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Spatial Distribution of Oligochaetes (Annelida: Clitellata: Oligochaeta) in Lakes of the Naroch System (Belarus) Differing in Trophic Status

M. A. Baturina[a], *, O. A Makarevich[b], T. V. Zhukova[b], and B. V. Adamovich[b]

[a] *Institute of Biology, Komi Scientific Center, Ural Branch, Russian Academy of Sciences, Syktyvkar, 167982 Russia*

[b] *Belarus State University, Minsk, 220030 Belarus*

**e-mail: baturina@ib.komisc.ru*

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Abstract—The species diversity of oligochaetes and their spatial distribution have been studied in three lakes of the Naroch system (Belarus) differing in trophic status. The results show that, irrespective of lake trophic status, the abundance, biomass, and species diversity of oligochaetes decrease toward the profundal zone. The species composition of oligochaetes is most diverse in the littoral zone of an oligomesotrophic lake. Dominant species of the subfamily Tubificinae show preferences for certain types of lake biotopes.

Keywords: Oligochaeta, spatial distribution, biotope preference, lake trophic status, Naroch lakes

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The pattern of spatial distribution of species depends on the geographic and ecological factors of the environment, biological characteristics of species, and their properties [1]. Natural abiotic factors influencing the diversity and distribution of invertebrates in the benthal include morphometric characteristics of a water body—its size, depth, and type of bottom ground [2–5]—and the distribution of organic matter constituting an accessible food resource [6].

Oligochaetes are one of the most abundant and diverse groups in the benthic communities of different types of water bodies [7–9]. Their biomass is an important link in the trophic net, as they serve as food for fishes and invertebrates [10]. Their low mobility, long life cycle and dependence on substrate make them sensitive to the influence of environmental conditions, mainly of bottom sediments. Therefore, oligochaetes are regarded as the most informative group for studies on pollution of water bodies [12]. Changes in the structure of their communities provide information about trophic variability of the environment over long periods of time [13].

A large amount of data on the hydrology, morphology, and biology of lakes included into the Naroch system (Narochanskie lakes)—Naroch, Myastro, and Batorino [14, 15, etc.]—makes them a convenient model for comparative research on the distribution of oligochaete species in contrasting trophic conditions. Long-term hydrobiological monitoring of these lakes has been performed, with its purposes including evaluation of the dynamics of qualitative parameters characterizing the development of macrozoobenthos [15]. The first survey of the oligochaete fauna in Belarusian lakes was performed by Sokol'skaya [16] in 1947–1948. She described 38 oligochaete species in eight lakes, including 24 species from Lake Naroch. Species lists of oligochaetes from lakes Naroch, Myastro, and Batorino were subsequently amended by N.P. Finogenova in 1972 and C.I. Gavrilov in 1978. The authors noted specific features of the vertical distribution of oligochaetes in some lakes, such as the expansion of many species to deep-water zones and lower parameters of oligochaete biomass, compared to those in lakes of the Russian Plain [14].

The purpose of our work study was assess the composition, species richness, and abundance of oligochaetes as one of the dominant groups of zoobenthos in lakes of different trophic status and reveal the patterns of their spatial distribution in these lakes based on long-term data.

MATERIAL AND METHODS

Studies on benthic communities in Narochanskie lakes were started in 1947, but it is only since 1997 that observations on the zoobenthos of lakes Naroch (54°51′18.24″ N, 26°46′1.03″ E), Myastro (54°52′0.91″ N, 26°52′49.86″ E), and Batorino (54°50′47.94″ N, 26°58′3.36″ E) have been performed on a regular basis (two to three times a year) and by the standard procedure [15, 17]. The research program in some years is supplemented by additional sampling from different

Table 1. Characteristics of biotopes in lakes of the Naroch system

Characteristic	Littoral zone	Sublittoral zone		Profundal zone
Lake Naroch	l_N	sbl_N_1	sbl_N_2	pr_N
Depth, m	0.1–2.0	2.1–8.0	2.1–8.0	8.1–over 25.0
Bottom ground	Light sand, slightly silty	Light silt	Light sand, slightly silty; clay (in places)	Dark olive silt
S_{bottom}, %	13.6	31.6		54.81
Macrophytes	Chara algae (sparse)	Chara algae (dense), waterweeds, hornwort (sparse)	Chara algae (sparse)	Absent
Lake Myastro	l_M	sbl_M		pr_M
Depth, m	0.1–2.0	2.1–4.0		4.1–over 11.0
Bottom ground	Light sand, moderately silty	Dark silt, shell ground		Dark olive silt
S_{bottom}, %	15.02	15.79		69.18
Macrophytes	Pondweeds, waterweeds, green hair algae, water-crowfoot, chara algae (sparse)	Waterweds, hornworts (sparse)		Absent
Lake Batorino	l_B	sbl_B		pr_B
Depth, m	0.1–1.0	1.1–2.0		2.1–over 5.0
Bottom ground	Gray sand, highly silty; detritus	Dark silt, detritus, peat		Dark olive silt, peat
S_{bottom}, %	14.44	16.41		69.15
Macrophytes	Chara algae (sparse)	Pondweeds (sparse)		Absent

biotopes in the littoral and profundal zones of the lakes.

