ephydridae (68% of total abundance), Chironomidae (31% of total abundance) and Syrphidae (1% of total abundance) were found in one sample (salinity of 122 g/L), Chironomidae (75% of total abundance) together with Ceratopoganidae (25% of total abundance) in one sample were found (salinity 140 g/L), and in other sample (salinity 150 g/L), Chironomidae and Ceratopoganidae had equal abundance.

A total of 14 Chironomidae genera and 21 species and forms were found (not all the larvae were identified to the species level), a list of which is given in Table 2, where data on salinity are also given. Nine of them had not previously been found in Crimea. No correlation between chironomid species presence and species richness with temperature and pH were found. Depending on the salinity, the number of Diptera families and chironomid species per sample varied in different degrees (Tab. 3). The total number of the chironomid species in different ranges of salinity decreased with increasing salinity (Fig. 2a), and it can be approximated by the equation ($R = 0.983$, $p = 0.0001$):

$$K = 15.68 - 2.63\,\mathrm{Ln}\,(S) \quad (1)$$

where K is the total number of species and S is the salinity, g/L.

The chironomid species number by sample in every salinity range taken significantly varied at salinity from 0 to 80–100 g/L. The average number of chironomid species found in one sample decreased with a salinity increase (Fig. 2b); this relation was not linear and can be approximated ($R = 0.958$, $p = 0.001$):

$$K_1 = 2.18 - 0.22\,\mathrm{Ln}\,(S) \quad (2)$$

where K_1 is the number of species in a sample.

Variability of the species number in one sample also decreased with the salinity increase. In the salinity range of 0–45 g/L, the average coefficient of variability (CV) of the species number in one sample was 0.64 (standard deviation = 0.086), and at salinity ≥100 g/L, it was 0.21 (standard deviation = 0.175). This difference in mean values is significant according to Student's *t*-test ($p = 0.001$).

A dominant–subdominant species varied and changed with a salinity increase (Tab. 4). Frequency of *C.* gr. *sylvestris* (Fabricius, 1794) and *B. noctivagus* were most common dominant species. *C.* gr. *sylvestris* dominance occurrence abruptly decreases with a salinity increase: from 57–60% of all samples in salinity range from 0 to 10 g/L to 0% in salinity higher than 15 g/L, with one exclusion at salinity of 62 g/L when it was alone. Frequency of *B. noctivagus* dominance occurrence increases starting from salinity of 15–45 g/L (in 33% of all samples) to 44% in salinity range of 50–80 g/L, and to 100% in salinity higher than 100 g/L.

The relative number of dominance variants (different dominant and subdominant species, according to abundance) per sample (the number of dominance variants/the number of samples in a salinity range) significantly decreases with increasing salinity (Tab. 3 and Fig. 2c); the relationship can be described by the equation ($R = 0.942$, $p = 0.005$):

$$Y = 0.737 - 0.08\,\mathrm{Ln}\,(S) \quad (3)$$

where Y is number of dominance variants and S is the salinity, g/L.

Table 2. List of the Chironomidae species and forms found in the Crimean water bodies during the study.

Species	Frequency of occurrence, %	Range of salinity, g/L	Type of a water body	Noted earlier in Crimea
Baeotendipes noctivagus (Kieffer, 1911)	67.0	7–300	Saline lakes, ponds	Ivanova *et al.*, 1994; Shadrin *et al.*, 2017
Chironomus sp.	3.5	0–10	River Bodrak	Baranov, 2011a
Cricotopus sp.	1.5	24	Pond	
C. gr. *cylindraceus* (Kieffer, 1908)	12.0	0–190	Saline lakes, ponds	Shadrin *et al.*, 2017
C. gr. *obnixus* (Walker, 1856)	1.5	13	Pond	No
C. gr. *sylvestris* (Fabricius, 1794)	13.0	0–62	Lakes, ponds, River Bodrak	Baranov, 2011a
Dicrotendipes nervosus (Staeger, 1839)	1.5	10	Pond	Baranov and Ferrington, 2013
Eukiefferiella gr. *claripennis* (Lundbeck, 1898)	1.5	0	River Bodrak	Baranov and Ferrington, 2013
Glyptotendipes gripekoveni (Kieffer, 1913)	1.5	2	Lake Kyzyl-Yar	No
G. (Phytotendipes) paripes (Edwards, 1929)	1.5	5	Lake Sakskoye	No
Halocladius fuscicola (Edwards, 1926)	1.5	33	Permanent pool	No
Metriocnemus sp.	1.5	15	Lake Kuchuk-Ajigol	Baranov and Ferrington, 2013
Orthocladius (Euorthocladius) sp.	1.5	0	River Bodrak	Baranov and Ferrington, 2013
Paratanytarsus confusus Palmen, 1960	4.0	0–60	Saline lakes, ponds	No
Polypedilum sp.	1.5	5	Lake Kuchuk-Ajigol	G. Prokopov, personal communication, March 16, 2018
P. (Polypedilum) nubifer (Skuse, 1889)	1.5	10	Pond	No
P. (Tripodura) tetracrenatum Hirvenoja, 1962	1.5	0	Lake Kuchuk-Ajigol	No
Psectrocladius sp.	4.5	0–2	Ponds	G. Prokopov, personal communication, March 16, 2018
Tanypus kraatzi (Kieffer, 1912)	1.5	2	Lake Kyzyl-Yar	No
Tanytarsus mendax Kieffer, 1925	3.0	30–180	Saline lakes	Shadrin *et al.*, 2017
T. veralli Goetghebuer, 1928	1.5	0.5	Pond	No