Oligochaetes were selected directly from samples of macrozoobenthos. The collected material was fixed in 4% formaldehyde, and preparations embedded in glycerol were analyzed under a Micromed MC-4-ZOOM LED stereomicroscope and a MIKMED-6 microscope. Species identification was performed in accordance with [18, 19]. Some oligochaetes were not identified to the specie level, including of the family Enchytraeidae and juvenile Tubificinae. They were taken into account in calculating the average abundance and biomass of oligochaetes, but were excluded from the calculation of diversity indices based on the species composition of the group. Special faunistic samples were also collected in the littoral zone of Lake Naroch in 2018 using various techniques (a hydrobiological scraper or washings from dense grounds and plants). Meiobenthic oligochaete species reveled in this course were not included in quantitative analysis.

The three lakes (Fig. 1) differ in morphological (depth, surface area), physical, and chemical parameters [14]. Their trophic status, evaluated based on Carlson's trophic state index (TSI) [20], has remained unchanged since 1997 and was determined as follows: Lake Naroch, oligomesotrophic; Lake Myastro, mesotrophic; Lake Batorino, eutrophic [20–22]. Water depth in the profundal zones of the lakes varied within the range of 5.5–20.0 m, reaching a maximum of about 25 m in Lake Naroch; in the littoral zones, within the range of 0.1–2.0 m. Different biotopes in each lake were distinguished based on analysis of water depth and the type of bottom ground (Table 1).

For quantitative characterization of the oligochaete community in different lakes and biotopes, we calculated the occurrence frequency, total and average abundance for the group as a whole and for each individual species, and their total and average biomass [23]; the index of dominance by biotopes (D, %) to estimate the significance of individual species in biocenosis and identify the complexes of oligochaetes [24, 25]; and the index of biotope preference F_{ij} to estimate the degree of species selectivity in choosing a biotope [26, 27]. The biological diversity of communities (alpha-diversity) was evaluated using conventional parameters: species density, Shannon's diversity index (H_N), Simpson's index (D_S), Margalef's index of species richness (d), and similarity indices by Jaccard (I_J) and Czekanowski–Sørensen (I_{CS}, for quantitative samples only) [26]. The data were processed statistically and visualized in the R environment (https://www.R-project.org/) using Statistica 6.0 для Windows (StatSoft Inc.). Cluster analysis

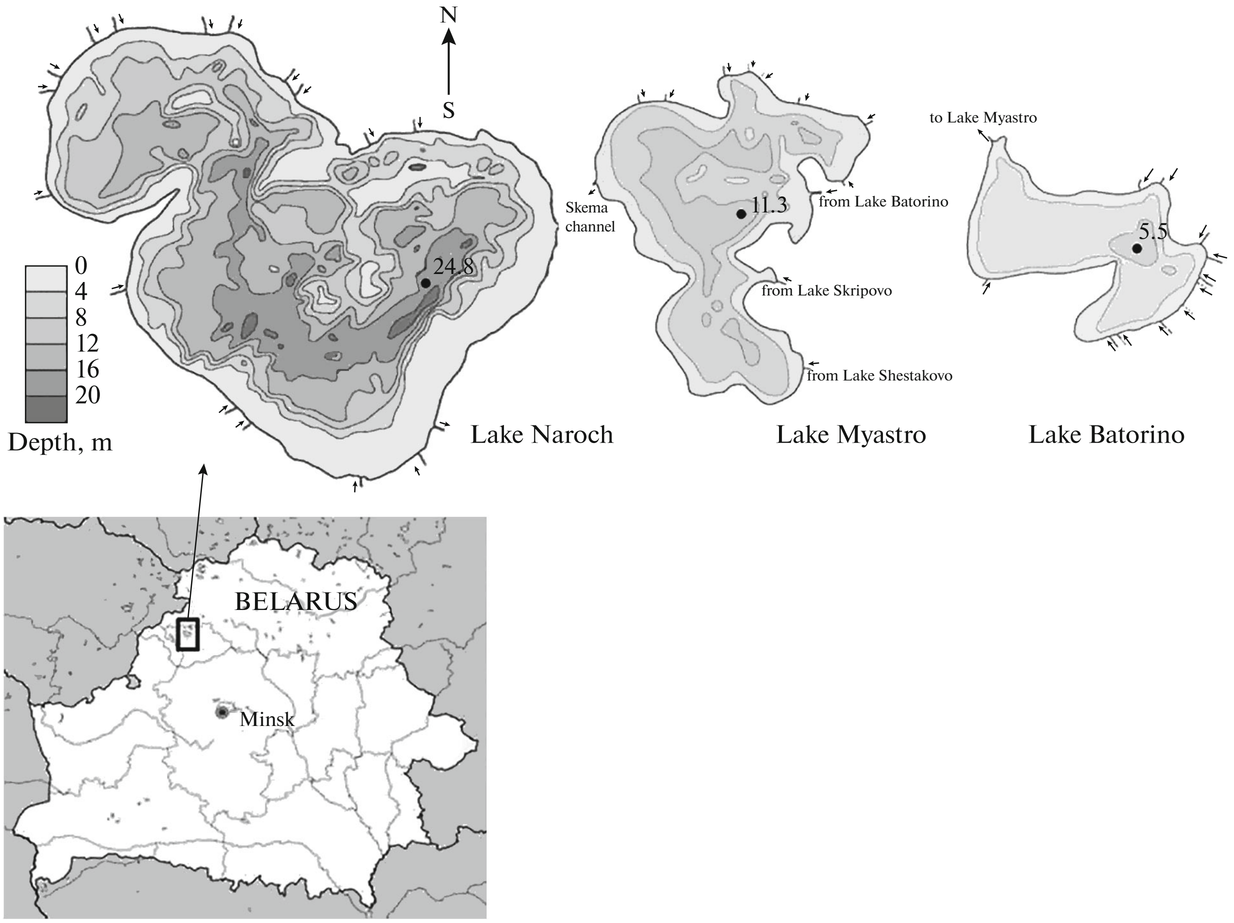

Fig. 1. Schematic map of three lakes of the Naroch system.

with Euclidean distance as the metric was performed by Ward's method. Dendrograms were plotted based on the initial parameters of abundance and species composition.

RESULTS AND DISCUSSION

Quantitative Parameters of Oligochaete Development

Oligochaetes play a prominent role in the benthic communities of Narochanskie Lakes. Since the beginning of regular monitoring in 1997 [17, 28], they have been found in more than 50% of hydrobiological samples. They account for up to 35.9% of the total abundance of the benthos and up to 15.8% of its total biomass. The highest average values of these parameters were recorded in mesotrophic Lake Myastro and oligomesotrophic Lake Naroch, and the lowest values, in eutrophic Lake Batorino (Fig. 2).

A statistically significant increase in the quantitative parameters of oligochaete development against the background of decrease in the values of TSI index, concentration of nutrient elements and changes in certain of hydroecological parameters of lakes (water transparency; nitrogen, phosphorus and carbon concentrations; suspended matter content, chlorophyll concentration, BOD_5, electrical conductivity) was revealed in the series Batorino (eutrophic, TSI 56.89 ± 2.71)—Myastro (mesotrophic, TSI 40.74 ± 1.24)—Naroch (oligomesotrophic, TSI 32.36 ± 1.35) [28]. This relationship between the trophic status of the lake and the diversity and biomass of oligochaetes is confirmed by the published data [9] according to which mesotrophic water bodies are more favorable for the development of these worms in comparison with eutrophic due to better oxygen conditions.

Analysis of the distribution of quantitative parameters of oligochaete development over lake water areas showed that their abundance and biomass reached the highest values at depths not exceeding 1 m in Lake Batorino, 2 m in Lake Myastro, and 4 m in Lake Naroch, decreasing significantly at greater depths. It has been noted [17] that higher aquatic vegetation in Lake Batorino covers 19.6% of the total bottom area to a depth of 1.5 m; the depth limit for the growth of macrophytes in Lake Naroch is 7 m (about 42.0% of the total bottom area); in Lake Myastro, where water transparency is lower, macrophytes spread to a depth of 4–5 m, covering more than 30.0% of the bottom.