Table 3. Salinity and Diptera community structure in the Crimean water bodies.

Salinity range, g/L	Number of Diptera families in a sample, range (average)	Chironomid species in a sample, range (average)	*A*	*A*/*n*
0–2	1–2 (1.5)	1–4 (2.0)	7	0.7
5–10	1–5 (1.8)	1–4 (1.9)	4	0.6
15–45	1	1–3 (1.2)	6	0.5
50–80	1	1–2 (1.1)	4	0.4
100–190	1–3 (1.1)	1–2 (1.05)	1	0.2
200–320	1–2 (1.05)	1–2 (1.05)	1	0.3

A – absolute number of dominance variants among chironomids in a salinity range; *A*/*n* – relative number of dominance variants among chironomids, where *n* is the number of samples in a salinity range.

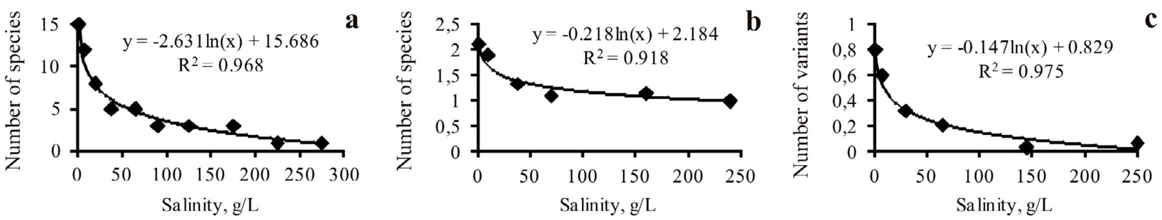

Fig. 2. Salinity influence on variability of chironomid community structure: (a) the total number of the chironomid species which live in the Crimean waters at different salinity (g/L); (b) the average number of the chironomid species in one sample in different intervals of salinity (g/L); (c) the relative number of dominance variants in chironomid community (the number of variants / total number of samples in a salinity range) in different intervals of salinity (g/L).

Table 4. Dominant and subdominant chironomid species in the different salinity ranges.

Salinity range, g/L	Dominant species			Other species
	Species	Frequency of dominance occurrence, %	Share in total abundance, %	
0–2	*C.* gr. *sylvestris*	40	100	–
	C. gr. *sylvestris*	10	38	*E.* gr. *claripennis* (31%), *Chironomus* sp. (8%), *Orthocladius (Euorthocladius)* sp. (23%)
	P. confusus	10	39	*C.* gr. *cylindraceus* (35%), *C.* gr. *sylvestris*
	P. (Tripodura) tetracrenatum + *B. noctivagus*	10	50 + 50	–
	Psectrocladius sp.	10	100	–
	Psectrocladius sp.	10	80	*T. veralli*
	T. kraatzi	10	56	*G. gripekoveni* (33%), *B. noctivagus*
5–10	*C.* gr. *sylvestris*	30	100	–
	C. gr. *sylvestris*	14		*G. (Phytotendipes) paripes*,
	C. gr. *sylvestris*	14		*Polypedilum* sp.
	C. gr. *sylvestris*	14	67	*D. nervosus*
	C. gr. *sylvestris*	14	50	*P. confusus* (28%), *Chironomus* sp. (15%), *P. (Polypedilum) nubifer* (7%)
	P. confusus	14	78	*C.* gr. *sylvestris* (20%), *B. noctivagus* (2%)
15–45	*B. noctivagus*	33	100	–
	C. gr. *sylindraceus*	25	100	–
	B. noctivagus	8.5	88	*Cricotopus* sp. (9%), *P. confusus* (3%)
	B. noctivagus + *C.* gr. *sylindraceus* + *Metriocnemus* sp.	8.5	33+33+33	–
	C. gr. *obnixus*	8.5	100	
	H. fuscicola	8.5	100	
	T. mendax	8.5	67	*C.* gr. *Cylindraceus*
50–80	*B. noctivagus*	44	100	–
	C. gr. *cylindraceus*	44		
	C. gr. *sylvestris*	11	100	
	P. confusus	11	100	
100–190	*B. noctivagus*	83	100	
	B. noctivagus	17	93	*C.* gr. *Cylindraceus*
200–320	*B. noctivagus*	100	100	–