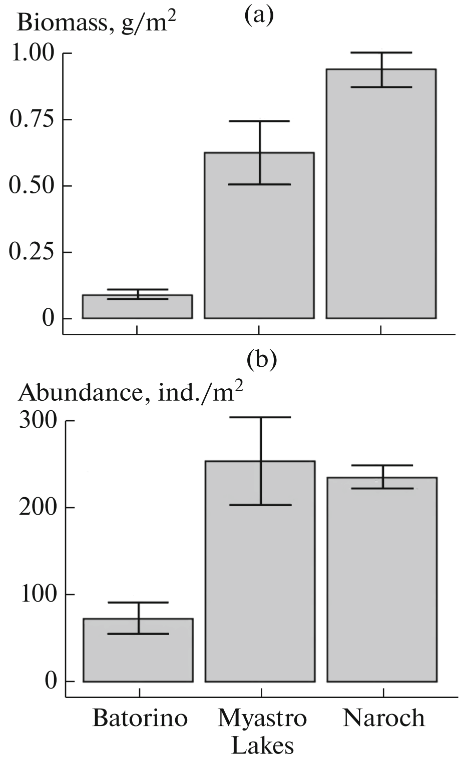

Fig. 2. Quantitative parameters of oligochaete development in three lakes of the Naroch system.

Conditions in the zone of macrophyte growth are most favorable for most taxa of aquatic invertebrates, including oligochaete species with various ecological preferences [11, 29]. The depth of the lake also has a significant influence on the biomass of invertebrates, oligochaetes in particular [30, 31]. Relatively high biomass values against the background of relatively low abundance of oligochaetes in the profundal zone is often explained by predominance of large tubificid worms in silted grounds of deep-water biotopes. In our studies, however, species of the subfamily Tubificinae accounted for 75 to 100% of the total oligochaete abundance in the macrozoobenthos of all zones of the lakes. Therefore, it appears that the decrease in the total abundance of worms from the littoral to the profundal zone (see Fig. 3) is a probable explanation to the general decrease in their biomass in the profundal zones of all lakes, regardless of their trophic status.

Species Composition of Oligochaetes

According to published data [14, 16] and the results of our studies, the fauna of Oligochaeta in lakes of the Naroch system includes a total of 48 species and supraspecific taxa, with 33 of them occurring in recent samples (1997–2018). Representatives of the subfamily Tubificinae comprise more than 30% of the present-day fauna. Since 1997, only five species (15.6% of the fauna) has occurred at different frequencies in samples from all the three lakes. These are *Limnodrilus hoffmeisteri* Claparède, 1862, *L. udekemianus* Claparède, 1862, *Potamothrix hammoniensis* (Michaelsen, 1901), *Psammoryctides barbatus* (Grube, 1861), and *Lumbriculus variegatus* (Müller, 1774). In previous studies [14, 16]. the proportion of species (including meiobenthic) common to all the lakes was estimated at 47%.

Analysis of quantitative and qualitative samples taken from oligomesotrophic Lake Naroch in 1997 to 2018 revealed 27 species and forms of oligochaetes. Four of them occurred only in this lake: *Lophochaeta ignota* (Štolc, 1886), *Spirosperma ferox* Eisen, 1879, *Rhynchelmis limosella* Hoffmeister, 1843, *Uncinais uncinata* (Ørsted, 1842). The authors of previous studies [14, 15] recorded 39 species in Lake Naroch. Thus, the similarity of the faunas between the periods was about 70%.

Recent hydrobiological samples from mesotrophic Lake Myastro contained 11 species, with single specimens of *Potamothrix bedoti* (Piguet, 1913), *Dero digitata* (Müller, 1774), and *Tubifex newaensis* (Michaelsen, 1903) occurring only in this lake. In the previous period [14], there were 32 species on the list, but they incuded highly diverse meiobethic forms of oligochaetes, which are not taken into account in monitoring studies by modern quantitative methods.

The present-day fauna of eutrophic Lake Batorino was represented by 10 species, including *L. claparedeanus* Ratzel, 1868, *L. profundicola* (Verrill, 1871), and *Isochaetides michaelseni* (Lastočkin, 1936) occurring only in this lake. Among 25 species recorded previously [14], more than 60% were meiobethic forms.

The similarity of present-day species composition of oligochaetes between macrozoobenthos samples from the three lakes was 60%.

Spatial Distribution of Oligochaete Species

The distribution of species over the water area previously described in oligomesotrophic Lake Naroch was uneven, with dominance of naidid worms in the littoral zone and their gradual replacement by tubificids at greater depths; *L. udekemianus*, *Psammoryctides albicola* (Michaelsen, 1901), and *L. variegatus* were indicated as prevailing species [14, 16]. In the recent study period, the dominant species complex included *P. albicola* (35% of the total abundance), *L. variegatus* (19%), and *P. barbatus* (17%) (Table 2). The composition of dominant species in the littoral (l_N) and sublittoral zones (sbl_N_1, sbl_N_2) was similar but sharply differed from that in silty grouds of the profundal zone (pr_N). The index of species diversity of oligochaetes in Lake Naroch was higher than in other lakes ($H_N = 0.98 \pm 0.1$), with its value decreasing from the littoral to the profundal zone along with decrease in the number of species (see Table 2) and species den-

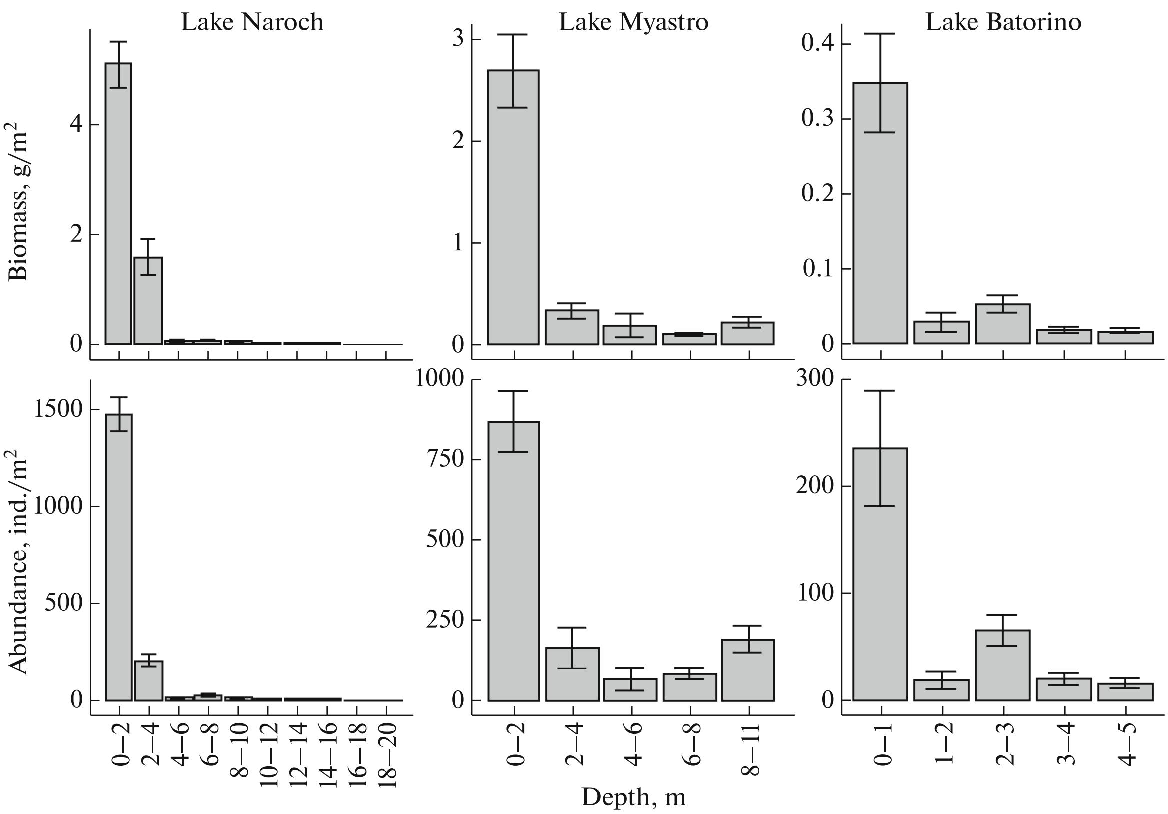

Fig. 3. Abundance and biomass distribution of oligochaetes in relation to lake depth.

sity (from 4.5 ± 0.4 in the littoral to 1.3 ± 0.3 in the profundal zone).

The degree of biotope preference in dominant oligochaete species was evaluated based on the proportion of a species in the community (Table 3). A high degree of preference for biotopes of the littoral zone was estimated for *P. barbatus*, and *S. ferox* also occurred only in this zone. Both *L. udekemianus* and *P. albicola* showed preference for the sublittoral zone, but for different biotopes: sandy grounds (sbl_N_2) were preferable for *L. udekemianus*, while *P. albicola* preferred fine detrital silts (sbl_N_1). Finally, the highest index of preference for the profundal zone was estimated for *P. hammoniensis*.