4 Discussion

As a result of this and earlier conducted researches (Baranov, 2011a,b, 2013; Baranov and Ferrington, 2013; Shadrin *et al.*, 2017; G. Prokopov, personal communication, March 16, 2018), up to 140 Chironomidae species have been found in Crimea. Calculations based on new and published data (Shadrin *et al.*, 2017; Belyakov *et al.*, 2017) showed that at salinity of 200–340 g/L, chironomid larvae were detected only in 32% of all collected samples, and only *B. noctivagus* was present. Taking in to account all the above, authors can propose that the chironomid fauna on the peninsula is still poorly understood, especially in a variety of water bodies with a salinity of 0–45 g/L.

The decrease of Diptera and Chironomidae diversity with increasing salinity in this study is not surprising. Many studies covering a broad salinity range have noted an inverse relationship between salinity/mineralization and animal taxa richness in saline lakes and rivers in general (Williams, 1998; Piscart *et al.*, 2005; Balushkina *et al.*, 2009; Belmonte *et al.*, 2012; Gutiérrez-Cánovas *et al.*, 2013; Shadrin and Anufriieva, 2018) and for specific taxon, as an example, Copepoda (Anufriieva, 2015; De los Ríos and Bayly, 2018). The review shows that larvae of 38 chironomid species can live at a salinity up to 45 g/L worldwide, 34 species – in the range of 50–100 g/L, 17 species – at 101–150 g/L, 3 species – at 151–200 g/L, and only *B. noctivagus* – at 201–320 g/L (Shadrin *et al.,* 2017). The general trend of a species richness decrease with a salinity increase may be overruled or masked by an influence of other factors. This may be a cause of relatively high variability of the species number in a sample taken in the salinity range from 0 to 80–100 g/L.

Reduction of the dominance variants in the community, as well as the number of chironomid species, with increasing salinity may indicate an increase of the salinity filtering role in the formation of the chironomid species composition. Considering the species composition of chironomids for different salinities, the conclusion can be made that there are two most stable states in Crimean waters with the dominance of *C.* gr. *sylvestris* (salinity range 0–10 g/L) and the dominance of *B. noctivagus* (salinity above 45–50 g/L). In salinity range from 15 to 45 g/L, *B. noctivagus* and *C.* gr. c*ylindraceus* demonstrated close frequency of dominance occurrence.

The species composition undergoes the most significant changes with salinity increase from 0–2 to 5–10 g/L, which agrees with the earlier conclusion (Williams, 1998; El-Shabrawy *et al.*, 2015; Shadrin *et al.,* 2016). With salinity above 10–15 g/L and up to 80–100 g/L, other factors contribute more to the variability of this composition and mask the effect of salinity. The role of salinity as an environmental filtering factor is quite significant and diverse. There are two mechanisms of salinity influence on Diptera taxonomic composition: (1) through the physiological limits of species halotolerance; (2) through interacting with biotic relations. Salinity influences biotic relationships, determining the outcome of competition and the presence of food and predators. Biotic factors can modify halotolerance of animals. As an example, high abundance of microalgae, which produce osmolytes, may allow chironomid larvae, as well as other animals, to exist at significantly higher salinities (Kokkinn, 1986; Shadrin *et al.*, 2017; Shadrin and Anufriieva, 2018). The proportion between these two mechanisms is a function of salinity, but there are not enough data to discuss this issue. It can also be assumed that when salinity is between 10 and 100 g/L, then a role of second mechanism is more pronounced. In this salinity range we may expect the importance of biotic interactions is higher than salinity, and then it decreases as salinity increases. Further studies are needed to detail and quantify the relationship between the salinity and composition of chironomid larvae in different types of water bodies.

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