The group of species currently dominating in mesotrophic Lake Myastro include *L. hoffmeisteri* (22% of the total oligochaete abundance), *P. barbatus* (19%), and *P. hammoniensis* (15%) (Table 2). This lake is on the second place with respect to the species diversity of the group (H_N = 0.77 ± 0.12). However, although Shannon's index decreased in the profundal zone, the number of species only slightly changed with depth, in contrast to species density that decreased from 3.1 ± 0.3 in the littoral to 1.5 ± 0.2 in the profundal zone. In Lake Myastro, high preference for sand–silt grounds overgrown by macrophytes in the littoral zone (l_M) was revealed for *L. hoffmeisteri* and *L. udekemianus* (Table 3); silty grounds in the sublittoral zone (sbl_M) were preferable for *L. variegatus* and *Tubifex tubifex* (Müller, 1774); and *P. hammoniensis* was the sole dominant species with a high index of preference for in the profundal zone (pr_M).

The most abundance group of species in our samples from eutrophic Lake Batorino included *L. hoffmeisteri* (34% of the total oligochaete abundance), *P. hammoniensis* (17%) and juveniles of the subfamily Tubificinae (24%), supposedly *P. hammoniensis* (Table 2). Shannon's diversity index has lower than in the other two lakes (H_N = 0.55 ± 0.13), and the index of species density also decreased from 2.1 ± 0.3 in the littoral to 1.2 ± 0.2 in the profundal zone. Higher preference for the littoral zone thinly overgrown by chara algae (l_B) was estimated for *P. albicola, L. variegatus,* and *L. udekemianus*; dark silts in the sublittoral zone (sbl_B) were preferable for *L. hoffmeisteri*; and *P. hammoniensis* in this lake also showed high preference to deep-water biotopes with silty grounds in the profundal zone (pr_B) (Table 3).

In the dendrogram of similarity in the species composition of oligochaete fauna (Fig. 4), all biotopes are grouped into two clusters: (1) the littoral and sublittoral zone and (2) the profundal zone. This confirms our asumption that the profundal zone in all lakes is faunistically distinct fromrelatively shallow zones. Within

Table 2. Numbers of species in quantitative samples (*n*), dominant species (D), and indices of species diversity for the oligochaete community in lakes of the Naroch system: H_N, Shannon's diversity index; *d*, Margalef's species richness index; D_S, Simpson's dominance index

Lake Naroch	l_N	sbl_N_1	sbl_N_2	pr_N
n	14	3	7	2
D	***L. variegatus*** ***P. albicola*** *L. hoffmeisteri* *P. barbatus* *S. ferox*	***P. albicola*** *L. variegatus* *L. hoffmeisteri*	***L. variegatus*** ***L. udekemianus*** *L. hoffmeisteri* *P. albicola*	***L. hoffmeisteri*** ***P. hammoniensis***
H_N, bit/ind.	1.29 ± 0.1	0.35 ± 0.1	0.51 ± 0.2	0.25 ± 0.25
d	0.9 ± 0.1	0.31 ± 0.1	0.39 ± 0.04	0.36 ± 0.36
D_S	0.5 ± 0.03	0.83 ± 0.06	1.0 ± 0.1	0.88 ± 0.1
Lake Myastro	**l_M**	**sbl_M**		**pr_M**
n	8	6		6
D	***L. hoffmeisteri*** *P. barbatus* *P. hammoniensis* *L. udekemianus*	***L. hoffmeisteri*** *T. tubifex*		***P. hammoniensis***
H_N, bit/ind.	1.09 ± 0.2	–		0.50 ± 0.2
d	0.69 ± 0.1	–		0.41 ± 0.2
D_S	0.69 ± 0.1	1		0.78 ± 0.07
Lake Batorino	**l_B**	**sbl_B**		**pr_B**
n	10	1		2
D	***L. hoffmeisteri***	***P. hammoniensis***		***P. hammoniensis***
H_N, bit/ind.	0.72 ± 0.2	–		0.16 ± 0.1
d	0.59 ± 0.15	–		0.07 ± 0.07
D_S	0.64 ± 0.08	1		0.92 ± 0.08

Bold italic type indicates dominant species (100 > *D* > 10); light italic type, subdominant species (10 > *D* > 1).

Table 3. Indices of preference for different biotopes (F_{ij}) of dominant Oligochaeta species

Species	Lake Naroch				Lake Myastro			Lake Batorino		
	l_N	sbl_N_1	sbl_N_2	pr_N	l_M	sbl_M	pr_M	l_B	sbl_B	pr_B
L. hoffmeisteri	0.2	–0.2	0.1	0.4	**0.6**	0.03	–1.0	–0.2	**0.7**	–1.0
L. udekemianus	0.5	–1.0	**0.8**	–1.0	**1.0**	–1.0	–1.0	**1.0**	–1.0	–1.0
L. variegatus	0.01	0.0	0.3	–1.0	–0.6	**0.9**	–1.0	**1.0**	–1.0	–1.0
P. hammoniensis	–1.0	–1.0	0.3	**0.9**	–0.5	–0.7	**0.7**	–0.8	–1.0	**0.9**
P. albicola	0.1	**0.5**	–0.5	–1.0	–	–	–	**1.0**	–1.0	–1.0
P. barbatus	**1.0**	–1.0	–1.0	–1.0	**1.0**	–1.0	–1.0	**1.0**	–1.0	–1.0
S. ferox	**1.0**	–1.0	–1.0	–1.0	–	–	–	–	–	–
T. tubifex	–0.3	–1.0	–1.0	**0.9**	–0.3	**0.7**	–0.7	–	–	–

High values of the preference index are in boldface. Its values may vary from –1 (the species is absent in a given biotope) to 1 (the species occurs only in a given biotope); $F_{ij} = 0$, the species is indifferent to the biotope (neither prefers nor avoids it); $F_{ij} < 0$, the species avoids the biotope; $F_{ij} > 0$, the species prefers the biotope [26, 27].

cluster 1, species compositions of littoral biotopes in relatively shallow lakes Myastro and Batorino are close to each other, and the second pair is formed by species in biotopes overgrown by macrophytes deeper Lake Naroch. Likewise, two large clusters are in the dendrogram based on the abundance of species (Fig. 4b). Oligochaete communities of overgrown littoral biotopes in lakes Myastro and Naroch (cluster 4) are probably grouped together due to relatively large numbers of dominant species in these biotopes (see Table 2).

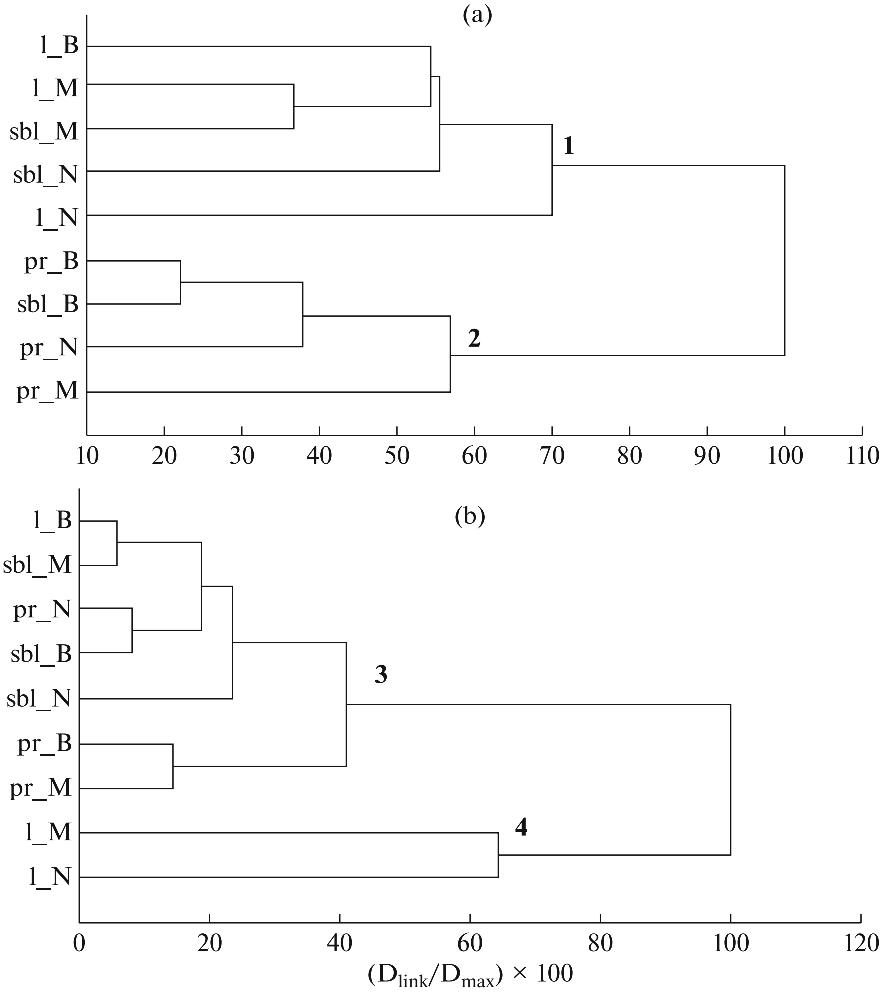

Fig. 4. Dendrogram of similarity in (a) qualitative composition and (b) species abundance of oligochaetes in different zones of lakes of the Naroch system (for characteristics and designations of zones see Table 1).

The pattern of dominance in these littoral zones is similar to some extent, since *L. variegatus, P. albicola, L. hoffmeisteri,* and *P. barbatus* are the most abundant species there. Within cluster 3, communities of biotopes with relatively small number and low abundance of species combine with each other: the thinly overgrown littoral zone of Lake Batorino with the sublittoral zone of Lake Myastro; the silted profundal zone of Lake Batorino with that in Lake Myastro; and the quantitative composition of species in the sublittoral zone of Lake Batorino is close to that in the profundal zone of Lake Naroch.

Thus, the littoral zones of all lakes are characterized by relatively high alpha-diversity expressed in species density, the highest values of diversity index and the lowest level of dominance (minimum $D_S = 0.5$). The profundal zones of all lakes are characterized by highly uniform conditions, regardless of trophic status. They are inhabited by a monodominant oligochaete community (with the highest D_S value) consisting of *P. hammoniensis,* and the index of species diversity for this biotope is the lowest. The specific separation of zones is manifested not only in the greater abundance and diversity of oligochaetes in littoral biotopes but also in the differences between the models of dominance. Species selectivity in choosing a biotope is also confirmed by the coefficient of biotope fidelity (*w*) [32], which characterizes biotopic specificity against the background of general level of species abundance and its variation between years.

The biological meaning of biotope fidelity is in the constancy of the species' presence in a particular habitat, which can be estimated from long-term data on its occurrence. Using such data available for the dominant oligochaete species, conditions they prefer have been identified (Table 4): the complex *L. variegatus*–*P. albicola*–*P. barbatus*–*S. ferox* has developed in sandy biotopes of the littoral zone, with the coefficient of biotope fidelity for these species being the highest in the oligomesotrophic lake; *L. hoffmeisteri* selectively

Table 4. Ecological characteristics of dominant Oligochaeta species in lakes of the Naroch system

Species	Coefficients of biotope fidelity (*w*)			Preferred biotope			
	Lake Naroch	Lake Myastro	Lake Batorino	biotope	depth, m	bottom ground	plants
L. hoffmeisteri	–0.41	**–0.24**	–0.67	l_M, sbl_B	0.0–2.0	s, sd, d, p	Pondweeds
L. udekemianus	**0.07**	–0.40	–1.94	sbl_N_2	2.1–8.0	s, sd	Chara algae
L. variegatus	**1.41**	–0.88	–0.80	l_N	0.1–2.0	s, sd	Chara algae
P. hammoniensis	0.01	**0.53**	–0.26	pr_N, pr_M	4.0–20.0	silt	Absent
P. albicola	**2.29**	–1.54	–1.03	l_N	0.1–2.0	s, sd	Chara algae
P. barbatus	**0.41**	–1.04	–2.23	l_N	0.1–2.0	s, sd	Chara algae
S. ferox	**0.18**	–0.12	–0.08	l_N	0.1–2.0	s, sd	Chara algae
T. tubifex	–0.05	**0.18**	–0.08	sbl_M	2.1–4.0	silt	Waterweeds (sparse)

High positive values of coefficient *w* (boldfaced) indicate species fidelity to a biotope; negative values, avoidance of a biotope [32]. Bottom ground: s, sand; sd, silt deposit; d, detritus; p, peat.

populates sand–silt soils overgrown by pondweed at depths of no more than 2 m; *T. tubifex* shows high selectivity toward silts at depths of 2 to 4 m; and silts at depths of 4 to 20 m are selectively populated only by *P. hammoniensis*, which also appears to prefer this biotope in the lake with mesotrophic status to those in the other two lakes.

In the literature [33, 34], the optimums for the majority of species dominating in the lakes studied are associated with eutrophic conditions. This inconsistency may be due to the absence of long-term data series in some studies. It is also not entirely clear to what extent the well-used terms “oligotrophic, mesotrophic and eutrophic lakes” correspond the actual trophic status of lakes determined on the basis of Carlson Index (TSI). This issue is discussed in studies on determining the ecological optimum for the species [34].

CONCLUSIONS

The results of this study show that the trophic status of lakes has an effect on quantitative parameters of oligochaete development, which decrease in the series oligomesotrophic–mesotrophic–eutrophic lake and on the composition of the oligochaete fauna, which is least diverse in the eutrophic lake. However, regardless of trophic status, the distribution pattern of these parameters in the lakes proved to be similar: the abundance and biomass of worms decreased in all lakes from the littoral to the profundal zone; the composition of species in the littoral zone of lakes with different trophic status was the most diverse and distinguished by the highest species density. Biotopic preferences and high selectivity toward certain biotopes in lakes of different trophic status were revealed for a series of oligochaetes dominating in lakes of the Naroch system.

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COMPLIANCE WITH ETHICAL STANDARDS

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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

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